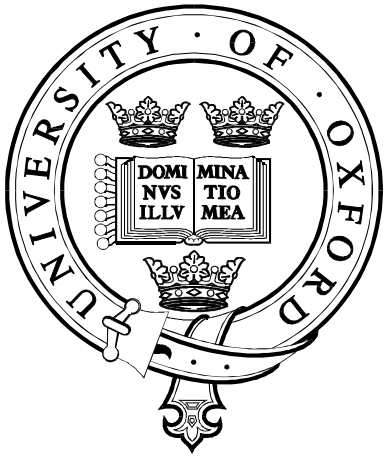
COMPETITIVE STRUCTURE AND THE OPERATION OF SEXUAL SELECTION



GRANT COLIN MCDONALD
ST CATHERINE'S COLLEGE
UNIVERSITY OF OXFORD

A thesis submitted for the degree of
Doctor of Philosophy
Trinity 2014

ACKNOWLEDGEMENTS

Firstly I would like to extend my gratitude to my supervisor Tommaso Pizzari, from whom I have learnt a great deal, for his patience, encouragement and guidance throughout my DPhil. I would like to Sozos Michaelides, Cedric Tan, Rosie Eccleston, Aitor Alvarez Fernandez, Stephen Georgiou, Jarrod Haddfield, Andy Gardner, Kellie Rance, Susannah Dart, Rachel Shephard, Tom Bregman, David Wilson and all other friends and colleagues who have all helped and supported me in a variety of ways. I thank my family for getting me here. A special thank you to Melanie Ghoul who was there from the very start to the very end. Finally, I am grateful to all the chickens that took part in this work, especially to the females, who are my favourites.

This thesis was funded by a CASE Scholarship between BBSRC and the industrial partners Aviagen®.

CONTENTS

THESIS ABSTRACT 4

CHAPTER 1 – INTRODUCTION 5-23

CHAPTER 2 – THE DETERMINANTS OF THE SOFTNESS OF SEXUAL SELECTION: IMPLICATIONS FOR STRUCTURED POPULATIONS 24-43

CHAPTER 3 – SEXUAL NETWORKS: MEASURING SEXUAL SELECTION IN STRUCTURED POLYANDROUS POPULATIONS 44-70

CHAPTER 4 – MATING BEHAVIOUR: SEXUAL SELECTION AND SEXUAL NETWORKS 71-96

CHAPTER 5 – POLYANDRY, MATING STRUCTURE AND THE DETERMINATION OF SEXUAL SELECTION 97-149

CHAPTER 6 – THE STRUCTURE OF SEXUAL NETWORKS IN RED JUNGLEFOWL (*GALLUS GALLUS*) AND ITS IMPLICATIONS FOR SEXUAL SELECTION 150-187

CHAPTER 7 – POSTCOPULATORY SEXUAL SELECTION AND THE ROLE OF COMPETITIVE ENVIRONMENT IN RED JUNGLEFOWL (*GALLUS GALLUS*) 188-215

CHAPTER 8 – ENVIRONMENTAL VARIATION AND ITS IMPLICATIONS FOR MATING SYSTEMS, SEXUAL SELECTION AND FEMALE FECUNDITY IN RED JUNGLEFOWL (*GALLUS GALLUS*) 216-277

CHAPTER 9 - DISCUSSION 278-288

REFERENCES 289-312

FULL AUTHOR LIST AND AUTHOR CONTRIBUTIONS

Grant C McDonald (GCM)
Tommaso Pizzari (TP)
Richard James (RJ)
Jens Krause (JK)
Dave Richardson (DR)
Lewis Spurgin (LS)
Ellie Fairfield (EF)

Chapter 2 The determinants of the softness of sexual selection and its implications for structured populations

- GCM conceived the idea and wrote the chapter.

Chapter 3 Sexual networks: measuring sexual selection in structured polyandrous populations

- GCM and TP conceived the idea, GCM led the simulation work in collaboration with DJ and conducted all statistical analyses. GCM wrote the paper in collaboration with DJ, JK and TP.

Chapter 4 Mating behaviour: Sexual selection and sexual networks

- GCM and TP conceived the idea, GCM led and designed simulations with TP, conducted all simulations and statistical analyses and wrote the chapter in collaboration with TP.

Chapter 5 Polyandry, mating structure and the determination of sexual selection

- GCM conceived the idea, GCM led and designed simulations in collaboration with TP, conducted all simulations and statistical analyses and wrote the chapter in collaboration with TP.

Chapter 6 The structure of mating networks in Red junglegowl (*Gallus gallus*) and its implications for sexual selection

- GCM and TP conceived the idea, GCM led the research collected all behavioural data and tissue samples, carried out all statistical analyses and wrote the chapter in collaboration with TP. DR, LS and EF designed and conducted all molecular work and parentage assignment.

Chapter 7 Postcopulatory sexual selection and the role of competitive environment in Red junglefowl (*Gallus gallus*)

- GCM conceived the idea, GCM led the research, collected all behavioural data and tissue samples, carried out all statistical analyses and wrote the chapter in collaboration with TP. DR, LS and EF designed and conducted all molecular work and parentage assignment.

Chapter 8 Environmental variation and its implications for mating systems, sexual selection and female fecundity.

- GCM and TP conceived the idea. GCM led the research, GCM designed experiments with TP, collected all behavioural data and tissue samples, carried out all statistical analyses and wrote the chapter in collaboration with TP. DR, LS and EF designed and conducted all molecular work and parentage assignment.

THESIS ABSTRACT

In this thesis I set out to further our understanding of two broad questions, 1) why it is that sexual selection favours the evolution of particular traits and 2) why do the patterns of sexual selection on such traits differ between groups and populations? Specifically, I focused on the role that variation in intrasexual competitive structure, the non-random distribution of socio-competitive environments across individuals, plays in shaping variation in the operation of sexual selection both within and across populations. I explore the roles of three main determinants of competitive structure, namely; population structure, polyandry and non-random variation in the distribution of the intensity of competition. To achieve this, I used a combination of empirical and theoretical tools, using the model system Red junglefowl, *Gallus gallus*. Throughout this thesis I both develop and employ network quantitative tools as a framework to describe variation in intrasexual competitive structure. Overall, this thesis demonstrates a complex relationship between competitive structure and the operation of sexual selection. This structure can modify the strength and direction of sexual selection operating on phenotypic traits, obscure the operation of selection at the population level and influence the relative roles of pre- and post-copulatory sexual selection. Furthermore, this thesis explores how differences in local ecology can shape competitive structure itself and in turn shape sexual selection. In doing so, this thesis sheds some light on the role that variation in competitive structure may play in shaping the operation of sexual selection both within and between populations and generating the great diversity in sexually-selected traits and behaviours between populations.

CHAPTER 1. INTRODUCTION

"*Our difficulty in regard to sexual selection lies in understanding how it is that the [individuals] which conquer other [rivals], or those which prove the most attractive to the [the opposite sex], leave a greater number of offspring to inherit their superiority than their beaten and less attractive rivals*"
p-567

"*many male birds are highly pugnacious, and some are furnished with special weapons for fighting with their rivals […] They are frequently ornamented with combs, horns, wattles and plumes of the most diversified kinds, and are decorated with beautiful colours, all evidently for the sake of display […] In other groups both sexes are equally plain-coloured and unornamented […] We shall often find, in the same group of birds, every gradation from no difference between the sexes, to an extreme difference."*
p-868

Darwin, Charles (1871). *The Descent of Man, and Selection in Relation to Sex*.

SEXUAL SELECTION

Sexual selection is the selective process that operates on variation in reproductive success generated by competition between members of one sex over access to the gametes of the opposite sex (Darwin 1871; Andersson 1994). Due to the sex difference in the rate of gametogenesis, sexual selection is typically thought to act more strongly in males, however the definition above may apply equally to both sexes (Bateman 1948; Shuster and Wade 2003; Kokko et al. 2012). Originally, intrasexual competition was thought to be restricted to competition before mating, over the number and quality (i.e. fecundity) of mating partners (precopulatory sexual selection) through either direct male-male competition or via female choice (intersexual choice) (Darwin 1871; Eberhard 2009; Parker and Birkhead 2013). However, it is now well established that intrasexual competition can also occur after copulation, driven by the realization that when females mate multiply (polyandry), the ejaculates of several males can compete to fertilise female ova (postcopulatory sexual selection) (Parker 1970; Eberhard 2009). Postcopulatory sexual can similarly operate

via direct male-male competition (i.e. sperm competition, Parker 1970; Parker and Pizzari 2010) and intersexual mechanisms (cryptic female choice, Childress and Hartl 1972; Thornhill 1983). Sexual selection is now recognized as a fundamental force in evolution, driving the evolution of extravagant secondary sexual characters, speciation and as a driver of conflict over reproductive decisions between the sexes (Andersson 1994; Higashi et al. 1999; Boughman 2001; Chapman et al. 2003; Shuster and Wade 2003; Seddon et al. 2008, 2013).

Despite an incredible wealth of research, nearly 150 years since the publication of Darwin's *The Descent of Man*, the observations at the beginning of this chapter highlight two of the fundamental questions that remain unresolved, namely; 1) How and why particular traits confer a reproductive advantage in intrasexual competition? and 2) Why patterns of sexual selection on such traits differ between groups, populations and species? Sexual selection is fundamentally a form of sex-specific social competition because the reproductive success of individuals depends not only on their own competitive ability but on the competitive ability of those individuals with which they compete, and the success of one individual necessarily results in the failure of others (i.e. fertilization of a gamete by one individual necessarily removes the opportunity for fertilization by another). Therefore, variation in the mechanisms and patterns of intrasexual competition may have knock-on effects for the operation of sexual selection and subsequent phenotypic evolution. Research aiming to understand why some traits predict reproductive success and others not, and why sexual selection on such traits varies across groups and populations, should seek to understand how the pattern of intrasexual competition, the driving force of sexual selection, varies and how this modifies the strength and form of selection and the relative roles of pre- and post-copulatory sexual selection.

COMPETITIVE STRUCTURE

Competitive structure describes the organization of social competitive environments across conspecific individuals within a population. The competitive environment of an individual characterizes the number of competitors, the frequency of interactions and the distribution of phenotypes across that individual's rivals. In terms of sexual selection, there are a number of axes in which competitive structure can vary, and that are expected to have important implications for the operation of sexual selection, including: i) population structure, ii) polyandry, and iii) non-random variation in the intensity of competition.

I) POPULATION STRUCTURE

When interactions between individuals occur randomly across a population with respect to phenotypes, then populations are not structured. Population structure arises when individuals interact non-randomly. For example, restricted dispersal results in an increased similarity between neighbouring individuals (i.e. populations are viscous), leading to greater similarity/relatedness between locally competing conspecifics (e.g. Johannesen and Lubin 2001; Duncan et al. 2010). Alternatively, dispersal may not be limited but individuals may preferentially interact with conspecifics of specific phenotypes, such preferential interactions can arise either passively or actively (O'Hara and Blaustein 1985; Formica and Tuttle 2009; Oh and Badyaev 2010; Formica et al. 2011). For example, both experimental work and studies of natural populations have demonstrated that males may actively choose to associate with less attractive rivals that increase their own relative attractiveness (Oh and Badyaev 2010; Gasparini et al. 2013), although in practice it may often be a

challenge to differentiate between active and passive mechanisms (Formica and Tuttle 2009). Regardless, the result of both passive and active mechanisms is to either increase the similarity of locally competing individuals or alternatively increase the variation in phenotypes between competitors (Formica et al. 2011). Population structure can therefore drive variation in competitive environments both within and between populations. This non-random assortment of phenotypes can have strong implications for the evolutionary trajectories of populations. For example, imagine a population that is structured due to low dispersal. This may result in an increase in the relatedness between individuals within local groups. This increase in relatedness between interacting individuals may be expected to increase the potential for kin selected behaviours (i.e. altruism) to evolve by facilitating interactions between kin (Hamilton 1964a,b, 1971). Crucially, however, this expectation depends on the scale of competition. If, despite population structure, competition occurs globally then fitness will be governed by population-level competition and our expectations for social evolution may be correct. However, when competition occurs locally and therefore fitness is determined locally, this will result in kin competing strongly with each other within their local groups, cancelling out the potential benefits of cooperation and limiting the evolution of kin selected strategies (Queller 1992; Taylor 1992; West et al. 2002). This demonstrates the importance of competitive structure in shaping phenotypic evolution.

Another potentially important generator of non-random competitive environments arises via a process known as indirect genetic effects (IGEs). Traditionally, the expression of any trait is expected to be influenced not only by the expression of its genetic component (*a*) but also by environmental influences (*e*) (Falconer and Mackay 1996) (fig. 1a). Selection then operates on the relative fitness

(ω) associated with these phenotypes. Indirect genetic effects however, describe the effects of the genotype of one individual affecting the expression of a phenotype in another individual (Moore et al. 1997; Wolf et al. 1998) (fig, 1b). Consider a hypothetical example of individual aggressiveness. If an individual's level of aggression affects the level of aggression expressed by conspecifics then an increase in an individual's aggression may also increase the aggression of conspecifics. A change in focal phenotype will therefore cause changes in the social environment, and this effect can then feedback onto the focal phenotype, increasing the rate of evolutionary change. For example, in group-housed mink, *Neovison vison*, the tendency for focal individuals to display bite marks correlates with the tendency for interactants to similarly display bite marks (Alemu et al. 2014). This suggests that minks fight in a reciprocal way and a change in tendency to fight in either the focal individual or the social environment will have impacts for the trait expression in the other.

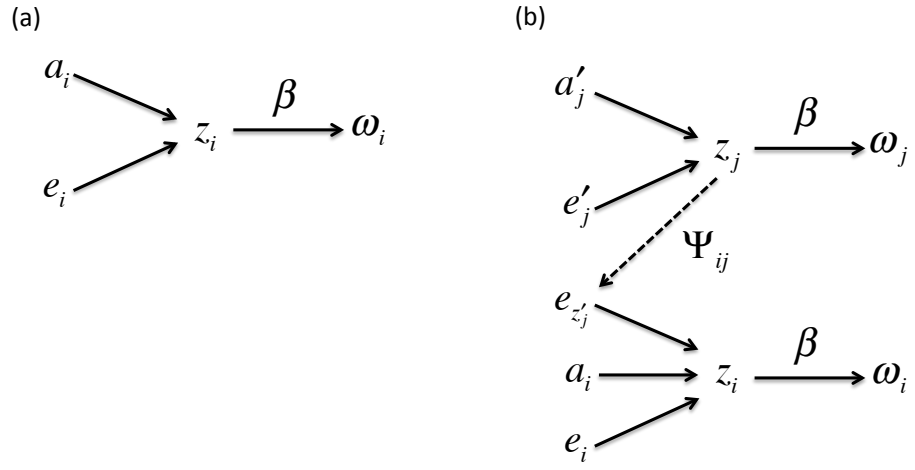


Figure 1[a] Shows the classic representation of the determination of a focal individuals phenotype (z) via additive genetic components (a) and environmental influences (e). [b] Shows how the phenotype of an interacting individual can modify the phenotype of another individual through the effect of its phenotype on the expression of the focal phenotype (Ψ). β represents selection and ω is the relative fitness of the individual.

Indirect genetic effects may have broad implications for trait evolution in structured populations (Agrawal et al. 2001) and for sexual selected traits (Bailey and Moore 2012). Firstly, because the phenotype of one individual influences the expression of a trait in another, indirect genetic effects can modify the covariance between the trait value of interactants, potentially increasing the phenotypic similarity between individuals even if interactions are initially random, therefore acting as a driver of competitive structure itself (Wolf et al. 1999). A second but important consequence of indirect genetic effects is their potential to vary the rate of evolutionary change between populations. This arises because changes in social environment feedback into changes in focal traits and this can accentuate or diminish the effect of selection on focal traits. Differences in the strength of indirect genetic effects (Ψ) across populations may drive variance in the rate of evolutionary change in sexually-selected traits across populations. For example, in the field cricket, *Teleogryllus oceanicus*, females of different populations responded differently to experimental manipulations of the social environment in terms of male calling rate (Bailey and Zuk 2012). When male calling rate was high, in some populations females increased choosiness whereas in other populations females decreased choosiness and *vice versa*. This example shows that variation in the competitive environment of males can modulate female behaviour, potentially generating strong differences in selection regimes between populations and inter-population divergence in traits.

II) POLYANDRY

Polyandry occurs when females mate with multiple males. Throughout this work I define polyandry as females mating with more than one male such that the

ejaculates of those males overlap at the time of fertilization (Pizzari and Wedell 2013). Polyandry is widespread throughout nature (Taylor et al. 2014) and not only affects the distribution of mating success across males but also determines the postcopulatory competitive environment (Collet et al. 2012; Kvarnemo and Simmons 2013). Importantly, polyandry varies both within and between populations (Taylor et al. 2014) and in doing so, polyandry can shape variation in population-level competitive structure between populations and groups, as well as the relative intensity of intrasexual competition in both pre- and post-copulatory episodes. For example, in a captive population of Red junglefowl, *Gallus gallus*, inter-group differences in average polyandry are associated with changes in the intensity of sperm competition, the patterns of phenotypic selection on male traits and the relative importance of postcopulatory sexual selection (Collet et al. 2012). Whereas in the harvestman, *Serracutisoma proximum*, variation in female polyandry within a population changes the intensity of sperm competition experienced by males expressing different alternative mating tactics (Muniz et al. 2014).

III) NON-RANDOM VARIATION IN THE INTENSITY OF COMPETITION

The intensity of competition that an individual faces describes the magnitude of competition, in terms of the number of competitors, with which an individual competes. Both across and within populations individuals are likely to vary in the number of intrasexual competitors, and such variation in the number of competitors may affect the operation of sexual selection, potentially changing its strength and direction on phenotypic traits (Krupa and Sih 1993). For example, in the myobatrachid frog, *Crinia georgiana*, among population variation in breeding male density correlates with variation in testes size, suggesting a role for local competitor

density to influence between-group patterns of selection on males due to increased risk and/or intensity of sperm competition (Dziminski et al. 2010). When variation in such intensity is spread non-randomly across phenotypes in the population (e.g. more attractive males must compete with more competitors), this may result in reduced or increased reproductive success for males of a given phenotype, and depending on whether they face consistently lower or higher competitive intensity, potentially accentuating or abating the evolution of those phenotypes.

This thesis focuses on how variation in the structure of male-male sexual competition, affects the operation of sexual selection and the implications for the methodology used to measure sexual selection.

RESEARCH APPROACHES AND A UNIFYING PERSPECTIVE: SEXUAL NETWORKS

Throughout this thesis I utilise the concept of sexual networks as descriptors of the patterns of sexual interactions within populations. I use this approach across a combination of theoretical tools and experimental research. Across both theoretical and empirical work I use a variety of approaches from quantitative sexual selection theory to estimate both the potential for sexual selection to operate and the strength of selection operating on phenotypic characters. Below I first outline i) the concept of sexual networks and then ii) the utility of using both theoretical and experimental approaches and finally, iii) introduce the quantitative selection theory and highlight the main approaches I use to quantify the potential strength of sexual selection.

I) SEXUAL NETWORKS

Within any population both inter- and intrasexual behaviours may create a complex system of interactions. This complexity can be captured as a web of sexual links, i.e. sexual networks. A sexual network is a collection of nodes (male and/or female individuals) connected by edges that may represent any behaviour of interest, such as copulations (fig. 2) and male-male competitive interactions. Originally developed as a tool to characterize social interactions in general, social network analysis as applied to sexual systems is an approach to characterize how local interactions between individuals create population-level patterns and has been used extensively to study the dynamics of disease transfer in sexual populations (e.g. Gupta et al. 1989; Liljeros et al. 2001; Schmid and Kretzschmar 2012). The use of networks as descriptors in the context of sexual selection, not only provides a conceptual framework to visualise the pattern of interactions between individuals, but also provides a variety of quantitative network analytical tools that can describe variation in inter- and intrasexual interactions (Krause et al. In Press, 2007; Croft et al. 2008; Whitehead 2008). By building population structure as an emergent property from individual interactions, sexual networks enable the identification of unique competitive environments for individuals, potentially revealing detail beyond population-level measures of social structure, such as population density and operational sex ratio (Krupa and Sih 1993; de Jong et al. 2009; Kokko et al. 2012; Aronsen et al. 2013; Wacker et al. 2013). I utilise the concept of sexual networks as descriptors of the patterns of sexual interactions across both theoretical and empirical approaches.

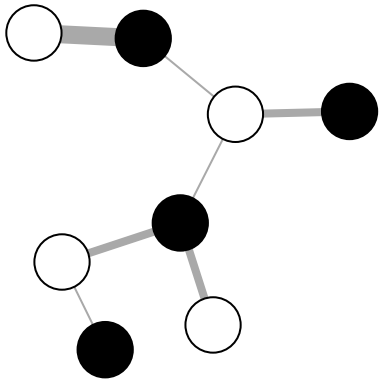


Figure 2. A hypothetical sexual network where nodes represent males (black) and females (white). Edges between nodes can represent any sexual interaction including courtship or mating. Thickness of edges can represent the frequency or intensity of the interaction.

II) SIMULATIONS AND EMPIRICAL APPROACHES

Using a mixture of simulation techniques and empirical approaches has two key benefits. Firstly, simulation techniques allow me to tackle the role of competitive structure in modifying sexual selection under more controlled hypothetical biological conditions and generate predictions across more idealized range of structure (Chapters 3-5). Secondly, the empirical approach allows me to test these predictions under more realistic biological conditions (Chapters 6-8). For all the empirical work I use Red junglefowl (*Gallus gallus*) as a model system.

III) QUANTITATIVE SEXUAL SELECTION APPROACHES

As detailed above, sexual selection arises via intrasexual competition over access to the gametes of the opposite sex, and operates on the resulting intrasexual variation in the number of fertilised gametes (Darwin 1871; Andersson 1994; Shuster and Wade 2003). Those traits that confer advantage in intrasexual competition will be selected for by facilitating an increase in reproductive success relative to that of competitors. Selection on any phenotypic trait can therefore be measured statistically as an association between the trait and reproductive success (Price 1970; Lande and

Arnold 1983). Within one sexual selection event, (e.g. one single breeding season), the reproductive success of an individual for that period can be quantified as the number of gametes fertilised (*T*). In a polyandrous population, the reproductive success of a male (*T*) will be determined by his number of mating partners (*M*), their average fecundity (*N*) and, because other males also mate those females, the proportion of those gametes that he fertilises (*P*). This relationship can be formally given as:

$$T = M * N * P + \varepsilon \quad (1)$$

where ε is an error term. Traits that affect the first two multiplicative components (*M* and *N*) relate to precopulatory sexual selection and those that affect the final term (*P*) relate to postcopulatory sexual selection (i.e paternity share). For any given selection event, or across multiple events (e.g. multiple breeding seasons or total lifetime reproductive success), the expected evolutionary trajectory of a trait (i.e. the response to selection, R) may be predicted by combining the relationship between trait and fitness at the population level and the additive genetic variance in the trait as:

$$\mathrm{R} = h^2 S \quad (2)$$

where, h^2 is the additive genetic variance of the trait and *S* is the selection differential, described by the covariance between trait and relative fitness at the population level. Equation 2 is the classic breeders equation (Falconer and Mackay 1996) and highlights two key points, firstly that selection is a process that can be studied separate from the response to selection. Secondly, it highlights that to understand trait evolution at the population-level we need to understand both the heritability of traits and the relationship between traits and relative reproductive success, at the population level. Therefore, when predicting changes in trait means across generations and selective episodes, ultimately the relationship between a trait and fitness (*S*) at the

level of the population is key. There are a number of techniques to quantify both the potential strength of sexual selection and its strength operating of phenotypic characters at the population level. These two approaches broadly fit within either variance based approaches or regression based approaches. Variance based approaches begin with a metric known as the opportunity for selection (I_T). The opportunity for selection is the variance in relative reproductive success calculated as:

$$I_T = \frac{\sigma_T^2}{\bar{T}^2} \quad (3)$$

where σ_T^2 is the variance in reproductive success and $\bar{T}$is mean reproductive success, both calculated at the population level. Fisher's fundamental theorem states that the maximum rate of increase in fitness for a population is proportional to the variation in relative fitness (Fisher 1930). Therefore, for those traits that determine fitness, the maximum rate of change in those traits is proportional to the variance in relative fitness i.e. the opportunity for selection. This approach does not therefore quantify selection *per se* but provides an understanding of the potential for selection to operate on traits by providing us with an estimate of the maximum covariance between traits and relative reproductive success (i.e. *S*). We can further develop this method to understand the opportunity for selection for each component of reproductive success (i.e. *M*, *N* and *P*) and the extent to which pre- and post-copulatory processes contribute to the total variation in *T*, providing a means to explore changes in the potential strength of selection across different socio-ecological parameters and through which selective episodes (i.e. pre and post) selection may operate most strongly. The second, regression based, population-level approach can be used to understand the strength and direction of selection operating on phenotypic traits. This approach uses a regression of fitness on trait values (both standardised at the level of

the population) to calculate selection gradients (β), that estimate the strength and direction of selection acting on a trait as:

$$\omega_i = \beta z_i + \varepsilon \quad (4)$$

where ω_i and z_i are relative fitness and trait value for the i_{th} individual respectively, β is the slope of the regression of relative fitness on trait value and ε represents the residual error. Using this approach we can investigate selection across both pre- and post-copulatory selection episodes using *T* as our measure of reproductive success or via individual components *M*, *N* or *P*, which may reinforce or oppose each other to contribute to total selection on the trait at the population level through T. Although such approaches have been key in understanding how selection operates on phenotypic characters and provide the means to measure selection when characters are correlated, such methods do have some limitations. For example, measuring selection on traits requires *a priori* knowledge of which traits are functionally relevant, and measuring such traits either in experimental or natural systems may be prohibitively challenging, resulting in errors in measurement or an inability to measure the trait at all. Furthermore, when important fitness related traits are not measured this may result in erroneous assumptions regarding the targets of selection (Mitchell-Olds and Shaw 1987; Price et al. 1988; Hadfield 2008). A related problem also regards those individuals in for which measurements are not possible. When particular sections of a population are consistently missed when measuring traits and fitness, this may result in a bias in the distribution of trait values measured, e.g. measuring natural selection on a phenotypic character after selection has already operated on this trait through viability selection (Hadfield 2008).

Finally, because this thesis is interested in understanding not only population-level selection, but is also interested in how population-level selection patterns

themselves are shaped by the structure of competition working at the local level, I also utilise other selection techniques that incorporate local variation in competitive environments. One key approach is multilevel selection and is an extension of that provided in equation 4 (Nunney 1985; Heisler and Damuth 1987; Okasha 2004). This can be used to measure selection in structured populations and allows the estimation of selection operating on a focal trait (e.g. tail length) controlling the local competitive environment (e.g. mean tail length of competitors). A simple multilevel selection analysis is given by

$$\omega_{ij} = \beta_1 z_{ij} + \beta_2 Z_j + \varepsilon \quad (5)$$

where ω_{ij} and z_{ij} are the relative fitness and trait value of the i_{th} individual in the j_{th} group and Z_j is the trait value for the j_{th} group. Using this approach it is possible to ask questions regarding how selection operates at the level of the local competitive group taking into account population structure. A key point here is that predicting evolution at the population-level still requires an understanding of population-level selection. However, multilevel selection techniques provide estimates of selection working at the local level and can reveal how population structure modulates population-level selection. A key example relates to the distinction between hard and soft selection. Imagine a population where individuals are segregated into multiple subgroups and groups are structured such that more similar individuals are found within the same group (i.e. small individuals compete with small competitors and large with large competitors). Within each group, individual males compete for mates and those individuals that are larger attain higher reproductive success than their local group competitors (i.e. competition is local and there is positive sexual selection on male size at the local scale). If groups also vary in their mean reproductive success, i.e. groups with on average larger males produce more offspring, then measuring

selection at the population level may reveal a positive relationship between body size and relative fitness (hard selection). However, if groups are equally productive regardless of average group male body size (soft selection) e.g. because female fecundity is similar across groups, the covariance between trait and fitness may be strong at the local level but due to population structure this covariance may be obscured at the population level (fig 3). Therefore, when selection is soft, sexual selection at the population level may seem weak or zero but at the local level remain strong. This example highlights that although predictions for trait evolution rely on population-level selection measures, multilevel level selection analyses provide an understanding of how such population-level selection patterns are generated. I use combinations of all the above approaches and further develop these methods throughout my thesis in both theoretical work and the empirical model system red junglefowl (*Gallus gallus*).

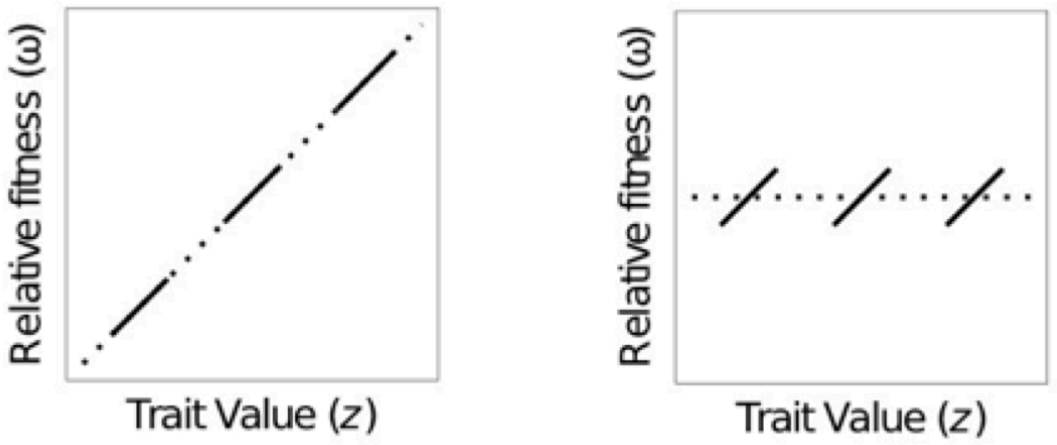


Figure 3. Panels show two hypothetical patterns of sexual selection on a competitive trait *z* in a population subdivided into three competitive subgroups (LHS = Hard selection, RHS = Soft selection). Dotted lines represent population-level selection patterns and solid lines represent selection at the level of the local group. For both the hard and soft selection individuals compete within local groups and an individual's absolute fitness depends on its own competitive quality relative to that of its local competitors. In the LHS panel, groups vary in their productivity according to mean group trait whereas as in the RHS panel groups are constrained to have equal productivity. This means that the best competitor in a low quality group will have the same reproductive success as the best competitor in a high quality group, obscuring population level covariance between trait and fitness.

THE EMPIRICAL MODEL SYSTEM: RED JUNGLEFOWL (*GALLUS GALLUS*)

Red junglefowl are a sexually dimorphic species where males are larger and display relatively more extravagant ornamentation than females. This species has been shown to demonstrate population structure (Collias et al. 1966), and free-ranging populations live in relatively small social groups ranging from at least 2-28 individuals (Collias and Collias 1996). Females of this species and feral populations of its domestic subspecies, the domestic chicken, *G. g. domesticus*, are polyandrous and both pre- and post-copulatory episodes of sexual selection are well documented (Pizzari et al. 2002). For example, studies have demonstrated strong precopulatory sexual selection on males, because male reproductive success is strongly associated with male mating success (Collet et al. 2014). Furthermore, because female junglefowl are polyandrous, this system provides opportunity for postcopulatory sexual selection through sperm competition and, because females can bias paternity by ejecting the sperm of disfavoured males, also cryptic female choice (Pizzari and Birkhead 2000; Dean et al. 2011; Collet et al. 2014). Together, these provide scope to explore the operation of sexual selection both before and after mating (Pizzari and Birkhead 2000; Pizzari et al. 2002; Collet et al. 2012, 2014). Finally, a large body of literature has characterized which male traits are expected to predict variation in reproductive success facilitating studies of sexual selection on phenotypic traits (Pizzari and Birkhead 2000; Johnsen et al. 2001; Pizzari et al. 2002; Dean et al. 2010, 2011; Collet et al. 2012).

I studied a semi-natural population of Red junglefowl, kept at the field station of the University of Oxford (refer to previous work that describes this population e.g. Collet et al. 2012, 2014). This population allows the collection of fine-scaled data on

reproductive behaviour from behavioural observations, which, in combination with molecular parentage and *a priori* knowledge of those traits important in determining reproductive success in sexual competition, provide an accurate description of both pre- and post-copulatory sexual behaviour and its outcome in terms of individual reproductive success. Using this system for empirical work does however have some limitations. Measures of lifetime reproductive success would be extremely challenging and therefore measures of reproductive success and estimates of selection correspond to relatively short periods of individual's reproductive life in this thesis. Although this does not impinge on the estimates of selection within that period, it limits the ability to predict trait evolution in the long term. This is because although selection on a phenotypic trait within the short period measured may be, for example, strong and positive, lifehistory trade-offs may result in opposing selection on phenotypic traits later in life, reducing the effect of selection on those traits across multiple reproductive events. However, much of the work in this thesis regards how competitive structure itself may modify patterns of selection rather than predicting trait evolution within the population and so in general the relative short periods in which selection is measured are relevant to test the ideas of competitive structure on the shape of selection.

THESIS OVERVIEW

My research investigates the influence of competitive structure on the strength and direction of sexual selection in the following seven chapters:

Chapter 2 firstly introduces the operation of sexual selection in structured populations. We introduce the concept of soft selection and discuss how variation in

the "softness" of sexual selection may have broad implications for the operation of sexual selection in structured populations and outline the potential forces that will modify the softness of sexual selection.

Chapter 3 uses a simulation approach to show how polyandry can modify population competitive structure. It then details the potential implications for the measurement of selection in structured polyandrous populations and introduces a novel approach based on network analysis to capture variation in population structure and improve our understanding of the operation of sexual selection in structured populations.

Chapter 4 builds on the work presented in chapter 3 and -using a simulation approach- aims to demonstrate how population structure can not only complicate cross-population measures of sexual selection but also obscure the relative roles of both pre- and post-copulatory sexual selection. This chapter then further develops the use of network analysis, not only as conceptual framework but also as quantitative tool to measure variation in competitive structure.

Chapter 5 firstly uses simulations to explore in detail how variation in polyandry, as a modulator of the intensity or pre- and postcopulatory sexual selection, can shape the relative roles of both processes, and how this in turn affects the total operation of sexual selection. Building on this work and the quantitative techniques proposed in chapter 4, this chapter then demonstrates how non-random variation in the intensity of postcopulatory sexual selection can modify the effect of polyandry on pre- and postcopulatory sexual selection.

Chapter 6 moves beyond simulation work and tests the quantitative predictions generated in chapter 5 in experimental groups of Red junglefowl. We explore how variation in polyandry, within and among groups, shapes the intensity pre- and postcopulatory sexual selection. Furthermore, we use quantitative network measures

from chapter 4 to test how non-random variation in competitive intensity across males modifies the operation of pre- and post-copulatory sexual selection

Chapter 7 builds on chapter 3 by using the quantitative methods presented there in an empirical study of Red junglefowl. We use these methods to estimate the role of structure in the sperm competitive environment in shaping phenotypic selection in male Red junglefowl. Furthermore, we expand and develop on the methods from chapter 3 and show the potential for these techniques to be expanded and to provide more accurate measures of selection in structured populations.

Finally, **Chapter 8** explores classic predictions of sexual selection theory, explaining patterns of variation in mating systems and sexual selection as function of variation of the abiotic environment. This chapter takes a step back from competitive structure *per se* and asks how the environment can modulate competitive structure, and considers the implications that this may have for the strength and direction of sexual selection. I use replicate groups of red junglefowl to test the effect of the structural complexity of the environment and resource distribution on mating systems (polyandry), the pattern of intrasexual competition and the operation of sexual selection on males.

CHAPTER 2. THE DETERMINANTS OF THE SOFTNESS OF SEXUAL SELECTION: IMPLICATIONS FOR STRUCTURED POPULATIONS

Grant C. McDonald

Edward Grey Institute, Department of Zoology, University of Oxford, Oxford, UK.

ABSTRACT

Sexual selection is a form of social selection because the reproductive success of any individual is dependent on the competitive ability of its rivals. When populations are structured this results in social competition within local subdivisions rather than globally across the population, leading to the expectation that reproductive success will be determined locally (soft selection) rather than globally (hard selection). Whether selection is soft or hard will have repercussions for a variety of fundamental evolutionary phenomena including mutation load, the maintenance of variation and kin selection. Despite these key implications, the potential for the softness of sexual selection to be dynamic has rarely been discussed. In this work we address this by firstly introducing sexual selection within the soft/hard selection paradigm. We then outline the key forces with potential to influence variation in the softness of sexual selection. Finally, we discuss the consequences for such variation for structured sexual populations.

INTRODUCTION

Sexual selection arises from competition between members of one sex, to access the gametes of the opposite sex (Darwin 1871; Andersson 1994). This intrasexual competition is inherently social, because the reproductive success of individuals depends not only on their own competitive ability but on the competitive ability of those individuals with which they compete (Lyon and Montgomerie 2012). Since Darwin outlined this process in 1871, sexual selection has been recognized as a powerful force in evolution, influencing macro- and microevolutionary processes (Darwin 1871; Andersson 1994; Coyne and Orr 2004; Jones and Ratterman 2009).

How sexual selection shapes the evolutionary trajectories of sexual populations depends on its strength and form (e.g. directional versus disruptive selection) (Lande and Arnold 1983; Hunt et al. 2009). Among the mechanisms that underpin variation the strength and form of sexual selection are both ecological and demographic factors (Emlen and Oring 1977; Shuster and Wade 2003; Aronsen et al. 2013; Wacker et al. 2013). One such key parameter is population structure, which arises when individuals interact non-randomly and results in intrasexual competition being played out locally within restricted subsets of the population. For example, when dispersal is limited, populations become more viscous, increasing interactions between the non-dispersing related individuals.. This competitive structure, in combination with the social nature of sexual selection, leads to the prediction that the reproductive success of any individual in the population depends on the competitive ability of its local competitors (soft selection) rather than that of the population as a whole (hard selection) (Wallace 1975; Lyon and Montgomerie 2012; McDonald et al. 2013) (Box.1). This intuitive prediction describes the "softness" of selection and, although deceptively simple, has important ramifications for a variety of fundamental evolutionary phenomena (Wallace 1968, 1975; Whitlock 2002; Laffafian et al. 2010).

Population structure is central to providing the opportunity for soft selection to occur (Wallace 1975), however, population structure alone does not guarantee soft selection. Recent empirical and theoretical work suggests that whether selection is soft or hard may be highly dynamic and itself dependent on numerous factors; including interspecific competition and differential mortality regimes between local groups (Agrawal 2010; Laffafian et al. 2010; Eldakar and Gallup 2011; Ho and Agrawal 2012). Therefore, classic implicit assumptions regarding the softness of sexual selection within structured populations may, under many circumstances, be

misleading. Modifications of the softness of selection can have knock-on effects on a variety of processes such as population mutation load, the maintenance of variation in quantitative traits, as well as how estimates of phenotypic selection should be interpreted (Goodnight et al. 1992; Whitlock 2002; Agrawal 2010). Furthermore, by defining the resolution at which competition occurs, the softness of selection is expected to have consequences for the intensity of competition between kin. The potential for the evolution of kin selected behaviours therefore depends on population viscosity and the softness of selection (e.g. when competition is completely local between kin this is expected to limit the evolution of kin selected behaviours (Taylor 1992a)). Despite these key implications, sexual selection has itself rarely been considered through the lens of the soft/hard selection paradigm and the extent to which the softness of sexual selection may be dynamic remains little understood.

In this paper, we address this shortcoming by firstly placing sexual selection within the soft/hard selection framework. We then detail several forces with the potential to influence the softness of sexual selection namely, i) multiple mating by females (polyandry), ii) interspecific reproductive interference, and iii) sexual conflict. In each case we highlight the evolutionary implications for structured populations. Finally, we outline the main consequences of variation in the softness of sexual selection for structured sexual populations, namely i) the maintenance of variation, ii) mutation load, iii) the evolution of kin-selected reproductive strategies, iv) the measurement of sexual selection and v) the relative roles of pre- versus postcopulatory sexual selection in phenotypic evolution.

SEXUAL SELECTION AND THE SOFT/HARD SELECTION PARADIGM

In structured populations not all traits are expected to experience the same softness of selection. For example, even when populations are split into distinct subunits, if dispersal between subunits occurs before competition, selection on those competitive traits involved may be hard. Furthermore, for traits with which social competition plays only a relatively minor role, selection may remain hard despite population structure. For example, consider two traits that determine the ability of individuals to attain resources and efficiently process those resources, respectively. If resources are limited then social competition to attain resources may be strong and those individuals better at acquiring those resources do so at the expense of other individuals. If competition occurs locally, selection on attaining resources will be soft (Box 1). Importantly however, the ability to efficiently process these resources does not necessarily depend on the processing ability of other individuals in the local competitive group. Therefore, despite population structure, selection on processing efficiency may remain hard (Laffafian et al. 2010). Traits under sexual selection are necessarily subject to social competition. This has led to the tacit assumption throughout the sexual selection literature that in structured populations sexual selection is soft (Wolf et al. 1999; Lyon and Montgomerie 2012; McDonald et al. 2013). This expectation was detailed by Wallace (1975) in his inception of the soft/hard selection framework where he explained how, in sexual contexts females would "*correspond to the pupation sites or territories*" [that comprise the limited resource] in non-sexual contexts. In this study we make one explicit differentiation to Wallace's statement and define the limited resource as the gametes of the opposite sex rather than individuals. This allows us to incorporate postcopulatory sexual selection

within the soft/hard selection framework. For example, the fertilization success of a male engaging in sperm competition will depend on the sperm competitive ability of the other males that have inseminated the focal female. Importantly, this competition forms a zero sum game, where the fertilization of one ovum by one individual directly removes the chance of fertilization by other individuals and is therefore ultimately the limiting resource for which competition occurs. For simplicity of discussion, we will assume throughout that the limited resource is female ova and sexual selection is operating more strongly in males, however the logic applies equally to sex role-reversed species in which females compete to access males and their gametes.

Box.1 **Variability in the softness of selection**

The softness of selection describes a property of selection, beyond the strength and direction with which it operates on phenotypic characters. Instead, the softness of selection refers to a continuum that describes the scale at which selection operates within a population.

Soft Selection: When selection is completely soft individuals compete within local groups and an individual's absolute fitness depends on its own competitive quality relative to that of its local competitors [1-2]. This means that the best competitor in a low quality group will have the same reproductive success as the best competitor in a high quality group when their competitive advantage relative to their local competitors is similar (fig.1). Furthermore, groups composed of on average higher quality individuals are not more productive than groups of lower mean quality.

Hard Selection: When selection is hard the absolute fitness of an individual depends on its competitive ability relative to that of the entire population [1-2]. This means that individuals of higher competitive ability have higher fitness relative to their local competitors but also groups of higher mean quality also have higher absolute fitness than poor quality groups.

Supersoft selection: When developing individuals die prior to maturity, the resources (e.g. food) they used unitil death are essentially wasted as individuals die before reproduction. If such mortality occurs earlier in lower-quality genotypes than higher-quality genotypes, fewer resources will be wasted in groups with a lower mean genetic quality, resulting in more per capita resources left over for the remaining individuals [3]. This has the overall effect of increasing the productivity of groups with lower mean competitive ability compared with groups with a higher mean competitive ability. This results in greater reproductive success for higher quality individuals within groups but overall higher productivity for groups composed of lower genetic quality individuals. This pattern is referred to as supersoft selection and has been both theoretically and subsequently experimentally demonstrated [3-4]. Supersoft selection can therefore be viewed as another way of hardening selection but where within-group competitiveness is at odds with group-level productivity.

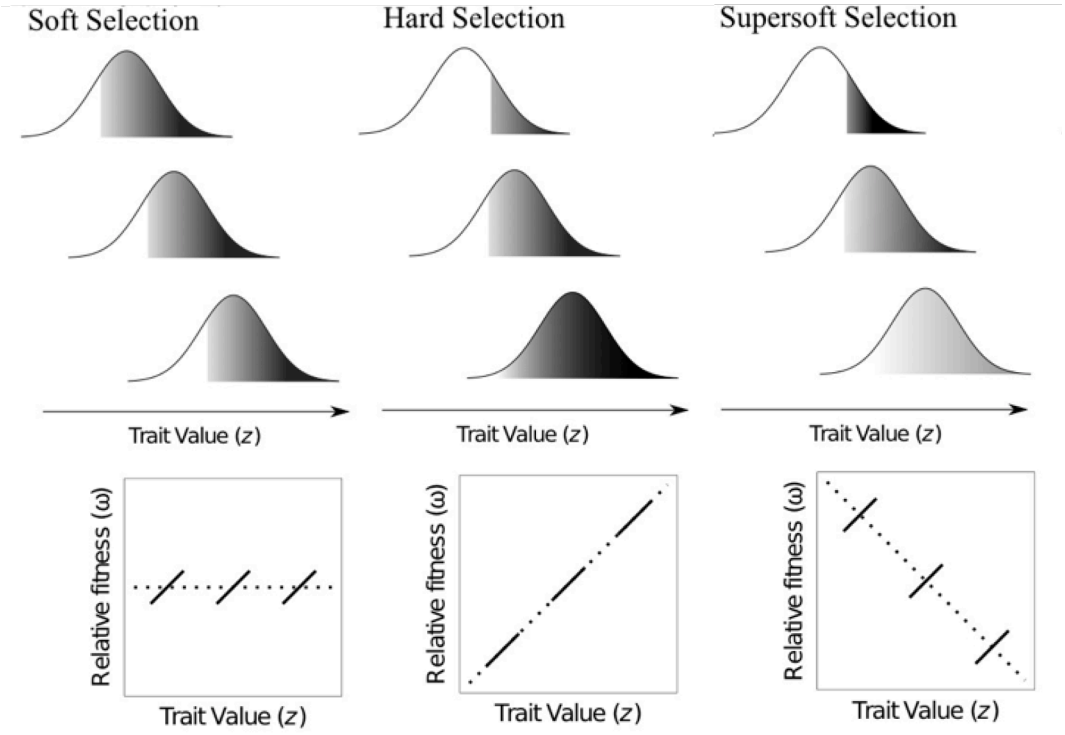


Figure 1. Bell curves represent the distribution of traits in local subpopulations. Shaded areas represent trait values that reproduce successfully and the intensity of shading represents the productivity of those traits. Dotted lines represent population level selection gradients and solid lines represent the operation of selection within local subpopulations

References

[1] Wallace, B. (1968) Polymorphism, population size, and genetic load. In Population biology and evolution, pp. 87–108, Syracuse University Press

[2] Wallace, B. (1975) Hard and Soft Selection Revisited. Evolution 29, 465

[3] Agrawal, A.F. (2010) Ecological determinants of mutation load and inbreeding depression in subdivided populations. Am. Nat. 176, 111–22

[4] Laffafian, A. et al. (2010) Variation in the strength and softness of selection on deleterious mutations. Evolution. l. 64, 3232–3241

THE DETERMINANTS OF THE SOFTNESS OF SEXUAL SELECTION

POLYANDRY AND POPULATION STRUCTURE

Population competitive structure is a prerequisite for soft selection. When population structure is absent and competition occurs randomly across the whole population then selection is hard. In sexual systems the potential for intrasexual competition to occur both before and after copulation adds a further layer of complexity. When females mate multiply, such that the ejaculates of multiple males overlap across the period at which fertilization occurs (i.e. polyandry), a second round of social competition between the ejaculates of several males can occur (Parker 1970; Thornhill 1983). In polyandrous populations, precopulatory competition between individuals may occur randomly at the population level. When this occurs sperm competition will remain local among the ejaculates of the males that mated with each female. In such cases precopulatory competition is expected to be harder or as hard as postcopulatory competition. However, when males are structured in local non-randomly assorted groups, female movement among patches between mating events will result in precopulatory intrasexual competition occurring locally within a patch (soft selection) but postcopulatory sexual selection will become increasingly global as females sample more patches between mating events. The potential for such patterns to occur in nature is large, for instance female pine engravers (*Ips pini*) move between patches of males varying in mean quality and accept mates from multiple patches, i.e. precopulatory sexual selection occurs within male groups of varying quality but postcopulatory sexual selection occurs across groups (Reid and Stamps 1997). Furthermore, the great variety of lekking structures across taxa provide scope for a great variety of both visitation and mating patterns (Höglund and Alatalo 1995). The

combination of female sampling patterns, polyandry and population structure therefore has the potential to modify softness of both pre- and postopulatory sexual selection and demonstrates a potential mismatch between the scales and softness of selection for pre- and postcopulatory episodes of sexual selection, modulated by population structure.

INTERSPECIFIC REPRODUCTIVE INTERFERENCE

The expectation that local social competition generates soft selection refers specifically to intraspecific competition (Wallace 1975). Interspecific competition is instead expected to have the opposite effect and harden selection (Agrawal 2010). This is because individuals of low competitive ability must compete for resources not only with other conspecifics of low competitive ability, but also against interspecific competitors. Therefore, in competitive groups composed of low quality individuals, resources not acquired by the focal species will not be surrendered to other low genetic quality conspecific neighbours but instead to interspecific competitors. When this happens the fitness of low quality focal competitors is expected to be compromised to a greater extent by interspecific competitors than that of their high quality counterparts, resulting in differential group reproduction and the globalisation (hardening) of selection. In a sexual context, the potential for interspecific competition is realised through interspecific reproductive interference. Reproductive interference occurs when the individuals of one species engage in reproductive activities with members of another species (Gröning and Hochkirch 2008; Burdfield-Steel and Shuker 2011). For sexual competition, the limited resource at the centre of competitive interactions is the gametes of the opposite sex. For reproductive interference to vary the softness of selection, the reproductive behaviour of the

interspecific competitor must remove the opportunity for fertilisation from individuals of the focal species, therefore reducing their fitness. Over the past years, increasing work has shown that the potential for reproductive interference to occur is substantial (Burdfield-Steel and Shuker 2011).

A potentially relevant example of interspecific reproductive interference influencing the softness of sexual selection comes from studies of sexual selection in plants. There is now growing consensus over the importance of sexual selection in shaping non-selfing plant evolution (Arnold 1994; Willson 1994; Skogsmyr and Lankinen 2002; Moore and Pannell 2011; Pannell and Labouche 2013) which may occur both before and after gamete dispersal (Willson 1994; Skogsmyr and Lankinen 2002; Pannell and Labouche 2013). Approximately 90% of terrestrial plant species are composed of flowering plants, of which more than 85% are pollinated by animals (Crepet and Niklas 2009; Ollerton et al. 2011). Pollinators therefore represent an important resource and provide an arena for sexual selection to operate on traits that increase reproductive success, e.g. greater pollen visitation, pollen uptake and dispersal (Skogsmyr and Lankinen 2002). In natural plant communities one to one plant-pollinator relationships are the exception rather than the rule, with many species of plants sharing pollinators (Olesen and Jordano 2002; Kay and Sargent 2009). This is demonstrated by heterospecific pollen transfer, which is now broadly documented (Galen and Gregory 1989; Caruso 2000; Brown et al. 2002; Sargent and Ackerly 2008; Flanagan et al. 2009; Mitchell et al. 2009; Muchhala and Thomson 2012; Fang and Huang 2013). Competition over pollinators can reduce both female fecundity and male reproductive success via a variety of mechanisms, e.g. by reducing the amount of conspecific pollen reaching female stigma, interfering with heterospecific pollen germination, and fertilization of ova by heterospecific pollen resulting in hybrid

inviability (Galen and Gregory 1989; Caruso 2000; Bell et al. 2005; Moccia et al. 2007; Flanagan et al. 2009; Mitchell et al. 2009; Muchhala and Thomson 2012). Plant-pollinator relationships therefore provide extensive opportunity for interspecific competition to modify the softness of sexual selection in animal-pollinated plants. In animals, studies of contemporary populations have demonstrated incomplete prezygotic isolation, with individuals of different species engaging in reproductive behaviours (Kozak et al. 2012). This provides the potential for interspecific competitors to remove fertilisation opportunities from focal species, which will act to harden sexual selection on the focal species. A lack of such prezygotic isolation may result from factors such as male-driven hybridization where the costs of heterospecific matings to males are outweighed by strong selection on indiscriminate male mating behaviours (Parker and Partridge 1998). In these systems selection for mate discrimination may operate more strongly in females but still result in heterospecific mating, especially if females lack complete control over mating decisions and sexual conflict occurs over mating decisions (see below).

INTERLOCUS SEXUAL CONFLICT

Interlocus sexual conflict emerges as a consequence of the difference between males and females in the optimal result of intersexual interactions (Rice and Holland 1997; Chapman et al. 2003). For example, if mating is costly to females and males have a higher optimal mating rate than females, this will generate sexual conflict between the sexes and impose antagonistic selection with respect to mating rate (Arnqvist and Nilsson 2000). When this conflict between the sexes results in reduced female fecundity, for example when males transfer harmful compounds (Wigby and Chapman 2005) or physically damage females (Galliard et al. 2005) this can result in

a "tragedy of the commons", where individual competition reduces the fitness of the entire group. Population structure provides the potential for variation between groups in the extent of sexual conflict and the associated reduction in female fitness. When female fecundity is reduced to a greater extent in groups composed of more highly competitive males (a "sexual tragedy of the commons" (Rankin et al. 2007)), the resulting variation in group-level productivity associated with male competitive traits, will harden selection on such traits even though intrasexual competition is entirely local. A similar mechanism has recently been developed theoretically and empirically demonstrated in non-sexual contexts and has been termed "supersoft" selection (Agrawal 2010; Laffafian et al. 2010) (Box.1). Supersoft sexual selection requires a local tragedy of the commons, where the most successful strategy within the group is not the best strategy for the group. Such local tragedy of the commons has recently been demonstrated in multigroup populations of water striders, *Aquarius remigis*, where male aggressiveness positively predicts male mating success but negatively predicts group level mating success by inducing the migration of females away from aggressive males to groups containing less aggressive males (Eldakar et al. 2009a,b, 2010) and, crucially, results in differential productivity between groups based on group level aggression (Eldakar and Gallup 2011). Supersoft selection is essentially another mechanism of hardening selection, however in this case; the direction of selection operating on differential group fitness is in opposition to that of selection on individual reproductive success within groups. In other words, selection on the focal trait operates at the population level due to differential reproductive success of groups, as expected under hard selection, but selection on the focal trait within groups acts in the opposite direction (Box.1). This final point highlights the similarities between for supersoft sexual selection and evolution of altruistic behaviours. In both

cases, traits negatively associated with within group competitive ability are positively associated with group level productivity. The prediction that sexual conflict may mediate social evolution in structured populations is therefore clear, however only recently has this potential mechanism been demonstrated (Carazo et al. 2014). Furthermore, the potential for tragedy of the commons driven by sexual conflict to interact with population structure and drive supersoft selection has yet to generalized across systems.

THE SOFTNESS OF SEXUAL SELECTION AND ITS IMPLICATIONS FOR SEXUAL POPULATIONS

I) THE MAINTENANCE OF VARIATION

A long standing problem in evolutionary biology is to explain the maintenance of variation in the face of constant directional selection which is expected to erode variation in fitness related traits, in sexual selection this is known as the "lek paradox" (Kirkpatrick and Ryan 1991). Among various potential resolutions to the lek paradox, the softness of selection is also expected to play a role in the maintenance of variation. When a trait is causally associated with fitness there is expected to be a non-zero covariance between relative fitness, and the trait. The second fundamental theorem of natural selection states that the larger the covariance between trait values and relative fitness, the faster the expected rate of increase in trait mean across generations (Fisher 1930; Robertson 1968). However, when populations are structured and selection is soft this covariance is largely restricted to the local scale but is obscured at the level of the population (Benton and Evans 1998; McDonald et al. 2013). This happens because local competition reduces the total variation on which selection can act at any one time, resulting in greater relative reproductive success of

individuals in groups with low mean genetic quality than would be expected if competition was global. By reducing the population-level covariance between relative fitness and trait values, soft selection effectively reduces the rate of phenotypic evolution at the population level and adds to the maintenance of variation in sexual traits. We therefore predict that populations experiencing softer sexual selection will have greater additive genetic variation in sexually selected traits, while populations under hard selection should experience a faster rate of erosion in additive genetic variance in fitness related sexual traits.

II) MUTATION LOAD

Classic population genetic theory predicts that population viscosity will increase population level homozygosity because mating locally increases the chances of inbreeding (Charlesworth and Willis 2009). This increase in homozygosity will initially result in an increase in variation in fitness at the population level which itself increases the efficiency of selection at removing deleterious mutations from the population (Crow and Kimura 2009). When selection is soft however, those individuals of poor genetic quality compete only with similarly poor neighbours. Therefore, under a soft selection regime, population structure results in the sheltering of deleterious mutations from selection (Whitlock 2002). This effect on deleterious mutations may be wide-ranging and not limited to purely sexual traits. A recent review has highlighted that many traits important for the general health and viability of an individual also play an important role in a sexually-competitive context (Whitlock and Agrawal 2009). It has thus been argued that sexual selection on males may facilitate the removal of deleterious mutations from populations due to the reinforcing effect of both sexual and natural selection (Whitlock and Agrawal 2009).

More recently, the potential for sexual selection to remove deleterious mutations from populations has been empirically investigated, although empirical studies have produced mixed results (Radwan 2004; Radwan et al. 2004; Hollis and Houle 2011; Plesnar et al. 2011; Almbro and Simmons 2014). Therefore, dynamic changes in the softness of sexual selection may have important implications for mutation load on traits subject to both sexual and natural selection even when the competitive scale associated with such general health traits remains local (i.e. soft selection). Such effects may result in increased risk of population extinction due to greater susceptibility to environmental and demographic stochasticity (Gabriel et al. 1991; Higgins and Lynch 2001).

III) KIN SELECTION

A major advance in evolutionary theory was the recognition that individuals can act to maximise their fitness not only by increasing their own reproductive success (direct benefits) but also by increasing the reproductive success of others (indirect benefits). Whether or not these indirect benefits are sufficiently large to validate the cost of helping others, depends on the level of relatedness between the individuals (Hamilton 1964a,b). Both indirect and direct fitness benefits sum together to form an individuals total inclusive fitness (Gardner et al. 2011). Selection acting on indirect benefits (kin selection) provides an explanation for the evolution of altruistic behaviours, because even by forgoing reproduction, a focal individual can still pass on its genes by increasing the reproductive success of its relatives. In sexual systems this can drive the evolution of cooperative reproductive strategies, for example in Wild turkeys (*Meleagris gallopavo*) males form cooperative courtship coalitions, where helper males do not reproduce but increase their inclusive fitness through

increasing the reproductive success of relatives (Krakauer 2005). Population structure is expected to influence social evolution by increasing the relatedness of those individuals that interact (Hamilton 1971). The result of this increase in within-group relatedness is to align the interests of the group as a whole and its individual members. However, such population structure also increases the chances of competition between kin, which is expected to undermine the evolution of altruistic behaviours by cancelling out the indirect benefits of helping (Taylor 1992a,b; West et al. 2002). Therefore, in structured populations the evolution of altruism will depend on the extent to which competition is local and the absolute fitness of an individual is dependent on the genetic quality of its local competitors i.e. whether selection is hard or soft. Factors that harden selection and therefore increase the scale at which reproductive success is determined will consequently have important implications for the potential evolution of kin selected reproductive strategies. For example, by hardening selection interspecific reproductive interference may play an important role in driving the evolution of kin selected behaviours in structured populations.

IV) MEASURING SEXUAL SELECTION

Classical approaches to measure sexual selection on phenotypic traits are applied at the population level (Lande and Arnold 1983). These approaches use regression to estimate the slope of the relationship between relative fitness and phenotypic traits standardized at the population level. In doing so, these measures assume that competition occurs randomly across the population and that competition at the local scale is representative of competition at the global scale. However the realisation that, in many populations, competition is structured non-randomly undermines this assumption. When for example, population structure results in a

covariance between the trait values of competitors (e.g. more similar individuals tend to compete), this can conceal the relationship between relative fitness and trait values at the population level despite strong selection at the local level, leading to the erroneous assumption that particular traits are not under selection. To overcome this, a variety of methods have been developed that allow the estimation of phenotypic selection on traits, controlling for the effect of population structure (Nunney 1985; Heisler and Damuth 1987; Wolf et al. 1999; Okasha 2004; McDonald et al. 2013). These methods allow the estimation of the effect of individual trait values on relative fitness as well as the effect of an individual's local competitive group on fitness. When selection is completely soft, the effect of the local competitive environment on reproductive success will be equal and opposite to selection on individual trait value, a pattern that will completely remove the covariance between trait value and fitness at the population level (Goodnight et al. 1992). Elucidating the reasons for differences in selection measures between populations can be further complicated despite population structure remaining unchanged. For example, when interspecific competitors vary the softness of selection on traits between populations (via interspecific reproductive interference) despite similar patterns of structure across populations. We argue that this additional layer of complexity provides the need for caution when delineating the mechanisms causally related to differences in selection regimes between populations.

V) THE RELATIVE ROLES OF PRE- AND POSTCOPULATORY SEXUAL SELECTION

As previously discussed, in structured polyandrous populations, precopulatory and postcopulatory competition may occur at differing scales depending on patterns of female movement and the extent of polyandry. Because postcopulatory selection

either occurs after or simultaneously with precopulatory sexual selection, variation in traits exposed to postcopulatory sexual selection is expected to be reduced from that at the population level due to the selection of non-random subsets of males by precopulatory selection. This results in the expectation that evolution of precopulatory traits may respond faster to sexual selection than postcopulatory traits. However, when precopulatory intrasexual competition occurs locally within non-randomly assorted male groups (soft selection) but females move among patches between mating events, postcopulatory sexual selection becomes increasing global (hard selection), and is delivered a potentially greater distribution of fitness related male traits. This may increase the potential importance of postcopulatory sexual selection in shaping phenotypic evolution relative to precopulatory selection. Viewing pre- and postcopulatory sexual selection through the lens of soft/hard selection opens new perspectives on the operation of pre- and post-copulatory sexual selection and their interaction with population structure. For example, recent work has revealed the potential importance of polyandry in shaping sexual selection on male phenotypic traits (Collet et al. 2012). However, for any given level of polyandry, population structure may further modify these patterns, increasing the relative efficiency of selection in on postcopulatory traits in populations with one pattern of population structure and precopulatory traits in another.

BROAD IMPLICATIONS AND FUTURE DIRECTIONS

Recent experimental work on the ecological determinants of the softness selection has highlighted the need for classic assumptions to be tested empirically (Laffafian et al. 2010; Ho and Agrawal 2012). Despite decades since Wallace coined the terms soft and hard selection the presumption that sexual selection will largely be

soft has not been tested and the potential for modification by the parameters highlighted in this work are yet to be explored. This is in part due to the difficulty in many systems to recreate natural population structure in an experimental context. However, growing evidence demonstrates the importance of including such population structure into sexual selection research (Eldakar et al. 2010; Carazo et al. 2014). By including such reality in experiments it will be possible to test the role of potential modulators of soft selection and the consequences for sexual populations. For example, experimental evolution approaches in combination with varying the intensity of interspecific competitors provide exciting opportunities to test predictions on changes in population fitness, mutation load and the consequences for the subsequent susceptibility of populations to environmental stress and stochasticity. Such experiments may play a key role in understanding how the introduction of invasive species and the current reduction of pollinators may drive an increase in interspecific competition for pollination (Traveset and Richardson 2006) and the effects this can have on population viability and the evolutionary trajectories of natural populations. Beyond simply exploring potential impacts of variation in the softness of sexual selection on experimental and contemporary populations, the work presented here raises potential hypotheses regarding broad scale evolutionary patterns. For example, interspecific reproductive interference is expected to strengthen sexual selection at the population level, increasing the rate of phenotypic evolution. One interesting perspective relates to the established relationship between species diversity and the strength of sexual selection (Seddon et al. 2008). If species richness within clades results in high incidence of interspecific interactions and potentially reproductive interference, sexual selection may be hardened in areas with high species richness (e.g. tropic regions), improving the effectiveness of sexual

selection at the population level and accelerating rates of phenotypic evolution due to sexual selection. The extent that latitudinal gradients in the strength of sexual selection may be in part shaped by modifications of the softness of sexual selection due to increased reproductive interference provides an exciting avenue of future research. A further exciting area to test these predictions with comparative approaches is across broadcast spawning species. Broadcast spawners offer strong potential for interspecific interference due to the mixing of gametes of different species released close in time. Furthermore, many species of broadcast spawners live in areas with high species richness such as coral reefs, whereas other species live in relatively species poor environments, such as deep sea versus coral reef living species of echinoderms (Lawrence 2013), providing scope to test ideas regarding the strength of reproductive interference and the strength of sexual selection on phenotypic traits.

CONCLUSION

The softness of selection may have strong implications for the evolutionary trajectories of natural populations. In this perspective we introduced sexual selection within the soft/hard selection framework. In doing so, we have integrated ecological concepts with evolutionary processes by highlighting how the softness of sexual selection may be modified by a variety of fundamental processes including, the interaction between polyandry and population structure, sexual conflict and interspecific reproductive interference. In modifying the softness of sexual selection these processes will have implications for the maintenance of variation in sexual traits, genome wide mutational load, beyond purely sexually competitive traits and influence the evolution of kin selected behaviours. Finally, this discussion raises

practical concerns over the interpretation of selection measures for empiricists in populations that vary in the extent of interspecific competition.

CHAPTER 3. SEXUAL NETWORKS: MEASURING SEXUAL SELECTION IN STRUCTURED POLYANDROUS POPULATIONS

Grant C. McDonald[1], Richard James[2], Jens Krause[3,4], Tommaso Pizzari[1]

[1]*Edward Grey Institute, Department of Zoology, University of Oxford, Oxford, UK.*
[2]*Department of Physics, University of Bath, Bath, UK*
[3]*Leibniz-Institute of Freshwater Ecology and Inland Fisheries, Berlin, Germany*
[4]*Humboldt University, Berlin, Germany*

ABSTRACT

Sexual selection is traditionally measured at the population level assuming that populations lack structure. However, increasing evidence undermines this approach indicating that intrasexual competition in natural populations often displays complex patterns of spatial and temporal structure. This complexity is due in part to the degree and mechanisms of polyandry within a population, which can influence the intensity and scale of both pre- and post-copulatory sexual competition. Attempts to measure selection at the local and global scale have been made through multilevel selection approaches. However, definitions of local scale are often based on physical proximity, providing a rather coarse measure of local competition, particularly in polyandrous populations where the local scale of pre- and post-copulatory competition may differ drastically from each other. These limitations can be solved by social network analysis, which allows us to define a unique sexual environment for each member of a population: 'local scale' competition therefore becomes an emergent property of a sexual network. Here, we first propose a novel quantitative approach to measure pre- and post-copulatory sexual selection, which integrates multi-level selection with information on local scale competition derived as an emergent property of networks of sexual interactions. We then use simple simulations to illustrate the ways in which polyandry can impact estimates of sexual selection. We show that for intermediate levels of polyandry the proposed network-based approach provides substantially more accurate measures of sexual selection than the more traditional population-level approach. We argue that the increasing availability of fine-grained behavioural datasets provides exciting new opportunities to develop network approaches to study sexual selection in complex societies.

INTRODUCTION

Darwin defined sexual selection as a selective process operating on individual variation in the number and reproductive quality of reproductive partners due to intrasexual competition (Darwin 1871). Over the last 40 years, sexual selection has emerged as a potent agent of Darwinian evolution (Andersson 1994), and driver of fundamental evolutionary processes such as speciation (Higashi et al. 1999; Boughman 2001; Seddon et al. 2008), local extinction rates (Doherty et al. 2003), as well as rates of mutation and the tempo of molecular evolution (Dorus et al. 2004; Petrie and Roberts 2007). The way sexual selection affects these phenomena depends on the strength with which it acts on individuals within a population. Therefore, a main goal in evolutionary biology has been to both measure sexual selection and understand the forces that determine its strength (Shuster and Wade 2003).

A major catalyst in the study of sexual selection was the development of quantitative measures to determine the targets of selection and the strength and direction of selection acting on these traits (Jones and Ratterman 2009). This approach is based on the decomposition of variance in individual male reproductive success into three multiplicative components as thus:

$$T = (M \times N \times P) + \varepsilon \quad (1)$$

where T is the total variance in male reproductive success, M is variation in male mating success (number of females mated by a male), N is the average number of eggs produced by the females successfully mated by a male in a given time unit), P represents variation in the average proportion of a clutch of eggs fertilised by a male, and ε represents a source of error (Collet et al. 2012). Together, M and N contribute to generate variation in male mating success and thus opportunity for pre-copulatory

sexual selection. Finally, *P* arises from post-copulatory competition which occurs when individual females mate with, or obtain sperm from, multiple males, a mating system referred to as polyandry. This classical approach considers the relationship between individual variation in the expression of phenotypic traits and a given measure of reproductive success (*M*, *N*, *P* or *T*) standardized within a population, to generate selection gradients (Lande and Arnold 1983). Such standardized measure of selection enables critical comparisons of the operation of sexual selection across different populations, different traits and sexes. An important implicit assumption of this population-level approach to sexual selection is that mating interactions are not structured, i.e. a focal individual is just as likely to encounter the same frequency distribution of phenotypes of prospective partners and/or competitors in any section (e.g. social, spatial or temporal) of the population, so that intrasexual competition measured at the level of the population is representative of patterns of intrasexual competition at the local scale. However, a growing body of empirical work has recently challenged the assumption of population level homogeneity in competitive structure on grounds of two inter-related factors: i) population structure, and ii) polyandry.

I) POPULATION STRUCTURE

There is increasing evidence that most populations are not randomly assorted but display complex social and phenotypic structure. Sexual selection is driven by social competition and therefore likely to be profoundly affected by local structure in the social environment (Wolf et al. 2008). Benton and Evans (1998) used a simulation approach to show that in species where females employ a "*Best of n*" strategy when choosing mates, local spatial clumping of males by quality may obscure the

covariance between trait and fitness at the population level. This happens when females are limited in the number of males they can sample. Local spatial clumping increases the chance that females will sample a non-random collection of males i.e. choosing between only low quality or only high quality males. The effect of local variation in social environment on the strength and direction of sexual selection was demonstrated empirically in natural populations of the golden orb-web spider (*Nephila plumipes*), in which local differences in density and the operational sex ratio (ratio of reproductive males to females, OSR) result in fine-scale differences in the strength and direction of sexual selection on male morphology (Kasumovic et al. 2008). By correlating male quality at the population level, classical selection analysis implicitly assumes that any female could potentially select any male and therefore ignores the importance of local competitive structure resulting in biased estimates of sexual selection.

II) POLYANDRY

Polyandry has substantial repercussions for the operation of sexual selection. First, by forcing the ejaculates of different males to compete for the fertilization of a set of ova, polyandry introduces a post-copulatory source of variance in male reproductive success which gives rise to post-copulatory episodes of sexual selection: sperm competition (Parker 1970) and cryptic female choice (Thornhill 1983). Second, the fact that males must defend paternity can weaken sexual selection gradients on male remating rates (i.e. the Bateman gradient) (Collet et al. 2012). Finally, the introduction of an additional (i.e. post-copulatory) round of intrasexual competition adds complexity to the structure of mating interactions. For example, females may mate with a male at a local patch and then move to a new patch to mate with another

male. In this scenario, each male competes with his local neighbours to mate with a female, but his sperm will compete with the ejaculates of males from other parts of the population. Similarly, when females vary in number of mating partners, the intensity of sperm competition will also vary across females. If males who mate with many females also mate with the least promiscuous females (have higher exclusivity of mating) they may face lower sperm competition and this may increase variation in reproductive success within males (Sih et al. 2009). Such a pattern may steepen the relationship between number of mates and number of offspring produced. This would therefore be expected to increase the strength of pre-copulatory sexual selection on male competition for access to mates. Therefore, the structure of polyandrous mating patterns may have strong implications for the strength and direction of sexual selection. However, such patterns have been rarely quantified and this may be in part due to a lack of quantitative tools to characterize these patterns.

III) SOCIAL NETWORK ANALYSIS

Network analysis is an approach to characterize how local interactions between individuals create population-level patterns and provide a quantitative tool to capture sexual patterns and dynamics. Social network analysis has become a widespread approach in behavioural biology to shed light on the complex dynamics of social systems (Krause et al. 2007; Croft et al. 2008). Originally developed by sociologists to deal with the complexities of human behaviour (Scott 2000), the social network approach has increasingly been used to address evolutionary questions, especially when it was realized that the success of behavioural strategies of individuals can be dependent on the structure of the social network of which they are part (Ohtsuki et al. 2006; Santos et al. 2006b). Models that take social heterogeneity

explicitly into account in game theoretic analyses have made a number of interesting predictions (for example, regarding the evolution and maintenance of cooperation) that have yet to be tested (Ohtsuki et al. 2006; Santos et al. 2006a,b). The fact that network structure arises from individual interactions (bottom-up) but also selects for individual behavioural strategies (top-down) and therefore has important fitness consequences for individuals (Krause et al. 2009), is of particular relevance in the context of sexual interactions. We therefore focus here on 'sexual networks', networks composed of nodes representing individuals (males and/or females) and the connections between these individuals (edges) may represent any sexual interaction of interest, here defined to include copulations themselves.

IV) AIMS

The aims of this study are twofold. First, we develop a novel quantitative framework to measure sexual selection based on multi-level selection analysis in which local competition is defined by the sexual network. Second, we consider the impact that polyandry has on the operation of sexual selection in a structured population, and compare the sensitivity of our multi-level selection approach with that of the traditional population-level approach to the influence of polyandry on estimates of sexual selection. We show that multi-level selection approaches informed by network analysis can provide substantially more sensitive, unbiased estimates of sexual selection whenever populations display some degree of viscosity (i.e. limited dispersal) and polyandry. Finally, we discuss how the quantitative characterization of the structure of the mating network itself, the mating topology, can shed light on the operation of sexual selection in polyandrous populations.

MEASURING SEXUAL SELECTION IN STRUCTURED POPULATIONS

Several quantitative methods have been developed to estimate the fitness effects of an individual's local competitive environment, including the "contextual analysis" approach (Heisler and Damuth 1987; Okasha 2004a,b) and the "neighbour analysis" approach (analogous to social selection) (Nunney 1985; Wolf et al. 1999; Okasha 2004a; Formica et al. 2011). These methods represent hierarchical expansions of Lande and Arnold's classic selection analyses (Lande and Arnold 1983), where selection is measured at more than one level of hierarchy (i.e. individual phenotype and the phenotype of the group) and have therefore been termed "multilevel selection analyses". The goal of contextual analysis and neighbour analysis is to estimate both the effects of individual phenotype on fitness and the effects of an individual's socio-competitive environment (local group) on individual fitness. To achieve this, each method partitions the effects both of individual phenotype (e.g. individual ornament size) and group phenotype (e.g. mean ornament size of group members) on individual fitness (Goodnight et al. 1992; Okasha 2004a). Specifically, the contextual analysis employs a multiple regression model of individual phenotype and group phenotype on individual fitness,

$$\omega_{ij} = \beta_1 z_{ij} + \beta_2 Z_j + \varepsilon, \qquad (2)$$

where, ω_{ij} and z_{ij} are the fitness and trait values for the i_{th} individual in the j_{th} group respectively, Z_j is the trait value for the j_{th} group, and ε is the residual variance. The regression coefficient β_1 is therefore the partial regression of individual fitness on individual character, controlling for group character and β_2 is the partial regression individual fitness on group character, controlling for individual character. The fitness effects of an individual's local social group on individual fitness are therefore

described by β_2. The neighbour analysis is mathematically identical to the contextual analysis except for the calculation of what constitutes group phenotype (Z_i). In the contextual approach group phenotype for a focal individual is calculated as the mean trait value for all (n) individuals, *including* the focal individual, of the jth group, i.e.

$$Z_j = \frac{1}{n_j}\sum_{i=1}^{n} z_{ij} \tag{3}$$

The neighbour analysis instead calculates group phenotype of all individuals in the group *omitting* the focal individual. This approach has been extensively used in social selection studies (Wolf et al. 1999; Formica et al. 2011), where an additional term named the "interactant covariance" (C_{ij}) is also included, e.g.

$$\omega_{ij} = \beta_1 z_{ij} + C_{ij}\beta_2 Z_j + \varepsilon \tag{4}$$

where, C_{ij} is calculated as the covariance between individual phenotype and the group phenotype. The interactant covariance therefore describes the relationship between focal and group phenotypes and translates the fitness effects of group phenotype (β_2) into phenotypic selection at the population level. In practice, analyses using contextual or neighbour approaches to calculate group phenotype are likely to produce similar results (Okasha 2004a). However, there are a few key differences between the two approaches. For instance, if the researcher is also interested in selection on emergent group properties, such as the density of the focal individual's group, then the contextual approach may be more appropriate (Okasha 2004a). This is because the neighbour approach calculates group phenotype explicitly as the mean trait value of the focal individual's neighbours. Contextual and neighbour analyses represent valuable tools to measure selection in structured populations. Crucially however, the utility of either analysis depends on the identification of functionally relevant groups, which often represents a non-trivial challenge.

I) THE CHALLENGE OF DEFINING 'LOCAL'

Few populations have easy-to-define, spatially-structured groups with little between-group migration, such that individuals can be attributed to their respective groups with ease (Wilson 1975); in the majority of cases groups are likely to be difficult to define *a priori* and competition may be likely to lie somewhere between completely local and completely global (Cornwallis and Uller 2010). How then should the researcher investigating the effects of competitive context (group character) on individual fitness decide which individuals constitute a particular group? Despite the importance of this question little work has been done to provide quantitative methods for competitive group identification. Methods to identify functional groups often require assumptions regarding space use and home range limits to infer social interactions. For instance, probabilistic home range models have recently been used to estimate local social environments (Formica et al. 2011). However, these methods may not be appropriate to accurately identify functional groups of sexual interactions in species with complex within-range space use (Formica et al. 2010). In fact, even within groups with home range overlap there can be considerable variation in interaction patterns between individuals both within and between groups (Marsh et al. 2010). Some experimental studies of multilevel selection have adopted designs that allow for a more objective definition of groups (e.g. based on individual position on an experimental grid (Weinig et al. 2007) or the sharing of the same experimental pools (Eldakar et al. 2010)). However, these definitions can be somewhat contrived being dictated by the experimental layout rather than by the behaviour and ecology of the study organisms. Crucially, these approaches fail to consider that in polyandrous populations the scale of mate

competition may differ drastically from that of sperm competition. For example, in vector-pollinated species, the movement of pollinators will determine the local competition for fertilization (Fortuna et al. 2008). Another important aspect is that some individuals share the same group phenotype but may compete with vastly different numbers of individuals. This variation can be minimized in experimental studies of sessile organisms (e.g. ref. (Weinig et al. 2007)) but represents a significant challenge for studies of more labile social systems. It is clear therefore, that for multilevel selection approaches to provide unbiased estimates of sexual selection in natural populations, a method for determining groups is required that is explicitly based on patterns of individual behaviour and that provides the flexibility needed to capture such variation across multiple episodes of selection operating on vastly different scales.

II) A NETWORK-BASED APPROACH

Network data explicitly build up population structure using interactions between individuals. Networks capturing intra- and inter-sexual interactions can be used to describe which individuals are in local competition. Such local competitive environments are emergent properties of the network and therefore there is no requirement to define *a priori* an arbitrary local group. Below, we describe a simple approach to estimate the effects of social structure and local competitive environment on fitness, based on the application of information on local competition derived from networks to multilevel selection analyses. The use of network information to study sexual selection is not in itself new. For example, Oh and Badyaev (2010) used a network approach to determine local competitive environments in a wild population of house finches (*Carpodacus mexicanus*). The study used network techniques to

delineate "communities" in the social networks of the finch population, and investigated the role of population social structure as a modulator of an individual's attractiveness to partners relative to competitors in its local competitive environment. Communities were defined as collections of individuals that interact more often with each other than the rest of the population, and were identified using network modularity, which measures the level of compartmentalization of interactions/associations within a network (Newman 2006). To quantify selection on the individual taking into account the local competitive context, Oh and Badyaev (2010) standardized individual trait value at the level of the local group (community) rather than the population. The study found that individuals are capable of influencing the selection regime that they experience by modifying their own social context. In particular, males with low secondary sexual ornament elaboration, and hence lower attractiveness, were able to increase their relative attractiveness by modifying their local competitive context via increased social lability. This is a promising approach, which demonstrates how consideration of network structure can help reveal subtle reproductive strategies, and add substantial resolution to our understanding of sexual selection in a population. However, this approach too suffers from some rigidity. First, community detection techniques fail to capture variation in interactions within individual communities (fig. 1), which can be considerable (Wolf and Trillmich 2008; Marsh et al. 2010), and which may have a large impact on the variation in competitive environments experienced between individuals. Second, this approach does not explicitly account for weak ties between communities (fig. 1). The importance of such weak ties has long been recognized in sociological research (Granovetter 1973; Weimann 1983). Consider a hypothetical population with community structure similar to that in figure 1. Females inspect the attractiveness of a subset of the male

population and select the most attractive male(s) encountered. Community composition with respect to male attractiveness is non-random such that all communities have similar levels of variation in male attractiveness but each community differs in its mean attractiveness. Females tend to interact and inspect only males within their community and so mate with the most attractive male from that community. Global selection measures would therefore underestimate sexual selection for male attractiveness. However, local selection measures purely based on community membership ignore inter-community "bridges". These bridges lie somewhere between local, i.e. within the community, and global, and may be important determinants of reproductive success. For instance, if inter-community bridges are formed by choosy females then competition over such "bridge" females will comprise males from different communities while competition over less choosy females will be restricted within a community. The distribution of such bridges in the network could lead to biased estimates of selection, which may be particularly important in species where females employ adjustable thresholds when making mate choice decisions but also suffer high search costs (Reid and Stamps 1997). To achieve unbiased estimates of selection, group membership must therefore take into account individual variation in socio-competitive group.

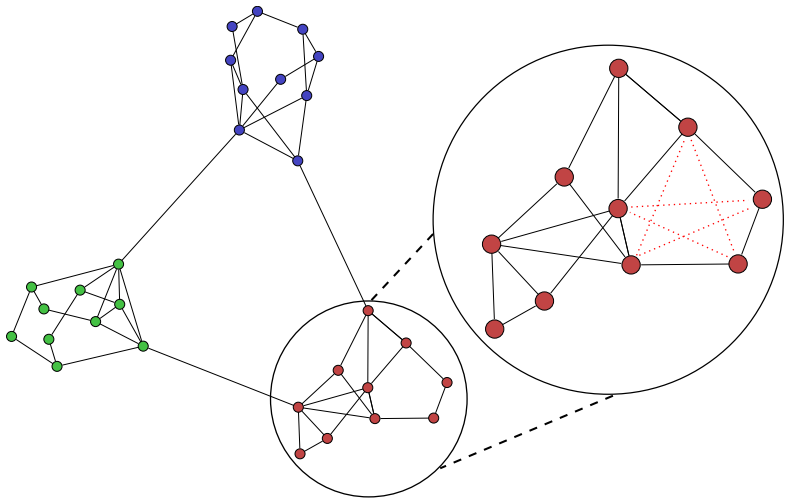


Figure 1. A network demonstrating heterogeneous interaction patterns. Colours represent community membership and red dotted lines represent the absence of interactions between community members.

To resolve these shortcomings and provide a framework for group identification of broad generality, we propose that the competitive environment of a focal individual is described by the pattern of the "connectedness" the focal individual shares with other individuals in a *competitive network*. An individual's competitive group can then be defined as the individuals with which it shares fitness-affecting interactions (Wilson 1975). Using such an approach, an individual's local social environment is defined based on social/competitive interactions, which may or may not correlate with the spatial assortment of individuals, potentially capturing "cryptic" social structure overlooked by spatial methods. Consider a hypothetical network capturing the pattern of ejaculate transfer from males to females (fig. 2a). Such a network enables one to explain *P* (variation in paternity share) by capturing patterns of post-copulatory competition. Edges are drawn between males and females for each independent set of ova for which the males compete for fertilization (e.g. batches of ova across different females or separate batches of ova produced successively by the same female). Two males are in sperm competition if they both transfer sperm to the same female within a certain time window such that their ejaculates co-occur at the time the same batch of ova is fertilized. Clearly, this time window is species-specific

and may vary widely with mode of fertilization, ovulation rates, and female sperm storage duration; from seconds to minutes in some broadcast spawners, to the entire lifetime of a female in some internally-fertilizing species. If two males compete to fertilize one batch of ova, then both males will be connected to the same female (fig.2a). In many cases, however, females will produce many independent batches of ova and males may mate with the same female but not compete for the same batches of ova. To deal with this complexity, mating networks can be more conveniently represented as a series of male nodes connected to nodes representing independent batches of ova (fig. 2c). If females only have one receptive period or produce a single batch of ova, these male-ova mating networks simplify into male-female mating networks. From these mating networks a male's competitive group is captured by all the male nodes that a focal individual is connected to via female or ova nodes (a male's *two step ego network*; fig. 2a & c). To calculate each male's local competitive group more directly it is possible to represent, *project*, the same mating network with female/ova nodes removed (fig. 2b & d). This provides us with a sperm competition network where edges between males represent events of sperm competition. The focal male's competitive context will then be described by his one-step ego network (fig. 2b & d). A similar approach can be used to build networks describing pre-copulatory inter- and intrasexual competitive interactions to explain *M* and/or *N*. Here, each edge may represent whether two males displayed to the same female (intersexual) e.g. within a lekking system, or direct male-male competition (e.g. fighting contests in horned beetles (Eberhard 1979)). In the case of direct male-male competition, the local competitive environment would be described directly by the focal individual's one-step ego network without the need for projection. When males mate only once at most, such as in species with male terminal investment (e.g. (Andrade and Kasumovic

2005)), it will be possible to represent the sperm competitive network using binary network data alone (i.e. presence or absence of an edge). When edges are binary, the contextual analysis using individual local group phenotype as determined by the focal's ego network would take the following form:

$$\omega_i = \beta_3 z_i + \beta_4 Z_{i(local)} + \varepsilon \quad (5)$$

Where ω_i and z_i are the fitness and trait value for the i_{th} individual respectively, ε is the residual variance and $Z_{i(local)}$ is the group phenotype for i_{th} individual's local competitive group, calculated as:

$$Z_{i(local)} = \frac{\sum_{t=1}^{n_t} z_t + z_i}{n_t + 1}. \quad (6)$$

Equation (6) is the mean of all individuals with which the focal individual competes including the focal individual; where z_i is the focal male's trait value and z_t is the trait value for the t_{th} individual with which the focal competes. In network nomenclature, z_i is the focal node's attribute and z_t is the attribute for the t_{th} node with which the focal node is connected. However, in many cases males mate multiply and may compete over several batches of ova. Therefore edges between males must be "weighted". These weights give value to the edges between males (e.g. fig. 2b & d) and represent the number of independent competitive events between males. For instance, when two males both transfer sperm to the same two females (or the same female but across two unique sets of ova) the edge drawn between these males will have a weight of two. Within the period of study (e.g. across a reproductive season), the edges between males can then be weighted to represent the number of independent competitive events. In the example in figure 2, weights indicate that although several males compete with each other, some do so to at greater or lesser extent than others.

To include this information when estimating selection gradients, the weight between two males (ρ_{it}) can formally be calculated as,

$$\rho_{it} = \sum_{q=1}^{n_q} \sum_{p=1}^{n_p} \theta_{it}^{pq} \tag{7}$$

where θ_{it}^{pq} represents whether the i_{th} and t_{th} males both competed for the q_{th} batch of eggs of the p_{th} female (0 when they did not compete, 1 if they did). These weights can then be used when calculating the mean trait value for a male's competitive group, by weighting male traits by the number of separate occasions in which the males competed. In this case, local mean trait value using contextual analysis would be calculated as follows:

$$Z_{i(localweighted)} = \frac{\sum_{t=1}^{n_t} (\rho_{it} z_t) + \sum_{j=1}^{n_j} (\rho_{ij} z_i)}{\sum_{t=1}^{n_t} \rho_{it} + \sum_{j=1}^{n_j} \rho_{ij}} \tag{8}$$

Equation (8) is the weighted mean of trait value of the focal i_{th} male's competitive group, where ρ_{it} is the number of competitive ties between the i_{th} and t_{th} male in the male-male competitive network (as in equation (7)), and $\sum_{j=1}^{n_j} \rho_{ij}$ is the total *strength* of male node i in the male –female sexual network (i.e. the sum of the edge weights between the i_{th} male node and all j_{th} female nodes). Note that the above calculations are equally applicable to neighbour analysis by omitting the focal individual's trait and node strength from group phenotype calculations.

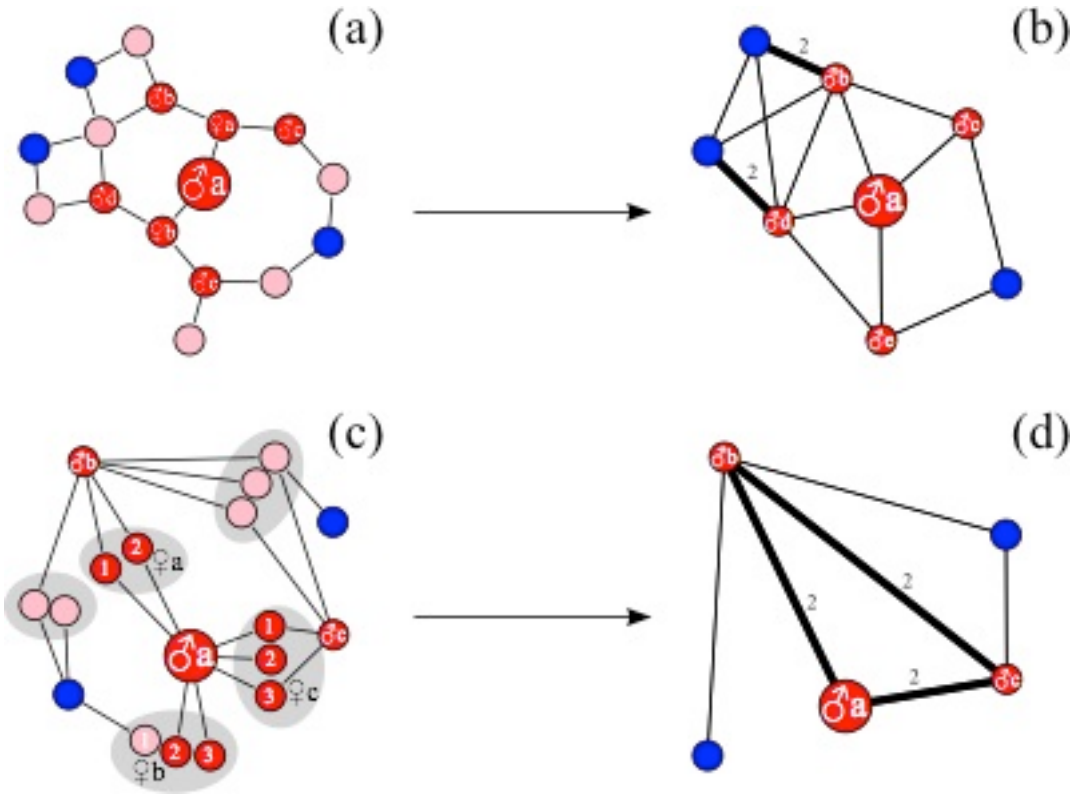


Figure 2. (a) An intersexual network showing the pattern of ejaculate transfer between males (blue) and females (pink). Red nodes highlight the two-step ego network for focal node male ♂a. (b) Male-male sperm competition network projection of (a) in which female nodes have been removed. Red nodes highlight the one step ego network for ♂a. Numbers over edges represent the number of independent competitive events between male nodes. The method of projection adopted here is the simple summation of shared ties between males. (c) An intersexual network showing the pattern of ejaculate transfer between males (blue) and females (grey) and their batches of ova (pink) for which the males competed. Red nodes highlight the two-step ego network for focal node male ♂a. Numbers over edges represent the number of independent batches of ova for which the male competed for fertilization. (d) Male-male sperm competition network projection of (c) in which female and ova nodes have been removed. Red nodes highlight the one step ego network for ♂a. Numbers over edges represent the number of independent competitive events between male nodes. The method of projection adopted here is the simple summation of shared ties between males.

III) WEIGHTING EDGES

So far, we have described weighting edges by the number of independent competitive events. However, edges could also be weighted by other factors with potentially strong impact on reproductive success and which might confound our estimates of sexual selection on male trait *z*. These factors may reflect the phenotype/genotype of the male (independent of *z*) or of the female, e.g. fecundity; the interaction between partners' phenotype/genotype, e.g. relatedness; social interactions among partners, e.g. the re-mating rates of an individual male with an

individual female, or the social environment, e.g. such as local variation in population density or OSR. Information on the number of individuals with which a focal individual competes can be derived from sufficiently detailed sexual networks. As networks are built based on interactions between individuals, emergent properties such as density or individual OSR can be calculated without the pseudoreplication of individuals and included in a contextual analysis model (Eldakar et al. 2010).

THE IMPACT OF POLYANDRY ON SEXUAL NETWORKS AND SEXUAL SELECTION – A SIMULATED EXAMPLE

I) CONTEXTUAL ANALYSIS

In the previous section we proposed a new quantitative framework, which integrates multi-level selection with network analysis. In this section we demonstrate the use of this approach in measuring changes in sexual selection under different regimes of polyandry. Specifically, we adopt simple simulations to change the degree of polyandry (i.e. the number of males mated by each female in a population), and measure the sexual selection gradient on a male trait *z*, using both standard population-level (global) selection analysis and the novel network-based multilevel selection approach proposed in the previous section to illustrate how these estimates are differentially sensitive to changes in polyandry.

We generated replicate sets of 1,000 populations of 100 individuals (50 females and 50 males). Males and females were placed randomly within a two-dimensional environment of fixed dimensions (1x1). In all populations males were provided with one phenotypic trait (*z*). To do so we used male position along one axis of the two-dimensional space, resulting in a continuous uniform distribution of male trait *z* values from left to right. This creates an environmental gradient in *z* such that

males to the left of the environment possess a lower phenotypic trait value compared to males on the right. For simplicity, we assumed that in all populations each female produced only one set of ova. In all cases we imposed post-copulatory sexual selection on z such that males mated to females fertilize a proportion of a female's ova, proportional to their trait value (i.e. males with a larger trait value fertilize a larger proportion of a set of ova). More explicitly, the proportion of the j_{th} female's ova fertilized by the i_{th} male (W_{ij}) that mated with her was calculated as:

$$W_{ij} = \frac{z_i}{\sum_{t=1}^{n} z_{it}^{j} + z_i} \tag{9}$$

where, z_i is the trait value for the i_{th} male, and z_{it} is the trait value for the i_{th} male's t_{th} sperm competitor within female j. We then calculated post-copulatory reproductive success for each male (W_i) as the mean proportion of eggs fertilized across all j_{th} females with whom he mated (Collet et al. 2012). Clearly, a similar approach can be used to focus on different episodes of sexual selection.

For each replicate set of populations we allowed females to mate with a set number (p) of their nearest males in the two-dimensional environment. We generated replicate sets for a distribution of levels of polyandry (p) ranging from p=2 (where females always mate with only their two nearest males, e.g. female degree=2) to p=50, where each female mates with all males in the population. This results in 1,000 replicate populations for each level of polyandry. For all populations at each level of polyandry we extracted both: the male-female sexual network and male-male sperm competitive network, with edges between males weighted by the number of independent competitive events (e.g. in this case a weight of 2 represents that both males mated the same two females, as calculated as in equation (7)). For each male we then calculated the mean phenotype of his competitive group ($Z_{(localweighted)}$) as in

equation (8) and obtained male reproductive success (W_i). We standardized all selection gradients by dividing male fitness by the population mean fitness and standardizing both male trait and male group trait to have a mean of zero and a standard deviation of one.

Figure 3 illustrates the way polyandry affects the sexual selection gradient (β) on z, measured using both population-level selection analysis and contextual multilevel selection analysis based on network information. As expected, β measured using population-level (global) selection analysis increases with the level of polyandry in the population, because polyandry increases the level of sperm competition and the covariance between the male trait z and male reproductive success at the population level. Estimates of sexual selection on z using network-based contextual analysis however, reveal a more nuanced pattern. This approach demonstrates that local selection on z is consistently higher than estimates of β based on traditional population-level selection analysis. Such discrepancy is due to the fact that population-level selection analysis fails to account for local variation in the sperm competitive environment experienced by the ejaculates of individual males, due to the environmental gradient in male z. The contextual approach instead explicitly estimates selection on a male's competitive group trait, which at all levels of polyandry shows negative selection (fig. 3). This means that male reproductive success is affected not only by the absolute trait value (z) but also by the composition of the social group within which a particular male (or his ejaculate) competes. In this case there is negative individual selection on competing in highly competitive groups, in other words males are selected against sharing females with other males with large z. It is this effect of male competitive group that, although often implicitly assumed using traditional population-level techniques, is not quantified resulting in biased

estimates of sexual selection in structured populations (Formica et al. 2011). The greatest discrepancy between post-copulatory sexual selection gradients (β) on male trait z estimated by traditional population-level selection analysis and by network-based contextual analysis is observed at low to intermediate levels of polyandry (i.e. where the positive correlation between male's trait value (z_i) and the trait value of his competitors ($Z_{i(localweighted)}$) is expected to be strongest). Only when polyandry is complete (i.e. each female mates with every male in the population, p=50) do we find complete agreement between both selection approaches, as expected (Wade et al. 1999). These results illustrate how population structure can obscure the effect of polyandry and bias estimates of sexual selection when this is measured purely at the global (population) scale.

II) NEIGHBOUR ANALYSIS

We can utilize the same simulation to measure changes in sexual selection using the neighbour approach based on equation (4). This approach also includes the interactant covariance term (C_{ij}) which was calculated as the Pearson product-moment correlations between male z and $Z_{(localweighted)}$. This analysis generates a pattern of local and global sexual selection gradients that are virtually identical to those generated by the contextual analysis for most of the parameter range of polyandry with the exception of very low polyandry, where contextual estimates of $|\beta|$ on male z exceed those of neighbour $|\beta|$ (fig. 3). This discrepancy is due to the fact that because the contextual analysis measures $Z_{(localweighted)}$ including the focal individual, the difference between male z and $Z_{(localweighted)}$ is lower than in the neighbour analysis. This means that in the contextual analysis a more limited amount of phenotypic variation (compared to the neighbour analysis) will result in the same change in

standardized fitness and thus in steeper selection gradients. As the level of polyandry increases however, the contribution of the focal individual to $Z_{(localweighted)}$ becomes more negligible because group size increases leading to a rapid alignment of contextual and neighbour estimates.

THE 'MATING TOPOLOGY' AND 'SEX WEBS'

In addition to providing information to determine the local structure of phenotypic competition, it has more recently become clear that network structure itself may modify the strength and direction of selection. By forcing the ejaculates of males to compete, polyandry may reduce the relationship between mating success and paternity and increase the importance of sperm competition intensity. A large body of work has focused on how males act to reduce the risk of sperm competition through mate guarding (Alonzo and Warner 2000; Preston et al. 2005) or by preferentially mating with females that pose low risk or intensity of sperm competition (Zahradnik et al. 2008). These behaviours can have a profound impact on the spatio-temporal distribution of mating within a population, *the mating topology*. Yet, we know very little about the way the mating topology generates local variation in the level of sperm competition (the sperm competitive environment) experienced by individual males. For instance, if males who mate with many females also mate with the least polyandrous females (i.e. have higher exclusivity of mating) they may face lower sperm competition and this may increase variation in reproductive success within males (Sih et al. 2009). In networks, this would be described as *negative degree assortment* between male and female nodes (males with many mating partners, high mating degree, tend to be connected to females with low mating degree and *vice versa*). In ecological networks such as that of plant-pollinator or plant-herbivore

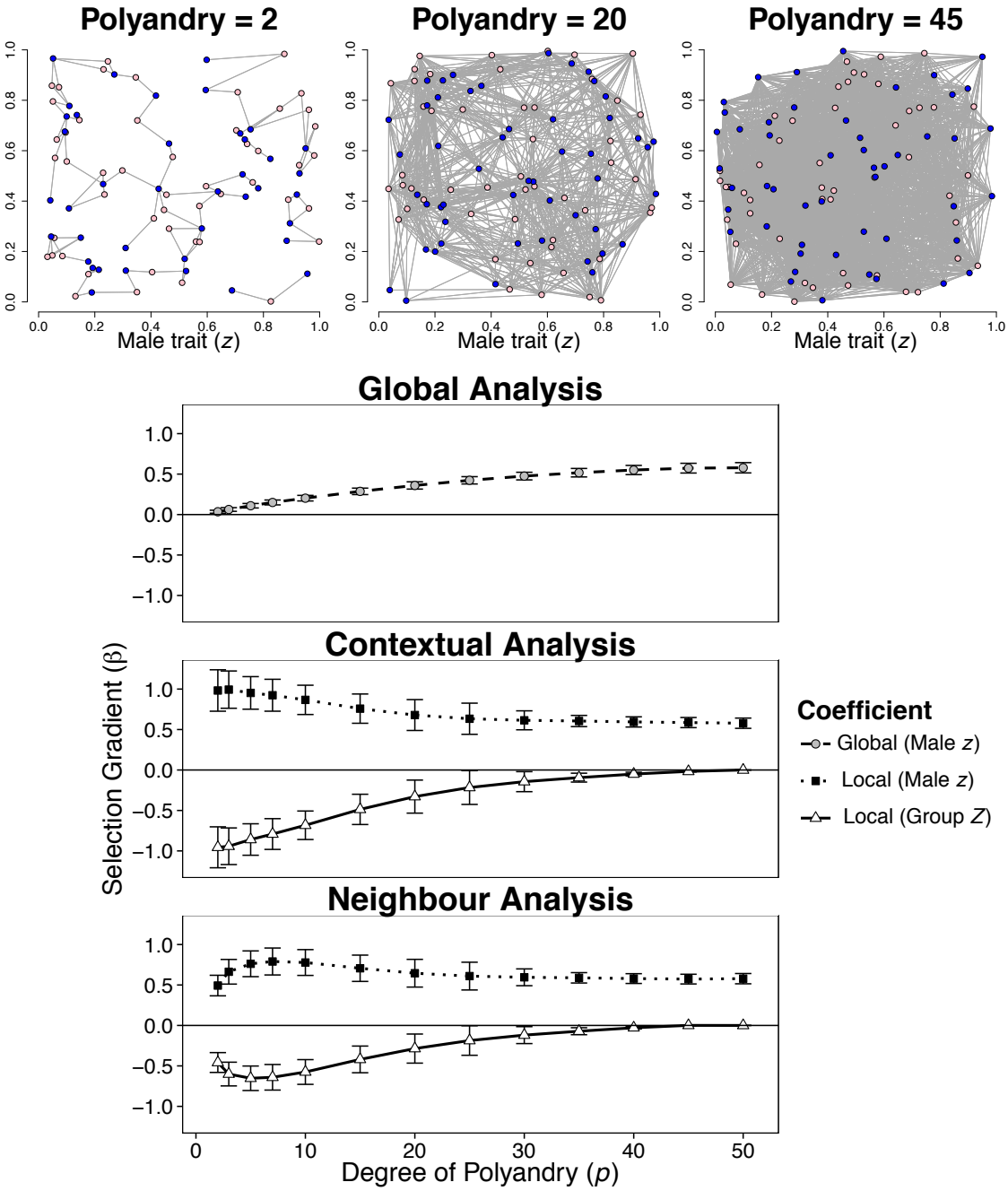


Figure 3. The relationship between the degree of polyandry in a population and estimates of sexual selection. The top three panels represent simulations of sexual networks under different degrees of polyandry, in populations comprising 50 males (blue) and 50 females (pink) arranged within a two-dimensional environment of fixed dimensions. Males are arranged according to the expression of a phenotypic trait (z), which affects the outcome of sperm competition. The bottom three panels illustrate the impact of polyandry on post-copulatory sexual selection gradient on male z, measured using traditional population level global selection analyses, contextual and neighbour analyses. Grey circles show global selection estimates, filled squares multilevel selection estimates on male individual trait, and open triangles show multilevel selection estimates on male group trait. Points show mean selection gradients based on 1,000 simulated populations ± 1SD.

systems where edges are drawn across different trophic levels or plants and their pollen vectors, negative degree assortment generates a nested pattern of interactions where "specialists" (those with few connections) from both sets of nodes tend to be connected to "generalist" nodes of the other set (fig. 4) (Bascompte and Jordano 2007; Ulrich et al. 2009). In a nested sexual network the "specialist" male nodes with few mating partners may suffer relatively high sperm competition whereas those males who mate with many females will have high exclusivity of mating across a subset of those females (fig. 4). Such a pattern may steepen the relationship between number of mates and number of offspring produced, thus increasing the strength of pre-copulatory sexual selection (i.e. selection on competition for access to mates). An alternative way to characterize such patterns of mating specialization is through network tools commonly used to study ecological specialization in food webs across trophic levels (Morris et al. 2004). Here, similar approaches can be utilized to characterize 'sex webs' and measure the degree of male and female specialization and their relationship. However, the mating topology has been rarely quantified and this may be in part due to a lack of adequate quantitative tools available. Characterization of the mating topology will allow for a more comprehensive understanding of how polyandry and population structure interact to determine both the strength and direction of pre- and post-copulatory sexual selection.

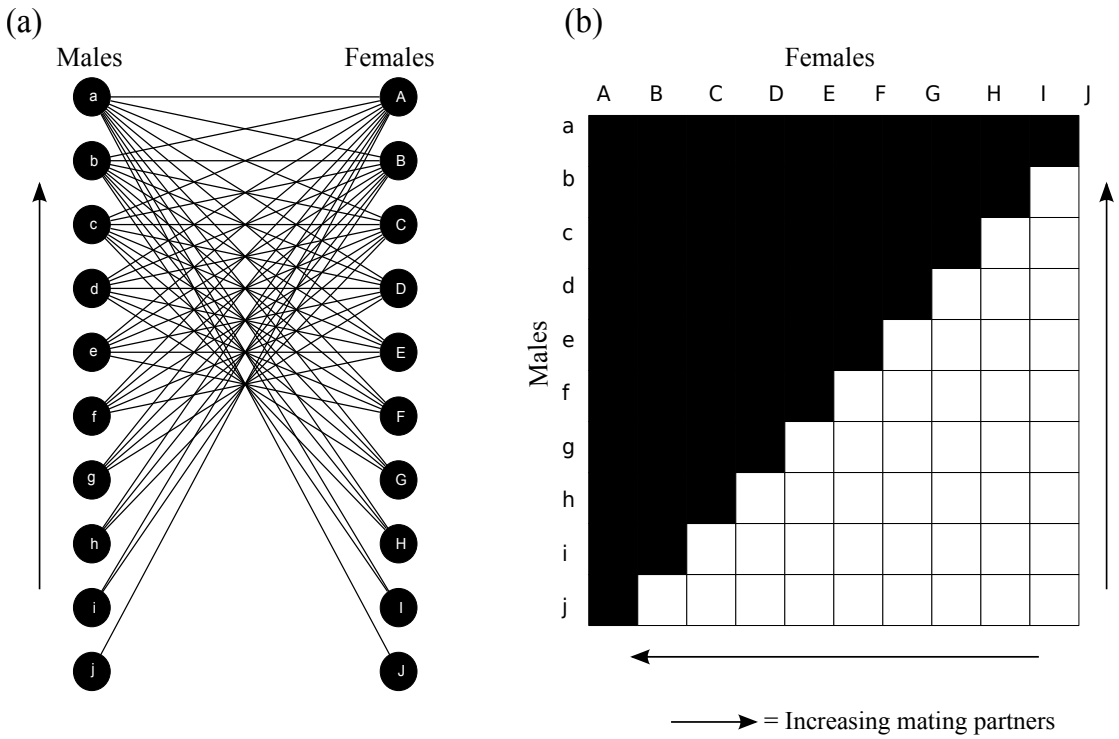


Figure 4. The mating topology. The same mating network between males [a-j] and females [A-J] demonstrating a nested pattern of copulations (density = 0.5), represented as: (a) a network, where nodes represent individuals and edges represent copulations, and (b) a matrix of males (rows) and females (columns), where filled squares represent copulations between individuals.

CONCLUSIONS

The realization that natural populations typically display degrees of both polyandry and viscosity means that the operation of sexual selection can differ markedly across the mating topology within the population. Standard quantitative tools to measure sexual selection at population level are often inadequate to capture such complexity. Here, we propose that a way to resolve this issue is to use a multilevel selection approach in which local and global scales are defined based on information independently derived as emergent property of sexual networks. The study of sexual networks can elucidate the fine-grained structure of intersexual interactions within populations, and its implications for variation in reproductive success and the operation of sexual selection.

The heuristic potential of this approach is vast and largely untapped. For example, how variation in female polyandry and complex behavioural processes such

as assortative mating govern the pre- and post-copulatory competitive landscape and its resulting effect on the operation of sexual selection represent exciting avenues of research that are yet to be fully explored. A particularly relevant future step emerging from the present work will be to investigate the effect of the 'scale' of polyandry; in other words, how far females and/or males move before females mate with a new male. It is plausible that even under relatively low levels of polyandry, patterns of local sexual selection will converge towards global sexual selection if substantial mixing (e.g. dispersal) occurs between mating events. Conversely, reduced dispersal might exacerbate the difference between local and global selection even under relatively high levels of polyandry.

Characterizing the sexual network of a population is a non-trivial challenge. The novel approach proposed here requires high-resolution behavioural data, comprehensive information of the mating and fertilization events representative of the whole population. However, studies of natural or semi-natural populations have become sufficiently sophisticated to generate this type of data. The application of recent technological advancement, such as complex visual recording systems (Branson et al. 2009; Rodríguez-Muñoz et al. 2010), as well as proximity loggers that allow the recording of interactions between individuals remotely (Guttridge et al. 2010; Psorakis et al. 2012; Rutz et al. 2012) and, importantly, also allow the inference of specific behavioural interactions (Krause et al. 2011) may facilitate obtaining such detailed interactions patterns in species where such observations may before been unfeasible in experimental and natural populations. Furthermore, experimental studies using semi-natural populations combined with network data will provide an opportunity to measure with exceptional accuracy the pattern of interactions and help

develop an understanding of how fine-scale assortment patterns may affect our measurement of selection using standard techniques.

CHAPTER 4. MATING BEHAVIOR: SEXUAL SELECTION AND SEXUAL NETWORKS

Grant C. McDonald and Tommaso Pizzari

Edward Grey Institute, Department of Zoology, University of Oxford, Oxford, UK.

ABSTRACT

A fundamental goal in biology is to understand how sexual behaviour translates into Darwinian fitness and evolves in response of sexual selection. Traditional quantitative frameworks to measure sexual selection and categorise mating systems often assume that populations are panmictic, an assumption undermined by recent work that shows that natural populations are often highly structured. In this chapter, we review recent theoretical and empirical work to illustrate how social network analysis can help biologists to study the complex patterns of sexual dynamics in structured populations.

INTRODUCTION

In non-selfing organisms, reproduction is mediated by interactions between individuals. These interactions represent a social trait, in which the sexual behaviour of an individual has the potential to affect not only its own fitness but also that of other individuals, through its influence on mating and fertilization (Pizzari and Gardner 2012). Differential sexual interactions among members of a population often constitute a major source of variance in individual fitness, creating opportunity for selection underpinning rapid and strong evolutionary change (Shuster and Wade 2003). However, studying the fitness implications of sexual behaviour is not trivial. The outcomes of sexual interactions are often complex because of three main reasons. First, the sexual behaviour of an actor has ramifications, which transcend dyadic interactions, by influencing the fitness of multiple recipients. Second, both the behaviour and its fitness outcomes can change plastically with the phenotype of the interactants. Third, individuals typically interact non-randomly within a population.

This can be due to multiple ecological and behavioural factors, which include spatio-temporal variation in resource distribution, constraints on dispersal, and assortative or disassortative matching. The goal of this chapter is to discuss how social network theory can help biologists better understand the evolutionary significance of sexual behaviour, by addressing these complexities. Recent years have witnessed an explosion of studies utilizing social network approaches to study animal behaviour. Our focus is to elucidate how these approaches can be harnessed to complement more traditional approaches and provide novel information on the evolutionary ecology of sexual behaviour. Below, we briefly introduce the principles of sexual selection theory, the Darwinian framework to understand the functional significance of sexual behaviour, and then present social networks within this context, i.e. sexual networks.

SEXUAL SELECTION

The functional significance of much of the diversity of sexual behaviours can be understood in light of two social mechanisms: (i) competition among members of the same sex over access to reproductive opportunities, and (ii) selection of reproductive partners by members of the opposite sex. Darwin (1859; 1871) intuited that these mechanisms arise from variation in individual reproductive success, generating opportunity for two distinct episodes of selection, namely intra- and inter-sexual selection. The realization of sexual selection resolved a number of outstanding issues in Darwinian theory: the evolution of extravagant traits, which was unexplained by viability selection, the evolution of sexual dimorphism, and the rapid evolutionary divergence of related species (Darwin 1871; Andersson 1994; Shuster and Wade 2003). Modern sexual selection theory developed this Darwinian framework in two fundamental ways.

First, while the Darwinian view of sexual selection was limited to variation in mating success, Parker (1970) realized that in several insect species sexual selection can in fact continue after mating because females mate with multiple males (polyandry) in a way that forces their ejaculates to compete for the fertilization of a single set of ova, a process that became known as sperm competition. Others noted that polyandry can also enable females to systematically bias the outcome of sperm competition in favour of the ejaculates of certain males, a process known as female sperm selection or cryptic female choice (Childress and Hartl 1972; Thornhill 1983; Eberhard 1996). Sperm competition and cryptic female choice represent episodes of intra- and inter-sexual selection respectively, which arise after copulation from variance in the share of paternity within a single set of eggs. The last decades have demonstrated that polyandry is near ubiquitous and that episodes of postcopulatory sexual selection can be powerful agents of evolutionary change (e.g. Birkhead and Pizzari 2002). Clearly, understanding the operation of sexual selection requires an appreciation of both pre- and postcopulatory processes. Despite this, however, only recently has a full appreciation for the potential for the interaction between both pre- and postcopulatory processes begun to develop (Collet et al. 2012; Kvarnemo and Simmons 2013; Parker and Birkhead 2013). Collet *et al.* (2012) investigated the relationship between the level of polyandry and the relative strength of both pre- and postcopulatory selection in red junglefowl, *Gallus gallus*, and showed that increasing levels of polyandry are associated with a decrease in the overall strength of sexual selection but that this decrease was chiefly the result of a reduction in the magnitude of the pre- (rather than post-) copulatory sexual selection. This study highlights how mating dynamics can not only affect the overall strength of selection but also

differentially modulate the roles of pre- vs. post-copulatory episodes, and thus the evolutionary trajectories of populations.

Second, a quantitative framework has been developed to formally define the opportunity for sexual selection and the strength of its episodes on individual phenotypic traits (Lande and Arnold 1983). The total reproductive success of an individual can be decomposed into three multiplicative constituents as follows:

$$T=(M*N*P)+\varepsilon \quad (1)$$

where M is the number of mating partners, N represents their fecundity, P the proportion of eggs fertilized (paternity share), and ε is an error term with 0 mean. M and N contribute reproductive success through mechanisms leading up to mating (i.e. pre-copulatory: intrasexual competition over mates and mate choice), while P captures post-copulatory mechanisms (sperm competition and cryptic female choice). The maximum potential strength of sexual selection is proportional to the variance in T, which is determined by the independent variance in each constituent and their covariances. The opportunity of sexual selection I, can therefore be expressed as the amount of standardized variance in each constituent (Shuster and Wade 2003) as follows:

$$I_X = \frac{\sigma^2}{\bar{X}} \quad (2)$$

where $\bar{X}$ represents the population average of a given constituent of reproductive success (T, M, N or P) and σ^2 its variance. Therefore, I is proportional to the maximum strength that sexual selection can exert on a given trait, z, which causally co-varies with reproductive success. This approach entails the estimation of the relationship between a measure of individual reproductive fitness and measurements of a phenotypic trait (e.g. tail length, body size, display rate) as follows:

$$\omega_T = \beta_z + \alpha \quad (3)$$

where ω_T represents reproductive fitness and α represents the intercept of the regression. Selection on such traits can then be formalized as β_z, the slope of the regression of ω_T on z, where ω_T and z are standardized to the mean of the population as a whole (Arnold and Wade 1984). This *selection analysis* provides researchers with an estimate of both the strength and direction of selection operating on the phenotypic traits measured, termed *selection gradients*. These selection gradients can be used to estimate both directional, as well quadratic selection such as stabilizing or disruptive relationships between fitness and the phenotypic trait (Brodie III et al. 1995; Hunt et al. 2009). Selection analysis has allowed researchers to determine the targets of sexual selection, understand its relative strength and form on different traits, as well as test how selection varies across populations and fitness components (Kingsolver et al. 2001; Kingsolver and Pfennig 2007; Siepielski et al. 2011).

SEXUAL SELECTION IN STRUCTURED POPULATIONS

The implicit assumption of these selection analysis studies, which measure both fitness and trait value relative to the population average, is that competitive and selective contexts are homogenous across the population (Formica et al. 2011; McDonald et al. 2013). However recently, studies taking a more sophisticated approach have undermined this assumption by showing how sexual selection may vary across a variety of ecological axes in both time and space (Cornwallis and Uller 2010). Both spatial and temporal variation in the ecological context of competition can have large effects on the strength and form of sexual selection. Furthermore, there has been increasing focus on how local variation in the social environment determines the force of selection on phenotypic traits and the evolution of sexual strategies. For example, Arnqvist (1992) found that variation in population density across three

populations of the water strider, *Gerris odontogaster*, not only changed the strength and form of sexual selection on male phenotypes but also qualitatively changed the targets of sexual selection. Similarly, Gosden and Svensson (2008) investigated both temporal and spatial variation in sexual selection regimes in the damselfly, *Ischnura elegans,* across an ecological gradient, and found a complex and variable mosaic of disparate sexual selection pressures on male body size, determined by fine-scale differences in densities of female colour morphs and body size. These studies call into question the assumption of classical population-level approaches that intrasexual competition at the population level (global scale) is representative of patterns of intrasexual competition and the social competitive context at the local scale (i.e. the structure of the competitive environment is homogenous across the population). Using simulations, Benton and Evans (1998) showed how such local structure can result in bias estimates of sexual selection. In populations where females select males following a "Best of n" strategy, local grouping of males by attractiveness can blur the relationship between male attractiveness and fitness at the population-level. To account for this, several quantitative methods have been developed to explicitly estimate the effect of the local socio-competitive group on individual fitness (Nunney 1985; Heisler and Damuth 1987; Wolf et al. 1999). These approaches, known as multilevel selection analyses, embody a hierarchical extension of classical selection analyses where selection is measured both at the level of individual phenotype and the level of the group phenotype (Okasha 2004a,b). The main multilevel selection approaches include contextual analysis and neighbour analysis (Nunney 1985; Heisler and Damuth 1987; Okasha 2004a). The explicit goals of these analyses are to estimate the relationship between individual fitness and the individual phenotypic trait

and the effects of the local competitive context on individual fitness. More formally, contextual analysis takes the form:

$$\omega_{ij} = \beta_1 z_{ij} + \beta_2 Z_j + \varepsilon, \quad (4)$$

where, ω_{ij} and z_{ij} are the fitness and trait values for the *i*th individual in the *j*th group, respectively, Z_j is the trait value for the *j*th group and ε is the residual variance. The regression coefficients β_1 and β_2 are, therefore, the partial regression of individual fitness on individual character, controlling for group character, and the partial regression individual fitness on group character, controlling for individual character, respectively. The fitness effects of an individual's local social group on individual fitness are captured by β_2. The neighbour analysis differs from contextual analysis only in the calculation of what constitutes group phenotype (i.e. Z_j, see McDonald *et al*. 2013). In practice, both methods are likely to give similar results, however larger differences between the two methods are expected when group size is low (Okasha 2004a; McDonald et al. 2013). A third common multilevel selection approach, social selection analysis, identifies local group in the same way as neighbour analysis (Wolf et al. 1999; Formica et al. 2011). However, this approach differs slightly from equation 4 in that it also includes a term called the interactant covariance, $C_{ij,}$ estimated using the Pearson product correlation between z_{ij} and $Z_{j.}$ This term measures the correlation between individual phenotypes and the phenotypes of their local group and is included thus:

$$\omega_{ij} = \beta_1 z_{ij} + C_{ij}\beta_2 Z_j + \varepsilon. \quad (5)$$

When positive, C_{ij} demonstrates that individuals of similar phenotypes tend to share group membership, whereas negative phenotypic assortment results in negative values of C_{ij}. By multiplying β_2, the term C_{ij}, translates local group effects into population-level selection on the focal trait. By estimating explicitly the effect of local

group on individual fitness, multilevel selection provides a powerful tool to understand how local competitive structure affects the evolutionary trajectories of phenotypic traits. Furthermore, such methods bring to the fore the opportunity for selection to act on alternative social strategies that individuals may use to alter their own selection regimes by manipulating their own competitive context (Formica et al. 2011).

Despite the appeal of the multilevel selection approach, however, its utility relies on the identification of individuals' local competitive group. This has often posed a non-trivial challenge (West et al. 2002), with local competitive context often approximated by using spatial proximity (Kasumovic et al. 2008; Eldakar et al. 2010). In many systems such measures of "local" may often require arbitrary decisions and may not reflect the scale of competition. Difficulties in determining local group have resulted in a bias in systems studied, towards more tractable invertebrate (Tsuji 1995; Eldakar et al. 2010) and sessile systems, where an individual's nearest neighbours are used to define local competition (Stevens et al. 1995; Donohue 2003, 2004; Weinig et al. 2007), with only one recent study in vertebrates (Laiolo and Obeso 2012). More complex analyses have been developed that use home-range overlap to infer the extent of competition between individuals (Formica et al. 2010). While these estimation methods may prove powerful especially in cases with largely indirect competition, they may not be appropriate to accurately identify functional groups in species with complex within-range space use (Formica et al. 2010). In fact, even within groups with home-range overlap there can considerable variation in interaction patterns between individuals both within- and between-groups (Wolf et al. 2007; Marsh et al. 2010).

THE LOGIC OF SEXUAL NETWORKS

Here we argue that the use of network data explicitly built upon interactions between individuals provides a powerful framework in which to characterize intra- and intersexual interactions and can be used to describe which individuals are in local competition. For example, in a wild population of house finches, *Carpodacus mexicanus*, Oh and Badyaev (2010) used network analysis to characterize the local competitive environments of individuals and its effects on male reproductive success. To achieve this, Oh and Badyaev (2010) first used network algorithms to define subsets of individuals (communities) within the finch social network, which interact with each other more often than the rest of the population. They then quantified selection on male ornamental traits by standardizing male trait value at the level of the community rather than that of the population. The study found that individual males can modify their own selective context, such that males of lower attractiveness were able to increase their relative attractiveness by moving between different socio-competitive groups. However, when measuring selection, community approaches may provide relatively coarse measures of individual competitive group. This is because community detection techniques do not account for variation in the structure of interactions within communities and do not account for weak ties between communities. An example population with a modular community structure is provided in figure 1. In this population, females inspect males and then choose to copulate with males based on their relative attractiveness. If the phenotypic distribution of males within this population were structured, such that males within communities were more similar, females would tend only to inspect a biased representation of the population phenotypic distribution due to the tendency of individuals to interact within rather than between communities. For this reason,

traditional population-level selection analyses would provide biased estimates of sexual selection on male attractiveness, by ignoring non-random female sampling regimes. Selection measures based purely on community membership would also ignore the between-community ties that themselves may have important repercussions for the estimation of phenotypic selection.

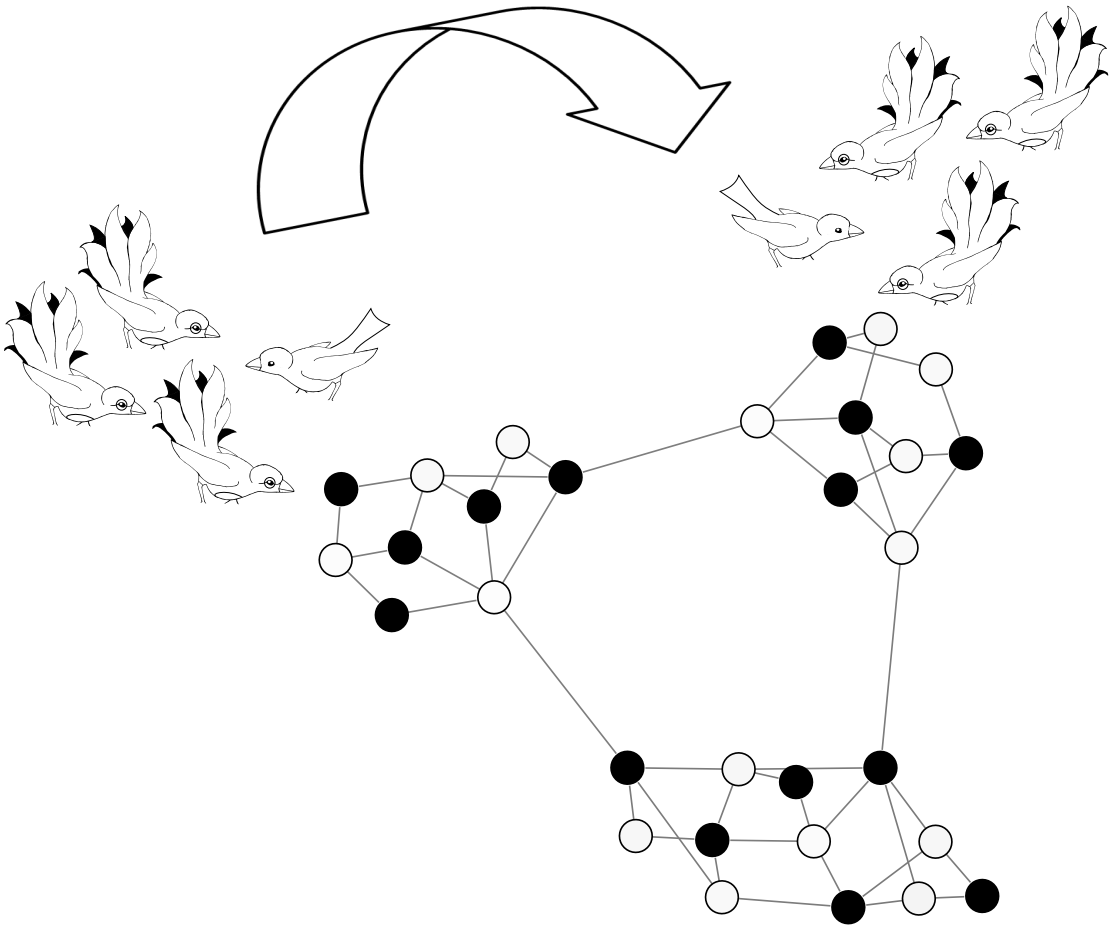


Figure 1 A hypothetical modular intersexual network with both males (black nodes) and females (white nodes). An edge (link) between a male and a female node represents courting between these two individuals The diagram highlights bridge females moving between communities to inspect males.

A key benefit of the network approach is that it allows a common framework that can be applied across different episodes of selection, which may operate on vastly different social and spatial scales. In the following sections we illustrate how sexual networks can be utilized to: (i) measure sexual selection, (ii) characterize patterns of intersexual interactions and their consequences for sexual selection, (iii) develop a

fuller understanding of mating systems, and (iv) study the functional significance of individual sexual network measures.

INTRASEXUAL INTERACTIONS AND THE MEASUREMENT OF SEXUAL SELECTION

McDonald *et al.* (2013) developed a quantitative framework based on network data of competitive interactions, which allows unique competitive environments for each member of the population. This method utilizes 'competitive networks' to capture the structure of intrasexual competition in sexual populations. Competitive networks can be derived from direct intersexual competitive interactions such as male combat or via intersexual male-female networks e.g. male-female courtship or copulations. These networks consist of individuals connected by edges representing unique competitive events. For instance, if two males within a lek display to the same female, both males have competed to mate that female and to fertilize her batch of ova and hence will share a competitive tie in a competitive network. Similarly, if two males inseminate a female sufficiently close in time such that their ejaculates overlap at the time of fertilization, then these males share a competitive tie through sperm competition. Following this pattern we can construct a complex web of interactions building competitive structure as an emergent property of individual interactions. Further resolution can be added to these networks, by adding value to the edges of the network, such that if two males compete over two unique females or for two batches of ova within the same female the competitive edge between these males will carry an edge weight of two. In figure 2a, we show how intrasexual competitive networks can be derived through the projection of intersexual courtship or copulation networks for dioecious animals. This approach is not limited to dioecious animals, and can be

extended to encompass sexual competition networks in hermaphrodites (Fig 2b) as well as pollen competition networks in flowering plants (Fig 2c).

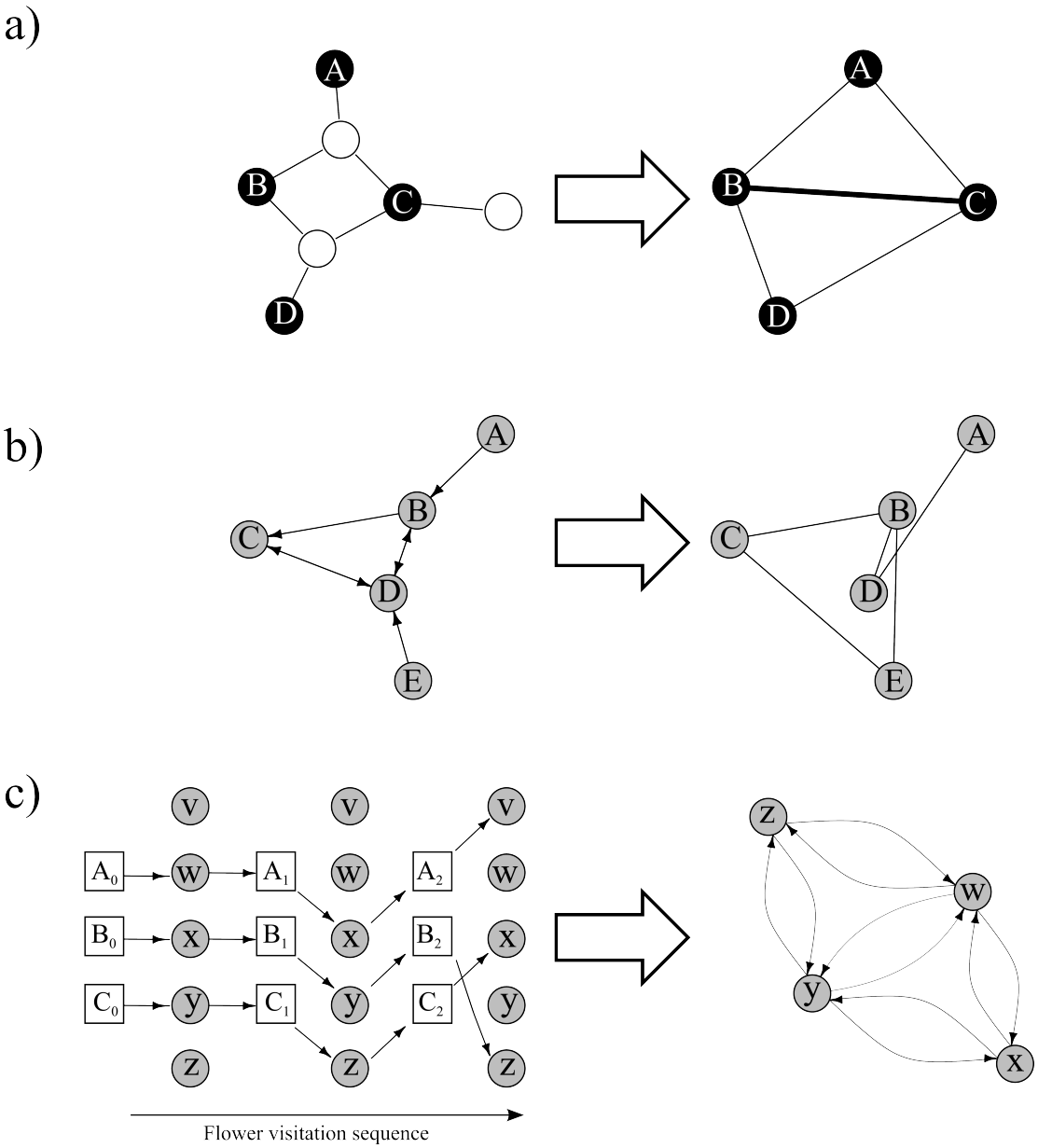


Figure 2. a) Intersexual network with males (black) and females (white) nodes projected into a male-male competitive network. b) A simultaneous hermaphrodite mating network where edges represent copulations. This network is directed with black arrows showing the direction of ejaculate transfer. This network is then shown projected into a male-male competitive network. c) Network of simultaneous hermaphroditic plants (circles) and pollinators (squares). Network shows pollinators visiting three successive plants each, and projection represents the pollen competition network between plant individuals.

Here, we extend the analysis of McDonald *et al.* (2013) and explore how the scale of competition may vary for both pre- and postcopulatory selection. We use a

simulation approach where males are organised into "leks" and females visit multiple leks forcing males to compete both before and after mating within and across leks. Specifically, we generated replicate populations of 500 males and 500 females. Individuals were placed randomly within a circle with radius 0.5. Each male was allocated a phenotypic trait *z*, ranging from 0 to 1, that positively influences both his pre- and postcopulatory reproductive success. Male trait *z* was allocated in direct relation to the position of males on the horizontal axis across the circle diameter, such that males on the left of the circle have lower values for *z* and males on the right of the circle have higher values of *z*. This generates an approximately normal distribution of male trait values for each population. Males were organized into 100 "leks" of 5 males each. Lek composition was determined with respect to *z* values, such that the first lek contains the 5 males with the lowest *z* and the last lek, the 5 males with the highest *z* values. Females visit a total of *n* leks, inspect all associated males and mate with the two males with the highest *z* within each lek, before moving onto the next lek. We generated 1,000 replicate populations each, for a range of values of *n* from 2-10. This creates a scale ranging from low levels of *n* where females are limited in the trait space they can inspect, up to *n*=10 where females could potentially inspect males from across the entire trait space. Lek composition was fixed for each male across all female movements. This process generates a competitive structure such that males only engage in precopulatory competition within their lek, but postcopulatory competition instead could occur within and between leks. For every male we calculated his mean mating success as the mean probability that an inspection would result in a successful copulation. Postcopulatory reproductive success was calculated as mean probability of fertilizing ova across all females mated where the probability of fertilization was calculated as the focal male's *z*, proportional

to the sum of all male *z* values that mated the particular female. For every replicate we generated both pre- and postcopulatory competition networks. We estimate the gradient of pre- and postcopulatory sexual selection on male phenotype (*z*) using network based multilevel selection analyses; for each model we included male trait *z* and the weighted mean group trait for each male as calculated from intrasexual competitive networks. We show how this approach provides a substantially more accurate representation of selection compared with classical population-based methods which quantify selection on a global scale.

PRECOPULATORY SELECTION

Across a gradient of number of leks visited by females, classical population-based methods fail to identify directional selection on male trait *z,* despite strong precopulatory selection imposed by our simulation protocol (Fig 3). This is because competition is highly local within the male leks and lek composition is positively assorted by *z* values. This obscures the correlation between precopulatory mating success and *z* at the population-level. Multilevel selection approaches using network information take into account this population structure. By identifying the scale at which competition occurs, both contextual and neighbour analyses identify strong positive precopulatory selection on *z* across all levels of *n*. The strong positive selection on male *z* is also closely mirrored in multilevel selection analyses by the strong negative selection on mean competitive group *z* values. This indicates strong selection against competing with males that have high relative *z* values. This result highlights the potential for selection not only to operate on increasing values of *z* but also on social strategies that may allow males to modify their competitive context, a

behaviour which has recently been identified in a number of studies (Oh and Badyaev 2010; Gasparini et al. 2013).

POSTCOPULATORY SELECTION

Similar to precopulatory selection results, multilevel selection analyses show consistently higher selection estimates on male trait *z* than global selection analyses. This difference is strongest when females visit few leks and polyandry is low. Global estimates of selection increase with the number of leks visited. This result is due to the combined effect of polyandry and the increase in the breadth of trait values that females sample, increasing the covariance between male trait *z* and fertilization success. These results are consistent with McDonald *et al* (2013) showing that for low to intermediate levels of polyandry multilevel selection analyses measure selection more accurately than classical approaches. As competition becomes more global due to female promiscuity both multilevel and global estimates of selection on *z* begin to converge.

Overall, these results highlight how both pre- and postcopulatory selection can operate on vastly different scales, both social and spatial, and that this provides opportunity to misinterpret measures of both pre- and postcopulatory selection. Specifically, the nature of competition through both episodes of selection and the scale at which they occur will determine the extent to which classical measures of selection provide biased estimates of phenotypic selection. Furthermore, these results demonstrate the power of multilevel selection techniques informed by sexual competitive networks to provide a common framework in which to measure sexual selection across disparate episodes of selection operating on different scales. Although we have focused on sexually competitive traits that result in negative group effects on individual fitness, more complex fitness implications may arise. For

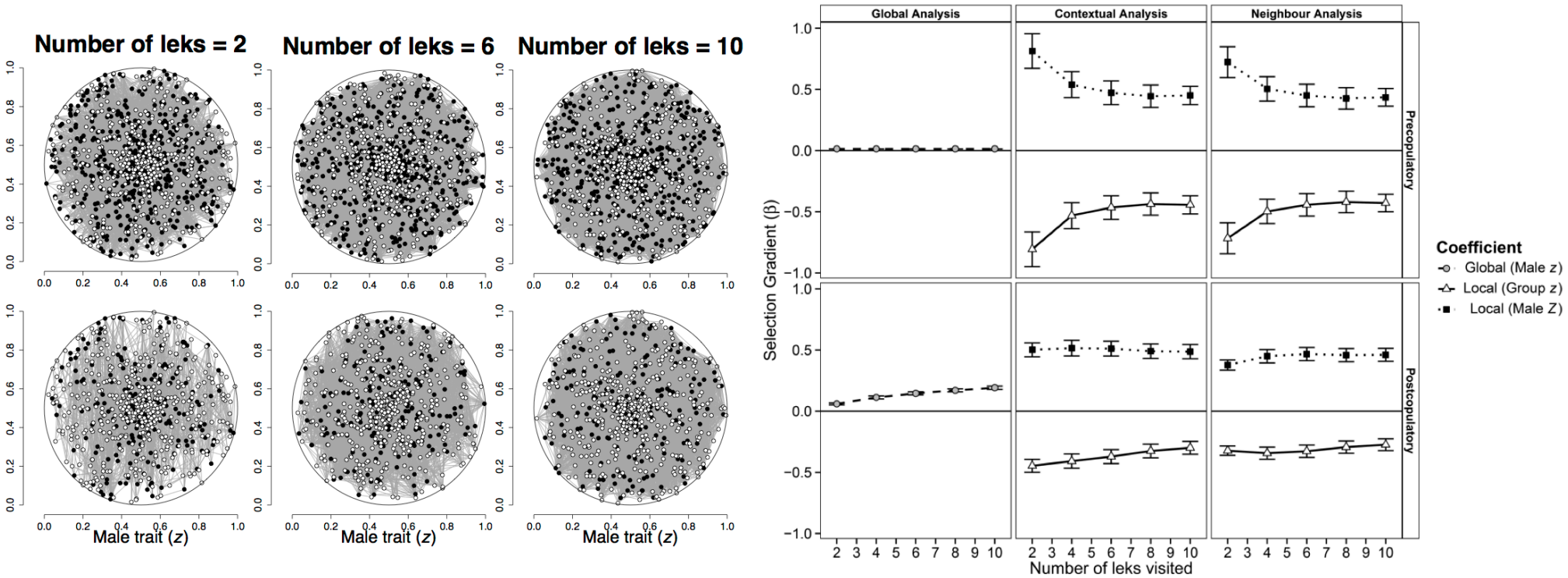


Figure 3. Simulation results. Network diagrams on the left show representative simulation results for six replicate populations of 500 males (black nodes) and 500 females (white nodes), where females inspect and mate males from 2, 6 and 10 different leks. The top row shows precopulatory networks where edges represent mate inspection events and the bottom row shows mating networks where edges represent copulations between pairs. The right-hand panel provides selection estimates on male trait *z* using population (global) selection analyses and two multilevel selection techniques based on network information (contextual and neighbour analyses). Selection gradient estimates show mean values across 1000 replicate populations ±1SD.

instance, competing in highly competitive groups may reduce the fitness of relatively poor competitors, but these individuals may also gain benefits if more females visit groups containing highly attractive males. It may be possible to measure such effects by partitioning the group component of multilevel selection analyses into a component which consists of the focal phenotype *relative* to mean competitor phenotype, and one which is explained by mean competitor phenotype alone. Finally, the methods presented above if combined with information on genetic relatedness may provide a useful tool to understand the extent to which related individuals compete directly and how this mediates the potential for kin selected behaviours.

INTERSEXUAL INTERACTIONS, MATING PATTERNS AND THE OPERATION OF SELECTION

In this section we explore how the mating patterns of a population, the 'mating topology', captured by network analysis can further elucidate the operation of pre- and postcopulatory sexual selection. In a mating network the number of mating partners an individual has is described by its node degree. In species where females do not mate multiply, i.e. no opportunity for postcopulatory selection, node degree may be sufficient to explain individual reproductive fitness (number of offspring). However, when females are polyandrous, a male's reproductive success also depends on the risk and intensity of sperm competition across the females with which he mated (Parker 1998). Under sperm competition, mating and sperm transfer may not necessarily result in the fertilization of ova (Preston et al. 2001). Copulations with polyandrous females may therefore represent decreasing returns in terms of fertilization success because they are associated with intense sperm competition (Parker and Pizzari 2010). Male reproductive success is thus tempered by the extent to which female partners mate with additional males. How such sperm competitive environments are distributed across males in the network may have strong repercussions for the distribution of reproductive success and the strength of selection.

If the males with the highest number of mating partners also tend to mate with females with the highest number of mating partners (i.e. the most polyandrous females) this may reduce the benefits, in terms of fertilization success, of high degree males as those males will also face on average the highest intensity of sperm competition (Sih et al. 2009). Indeed, if males that mate with many females also tend to face the most intense sperm competition then the number of mating partners may indeed negatively correlate with reproductive success. Importantly, this pattern would be expected to reduce the strength of precopulatory selection on mating success (i.e. β_M, the 'Bateman gradient'). This is because males with high mating success also on average face high sperm competition intensity, flattening the relationship between mating success and reproductive fitness. In network terminology this pattern is described as positive assortativity by degree (i.e. nodes of similar degree are positively associated; Fig 4a & b; Newman 2002, 2003). Degree assortment in networks has been explored in a variety of contexts including human and animal social networks (Newman 2002; Croft et al. 2005) as well as ecological networks (Abramson et al. 2011). In ecological networks such as that of plant-pollinator or plant-herbivore systems, edges are drawn across different trophic levels or between plants and their pollen vectors. Similar to male-female sexual networks, these networks are bipartite i.e. there are two sets of nodes such as consumers and producers, and edges are only drawn between these sets and never within. Such networks tend to show negative degree assortativity (disassortativity) where generalist species (those with many connections) tend to be connected to specialist nodes of the other set and *vice versa* (Bascompte et al. 2003). These disassortative networks also tend show a nested structure of interactions where specialist nodes only interact with a restricted subset of more generalist nodes (Bascompte et al. 2003; Bascompte and Jordano 2007; Ulrich et al. 2009; Joppa and Pimm 2010) (Fig 4c & d). This can generate a strong core-periphery structure, where a well connected core of individuals are strongly connected to each other, whereas peripheral nodes are weakly connected to the core and sparsely connected to each other (Bascompte and Jordano 2007; Ulrich et al. 2009) (Fig 4c & d). In the context of mating networks, such disassortative nested mating patterns, may

have strong implications for variation in the level of sperm competition intensity experienced by different males. This is because males with few partners face on average higher sperm competition than those with many mating partners who instead tend to experience higher exclusivity of mating (Sih et al. 2009). This scenario may increase the variation in male reproductive success and the Bateman gradient (Sih et al. 2009; McDonald et al. 2013).

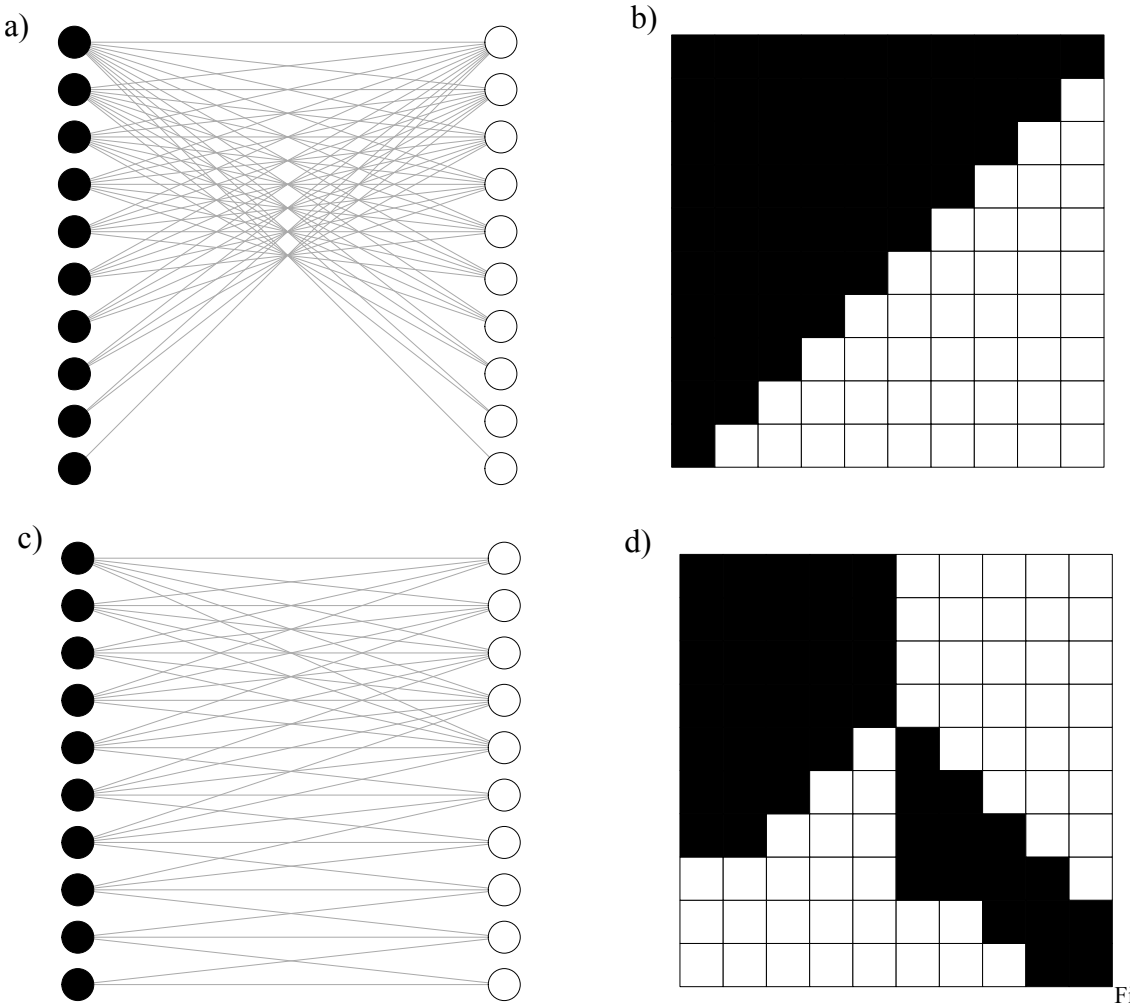


Figure **4**a-d. Two hypothetical mating networks each presented by two alternative graphical representations. Networks a & b provide network and matrix representation of a mating network characterized by positive degree assortment respectively. Networks c & d provide network and matrix representation of a mating network characterized by positive degree assortment respectively. Black nodes and rows represent males and white nodes and columns represent females. Edges and filled squares indicate a pair copulated. Males and females are ordered by number of mating partners.

Using existing network techniques it is possible to quantify degree assortativity in mating networks. Newman (2002) introduced a quantitative measure of degree assortativity in networks, now termed the Newman assortativity coefficient *r*. This measure is the standard Pearson

correlation coefficient between the degree of a node and the mean degree of its neighbours and ranges from -1 representing negative assortativity to 1 demonstrating high positive assortativity with 0 representing no relationship. Similarly, there has been a proliferation of nestedness measurements (Ulrich et al. 2009). One of the most widely used methods is that of Almeida-Neto et al. (2008) named NODF. This method is based on the rules of percentage overlap and decreasing fill between pairs of rows and columns and measures the nestedness of the whole network ranging from 100 (perfect nestedness) to zero (no nestedness). A strong positive relationship between nestedness and disassortativity is clear (May et al. 2008; Abramson et al. 2011; Gonzalez et al. 2011; Koenig et al. 2012), however, disassortativity is best regarded as a more general property of degree correlations also found in one-mode networks, whereas nestedness is restricted to bipartite (two-mode) networks (Sugihara and Ye 2009). Furthermore, generally positively degree assortative networks can show some degree of nestedness (Abramson et al. 2011). Despite its clear implications for sexual selection however, the way in which such population-level patterns of mating behavior (the mating topology) generate variation in the level of sperm competition experienced across males has rarely been quantified (Sih et al. 2009).

So far, we have discussed binary ties, where an edge is present or absent (i.e. the pair either mated or not). However, in many systems multiple mating events between the same individuals may be an important determinant of reproductive success (McNamara et al. 2008; Collet et al. 2012). In such cases, the frequency with which a male mates with individual females also needs consideration. This can be achieved using weighted networks, where edges are weighted by the number of repeat mating events between individuals. With respect to disassortativity and nestedness there has been effort to develop methods that utilize such quantitative interaction data (Galeano et al. 2009; Almeida-Neto and Ulrich 2011). A perfectly nested weighted network similar to that provided by Almeida-Neto and Ulrich (2011) is shown in figure 5a. In this network males are represented by rows and females by columns. Individuals are ranked based on the total number of unique partners (node degree) and the total number of copulations across all partners (node

strength). In the perfectly nested example the male with the highest number of mating partners also has the highest remating rate with the most polyandrous females and male remating rate decreases with female polyandry for all males, demonstrating that males re-mate with individual females based on their polyandry. Such a pattern would be expected to produce high variation in reproductive success among males as the males with the highest number of mating partners also have the highest relative sperm investment across females mated. As in binary nested networks we can again quantify the extent that the interaction patterns of nodes with few connections tend to represent subsets of those nodes with higher mating rates. Interestingly, the nestedness contribution for males and females can also be calculated separately, to understand whether the pattern of ejaculate transfer is nested across males (Fig. 5b) or across females (Fig. 5c). Quantitatively distinguishing between such population-level mating patterns may facilitate tests of optimal sperm allocation strategies across males and the influence of group-level patterns of such strategies for variation in male reproductive success.

More recently, further quantitative methods have examined the nestedness of ecological interaction networks such as host-parasitoid networks (e.g. Staniczenko et al. 2013). This work has revealed that although networks were binary nested, weighted networks tended to be non-nested, suggesting that interaction strengths between species are organized to reduce competition. Exploring such patterns in mating networks may provide an interesting complement to traditional techniques to understand how mating behaviour contributes the operation of selection and variation in reproductive success. Furthermore, it may be enlightening to investigate how such competitive structures vary in response to other factors such as group relatedness. Finally, degree assortativity in the mating topology will also have ramifications beyond sperm competition. For instance, classic research in the patterns of sexual behaviour in humans demonstrated how assortative mixing by degree will affect the transmission of sexually transmitted diseases (May 2013). In negatively assorted networks, epidemics may spread more slowly but reach a higher level of infection than in similar but positively assorted networks (Gupta et al. 1989).

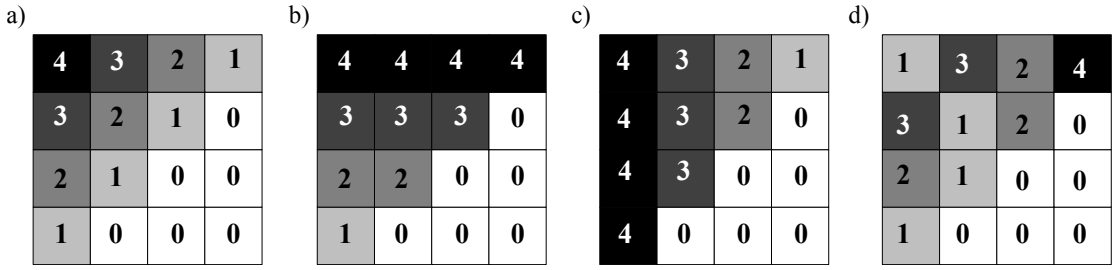


Figure 5 Four mating networks where rows represent males and females are represented by columns. Filled squares indicate mating between pairs. Numbers and shading intensity represent the number of copulations between pairs. Rows and columns are ordered by number of mating partners and total number of copulations. Networks demonstrate [a] perfect weighted nestedness, [b] weighted row nestedness, [c] weighted column nestedness, [d] high binary nestedness but relatively low weighted nestedness.

MATING SYSTEM ECOLOGY

Here we discuss how patterns of intersexual interactions captured by network analysis can help biologists understand how ecology shapes mating systems and the evolutionary ecological implications of such patterns. Classical characterizations of animal mating system employed coarse categories based on male and female promiscuity (e.g. monogamy, polygamy; Thornhill and Alcock 1983; Rubenstein and Wrangham 1987; Shuster and Wade 2003). In their seminal work, Emlen & Oring, (1977) linked mating system definitions to the ecological context of competition. This provided a predictive framework for variation in mating system based on the ecology of the population. A key prediction is that the spatial and temporal distribution of resources determine the ability of individuals to monopolize access to mates (Emlen and Oring 1977). For instance, where resources are spatially clumped and more easily defendable, a subset of the population may be able to better control access to this resource and therefore access to mates that utilize these resources. Therefore, variation in resource availability may affect the variation in reproductive success and the potential strength of sexual selection among populations. Similarly, the ratio of reproductive males to females (OSR; operational sex ratio) as well as the spatial and temporal distribution of mates (Emlen and Oring 1977; Clutton-Brock and Parker 1992) are expected to affect the strength of sexual selection acting in a population by varying the ability of individuals to monopolize reproductive opportunities. However, recent research has highlighted some of the limitations of this

classic categorization of mating systems. Bertram and Gorelick (2009) highlighted how vastly disparate mating patterns can result in the same variation in both male and female mating success. An example of this is illustrated in figure 6.

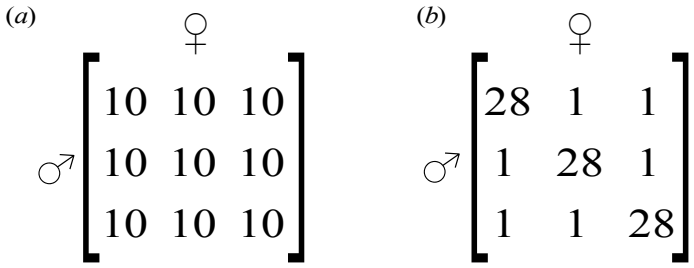


Figure 6 Matrices of two polygynandrous mating systems. Rows represent males and columns females, numbers in matrices represent the number of copulations between pairs. In both groups every male and female mates with three partners and so the opportunity for precopulatory sexual selection (I_s) is zero. Furthermore, every male and female has 30 total copulations and an average of 10 copulations per partner. Despite such similarities however, matrix *a* represents a highly promiscuous system, where every female mates equally across all males, leading to intense postcopulatory competition, whereas matrix *b* reflects clear preferential assortment between mates, generating relatively little postcopulatory competition.

In social network research, tools have been developed to distinguish such subtle but potentially important differences in interaction structure. For example, network clustering coefficients can be used to measure the extent that groups of nodes form smaller groups within the population with strong connections within the group and weak connections between groups (Watts and Strogatz 1998) and have been extended for use with weighted networks (Opsahl and Panzarasa 2009). Furthermore, a variety of community detection techniques allow the delineation of such strongly interconnected subgroups and have been used in variety of biological networks (Girvan and Newman 2002; Lusseau et al. 2006; Olesen et al. 2007). Such techniques may provide a useful tool to determine how demographic, genetic and ecological variables shape the non-random associations in sexual interaction networks. For example, Wolf *et al.* (2007) explored how factors such as demography and territoriality determine the structure of social networks in a colony of the Galápagos sea lion, *Zalophus wollebaeki*. Wolf *et al.* (2007) found that sea lion social structure was strongly hierarchical with sex and age determining population-level associations, while community structure was determined by individual space use. Such methods could be used to understand how mating structures change in fine-scaled ways according to different ecologies. For instance, do

different resource or nesting site distributions predictably alter the topology of mating interactions and the distribution of partner phenotypes available to individual males and females, and what are the resulting implications for the spread of sexually transmitted infections. Recent studies have investigated how changes in the availability of important food resources can alter the feeding regimes of different individuals, leading to variation in diet preferences and diet specialization (Araújo et al. 2008). Such preferences may result in consistent differences in associations between individuals (Ward et al. 2004, 2005) and have been shown to play a role in assortative mating (Snowberg and Bolnick 2008). How such assortativity is organized may have strong implications for the maintenance of alternative phenotypes and reproductive isolation. A recent theoretical treatment showed that whether the pattern of assortative mating acts in a graded fashion across the population or is realized in a more extreme (i.e. modular and stepwise) fashion can have important implications for speciation, as well as for the maintenance of distinct species during secondary contact (Kondrashov and Shpak 1998). Although assortative mating and incipient speciation have been documented (Reynolds and Fitzpatrick 2007), the extent to which this mating is stepwise and modular remains to be investigated empirically. We argue that network analysis provides a tool through which we can directly and empirically address these significant gaps in knowledge.

INDIVIDUAL NETWORK MEASURES

In addition to population- and group-level characterizations of sexual structure, network analysis also allows the identification and characterisation of the position of individuals nodes within networks (Newman 2010). How these individual-level metrics relate to the roles that individuals play within animal societies (Lusseau and Newman 2004), as well as the costs and benefits of individual network position have begun to be explored (Godfrey et al. 2009; Aplin et al. 2012). Here we discuss the potential for individual-level metrics within sexual networks to shed light on reproductive strategies and how the position of individuals within sexual networks can affect reproductive success.

The characterisation of individual node position, for example in terms of measures of network centrality, has been used to characterise the risk of sexually-transmitted infection of different individuals as well their role in disease spread (e.g. Corner et al. 2003; De et al. 2004; Christley et al. 2005; Fichtenberg et al. 2009). A number of studies have adopted a similar approach to investigate how individual position within social networks can affect individual mating and reproductive success (e.g. McDonald 2007; Ryder et al. 2008). The extent that such positional roles may represent reproductive strategies has recently been investigated by Oh and Badyaev (2010) in the house finch. Within this population, male social network position was measured in terms of individual *betweeness centrality* (Freeman 1978). Oh & Badyaev (2010) showed that individual males with the highest betweeness centrality, which may be expected to experience the greatest social diversity, had higher reproductive success. This is because, as discussed earlier, by moving between different groups, these males were able to modify their socio-competitive environment such that they increased their relative attractiveness to females compared to their competitors. This study provides an excellent example of how individual network measures can capture sexual strategies, which would be missed using more conventional methods.

In previous sections we discuss how a nested mating network may relate to group-level variation in the strength of precopulatory sexual selection. In such networks we may expect strong relationships between mating success and the level of sperm competition. Recent methodology developed in ecological research allows the calculation of each individual's contribution to the global level of nestedness in the network (Saavedra et al. 2011). Such methods may provide a useful characterization of sperm competition such that for any male with any given mating success, his contribution to the nestedness of the network may mediate his reproductive success. Recent research however, has shown that such higher-order network properties may be correlates of simpler measures (James et al. 2012). For example, Formica *et al.* (2012) used path analysis to explain male mating success in light of male position within male social networks in the forked fungus beetle, *Bolitotherus cornutus*, and showed that although some network metrics

independently predicted male reproductive success, measures of centrality were simply correlates of male activity level. Future work should therefore seek to establish how measurements such as individual contribution to nestedness add novel biological information to simpler approaches.

CONCLUSIONS

In this chapter we have outlined the diverse and complex array of social interactions arising from sexual reproduction. This complexity provides a challenge to researchers studying sexual selection. With this challenge in mind, we have discussed how network analysis may allow the characterization of complex web of socio-sexual interactions in both animals and plants. We then demonstrated how such quantitative network approaches may be utilized in combination with traditional sexual selection techniques to provide fertile ground for a more comprehensive view of reproductive behaviours and sexual selection.

CHAPTER 5. POLYANDRY, MATING STRUCTURE AND THE DETERMINATION OF SEXUAL SELECTION

Grant C. McDonald and Tommaso Pizzari

Edward Grey Institute, Department of Zoology, University of Oxford, Oxford, UK.

ABSTRACT

Multiple mating by females provides the opportunity for both pre- and post-copulatory sexual selection to operate on males. Empirical studies investigating the effects of polyandry on the strength of sexual selection have provided conflicting results, suggesting an intricate relationship between polyandry and sexual selection. This potentially complex relationship may be further complicated by recent work suggesting that the relationship between male and female promiscuity within a population may also shape sexual selection by influencing the reproductive value of male mating success. Understanding the causes of conflicting empirical results, and the mechanisms generating such differences between systems, requires quantitative predictions for the effects of polyandry under different biologically meaningful scenarios and for the role that the relationship between male and female promiscuity may have in further shaping the effects of polyandry. Here we use a simulation approach to generate predictions for the independent effects of polyandry and the relationship between male and female promiscuity. We demonstrate that the response of sexual selection to increasing polyandry depends on male promiscuity and the sperm competitive mechanisms of the system studied. Furthermore, we show that the relationship between male and female promiscuity has the potential to mediate or accentuate the effects of polyandry on the potential strength of sexual selection.

INTRODUCTION

Understanding the forces that generate variation in reproductive success is a fundamental goal in evolutionary biology. This variation sets the upper limit to the potential strength of selection operating on any trait that confers a competitive advantage (Crow 1958). In sexually reproducing species, the variation in reproductive success attributable to intrasexual competition over fertilisation opportunities determines the maximum potential strength of sexual selection (Bateman 1948;

Arnold and Duvall 1994; Shuster and Wade 2003). Within the classical Darwinian framework of sexual selection the total reproductive success of any individual male (*T*), was thought to be determined by two multiplicative components, the number of unique mating partners (*M*) and the average fecundity of those partners (*N*) (Darwin 1871) Over forty years ago, this simplistic view was overturned with the realisation that when females mate with more than one male (polyandry), intrasexual male competition can occur after mating, via sperm competition (Parker 1970) and cryptic female choice (Childress and Hartl 1972; Thornhill 1983). Polyandry allows for a "second round" of sexual selection and adds a further component, *P*, to our multiplicative model of male reproductive success. This final component defines the proportion of all the zygotes produced by the females, that are sired by the male that mated them (Webster et al. 1995), providing us with the final formalism,

$$T = M * N * P + \varepsilon \qquad (1)$$

where ε is the residual error. This conceptual framework, illustrates that polyandry reshapes male reproductive success (*T*) and its associated variation into the product of both pre- (*M* and *N*) and post-copulatory (*P*) processes.

Using the formal relationship in equation (1) it is possible to quantitatively partition the maximum potential strength of selection into its pre- and post-copulatory selection episodes (Webster et al. 1995). Specifically, the maximum potential strength of selection is determined by the variation in relative reproductive success, termed the opportunity for selection (I_T). Decades of intense research have aimed at understanding the traits and mechanisms through which reproductive success is determined through both pre- and post-copulatory mechanisms (Andersson 1994; Eberhard 2009). However, studies typically focused on either pre- or post-copulatory selection in isolation, whereas in natural polyandrous populations, both pre- and post-

copulatory sexual selection can operate together (Parker and Birkhead 2013; Shuster et al. 2013). More recently, an appreciation has begun to develop that, polyandry does not just simply provide the arena for post-copulatory sexual selection but can simultaneously and differentially modulate both pre- and post-copulatory episodes of sexual selection (Collet et al. 2012; Kvarnemo and Simmons 2013; Parker and Birkhead 2013; Shuster et al. 2013). This complexity arises because polyandry can influence all three constituents of male reproductive success (i.e. *M*, *N*, and *P*) and in differing directions. Furthermore, changes in the strength or direction of selection through one component of fitness may interact and alter the importance of another (Kvarnemo and Simmons 2013). Understanding how polyandry modulates the patterns, targets and strength of sexual selection hinges on resolving the way polyandry affects each component of reproductive success and how this sums to determine total variation in reproductive success.

Empirical studies have provided varying results, suggesting that polyandry may either increase or decrease the opportunity for selection (e.g. Whittingham and Lifjeld 1995; Freeman-Gallant et al. 2005; Collet et al. 2012; Schlicht and Kempenaers 2013; García-Navas et al. 2014). Reasons for such apparent inconsistencies may be due in part to the absolute level of promiscuity measured across studies. For example, in systems where polyandry is relatively low, i.e. the mating system is socially monogamous such as in the splendid fairy-wren (*Malurus splendens*), polyandry may provide the potential for increased variation in mating success which is otherwise minimized in strictly monogamous populations with an even operational sex ratio (Webster et al. 2007). However, such studies do not measure polyandry directly, but instead demonstrate the effects of some level of polyandry, revealed by the detection of extra-pair paternity, compared either

explicitly, or implicitly, to expectations under zero polyandry. This approach limits our ability to resolve the impact of a relative increase or decrease in polyandry. In non-socially monogamous systems, i.e. those systems that are both socially and genetically polygynandrous, results instead suggest that increasing polyandry may in fact reduce the overall strength of selection (Lesobre et al. 2010; Collet et al. 2012).

This potentially complex relationship between polyandry and sexual selection may be further complicated by recent work suggesting that the absolute level of polyandry is not the only potential axis in which variation in female promiscuity may affect variation in reproductive success (Sih et al. 2009). For any given level of polyandry, individual females may vary in the number of mates. This variation in polyandry may itself play an important role in determining the absolute strength of sexual selection and through which episodes selection may act most strongly. This is because polyandry tends to increase the intensity of sperm competition and accordingly, decrease average male paternity share (*P*). It is for this reason males of many species have developed complex traits such as mate-guarding and mating plugs to defend paternity share (Alcock 1994). Recent work suggests that the way female partners of varying promiscuity are distributed across males may have a large impact on the strength of sexual selection on males (Sih et al. 2009). For example, consider a situation where males that mate with many females (i.e. males with high *M*) also tend to mate with the most promiscuous females (females with high *M*). This pattern may result in the males most successful in precopulatory competition being the least successful in postcopulatory competition. This may weaken the strength of precopulatory sexual selection, as increasing average male *M* reduces reproductive gains of individual males by diminishing the proportion of ova fertilized by each male (*P*) (McDonald and Pizzari In press; Sih et al. 2009; McDonald et al. 2013). Such

patterns may be common in nature, for instance, in Soay sheep (*Ovis aries)*, high levels of polyandry result in a reduction in reproductive skew across males (Preston et al. 2001, 2003). However, it has also been shown in this system that those males that are least successful in attaining mates also tend to share the most promiscuous females (Preston et al. 2005), however this potential negative relationship between a male's mating success and that of his partners has not been quantified and so the extent to which this may temper the reduction in sexual selection caused by polyandry is not known.

In this study we use a simulation approach to assess the independent effects of polyandry per se and the relationship between male and female promiscuity on the total variance in male reproductive success (I_T) and its pre- and post-copulatory components (i.e. *M*, *N* and *P*). Specifically, our aims are to predict the effect of: (i) increasing polyandry and (ii) the relationship between male and female promiscuity on sexual selection ("mating structure"). We test the first aim for populations that are either originally monogamous or polygynous. To address the second aim we use recently proposed network analytical approach to determine the relationship between male and female *M*. Any sexually reproducing population can be visualised as a complex network of individuals nodes (males and females) connected by links (copulations), that not only defines the variation in mating success (*M*) but simultaneously defines the postcopulatory competitive landscape. Embracing this sexual network perspective, it is possible to quantify the extent to which male and female mating success covary. To measure this covariance we use two recently proposed measures, namely degree assortativity and nestedness (McDonald and Pizzari In press; Sih et al. 2009; McDonald et al. 2013). Specifically, degree assortativity measures the population-level correlation coefficient between the mating

success of a male and the mating success of his partners, whereas nestedness measures the extent to which males with fewer partners preferentially mate with females that preferentially mate with males with higher mating success (McDonald and Pizzari In press; McDonald et al. 2013). We provide qualitative and quantitative predictions for the effects of the average polyandry of a population and of its mating structure independent of polyandry on the operation of sexual selection on males.

METHODS

POLYANDRY SIMULATIONS

To assess the effect of polyandry on sexual selection we first simulated populations under two distinct starting mating systems, namely monogamy and polygyny. Each starting population was composed of 10 males and 10 females. In the monogamy simulation each male mates with one female each, such that all males and females have one mating partner each (average population polyandry = 1). In the polygyny simulation, one male mates with every female in the population and all other males have zero mating success (average population polyandry = 1; fig. 1b). Simulations were then carried out to increase the average polyandry of a population by adding randomly one copulation between a male and a female until every male mated with every female once (complete promiscuity, polyandry = 10; fig. 1b). For each increment in polyandry along this scale we generated 100 independent populations, totalling 9,000 populations starting with a monogamous mating system, and 9,000 starting with a polygynous mating system. To assess the impact that different mechanisms of sperm competition[1] may have on estimates of sexual

[1] Here we discuss sperm competition as the mechanisms for biasing paternity, however, such biases could equally be driven by cryptic female choice

selection, at each increment in polyandry we determined male reproductive success under several possible scenarios (fig. 1a). In total, we considered eight different scenarios, determined by whether a single male fertilises the entire set of ova (winner fertilizes all, WFA) or whether the most successful male fertilizes only a larger proportion of the ova than his competitors (winner fertilise some, WFS), whether males are equipped with a postcopulatory trait, *z*, that positively influences their fertilisation success (*P*), and for those scenarios with such a trait *z*, whether or not *z* positively or negatively covaries with male mating success (*M*). Of these eight scenarios, five fall under the heading WFA where, female clutch size equals one, and males successful in sperm competition necessarily fertilise all of a female's ova, and three are WFS, where females each produce 100 ova and all males that mate with a female fertilise some proportion of her ova. For both WFA and WFS, we ran one base simulation where males did not possess a postcopulatory trait *z*, (WFA:BASE and WFS:BASE, respectively) and so males that share a female have a probability of fertilizing an ovum, *p*, that is proportional to the number of males mating with the female (e.g. if four males mated with a female each male had a 25% chance of fertilising her single ovum or a 25% chance of fertilisation for each of her 100 ova, i.e. p=0.25). For simulations where males possess a postcopulatory trait *z*, males were allocated a trait value from a normal distribution with a mean of 5 and a standard deviation of 1. In each case *z* positively affected a male's probability of fertilisation such that the probability, *p,* that the i_{th} male fertilises any given ova produced by the *k*th female (whether females possess 1or 100 ova) is determined by:

$$p_i = z_i / \sum_{i=1}^{n_k} z_i \qquad (2)$$

where, n_k is the number of males that mated with the kth female. Because clutch size is kept constant across females within a simulation, the average p for each male, calculates as:

$$\bar{p}_i = \sum_{j=1}^{M_i} p_j / M_i \quad (3)$$

for i_{th} male who mates with M females, will correlate positively with P.

Using this basic framework we further modified the predictability of z in determining a male's overall P through its correlation with p, when clutch size was fixed at one. In the first scenario, P was determined such that the male with the highest p to mate with a female always fertilises this female's single ovum ($p \equiv 1$; WFA:Intense). In the second WFA scenario (WFA:Weak), males tend to fertilise the female's ovum proportionally to p, e.g. if p=0.75 then a male has a 75% chance of fertilisation. The third sperm competition mechanism was a WFS scenario (WFS:Weak), where paternity was determined such that a male had a probability of fertilising each individual ovum proportional to p, e.g. if p=0.75 then a male has a 75% chance of fertilising each independent ova. Finally, in all simulations with a postcopulatory trait z, we manipulated the covariance between a male's z and his mating success (M), such that z either tended to covary either positively or negatively with M (fig. 1b). For all eight simulation scenarios, we generated the 9000 matrices as described above, for both monogamous and polygynous populations and measured standardised total variation in male reproductive success (I_T), standardised variance components in mating success (I_M) and paternity share (I_P) and standardised univarite Bateman gradients (β'_M). For the six scenarios where simulations included a male trait z, we also calculated the standardised covariance between M and P ($Cov(M,P)$). All measures of sexual selection were calculated as presented in Webster et al. (1995).

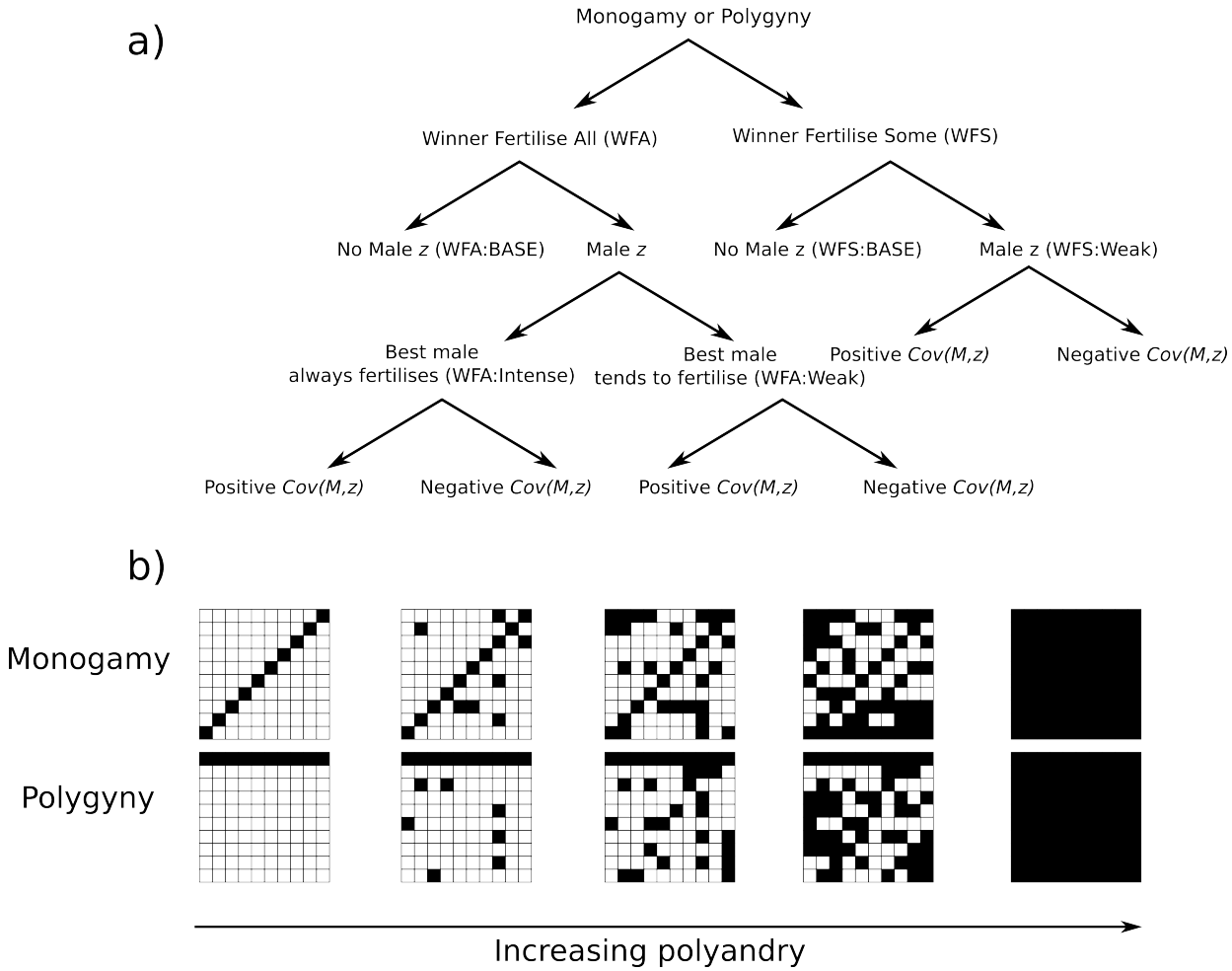


Figure 1. Panel (a) shows the different scenarios used to determine male reproductive success from simulated mating populations. Each terminal end of the tree represents one of the eight simulation scenarios. Panel (b) shows the two starting conditions, monogamy and polygyny, used in all simulations. Each matrix represents an individual mating population where column are females and rows are males, filled squares represent copulations and empty squares demonstrate male and female pairs that have not mated. This panel shows matrices may be randomly filled with copulations across a range of polyandry values.

MATING STRUCTURE SIMULATIONS

To assess the effects of population mating structure (relationship between a male's M and the average M of his partners) we use network approach that conceptualises the mating patterns of a population as individual nodes (males and females) connected by links (copulations). This approach allows us to quantify the extent to which male and female mating success covary using two recently proposed measures, namely degree assortativity and nestedness (Sih et al. 2009; McDonald et al. 2013, McDonald & Pizzari *in press*). To achieve this we used an approach similar to that of Saavedra & Stouffer (2013), which visualizes interactions between two sets

of entities (originally plants and their mutualists, in this case males and females) as a network of interactions. We used 59 empirical plant-animal mutualistic networks that have been used previously in the literature (Rezende et al. 2007; James et al. 2012). These 59 mutualistic networks have the same structure as mating networks in dioecious species, in that there are two types of nodes animals (males) and plants (females) that share connections but no connections can occur within a node type (i.e. males cannot be connected to other males). For each network we use the raw empirical structure of the mutualistic network as a mating network assuming animals are males and plants are females and connections between nodes are copulations rather than interspecific interactions. This provides us with 59 hypothetical mating networks that vary in the number of individuals, sex ratio and the number of total copulations measured as network density (i.e. network density = total number mating pairs / total number of possible mating pairs). In general, these networks tend to be negatively assorted by degree (disassortative), that is, nodes with many connections tend to be connected to nodes with fewer connections and *vice versa*. For each of the 59 networks we used two complementary randomisation algorithms to generate randomised versions of the empirical networks. Both approaches hold density (and therefore, average polyandry) constant within each population, allowing us to assess the effects of network structure for a given level of polyandry. The first randomisation algorithm named the "probabilistic" algorithm generates randomised versions of the empirical network where the probability of drawing an interaction between any animal and plant is proportional to the degree of both the plant and the animal (Bascompte et al. 2003; Olesen et al. 2007; Fortuna et al. 2010; Saavedra and Stouffer 2013). The probabilistic method however does not maintain the same degree distribution, i.e. this method alters the variance in number of mates (degree) across

males and females and allows some males and females not to mate at all. Specifically, this method tends to reduce the variance in mating success across males and females (Saavedra and Stouffer 2013). Because variance in mating success forms part of the variance in reproductive success we also use another randomisation algorithm, "Fixed", that maintains the same variation in mating success as the original empirical network for each randomised version and therefore allows us to assess the effects of network structure independent of changes in variation in mating success and mean polyandry (Gale 1957; Connor and Simberloff 1979; Gotelli 2000; Fortuna et al. 2010; Saavedra and Stouffer 2013). Note that variation in mating success as measured by I_M is not held constant in Fixed simulations because the calculation of I_M is affected by mean P (I_P) (i.e. $I_M = \overline{N}^2\overline{P}^2 Var(M)$). It is for this reason that when analyses are interested in changes in variation in mating success independent of P we use the Opportunity for sexual selection (I_s), calculated as $I_s = Var(M)/\overline{M}^2$, as our measure of standardised variation in mating success as it is not confounded by changes in mean P. Using both algorithms we generated 250 randomised networks for each of the 59 empirical networks, resulting in 14750 networks that provide us with a range of different mating structures. For each algorithm, we then simulated reproductive competition using two different approaches, the first approach where females produce one ova each and males have a probability of fertilising a females ova proportional to the number of males mating the female e.g. if 4 males mated with a female each male had a 25% chance of fertilising her ova (WFA:BASE). The second approach females produced 100 ova and males had a probability of fertilising each ova proportional to the number of males mating the female e.g. if 4 males mated with a female each male had a 25% chance of fertilising ova independently (WFS:BASE). This resulted in a total of four separate simulations where randomised

matrices either controlled for variation in mating success (Fixed) or did not (Probabilistic), and were either WFA or WFS. For every randomised network we also measured a number of network parameters including network density (total number of mating pairs / total number of possible mating pairs), network magnitude (number of males × number of females). To measure the relationship between a males *M* and that of his partners we use two recently advocated measures, assortativity and nestedness. Assortativity was measured as the Pearson correlation coefficient (*r*) between a male's degree and the mean of his partner's degree minus one, and so *r* ranges from 1 to -1 (Newman 2002). Nestedness was calculated using one of the most widely used methods named NODF (nestedness measure based on overlap and decreasing fills, Almeida-Neto et al. 2008) which measures the extent to which those nodes with many connections tend to be connected to nodes with fewer mating partners. This method is based on the rules of percentage overlap and decreasing fill between pairs of rows and columns and thus quantifies extent to which the mating partners of individuals with low degree form subsets of the mating partners of those individuals with a higher degree (McDonald and Pizzari In press). NODF measures the nestedness of the whole network ranging from 100 (perfect nestedness) to zero (no nestedness).

For each of the four scenarios (i.e. probabilistic WFA, probabilistic WFS, fixed WFA, fixed WFS) we assessed the effects of nestedness (NODF) and assortativity (*r*) on several sexual selection measures using generalised linear mixed models (GLMMs) across the 14,750 simulated networks. For all analyses we treated NODF and assortativity as predictors in separate models because the high colinearity between these variables may reduce confidence in predictor estimates. Response variables in GLMMs included the standardised total variation in reproductive success (I_T), standardised variance components in mating success (I_M) and paternity share (I_P),

standardised covariance between *M* and *P* (*Cov(M,P)*) and standardised univariate Bateman gradients (β'_M). For the probabilistic simulations fixed effects included either NODF or assortativity and as covariates network density, network magnitude, and the number of females that produced offspring. Number of females that produced offspring was included as this simulation allowed some females not to mate and only mated females reproduced, which results in variation in the total number of fertilised ova in Probabilitsic simulations. Finally the ID of the original network was included as a random effect. For the fixed simulations fixed effects included either NODF or assortativity and as covariates network density, network magnitude, and the ID of the original network was included as a random effect.

We also assessed the relationship between NODF and assortativity of networks for Fixed and Probabilistic models with assortativity as the response, NODF as an explanatory variable and covariates as above for Fixed and Probabilistic models respectively and original network ID as a random effect. For these results we only used data from WFA simulations because we were only assessing the structure of mating networks before paternity was determined and therefore the sperm competitive mechanism would not affect results.

Finally, in an attempt to compare the utility of both measures of mating structure in explaining variation in reproductive success we ran individual linear models assessing the effects of either NODF or assortativity on the opportunity for selection (I_T) independently for each set of 250 simulated networks for all 59 empirical networks for both fixed and probabilistic simulations and for both WFA and WFS simulations. For fixed simulations these models simply included the opportunity for selection (I_T) as the response variable and either assortativity (r) or NODF as the explanatory variable and for probabilistic models we added the number of

reproducing females as a covariate. For each linear model we extracted the adjusted coefficient of determination (R^2) and then used GLMMs to compare whether models using assortativity or NODF to explain variation in I_T have higher R^2 values. We ran one GLMM for each combination of fixed/probabilistic and WFA/WFS, resulting in 4 GLMMs. Each GLMM had R^2 as the response variable and mating structure measure (i.e. NODF or assortativity (r)) as a main effect and we included the original network ID as a random effect. All statistical analysis was carried out using R statistical software (R Core Team 2014).

RESULTS

I) THE EFFECT OF POLYANDRY

Base scenarios simulations (WFA:BASE & WFS:BASE)

In initially monogamous populations, polyandry initially increased the overall opportunity for sexual selection but this increase was much reduced for WFS populations (fig. 2a, c). Instead for initially polygynous populations, polyandry tended to reduce the overall opportunity for sexual selection (fig. 2b, d). However, the increase in I_T for monogamous populations was far smaller than the respective decrease for polygynous populations. Changes in I_T were largely due to an increase or decrease in the variation in male mating success respectively (fig. 2i-l). For monogamous populations, I_M increased initially then slowly declined (fig. 2j, k), whereas in polygynous populations the reduction in I_M was much larger and for both WFS and WFA scenarios the rate of decline decreased asymptotically with increasing polyandry (fig. 2j, k). Standardised variance in paternity share (I_P) in all cases initially increased and remained relatively constant across further changes in polyandry (fig. 2m-p). However, for WFS:BASE, the magnitude of the increase in I_P was limited compared to WFA:BASE, as all males that mate gain some paternity share (fig. 2m-

p). Furthermore, in monogamous populations with WFS:BASE there is an additional peak in the variation in I_P across populations around polyandry values of 2-5. This is because within monogamous populations, at those levels of polyandry, many males may have exclusive pairs but some males may share many females, increasing the maximum potential I_P for originally monogamous populations compared to lower or high values of polyandry (fig. 2o). Throughout all levels of polyandry, pre-copulatory sexual selection as measured by the Bateman gradient (β'_M) was randomly and equally spread around zero in WFA:BASE and WFS:BASE with the largest variation at higher levels of polyandry (fig. 2e-h). This is due to the probabilistic nature in which reproductive success is determined meaning across populations, M may be positively, negatively or uncorrelated with P.

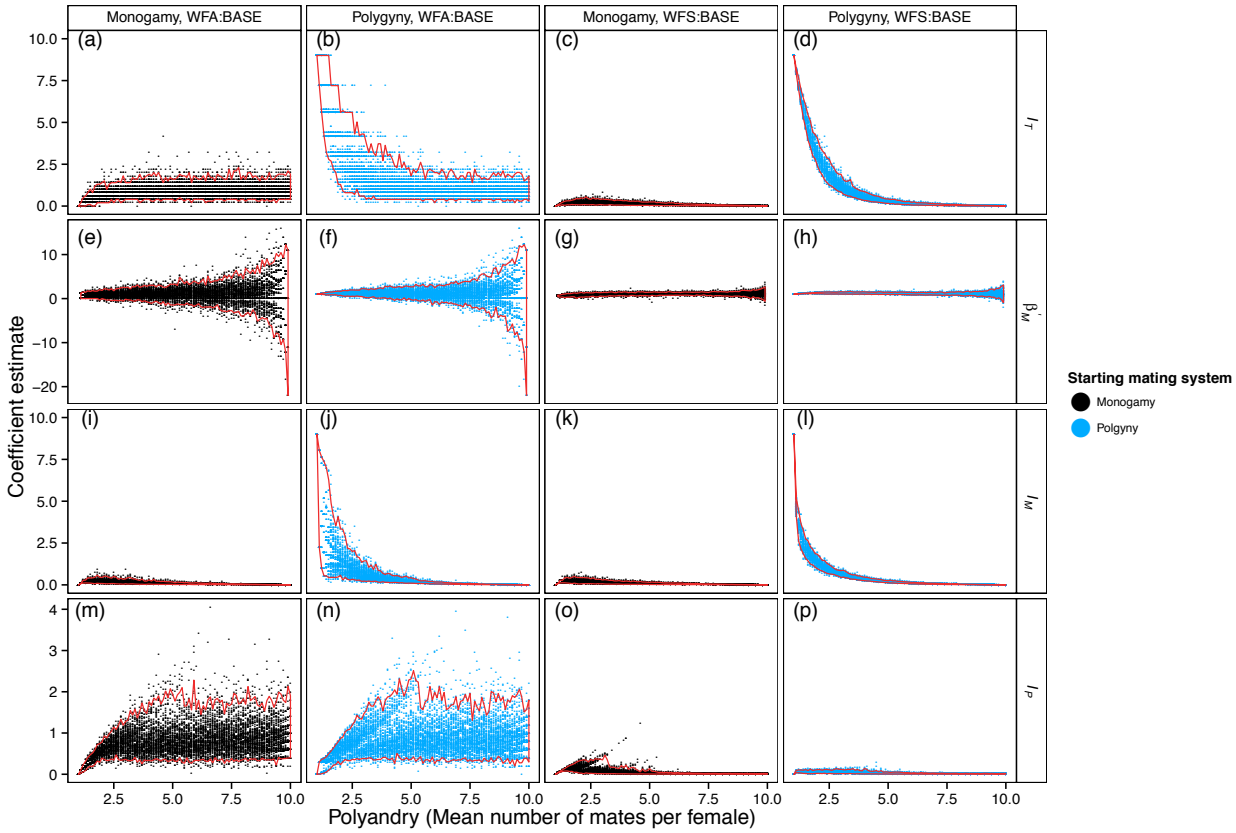


Figure 2. Simulation Results for WFA:BASE and WFS:BASE polyandry simulations where females produce one egg (Winner fertilise all) or 100 eggs (WFS). Each male that mated with a female had an equal chance to fertilise her ova. Panels show the effect of increasing polyandry on total standardised variation in reproductive success (I_T) (a-d), standardised univariate Bateman gradients (β'_M) (e-h), standardised variance in mating success (I_M) (i-l) and paternity share (I_P) (m-p). Each panel shows the results for 9,000 populations of 10 males and 10 females created from two distinct starting mating systems called "monogamy" (black) and "polygyny" (blue). For monogamy simulations each male mated with one female each at polyandry = 1, for the polygyny simulation one male mated with every female in the population at polyandry = 1. For each increment of polyandry graphs present results for 100 independent populations totalling 9000 populations per panel. Red lines represent upper and lower interquartile ranges.

Winner fertilise all with post-copulatory z scenarios (WFA:Intense & WFA:Weak)

When sperm competition follows a 'winner fertilise all' pattern and the best male always fertilises a clutch (WFA:Intense, fig. 3), the impact of polyandry on I_T depended on the covariance between M and z and on the initial mating system of the population. For monogamous populations I_T continued to increase with polyandry (fig.3a, b), as the males with highest z eventually mated with more females, increasing variance in reproductive success. In this case the negative covariance between M and z tended to reduce the rate at which polyandry initially increased I_T(fig.3 a & b). For polygynous populations, results are more complex. When the covariance between M and z is positive the best male already mates with all females and so there was no

change in I_T. (fig.3 c) However, for these populations the relative contribution of M and P did change as postcopulatory mechanisms became more important in determining T (fig. 3k, o). Instead, for polygynous populations with a negative covariance between M and z, there was an initial decrease in I_T as the better sperm competitors begin to mate with more females, stealing paternity from the males with higher M (fig. 3d). Eventually, this decrease was overturned and I_T increased to the maximum level as the best sperm competitors continued to increase their mating success (fig. 3d). In all cases, the variance in paternity share (I_P) increased steadily with polyandry (fig. 3m-o). Finally, the strength of precopulatory sexual selection as measured by the Bateman gradient initially begun at 1 for polygynous populations and 0.5 or 1 for monogamous populations (fig. 3e-h). Bateman gradients then increased or decreased with polyandry depending on whether the covariance between M and z is positive or negative, respectively, resulting in the strongest possible selection on mating success occurring when the variance in mating success is low. This is because Bateman gradients are calculated as $Cov(M,T)/Var(M)$, so reductions in variance in mating success combined with proportional increases in $Cov(M,T)$ (driven by the positive or negative covariance between M and P caused by z) result in maximum possible values for β'_M at higher levels of polyandry. Under winner fertilise all sperm competition (WFA:Weak, fig.4), results were typically more similar to WFA:BASE (fig.2) and I_T stabilised much earlier than WFA:Intense due to a lower maximum potential variance set by postcopulatory selection (fig.4 a-d).

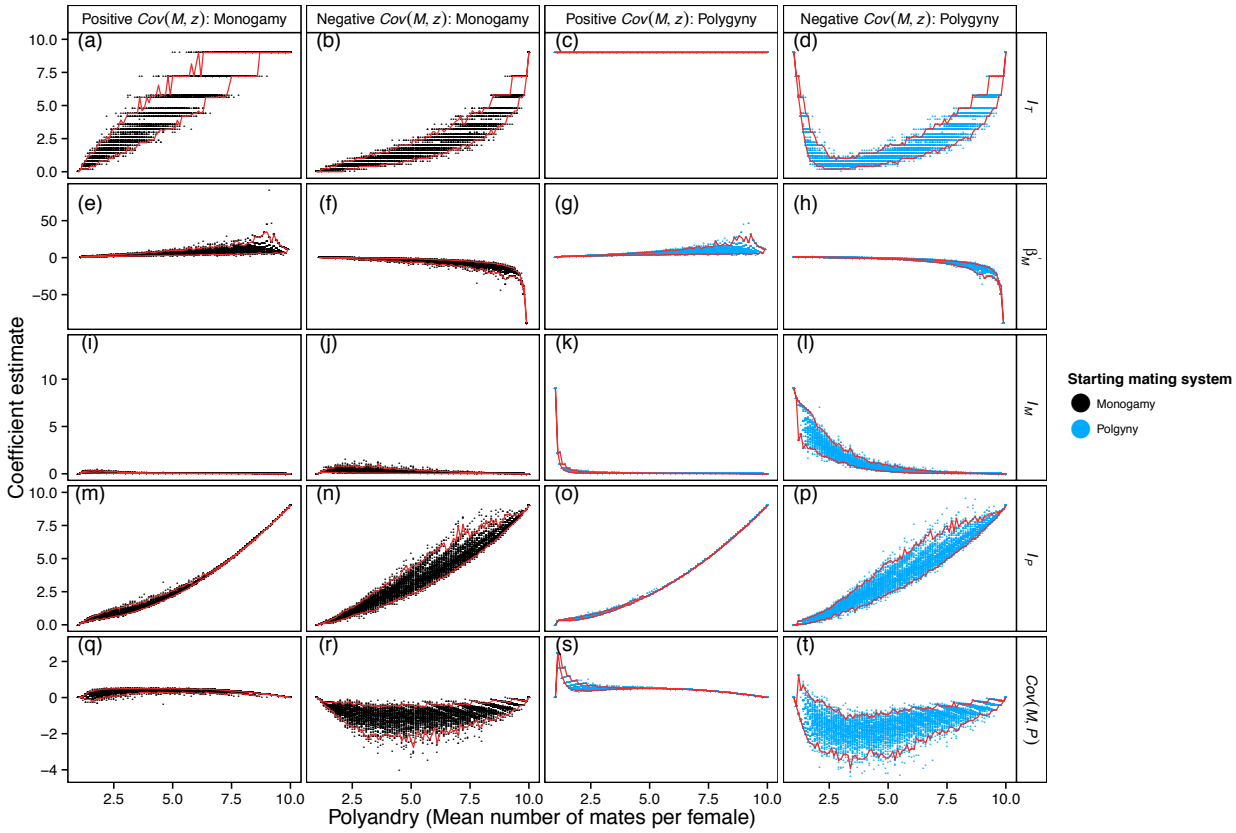


Figure 3. Simulation Results for WFA:Intense simulations where females produce one egg (Winner fertilise all) and the male with the highest post-copulatory trait *z* always fertilises the female's single ovum. Panels show the effect of increasing polyandry on total standardised variation in reproductive success (I_T) (a-d), standardised univariate Bateman gradients (β'_M) (e-h), standardised variance in mating success (I_M) (i-l), paternity share (I_P) (m-p) and the standardised covariance between M and P (*Cov(M,P)*) (q-t). Each panel shows the results for 9,000 populations of 10 males and 10 females created from two distinct starting mating systems called "monogamy" (black) and "polygyny" (blue). For monogamy simulations each male mated with one female each at polyandry = 1, for the polygyny simulation one male mated with every female in the population at polyandry = 1. For each increment of polyandry graphs present results for 100 independent populations totalling 9000 populations per panel. Red lines represent upper and lower interquartile ranges.

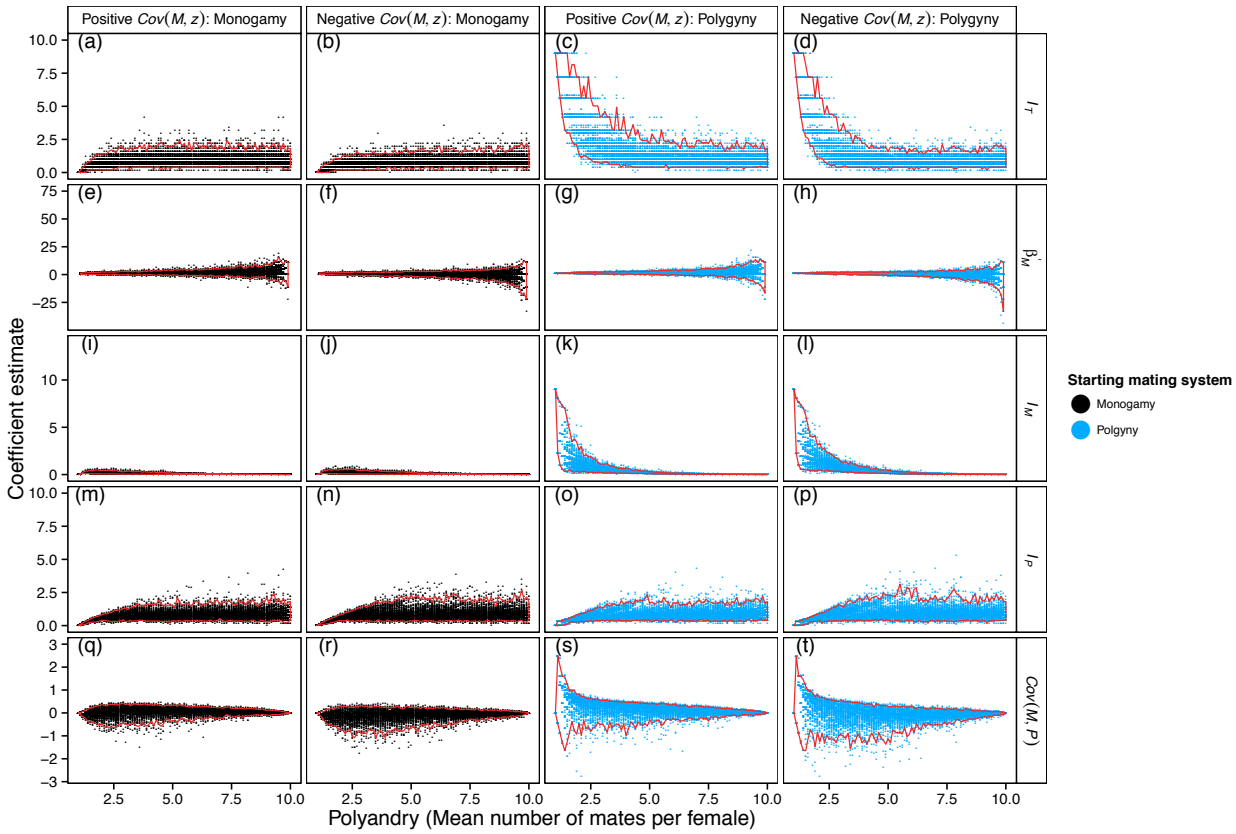


Figure 4. Simulation Results for WFA:Weak simulations where females produce one egg (Winner fertilise all) and the male with the highest post-copulatory trait *z* tends to fertilise the females single ova. Panels show the effect of increasing polyandry on total standardised variation in reproductive success (I_T) (a-d), standardised univariate Bateman gradients (β'_M) (e-h), standardised variance in mating success (I_M) (i-l), paternity share (I_P) (m-p) and the standardised covariance between M and P ($Cov(M,P)$) (q-t). Each panel shows the results for 9,000 originally monogamous (black) or polygynous (blue) populations (see above). Red lines represent upper and lower interquartile ranges.

Winner fertilise some with post-copulatory z scenario (WFS:Weak)

When sperm competition follows a "winner fertilizes some" pattern and selection on *z* is weak (WFS:Weak) (i.e. the best male does not always fertilise the majority of a females ova), I_T showed a small initial increase with increasing polyandry for monogamous populations due to increased variance in mating success. However, the overall pattern was a strong decline with further increments of polyandry (fig. 5a, b). This decrease occurred because the maximum variation set by postcopulatory sexual selection was lower in WFS than in WFA as all males attain some level of paternity. When all males mate with most females (polyandry is high), ova are shared out more equally in a WFS scenario than in a WFA scenario where

fertilization by one male means that all the other males mating with same female do not fertilize any of her ova. Again, variance in paternity share (I_P) initially increased for both monogamous and polyandrous populations (fig. 5m-p). However, for monogamous populations the initial increase was much higher as at these low levels of polyandry, within a given population many males may still mate with females who only mate once (i.e. p=1) but some males may share very polyandrous females, increasing the maximum potential variance in P within populations (fig. 5m, n). This pattern was not observed to the same extent in polygynous populations where individual males never had complete exclusivity over females (fig. 5 o, p). Standardised Bateman gradients tended to increase or decrease with polyandry depending the covariance between M and z as described in WFA scenarios (fig. 5e-h).

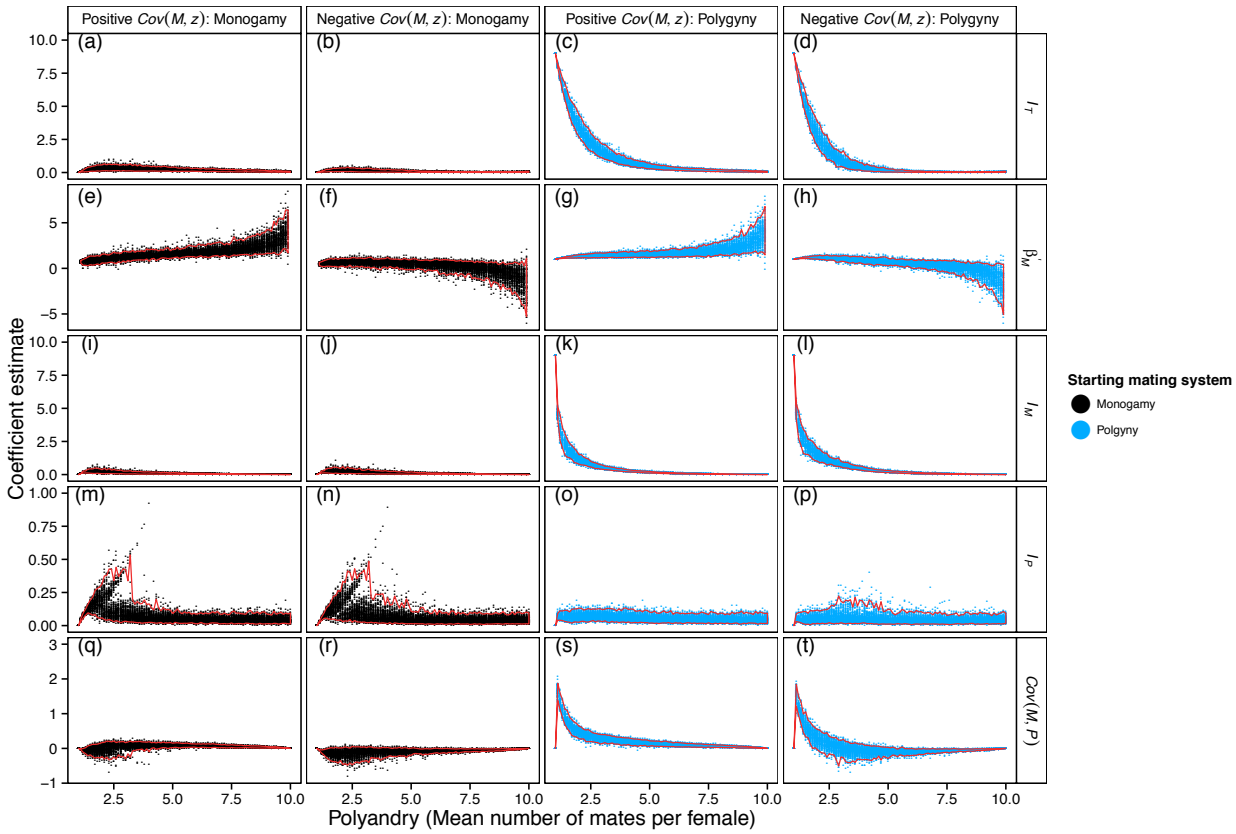


Figure 5. Simulation Results for WFS:Weak simulations where females produce 100 eggs (Winner fertilise some) and the male with the highest post-copulatory trait z tends to fertilises each of the females 100 ova. Panels show the effect of increasing polyandry on total standardised variation in reproductive success (I_T) (a-d), standardised univariate Bateman gradients (β'_M) (e-h), standardised variance in mating success (I_M) (i-l), paternity share (I_P) (m-p) and the standardised covariance between M and P ($Cov(M,P)$) (q-t). Each panel shows the results for 9,000 originally monogamous (black) or polygynous (blue) populations (see above). Red lines represent upper and lower interquartile ranges.

II) MATING STRUCTURE SIMULATIONS

Nestedness (NODF)

Nestedness as measured by NODF positively and significantly predicted standardised variation in reproductive success (I_T) across all simulations (Table 1; fig. 6a, d, g, i; see full model results Appendix§1 Table A1) and this effect was strongest using the fixed simulation algorithm and for WFA postcopulatory competition. The increase in I_T was accompanied by an increase in precopulatory sexual selection measured by the standardised Bateman gradients (β'_M) (Table 2; fig. 6b, e, h, k; Appendix§1 Table A2). This increase in β'_M was driven by an increase in the covariance between M and P ($Cov(M,P)$) (Table 3; fig. 6c, f, i, l; Appendix§1 A3).

Further relationships between NODF and I_P and I_M are presented in Appendix§1 Tables A4 & A5. In general the relationship between NODF and measures of sexual selection tended to be flatter for probabilistic simulations than for the fixed simulations (Tables 1-3, fig. 6). This difference in slope was potentially driven by lower average NODF for probabilistic simulations (fig.6) and because variation in male mating success, as measured by the Opportunity for sexual selection (I_s), is allowed to vary from the original empirical networks in probabilistic simulations and tends to be reduced (Saavedra & Stouffer, 2013). This tendency to reduce I_s across randomized networks may result in lower scope for NODF to increase variation in reproductive success, resulting in relatively lower increases in measures of selection for a standard increment of NODF in probabilistic versus fixed simulations, as evidenced by the relatively lower standard errors in GLMMs for probabilistic simulations (Tables 1-3).

Finally, estimated slopes for selection measures against NODF for WFS simulations tended to be similar or steeper than WFA simulations, potentially reflecting the greater consistency in the assignment of reproductive success to males in WFS systems than WFA systems (i.e. for any level of NODF and any network position of a male within that nested network, the relationship between his position and his reproductive success will be stronger in the WFS simulations than WFA simulations due to the consistency with which males achieve some reproductive success).

Table 1. GLMM results for the relationship between the opportunity for selection (I_T) and nestednesss (NODF).

Simulation type	Full model	Competitive mechanism	Parameter removed	Model number & comparison	Parameter estimate ± SE	AIC	LRT
Fixed	Opportunity for selection vs NODF (I_T)	WFA:BASE	None NODF	1 2 vs 1	- 0.06 ± 0.008	42227 42282	- $\chi2 = 57.135$, df = 1, p < 0.001
	Fixed effects: Density + NODF+ Magnitude, Random effects: Original network	WFS:BASE	None NODF	1 2 vs 1	- 0.05 ± 0.002	6532.3 7011.6	- $\chi2 = 481.25$, df = 1, p < 0.001
Probabilistic	Opportunity for selection (I_T) vs NODF	WFA:BASE	None NODF	1 2 vs 1	- 0.03 ± 0.002	29675 29834	- $\chi2 = 160.49$, df = 1, p < 0.001
	Fixed effects: Density + NODF+ *n* females + Magnitude Random effects: Original network	WFS:BASE	None NODF	1 2 vs 1	- 0.03 ± 0.0006	-7172.0 -4215.8	- $\chi2 = 2958.2$, df = 1, p < 0.001

Table 2. GLMM results for the relationship between the univariate Bateman Gradients (β'_M) and nestednesss (NODF).

Simulation type	Full model	Competitive mechanism	Parameter removed	Model number & comparison	Parameter estimate ± SE	AIC	LRT
Fixed	Standardised variance in univariate Bateman Gradients (β'_M) vs NODF	WFA:BASE	None NODF	1 2 vs 1	- 0.03 ± 0.002	14915 15058	- $\chi2 = 144.87$, df = 1, p < 0.001
		WFS:BASE	None NODF	1 2 vs 1	- 0.04 ± 0.001	-22080 -20023	- $\chi2 = 2059.1$, df = 1, p < 0.001
	Fixed effects: Density + NODF+ Magnitude, Random effects: Original network						
Probabilistic	Standardised variance in univariate Bateman Gradients (β'_M) vs NODF	WFA:BASE	None NODF	1 2 vs 1	- 0.01 ± 0.001	17732 17851	- $\chi2 = 121.03$, df = 1, p < 0.001
		WFS:BASE	None NODF	1 2 vs 1	- 0.016 ± 0.0003	-24194 -22077	- $\chi2 = 2118.8$, df = 1, p < 0.001
	Fixed effects: Density + NODF+ *n* females + Magnitude Random effects: Original network						

Table 3. GLMM results for the relationship between the standardised covariance between *M* and *P* (*Cov(M,P)*) and nestedness (NODF).

Simulation type	Full model	Competitive mechanism	Parameter removed	Model number & comparison	Parameter estimate ± SE	AIC	LRT
Fixed	Standardised covariance between mating success and paternity share (*Cov(M,P)*) vs NODF Fixed effects: Density + NODF+ Magnitude, Random effects: Original network	WFA:BASE	None	1	-	1897.2	-
			NODF	2 vs 1	0.03 ± 0.002	2161.7	$\chi 2 = 266.49$, df = 1, p < 0.001
		WFS:BASE	None	1	-	-36739	-
			NODF	2 vs 1	0.04 ± 0.0006	-32886	$\chi 2 = 3855.5$, df = 1, p < 0.001
Probabilistic	Standardised covariance between mating success and paternity share (*Cov(M,P)*) vs NODF Fixed effects: Density + NODF+ *n* females + Magnitude Random effects: Original network	WFA:BASE	None	1	-	2702.9	-
			NODF	2 vs 1	0.008 ± 0.0007	2829.4	$\chi 2 = 128.48$, df = 1, p < 0.001
		WFS:BASE	None	1	-	-38438	-
			NODF	2 vs 1	0.01 ± 0.0002	-35820	$\chi 2 = 2619.9$, df = 1, p < 0.001

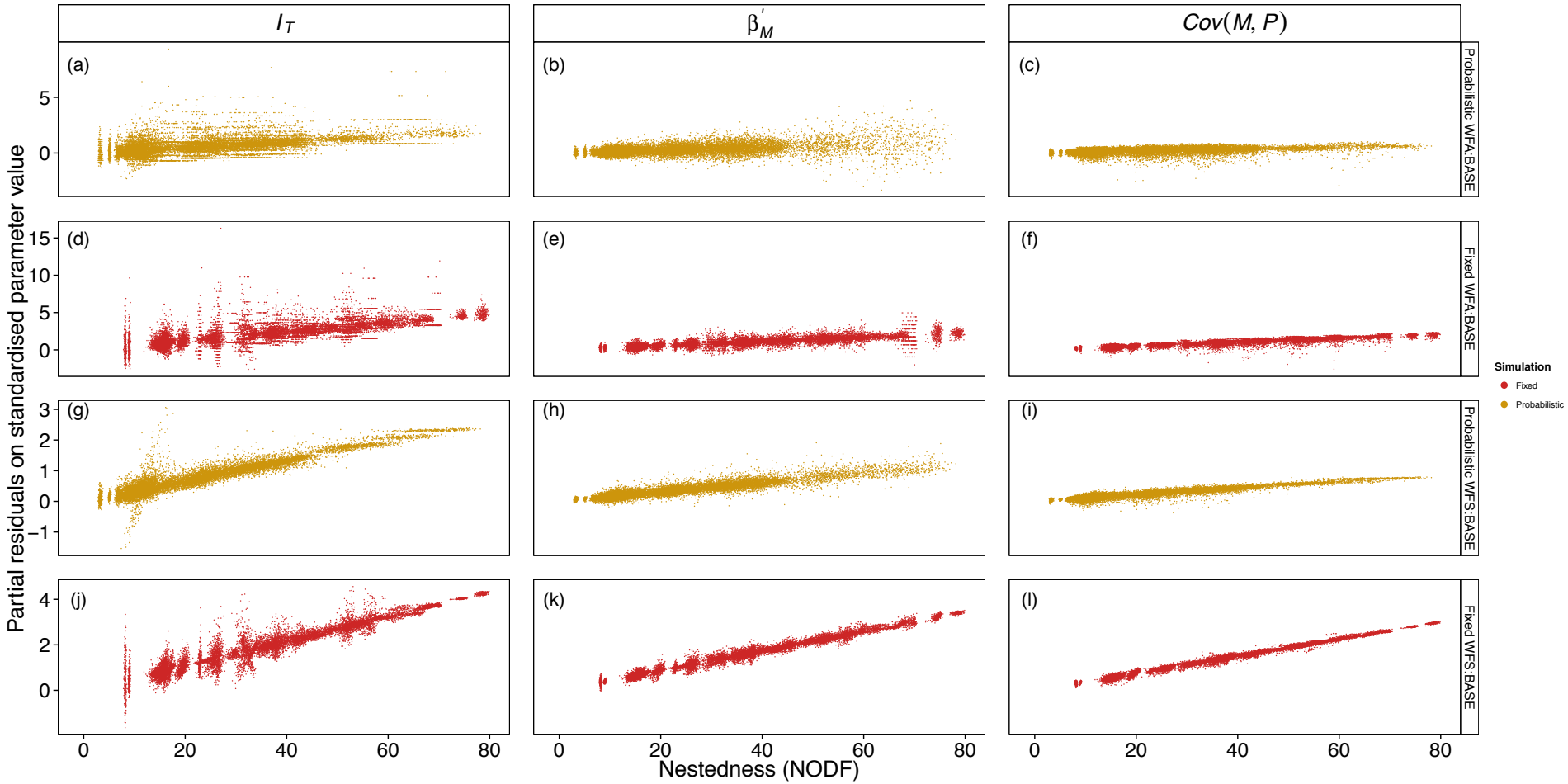


Figure 6. Plots show the relationship between the Nestedness (NODF) of simulated networks (generated using probabilistic and fixed models) and the standardized variation in reproductive success (I_T) (a, d, g & j), standardised Bateman gradients (β'_M) (b, e, h & k) and the standardised covariance between M and P ($Cov(M,P)$) (c, f, i & l) for the males of those networks. Partial residuals are estimated from GLMMs reported in tables 1-3.

Assortativity (r)

Assortativity negatively and significantly predicted standardised variation in reproductive success (I_T) across all simulations (i.e. the more disassortative the network, the higher the variation in I_T; Table. 4; fig. 7a, d, g, j; Appendix§2 A6) and this effect was strongest using the fixed simulation algorithm and for WFA postcopulatory competition. The increase in I_T with increasing disassortativity was accompanied by an increase in precopulatory sexual selection measured by the standardised univariate Bateman gradient (β'_M; Table. 5; fig. 7b, e, h, k; Appendix§2 Table A7), and driven by an increase in the covariance between *M* and *P* (*Cov(M,P)*; Table. 6; fig. 7c, f, I, l; Appendix§2 A8). Further relationships between I_P and I_M are presented in Appendix§2 Tables A9 and A10. In general the relationship between assortativity and measures of sexual selection tended to be flatter for probabilistic simulations than for the fixed simulations (Tables 1-3, fig. 6). This pattern was likely due to the same mechanisms that drive this pattern for NODF (see above). Estimated slopes for selection measures against assortativity for WFS simulations also tended to be similar or more negative than WFA simulations potentially reflecting the greater consistency in the assignment of reproductive success to males in WFS systems than in WFA systems as for NODF results above.

Table 4. GLMM results for the relationship between the opportunity for selection (I_T) and assortativity (r).

Simulation type	Full model	Competitive mechanism	Parameter removed	Model number & comparison	Parameter estimate ± SE	AIC	LRT
Fixed	Opportunity for selection (I_T) vs Assortativity Fixed effects: Density + Assortativity + Magnitude, Random effects: Original network	WFA:BASE	None Assortativity	1 2 vs 1	 -3.49 ± 0.26	42105 42282	 $\chi 2 = 178.81$, df = 1, p < 0.001
		WFS:BASE	None Assortativity	1 2 vs 1	 -3.23 ± 0.07	5241.8 7011.6	- $\chi 2 = 1771.7$, df = 1, p < 0.001
Probabilistic	Opportunity for selection (I_T) vs Assortativity Fixed effects: Density + Assortativity + *n* females + Magnitude Random effects: Original network	WFA:BASE	None Assortativity	1 2 vs 1	 -0.52 ± 0.09	29798 29834	 $\chi 2 = 37.522$, df = 1, p < 0.001
		WFS:BASE	None Assortativity	1 2 vs 1	 -0.53 ± 0.03	-4605.1 -4215.8	 $\chi 2 = 391.32$, df = 1, p < 0.001

Table 5. GLMM results for the relationship between the standardised univariate Bateman gradient (β'_M) and assortativity (r).

Simulation type	Full model	Competitive mechanism	Parameter removed	Model number & comparison	Parameter estimate ± SE	AIC	LRT
Fixed	Standardised variance in univariate Bateman Gradients (β'_M) vs Assortativity Fixed effects: Density + Assortativity + Magnitude, Random effects: Original network	WFA:BASE	None Assortativity	1 2 vs 1	 -1.79 ± 0.1	14725 15058	 $\chi 2 = 335.06$, df = 1, p < 0.001
		WFS:BASE	None Assortativity	1 2 vs 1	 -1.84 ±0.03	-23872 -20023	 $\chi 2 = 3851.7$, df = 1, p < 0.001
Probabilistic	Standardised variance in univariate Bateman Gradients (β'_M) vs Assortativity Fixed effects: Density + Assortativity + *n* females + Magnitude Random effects: Original network	WFA:BASE	None Assortativity	1 2 vs 1	 -0.29 ± 0.04	17819 17851	 $\chi 2 = 34.066$, df = 1, p < 0.001
		WFS:BASE	None Assortativity	1 2 vs 1	 -0.65 ± 0.014	-24082 -22077	 $\chi 2 = 2006.7$, df = 1, p < 0.001

Table 6. GLMM results for the relationship between the standardised covariance between *M* and *P* (*Cov(M,P)*) and assortativity (*r*).

Simulation type	Full model	Competitive mechanism	Parameter removed	Model number & comparison	Parameter estimate ± SE	AIC	LRT
Fixed	Standardised covariance between mating success and paternity share (*Cov(M,P)*) vs Assortativity Fixed effects: Density + Assortativity + Magnitude, Random effects: Original network	WFA:BASE	None	1		1749.1	
			Assortativity	2 vs 1	-1.33 ± 0.06	2161.7	$\chi 2 = 414.62$, df = 1, p < 0.001
		WFS:BASE	None	1		-37813	
			Assortativity	2 vs 1	-1.33 ± 0.02	-32886	$\chi 2 = 4929.8$, df = 1, p < 0.001
Probabilistic	Standardised covariance between mating success and paternity share (*Cov(M,P)*) vs Assortativity Fixed effects: Density + Assortativity + *n* females + Magnitude Random effects: Original network	WFA:BASE	None	1		2802.4	
			Assortativity	2 vs 1	-0.184 ± 0.03	2829.4	$\chi 2 = 28.959$, df = 1, p < 0.001
		WFS:BASE	None	1		-37872	
			Assortativity	2 vs 1	-0.41 ± 0.009	-35820	$\chi 2 = 2054.4$, df = 1, p < 0.001

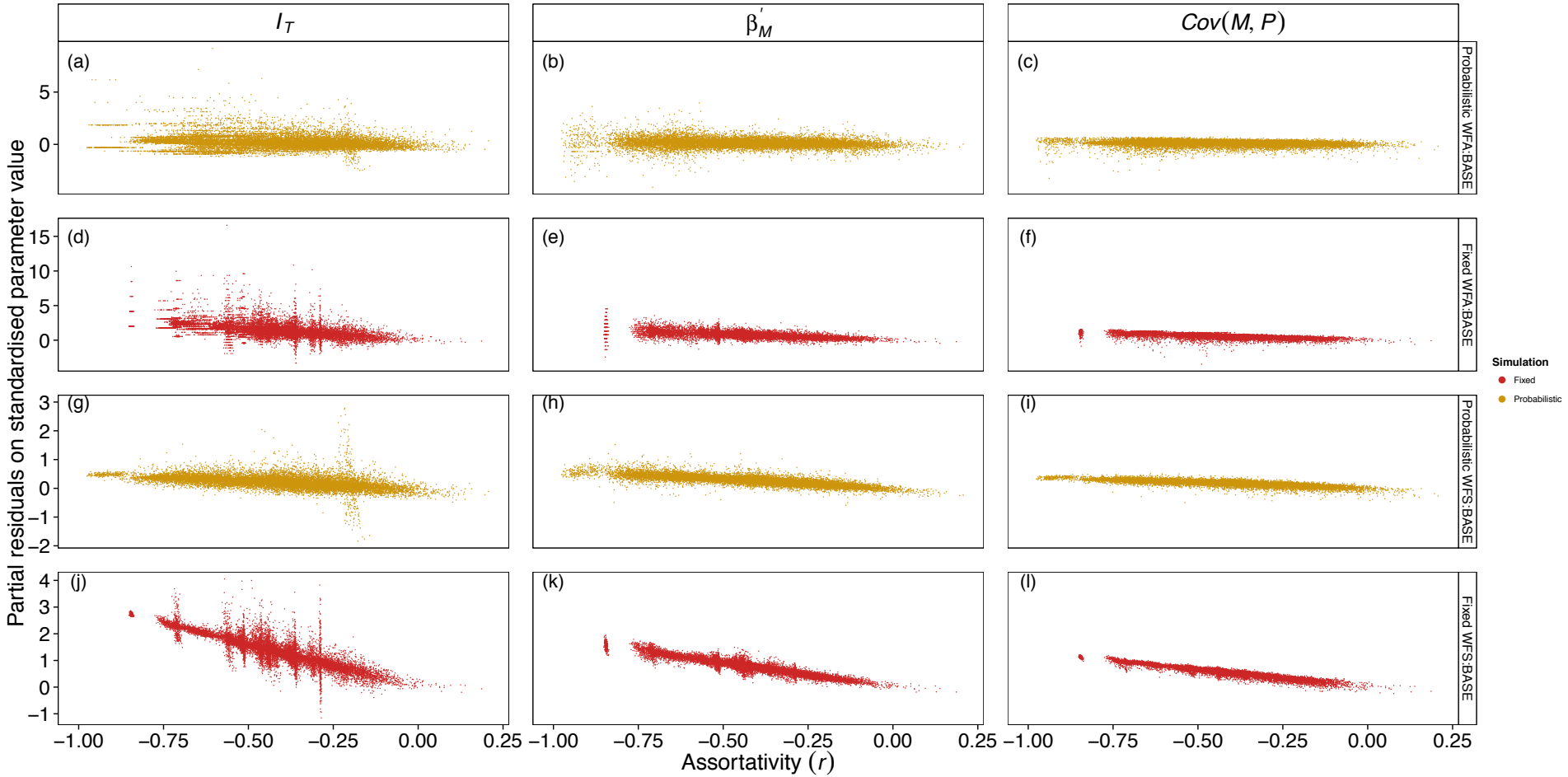


Figure 7. Plots show the relationship between the assortativity (r) of simulated networks (generated using probabilistic and fixed models),and the standardized variation in reproductive success (I_T) (a, d, g & j), standardised Bateman gradients (β'_M) (b, e, h & k) and the standardised covariance between M and P ($Cov(M,P)$) (c, f, i & l) for the males of those networks. Partial residuals are estimated from GLMMs reported in tables 4-6.

Nestedness (NODF) vs. Assortativity (r)

Assortativity and NODF were significantly negatively correlated such that as networks became more nested they also became more strongly disassortative (Table 7). This effect was stronger for the fixed simulations compared to probabilistic simulations (Table 7; Fig. 8). This demonstrates that although assortativity (r) and nestedness (NODF) are highly correlated both measures do not capture the exact same information. The disparity between the slope of assortativity against nestedness in fixed versus swap simulations is potentially explained by the greater variation in network structure allowed in probabilistic simulations. This is because fixed models hold constant the degree distribution of networks, and therefore I_s for both males and females, resulting in a smaller potential landscape of possible network structures available for fixed rather than for probabilistic simulations. This generates more variation in network structure and degree distributions (I_s) resulting in a potentially greater mismatch between the two measures (fig. 9).

Table 7 . GLMM results for the relationship between assortativity (*r*) and nestedness (NODF).

Simulation type	Full model	Parameter removed	Model number & comparison	Parameter estimate ± SE	AIC	LRT
Fixed	Assortativity vs. NODF	None	1		-61364	
		NODF	2 vs 1	-0.01 ± 0.0002	-59769	$\chi 2 = 1597.4$, df = 1, p < 0.001
	Fixed effects: Density + NODF+ Magnitude,	Density	3 vs 1	0.31 ± 0.14	-61362	$\chi 2 = 4.601$, df = 1, p < 0.05
	Random effects: Original network	Magnitude	4 vs 1	-0.19 ± 0.02	-61366	$\chi 2 = 0.885$, df = 1, p = 0.35
Probabilistic	Assortativity vs. NODF	None	1		-39219	
		NODF	2 vs. 1	-0.001 ± 0.0002	-39180	$\chi 2 = 40.816$, df = 1, p < 0.001
	Fixed effects: Density + NODF+ *n* females + Magnitude	Density	3 vs. 1	-0.7 ± 0.17	-39206	$\chi 2 = 14.952$, df = 1, p < 0.001
	Random effects: Original network	Magnitude	4 vs. 1	-0.011 ± 0.03	-39221	$\chi 2 = 0.203$, df = 1, p = 0.65
		n females	5 vs. 1	-0.001 ± 0.0005	-39219	$\chi 2 = 2.057$, df = 1, p = 0.15

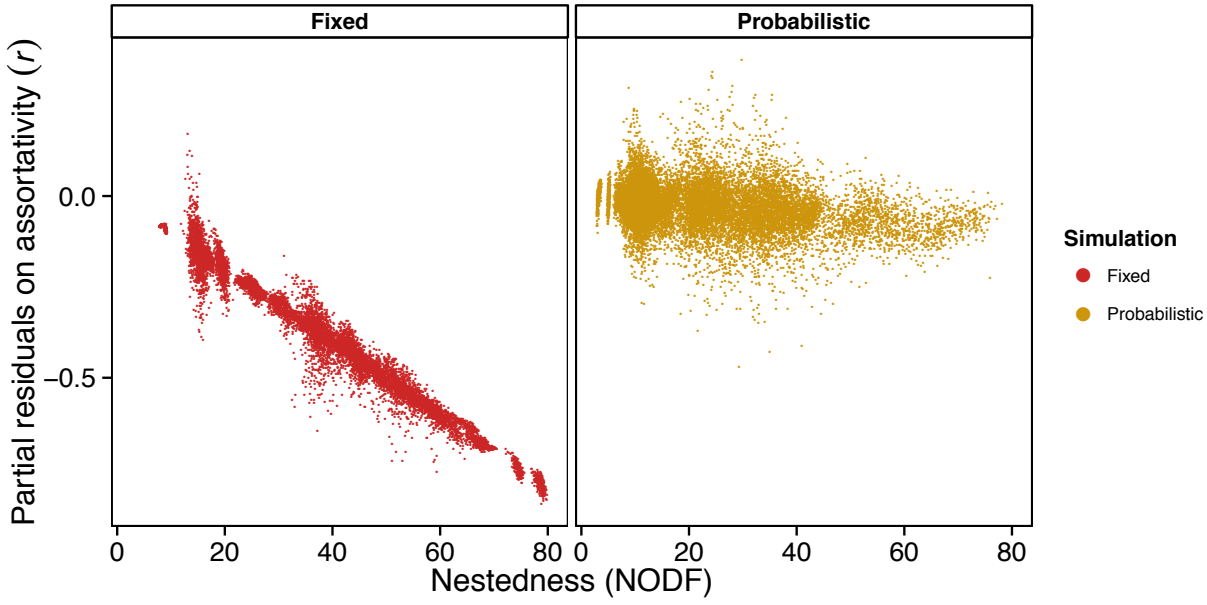


Figure 8. Plot shows the relationship between nestedness (NODF) and assortativity (*r*) for both fixed and probabilistic randomisation simulations derived from GLMMs.

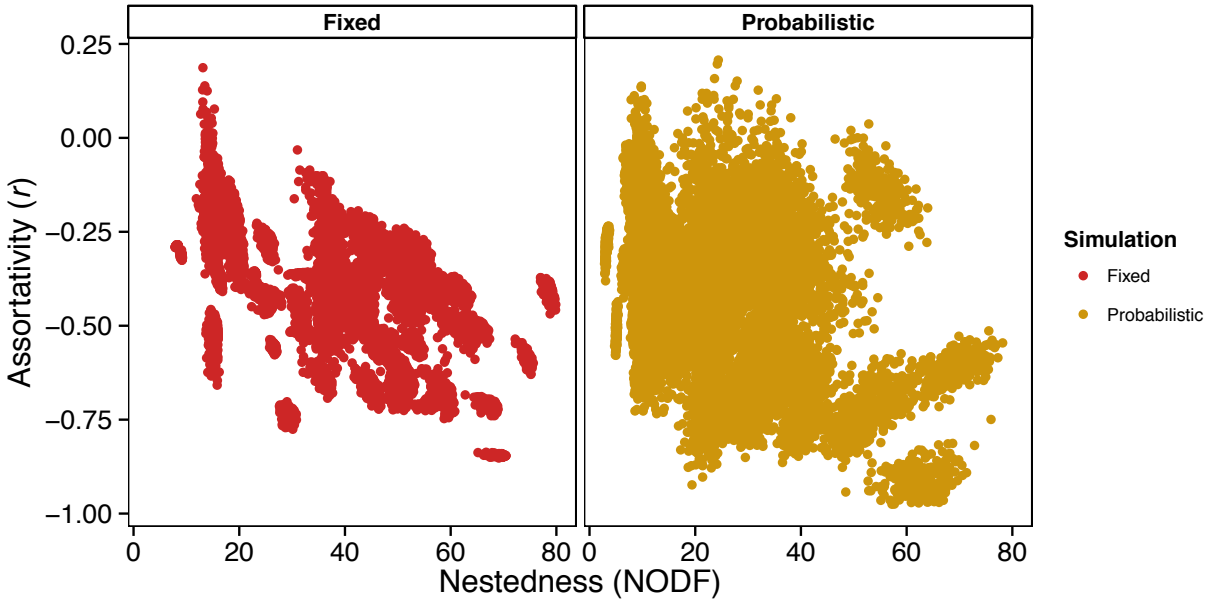


Figure 9. Plot shows raw assortativity (*r*) values for probabilistic and fixed simulations from the WFA simulations plotted against nestedness (NODF) values for all simulated networks.

Finally, GLMMs comparing the explanatory power of assortativity *vs*. NODF on the opportunity for selection (I_T) revealed consistent differences across the four simulations. Firstly, the explanatory power of both measures tended to be reduced for WFA simulations compared with WFS simulations driven by the lower consistency with which reproductive success is attributed to males of a given network position (Table 8). Secondly, NODF tended to have a higher explanatory power as measured by the adjusted R^2 in probabilistic simulations, whereas assortativity tended to have higher explanatory power in fixed simulations (Table 8). This second difference may be in part driven by the stronger relationship between NODF and the opportunity for sexual selection (I_s) compered with assortativity. This is because the NODF of simulated networks more strongly correlated with I_s than assortativity (Appendix§3 Table A11), and therefore a change in variation in mating success (I_s) results in a greater increase in NODF than assortativity. i.e. in probabilistic simulations the variation allowed in I_s will contribute more strongly to inter-network variation in I_T than in fixed simulations where I_s is held constant.

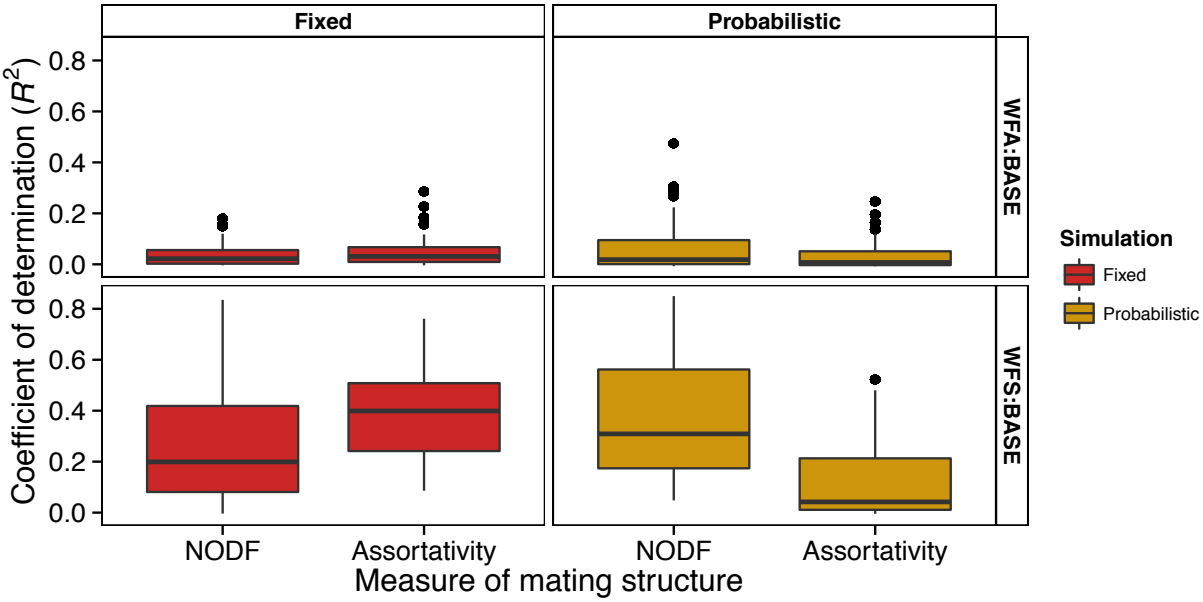


Figure 10. Boxplot shows adjusted R^2 values from linear models assessing the effects of either assortativity (r) or nestedness (NODF) on the opportunity for selection (I_T) across the 250 simulations for each of the 59 empirical networks.

Table 8. GLMM results comparing the amount of variation explained in the opportunity for selection (I_T) by two measures of mating structure; assortativity (r) and nestedness (NODF).

Simulation type	Full model	Competitive mechanism	Parameter	Parameter estimate ± SE	AIC	LRT
Fixed	Coefficient of determination (R^2) vs. Mating structure measure Fixed effects: Mating structure measure Magnitude, Random effects: Original network	WFA:BASE	NODF Assortativity	0.0342 ± 0.006 0.0452 ± 0.006	-104767	$\chi2$ =962.59 , df = 1, p < 0.001
		WFS:BASE	Nestedness Assortativity	0.251 ± 0.0211 0.374 ± 0.0211	-38306	$\chi2$ = 6738.2, df = 1, p < 0.001
Probabilistic	Coefficient of determination (R^2) vs. Mating structure measure Fixed effects: Mating structure measure Magnitude, Random effects: Original network	WFA:BASE	NODF Assortativity	0.071 ± 0.01 0.034 ± 0.01	-104845	$\chi2$ = 6943.9, df = 1, p < 0.001
		WFS:BASE	NODF Assortativity	0.37 ± 0.022 0.124 ± 0.37 t	-59256	$\chi2$ =32660 , df = 1, p < 0.001

DISCUSSION

In this study we used a simulation approach to assess separately the effect of polyandry and the relationship between male and female mating success on the total variance

in reproductive success (I_T) and it's operation through both pre- and post-copulatory components. In doing so, we generate predictions for the effect of polyandry and the relationship between male and female promiscuity on sexual selection. Overall our results are in line with previous predictions (Shuster and Wade 2003) but move beyond this to produce quantitative predictions that reveal potentially subtle differences in the effect of sperm competitive mechanisms on relative changes in the potential strength of sexual selection on through different fitness components and the role of mating system structure.

POLYANDRY

Our polyandry simulations demonstrate a complex relationship between polyandry and the potential strength of sexual selection and indicate that this complexity can arise for four reasons; i) the relationship between polyandry and any measure of sexual selection is not necessarily linear, ii) the relative changes in sexual selection depend on the extent of male promiscuity independent of female promiscuity (i.e. polyandry), iii) the relationship depends on the strength of sperm competitive exclusion i.e. whether successfully reproducing males fertilise all of a females ova (winner fertilise all, WFA), a proportion of her ova (winner fertilise some, WFS) and the consistency with which the best sperm competitors win a sperm competitive bout, and iv) whether sperm competitive ability covaries positively or negatively with mating success.

When a population is strictly monogamous, sexual selection on males is limited to variance in mate fecundity (I_N), which is zero in our simulations, resulting in zero variation in reproductive success for initially strictly monogamous populations. A marginal increment in polyandry results in an initial increase in I_M, which acts to increase the overall opportunity for sexual selection I_T. This confirms the potential for sexual selection to operate in socially monogamous species, such as many passerine birds, through extra-pair copulations.

However, the details of how this increase in I_M affects I_T depends on the variance in P and the relationship between the two. The initial increase in I_M in monogamous populations is followed by an eventual decrease as the mating table becomes increasingly saturated. This means that with further increases in polyandry I_T becomes increasingly dependent on I_P, at which point the mechanisms through which paternity is distributed across males become important in determining variance in male reproductive success. In the extreme case where paternity is WFA and the most competitive male always fertilises all of a female's ova, I_T continues to increase towards the maximum level as set by post-copulatory sexual selection where one male fertilizes every female's ova. Instead, if we introduce uncertainty in the probability that highly competitive males fertilise ova, that is a WFA scenario where the most competitive male's chances of fertilizing ova are a probabilistic function of his competitive ability rather than 100% certainty (i.e. selection on postcopulatory traits is reduced), I_T stabilises much earlier due to a lower maximum potential variance set by post-copulatory selection. Finally, under WFS, the initial increase in I_T is followed by an eventual decrease. This final point highlights the role of female clutch size in determining the magnitude of I_P. By allowing multiple males to sire offspring with the same female, many males can achieve some paternity across the females they mated, reducing the I_P to a level lower than WFA scenarios. Instead, when a population is already typically more promiscuous (i.e. polygyny), marginal increments in polyandry tend to reduce the overall opportunity of sexual selection or in extreme cases have no effect. This is largely due to a decline in the opportunity for precopulatory sexual selection (here restricted to I_M) as polyandry homogenizes mating success. The opportunity for post-copulatory sexual selection instead reveals a pattern more consistent with that of the monogamous populations. In general, I_P tends to increase with initial increases in polyandry. This initial increase then levels off and variance in P remains relatively stable across further increments of polyandry, at a level determined by the sperm

competition framework (i.e. WFA or WFS). As a result, increasing polyandry weakens the opportunity for total sexual selection (I_T) and simultaneously increases the relative importance of post-copulatory sexual selection. Here, sexual selection is strongest in the absence of polyandry, and decreases rapidly with increasing polyandry as the reduction in mating skew cannot be met by the resulting opportunity of postcopulatory sexual selection, which is consistent with the findings of typically more promiscuous species (Jones et al. 2001; Lesobre et al. 2010; Collet et al. 2012). Only in the extreme case of WFA where the most competitive male always fertilizes a clutch, does this pattern vary. In such cases, when M and P covary positively, variance in P continues to increase I_T across all levels of polyandry, to its maximum at saturation, whereas when M and P covary negatively, I_T firstly declines as M becomes more even across males, followed by an increase towards the maximum I_T set by postcopulatory sexual selection. These patterns reveal a fundamental difference between M and P: while mates are public (i.e. they can be shared across individuals leading to a saturation of the mating table), P is exclusive, fertilization of an ovum by a male, automatically excludes rivals and as such its variability is more resistant to erosion caused by polyandry.

While providing useful predictions for the effect of polyandry, the results presented here are necessarily a simplification of what may occur in nature. In more complex systems predicting how polyandry may affect different components of reproductive success and the strength of selection acting through those components may be more challenging. For instance, in our simulations males do not suffer from sperm depletion, which if present may affect our predictions. For example, sperm competition theory predicts that as the probability that males may face sperm competition with a potential mating partner (sperm competition risk) increases, males should increase investment in ejaculates per copulation, whereas, as the number of males competing for a given batch of ova increases (sperm competition intensity),

males should decrease their investment in ejaculates (Parker and Pizzari 2010). For any population, an initial increase from low to intermediate levels of polyandry may increase the importance of sperm competition risk and increase male ejaculate investment. However, a further increase towards high levels of polyandry may increase the importance of sperm competition intensity and reduce male ejaculate investment. When ejaculate production in males is limited, as has been increasingly documented (Levitan and Petersen 1995; Wedell et al. 2002), the former scenario with low levels of polyandry may reduce the ability and potential benefits to males of gaining additional matings, limiting the importance of precopulatory sexual selection. However, at higher levels of polyandry, lower male ejaculate investment per copulation, may allow males to increase the number of females with which they mate, increasing the potential importance of precopulatory sexual selection (Kvarnemo and Simmons 2013). This example highlights the importance of male sperm limitation and how different levels polyandry can feedback into male strategies and result in varying predictions of the potential importance of different components of reproductive success. Furthermore, it emphasizes the importance of quantitative predictions as in both scenarios above, the suggested predictions relate only to the potential importance of mating success, not the actual strength of precopulatory sexual selection. Indeed, in both scenarios it could be argued that net precopulatory sexual selection will increase, remain constant or decrease and makes no explicit predictions about the overall strength of sexual selection.

MATING STRUCTURE

Overall, our mating structure simulations demonstrate that the maximum potential strength of sexual selection operating on males, as determined through the standardized variation in reproductive success (I_T), is reliant not only on variation in mating success and levels of polyandry but also on the pattern with which males of varying M share females.

Specifically, our results demonstrate that when mating networks are negatively assorted by degree (i.e. males with high *M* tend to mate with females of low *M* and *vice versa*) total variation in reproductive success and therefore the maximum potential strength of sexual selection tends to in increase. These results are in line with informal predictions previously described in the literature (Sih et al 2009, McDonald et al 2013, McDonald & Pizzari, *in press*). By using two separate simulation techniques to vary the assortativity of mating networks (i.e. probabilistic and fixed) we are able to show that changes in I_T are not simply driven by higher variation in mating success (I_s) when networks are less assortative but that for any given I_s a more negatively assorted network increases I_T. Furthermore, our results show that this increase in the maximum potential for sexual selection is accompanied by an increase in the strength of precopulatory sexual selection on mating success as measured by the Bateman gradient (β'_M). This increase in β'_M is driven by an increase *Cov(M,P)*, which will in turn increase *Cov(M,T)* and therefore increase the Bateman gradient. Importantly this increase in Bateman gradients through *Cov(M,T)* occurs irrespective of increases or decreases in I_M. These results have important implications for comparisons of sexual selection between populations. For example, researchers comparing sexual selection between populations as a function of polyandry may assume that polyandry increases or decreases sexual selection. However, without considering the mating patterns of populations, any causal effect of polyandry may be obscured or even reversed by differences in mating structure. Despite this potential importance, it is yet unclear how common and widespread these may be (Sih et al. 2009). Mechanisms that may drive covariances in nature may include male driven mechanisms; for example the exclusion of subdominant males from mating with high quality females, results in several excluded males with low *M,* mating with low quality females with high *M,* whereas dominant males may have exclusivity across their mates (Preston et al. 2005). Similarly, high quality males with high *M* may preferentially mate with fecund

females who also therefore attain a high M, creating a positive covariance between male and female mating success (Gage 1995; Collet et al. 2014). This may be expected generate a positive covariance between male and female promiscuity and decrease precopulatory sexual selection while simultaneously eroding the benefits of mating with highly fecund females. Alternatively, such patterns could be female driven. For example, if males also provide direct and/or indirect benefits, selection may drive high quality females to mate multiply and preferentially with many high quality males (who also then attain high *M*) (e.g. Squires et al. 2012) creating a positive covariance between male and female promiscuity and decreasing precopulatory sexual selection. In such cases, low quality individuals may face a higher cost of mating, reducing their promiscuity and further strengthening the positive covariance between male and female mating success.

In this work our main aim was to show that mating structure can modify both the potential and actual strength of sexual selection, rather than provide an argument for or against a particular measure of mating structure. However, we have attempted to assess the relative predictive powers of the two alternative measures of mating structure. Mating networks can be both negatively and positively assorted by degree, however only the assortativity coefficient, *r*, captures this full range of variation. For this reason, combined with its more intuitive understanding as a correlation coefficient, assortativity is a potentially attractive measure. Nestedness (NODF) is instead a more restricted measure, focusing on disassortativity (May et al. 2008; Sugihara and Ye 2009). However, even positively assorted networks can show some level of nestedness as demonstrated both in the simulations presented here and in previous literature (Abramson et al. 2011). Suggesting both measures may provide potentially complimentary information. Our results do suggest that both measures my have differing predictive powers under differing circumstances. When variation in mating success (I_s) within groups is lower, NODF appears to capture more variation in I_T

than assortativity, however when I_s tends to be relatively larger, assortativity appears to capture more variation. Suggesting that for populations with more homogenous mating success across males NODF may be more sensitive to changes in the sperm competitive landscape.

IMPLICATIONS FOR STUDIES OF EMPIRICAL SYSTEMS

The simulation approach used in this work has provided hypothetical conditions in which the researcher has detailed and complete knowledge of the mating patterns of males and females. This high level resolution of data provides us with the scope to test the effects of variation in polyandry and mating structure. However, studies of natural populations and to a lesser extent experimental model systems will less commonly provide such comprehensive data. In any study, the extent of the conclusions that can be drawn will be limited by the quality of the data that can be collected. With this caveat in mind, we highlight some of the main data requirements of this method and data limitations that may impinge on the usage of the methods examined in this paper. Firstly, appropriately determining female polyandry and male mating success requires detailed behavioural observations of copulation behaviour. In many studies, the quantification of female polyandry is based on evidence of mixed paternity of clutches, where paternity is determined using genetic parentage assignment (Collet et al. 2014; Taylor et al. 2014). Calculating female polyandry and male mating success in this way can systematically underestimate the level of both male and female promiscuity, as under sperm competition males may copulate with females but sire no offspring (Collet et al. 2014). Using genetic data alone may result in overestimates of variance in male mating success and Bateman gradients (Collet et al. 2014). With reference to the estimation of mating structure as defined in this work, we expect that using genetic parentage as a means of determining male-female mating networks to also bias results. This is because measures of mating structure and

associated predictions relate to the distribution of matings in determining sperm competitive structure. When behavioural data are not available, genetically determined mating networks may not accurately characterize the distribution of sperm competitive environments across males and instead may better represent the results of sperm competition rather than the competitive landscape, potentially underestimating the disassortativity or nestedness of a given mating network. This problem may be exacerbated when female clutch size is low. An extreme example comes from cases where female clutch size is restricted to one egg. In this case, using genetic parentage assignment removes completely the potential for female polyandry and any level of mating structure, however the distribution of reproductive success across males may strongly depend on female polyandry and mating structure in such winner fertilise all cases, as demonstrated in our results. A similar issue relates to the accuracy of behavioural data when available. Missing data on given copulations between male and female pairs may result an underestimation of female polyandry and both over and underestimation of mating network structure, which may be particularly strong when males of a given mating success may be associated with a particular reproductive tactic that is consistently not recorded. The potential to cause noise in measures of mating structure may be larger when mating networks are sparse when small variations in network structure may cause large variation in network measures. We therefore advocate that use of such methods requires both genetic and detailed behavioural data on copulations, or genetic parentage assignment only, in those cases where genetic data is a strongly representative of behavioural mating patterns. Although these conditions may seem restrictive there is great potential to explore these methods in laboratory conditions where behavioural data is more readily available. Furthermore, there is great potential in some natural systems where detailed behavioural data sets are available in both vertebrate and invertebrate system (e.g. Preston et al. 2005; Rodríguez-Muñoz et al. 2010).

CONCLUSIONS

This study shows polyandry can modify the maximum potential of sexual selection and the relative importance of pre- and postcopulatory sexual selection. It clarifies conflicting results in empirical literature by demonstrating both positive and negative relationships between polyandry and the opportunity for selection and that the direction in which sexual selection responds hinges on the extent of male promiscuity independent of polyandry. Furthermore, it demonstrates that differing sperm competitive mechanisms can alter the direction and magnitude of changes in sexual selection with increments in polyandry. Finally, we use quantitative network measures to show that for any level of polyandry the relationship between male mating success and that of his partners, can further shape potential strength of total sexual selection and the strength of precopulatory sexual selection on male mating success.

APPENDIX§1

Table A1 GLMM results assessing the relationship between the opportunity for selection (I_T) and nestedness (NODF).

Simulation type	Full model	Competitive mechanism	Parameter removed	Model number & comparison	Parameter estimate ± SE	AIC	LRT
Probabilistic	Opportunity for selection (I_T) vs. Nestedness Fixed effects: Density + Nestedness + *n* females + Magnitude Random effects: Original network	WFA:BASE	None	1	-	29675	-
			Nestedness	2 vs. 1	0.03 ± 0.002	29834	$\chi 2$ = 160.49, df = 1, p < 0.001
			Density	3 vs. 1	-6.42 ± 1.7	29686	$\chi 2$ = 12.648, df = 1, p < 0.001
			Magnitude	4 vs. 1	1.03 ± 0.25	29688	$\chi 2$ = 14.361, df = 1, p < 0.001
			n females	5 vs. 1	-0.02 ± 0.005	29675	$\chi 2$ = 15.289, df = 1, p < 0.001
		WFS:BASE	None	1	-	-7172.0	-
			Nestedness	2 vs. 1	0.03 ± 0.0006	-4215.8	$\chi 2$ = 2958.2, df = 1, p < 0.001
			Density	3 vs. 1	-5.07 ± 0.45	-7111.6	$\chi 2$ = 62.464, df = 1, p < 0.001
			Magnitude	4 vs. 1	-0.04 ± 0.07	-7173.7	$\chi 2$ = 0.3455, df = 1, p = 0.56
			n females	5 vs. 1	0.006 ± 0.01	-7154.9	$\chi 2$ = 19.073, df = 1, p < 0.001
Fixed	Opportunity for selection vs. Nestedness (I_T) Fixed effects: Density + Nestedness + Magnitude Random effects: Original network	WFA:BASE	None	1	-	42227	-
			Nestedness	2 vs. 1	0.06 ± 0.008	42282	$\chi 2$ = 57.135, df = 1, p < 0.001
			Density	3 vs. 1	-12.87 ± 2.8	42244	$\chi 2$ = 18.458, df = 1, p < 0.001
			Magnitude	4 vs. 1	1.03 ± 0.38	42232	$\chi 2$ = 6.853, df = 1, p < 0.01
		WFS:BASE	None	1	-	6532.3	-
			Nestedness	2 vs. 1	0.05 ± 0.002	7011.6	$\chi 2$ = 481.25, df = 1, p < 0.001
			Density	3 vs. 1	-12.47 ± 2.16	6556.8	$\chi 2$ = 26.484, df = 1, p < 0.001
			Magnitude	4 vs. 1	0.54 ± 0.31	6533.4	$\chi 2$ = 3.082, df = 1, p = 0.07

Table A2 GLMM results assessing the relationship between the standardised univariate Bateman gradient (β'_M) and nestedness (NODF).

Simulation type	Full model	Competitive mechanism	Parameter removed	Model number & comparison	Parameter estimate ± SE	AIC	LRT
Probabilistic	Standardised variance in univariate Bateman Gradients (β'_M) vs. Nestedness Fixed effects: Density + Nestedness + *n* females + Magnitude Random effects: Original network	WFA:BASE	None	1	-	17732	-
			Nestedness	2 vs. 1	0.01 ± 0.001	17851	$\chi 2 = 121.03$, df = 1, $p < 0.001$
			Density	3 vs. 1	-1.28 ± 0.13	17826	$\chi 2 = 95.465$, df = 1, $p < 0.001$
			Magnitude	4 vs. 1	0.002 ± 0.008	17730	$\chi 2 = 0.0949$, df = 1, $p = 0.76$
			n females	5 vs. 1	0.0006 ± 0.0002	17738	$\chi 2 = 7.762$, df = 1, $p < 0.01$
		WFS:BASE	None	1	-	-24194	-
			Nestedness	2 vs. 1	0.016 ± 0.0003	-22077	$\chi 2 = 2118.8$, df = 1, $p < 0.001$
			Density	3 vs. 1	-1.54 ± 0.05	-24003	$\chi 2 = 192.45$, df = 1, $p < 0.001$
			Magnitude	4 vs. 1	0.008 ± 0.007	-24194	$\chi 2 = 1.5482$, df = 1, $p = 0.02$
			n females	5 vs. 1	0.0004 ± 0.0002	-24192	$\chi 2 = 4.2002$, df = 1, $p < 0.05$
Fixed	Standardised variance in univariate Bateman Gradients (β'_M) vs. Nestedness Fixed effects: Density + Nestedness + Magnitude Random effects: Original network	WFA:BASE	None	1	-	14915	-
			Nestedness	2 vs. 1	0.03 ± 0.002	15058	$\chi 2 = 144.87$, df = 1, $p < 0.001$
			Density	3 vs. 1	-2.96 ± 0.32	14994	$\chi 2 = 80.941$, df = 1, $p < 0.001$
			Magnitude	4 vs. 1	0.06 ± 0.03	14917	$\chi 2 = 4.2295$, df = 1, $p < 0.05$
		WFS:BASE	None	1	-	-22080	-
			Nestedness	2 vs. 1	0.04 ± 0.001	-20023	$\chi 2 = 2059.1$, df = 1, $p < 0.001$
			Density	3 vs. 1	-4.52 ± 0.33	-21993	$\chi 2 = 88.752$, df = 1, $p < 0.001$
			Magnitude	4 vs. 1	0.1 ± 0.04	-22077	$\chi 2 = 4.795$, df = 1, $p < 0.05$

Table A3 GLMM results assessing the relationship between the standardised covariance between *M* and *P* (*Cov(M,P)*) and nestedness (NODF).

Simulation type	Full model	Competitive mechanism	Parameter removed	Model number & comparison	Parameter estimate ± SE	AIC	LRT
Probabilistic	Standardised covariance between mating success and paternity share (*Cov(M,P)*) vs. Nestedness	WFA:BASE	None	1	-	2702.9	-
			Nestedness	2 vs. 1	0.008 ± 0.0007	2829.4	$\chi 2 = 128.48$, df = 1, p < 0.001
	Fixed effects: Density + Nestedness + *n* females + Magnitude		Density	3 vs. 1	-0.95 ± 0.078	2841.2	$\chi 2 = 140.31$, df = 1, p < 0.001
	Random effects: Original network		Magnitude	4 vs. 1	0.002 ± 0.009	2701.1	$\chi 2 = 0.1846$, df = 1, p = 0.67
			n females	5 vs. 1	0.0004 ± 0.0001	2708.1	$\chi 2 = 7.153$, df = 1, p < 0.05
		WFS:BASE	None	1	-	-38438	-
			Nestedness	2 vs. 1	0.01 ± 0.0002	-35820	$\chi 2 = 2619.9$, df = 1, p < 0.001
			Density	3 vs. 1	-1.23 ± 0.04	-38238	$\chi 2 = 201.52$, df = 1, p < 0.001
			Magnitude	4 vs. 1	0.01 ± 0.005	-38435	$\chi 2 = 4.4431$, df = 1, p < 0.05
			n females	5 vs. 1	-0.0001 ± 0.0001	-38438	$\chi 2 = 1.6098$, df = 1, p = 0.2
Fixed	Standardised covariance between mating success and paternity share (*Cov(M,P)*) vs. Nestedness	WFA:BASE	None	1	-	1897.2	-
			Nestedness	2 vs. 1	0.03 ± 0.002	2161.7	$\chi 2 = 266.49$, df = 1, p < 0.001
	Fixed effects: Density + Nestedness + Magnitude		Density	3 vs. 1	-2.93 ± 0.22	2009.7	$\chi 2 = 114.41$, df = 1, p < 0.001
	Random effects: Original network		Magnitude	4 vs. 1	0.05 ± 0.02	1900.4	$\chi 2 = 5.1428$, df = 1, p < 0.05
		WFS:BASE	None	1	-	-36739	-
			Nestedness	2 vs. 1	0.04 ± 0.0006	-32886	$\chi 2 = 3855.5$, df = 1, p < 0.001
			Density	3 vs. 1	-4.063 ± 0.25	-36637	$\chi 2 = 103.7$, df = 1, p < 0.001
			Magnitude	4 vs. 1	0.07 ± 0.03	-36737	$\chi 2 = 4.3381$, df = 1, p < 0.05

Table A4 GLMM results assessing the relationship between the standardised variation in mating success (I_M) and nestedness (NODF).

Simulation type	Full model	Competitive mechanism	Parameter removed	Model number & comparison	Parameter estimate ± SE	AIC	LRT
Probabilistic	Standardised variance in mating success (I_M) vs. Nestedness Fixed effects: Density + Nestedness + *n* females + Magnitude Random effects: Original network	WFA:BASE	None	1	-	-12992	-
			Nestedness	2 vs. 1	0.008 ± 0.0005	-12753	χ2 = 241.33, df = 1, p < 0.001
			Density	3 vs. 1	-1.64 ± 0.25	-12970	χ2 = 24.206, df = 1, p < 0.001
			Magnitude	4 vs. 1	-0.05 ± 0.04	-12993	χ2 = 1.4228, df = 1, p = 0.2
			n females	5 vs. 1	0.004 ± 0.001	-12975	χ2 = 19.338, df = 1, p < 0.001
		WFS:BASE	None	1	-	-25024	-
			Nestedness	2 vs. 1	0.007 ± 0.0003	-24596	χ2 = 430.07, df = 1, p < 0.001
			Density	3 vs. 1	-1.3 ± 0.32	-25012	χ2 = 13.496, df = 1, p < 0.001
			Magnitude	4 vs. 1	-0.12 ± 0.05	-25019	χ2 = 6.3263, df = 1, p < 0.05
			n females	5 vs. 1	0.008 ± 0.0008	-24943	χ2 = 82.194, df = 1, p < 0.001
Fixed	Standardised variance in mating success (I_M) vs. Nestedness Fixed effects: Density + Nestedness + Magnitude Random effects: Original network	WFA:BASE	None	1	-	-1058.54	-
			Nestedness	2 vs. 1	-0.03 ± 0.002	-811.68	χ2 = 248.86, df = 1, p < 0.001
			Density	3 vs. 1	1.26 ± 0.43	-1052.0	χ2 = 8.50, df = 1, p < 0.005
			Magnitude	4 vs. 1	0.11 ± 0.056	-1056.7	χ2 = 3.87, df = 1, p < 0.05
		WFS:BASE	None	1	-	-27319	-
			Nestedness	2 vs. 1	-0.03 ± 0.0007	-25702	χ2 = 1618.9, df = 1, p < 0.001
			Density	3 vs. 1	1.56 ± 0.412	-27308	χ2 = 13.05, df = 1, p < 0.001
			Magnitude	4 vs. 1	0.1 ± 0.06	-27318	χ2 = 3.0896, df = 1, p = 0.08

Table A5 GLMM results assessing the relationship between the standardised variation in fertilisation success (I_P) and nestedness (NODF).

Simulation type	Full model	Competitive mechanism	Parameter removed	Model number & comparison	Parameter estimate ± SE	AIC	LRT
Probabilistic	Standardised variance in paternity share (I_P) vs. Nestedness Fixed effects: Density + Nestedness + *n* females + Magnitude Random effects: Original network	WFA:BASE	None	1	-	42960	-
			Nestedness	2 vs. 1	0.006 ± 0.003	42961	$\chi 2 = 3.6$, df = 1, p = 0.058
			Density	3 vs. 1	-3.58 ± 1.722	42962	$\chi 2 = 4.1609$, df = 1, p < 0.05
			Magnitude	4 vs. 1	1.39 ± 0.26	42981	$\chi 2 = 23.454$, df = 1, p < 0.001
			n females	5 vs. 1	-0.03 ± 0.006	42981	$\chi 2 = 23.235$, df = 1, p < 0.001
		WFS:BASE	None	1	-	-28993	-
			Nestedness	2 vs. 1	0.004 ± 0.0003	-28794	$\chi 2 = 201.51$, df = 1, p < 0.001
			Density	3 vs. 1	-0.9 ± 0.07	-28908	$\chi 2 = 86.679$, df = 1, p < 0.001
			Magnitude	4 vs. 1	0.27 ± 0.02	-28988	$\chi 2 = 7.005$, df = 1, p < 0.01
			n females	5 vs. 1	-0.001 ± 0.0003	-28976	$\chi 2 = 19.054$, df = 1, p < 0.001
Fixed	Standardised variance in paternity share (I_P) vs. Nestedness Fixed effects: Density + Nestedness + Magnitude, Random effects: Original network	WFA:BASE	None	1	-	48220	-
			Nestedness	2 vs. 1	-0.07 ± 0.01	48270	$\chi 2 = 52.629$, df = 1, p < 0.001
			Density	3 vs. 1	5.97 ± 2.22	48225	$\chi 2 = 6.9703$, df = 1, p < 0.01
			Magnitude	4 vs. 1	1.12 ± 0.29	48231	$\chi 2 = 13.578$, df = 1, p < 0.001
		WFS:BASE	None	1	-	-610.5	-
			Nestedness	2 vs. 1	-0.05 ± 0.002	-48.16	$\chi 2 = 564.34$, df = 1, p < 0.001
			Density	3 vs. 1	3.38 ± 0.56	-582.21	$\chi 2 = 30.292$, df = 1, p < 0.001
			Magnitude	4 vs. 1	0.08 ± 0.08	-611.34	$\chi 2 = 1.1605$, df = 1, p = 0.28

APPENDIX§2

Table A6 GLMM results assessing the relationship between the opportunity for selection (I_T) and assortativity (r).

Simulation type	Full model	Competitive mechanism	Parameter removed	Model number & comparison	Parameter estimate ± SE	AIC	LRT
Probabilistic	Opportunity for selection (I_T) vs. Assortativity Fixed effects: Density + Assortativity + *n* females + Magnitude Random effects: Original network	WFA:BASE	None	1	-	29798	-
			Assortativity	2 vs. 1	-0.52 ± 0.09	29834	$\chi 2 = 37.522$, df = 1, p < 0.001
			Density	3 vs. 1	-3.97 ± 1.63	29802	$\chi 2 = 5.5194$, df = 1, p < 0.05
			Magnitude	4 vs. 1	0.93 ± 0.25	29809	$\chi 2 = 12.555$, df = 1, p < 0.001
			n females	5 vs. 1	-0.02 ± 0.005	29806	$\chi 2 = 10.284$, df = 1, p < 0.001
		WFS:BASE	None	1	-	-4605.1	-
			Assortativity	2 vs. 1	-0.53 ± 0.03	-4215.8	$\chi 2 = 391.32$, df = 1, p < 0.001
			Density	3 vs. 1	-1.71 ± 0.053	-4598.4	$\chi 2 = 8.6912$, df = 1, p < 0.001
			Magnitude	4 vs. 1	-0.18 ± 0.08	-4602.1	$\chi 2 = 5.0385$, df = 1, p < 0.05
			n females	5 vs. 1	0.012 ± 0.001	-4553.9	$\chi 2 = 53.174$, df = 1, p < 0.001
Fixed	Opportunity for selection (I_T) vs. Assortativity Fixed effects: Density + Assortativity + Magnitude Random effects: Original network	WFA:BASE	None	1	-	42105	-
			Assortativity	2 vs. 1	-3.49 ± 0.26	42282	$\chi 2 = 178.81$, df = 1, p < 0.001
			Density	3 vs. 1	-9.14 ± 2.58	42115	$\chi 2 = 11.396$, df = 1, p < 0.001
			Magnitude	4 vs. 1	0.9 ± 0.37	42109	$\chi 2 = 5.7734$, df = 1, p < 0.05
		WFS:BASE	None	1	-	5241.8	-
			Assortativity	2 vs. 1	-3.23 ± 0.07	7011.6	$\chi 2 = 1771.7$, df = 1, p < 0.001
			Density	3 vs. 1	-9.28 ± 2.21	5255.2	$\chi 2 = 15.401$, df = 1, p < 0.001
			Magnitude	4 vs. 1	0.43 ± 0.32	5241.7	$\chi 2 = 1.855$, df = 1, p = 0.17

Table A7 GLMM results assessing the relationship between the standardised univariate Bateman gradient ($\beta`_M$) and assortativity (r).

Simulation type	Full model	Competitive mechanism	Parameter removed	Model number & comparison	Parameter estimate ± SE	AIC	LRT
Probabilistic	Standardised variance in univariate Bateman Gradients (β'_M) vs. Assortativity	WFA:BASE	None	1	-	17819	-
			Assortativity	2 vs. 1	-0.29 ± 0.04	17851	χ2 = 34.066, df = 1, p < 0.001
	Fixed effects: Density + Assortativity + *n* females + Magnitude		Density	3 vs. 1	-0.17 ± 0.08	17821	χ2 = 4.1701, df = 1, p < 0.05
	Random effects: Original network		Magnitude	4 vs. 1	-0.016 ± 0.26	17819	χ2 = 1.677, df = 1, p = 0.2
			n females	5 vs. 1	0.001 ± 0.0003	17822	χ2 = 5.308, df = 1, p < 0.05
		WFS:BASE	None	1	-	-24082	-
			Assortativity	2 vs. 1	-0.65 ± 0.014	-22077	χ2 = 2006.7, df = 1, p < 0.001
			Density	3 vs. 1	-0.48 ± 0.13	-24071	χ2 = 13.128, df = 1, p < 0.001
			Magnitude	4 vs. 1	-0.01 ± 0.02	-24083	χ2 = 0.45, df = 1, p = 0.5
			n females	5 vs. 1	0.0003 ± 0.0005	-24084	χ2 = 0.256, df = 1, p = 0.6
Fixed	Standardised variance in univariate Bateman Gradients (β'_M) vs. Assortativity	WFA:BASE	None	1	-	14725	-
			Assortativity	2 vs. 1	-1.79 ± 0.1	15058	χ2 = 335.06, df = 1, p < 0.001
	Fixed effects: Density + Assortativity + Magnitude		Density	3 vs. 1	-1.4 ± 0.25	14748	χ2 = 25.384, df = 1, p < 0.001
	Random effects: Original network		Magnitude	4 vs. 1	0.007 ± 0.03	14723	χ2 = 0.0382, df = 1, p = 0.85
		WFS:BASE	None	1	-	-23872	-
			Assortativity	2 vs. 1	-1.84 ±0.03	-20023	χ2 = 3851.7, df = 1, p < 0.001
			Density	3 vs. 1	-1.44 ± 0.25	-23848	χ2 = 26.387, df = 1, p < 0.001
			Magnitude	4 vs. 1	0.007 ± 0.04	-23874	χ2 = 0.0391, df = 1, p = 0.84

Table A8 GLMM results assessing the relationship between the standardised covariance between *M* and *P* (*Cov(M,P)*) and assortativity (*r*).

Simulation type	Full model	Competitive mechanism	Parameter removed	Model number & comparison	Parameter estimate ± SE	AIC	LRT
Probabilistic	Standardised covariance between mating success and paternity share (*Cov(M,P)*) vs. Assortativity Fixed effects: Density + Assortativity + *n* females + Magnitude Random effects: Original network	WFA:BASE	None	1	-	2802.4	-
			Assortativity	2 vs. 1	-0.184 ± 0.03	2829.4	$\chi 2 = 28.959$, df = 1, p < 0.001
			Density	3 vs. 1	-0.27 ± 0.05	2823.2	$\chi 2 = 22.79$, df = 1, p < 0.001
			Magnitude	4 vs. 1	-0.01± 0.008	2801.8	$\chi 2 = 1.36$, df = 1, p = 0.24
			n females	5 vs. 1	0.0004± 0.0002	2804.6	$\chi 2 = 4.173$, df = 1, p < 0.05
		WFS:BASE	None	1	-	-37872	-
			Assortativity	2 vs. 1	-0.41 ± 0.009	-35820	$\chi 2 = 2054.4$, df = 1, p < 0.001
			Density	3 vs. 1	-0.45 ± 0.08	-37848	$\chi 2 = 26.049$, df = 1, p < 0.001
			Magnitude	4 vs. 1	0.009 ± 0.01	-37874	$\chi 2 = 0.5607$, df = 1, p = 0.45
			n females	5 vs. 1	0.0001 ± 0.0003	-37874	$\chi 2 = 0.004$, df = 1, p = 0.95
Fixed	Standardised covariance between mating success and paternity share (*Cov(M,P)*) vs. Assortativity Fixed effects: Density + Assortativity + Magnitude Random effects: Original network	WFA:BASE	None	1	-	1749.1	-
			Assortativity	2 vs. 1	-1.33 ± 0.06	2161.7	$\chi 2 = 414.62$, df = 1, p < 0.001
			Density	3 vs. 1	-1.25 ± 0.18	1783.0	$\chi 2 = 35.934$, df = 1, p < 0.001
			Magnitude	4 vs. 1	-0.005 ± 0.025	1747.2	$\chi 2 = 0.047$, df = 1, p = 0.8
		WFS:BASE	None	1	-	-37813	-
			Assortativity	2 vs. 1	-1.33 ± 0.02	-32886	$\chi 2 = 4929.8$, df = 1, p < 0.001
			Density	3 vs. 1	-1.2 ± 0.17	-37779	$\chi 2 = 36.014$, df = 1, p < 0.001
			Magnitude	4 vs. 1	-0.01 ± 0.02	-37815	$\chi 2 = 0.144$, df = 1, p = 0.7

Table A9 GLMM results assessing the relationship between the standardised variation in mating success (I_M) and assortativity (r).

Simulation type	Full model	Competitive mechanism	Parameter removed	Model number & comparison	Parameter estimate ± SE	AIC	LRT
Probabilistic	Standardised variance in mating success (I_M) vs. Assortativity	WFA:BASE	None	1	-	-12929	-
			Assortativity	2 vs. 1	0.27 ± 0.02	-12753	χ2 = 177.65, df = 1, p < 0.001
	Fixed effects: Density + Assortativity + *n* females + Magnitude		Density	3 vs. 1	-0.53 ± 0.29	-12928	χ2 = 2.704, df = 1, p = 0.1
	Random effects: Original network		Magnitude	4 vs. 1	-0.09 ± 0.04	-12927	χ2 = 3.652, df = 1, p < 0.001
			n females	5 vs. 1	0.007 ± 0.001	-12898	χ2 = 32.24, df = 1, p = 0.06
		WFS:BASE	None	1	-	-25054	-
			Assortativity	2 vs. 1	0.29 ± 0.013	-24596	χ2 = 460.86, df = 1, p < 0.001
			Density	3 vs. 1	-0.24 ± 0.37	-25056	χ2 = 0.411, df = 1, p = 0.52
			Magnitude	4 vs. 1	-0.16 ± 0.054	-25048	χ2 = 8.653, df = 1, p < 0.005
			n females	5 vs. 1	0.01 ± 0.001	-24927	χ2 = 129.2, df = 1, p < 0.001
Fixed	Standardised variance in mating success (I_M) vs. Assortativity	WFA:BASE	None	1	-	-1211.83	-
			Assortativity	2 vs. 1	1.2 ± 0.06	-811.68	χ2 = 402.15, df = 1, p < 0.001
	Fixed effects: Density + Assortativity + Magnitude		Density	3 vs. 1	-0.84 ± 0.32	-1207.2	χ2 = 6.68,, df = 1, p < 0.01
	Random effects: Original network		Magnitude	4 vs. 1	0.18 ± 0.04	-1200.3	χ2 = 13.56, df = 1, p < 0.001
		WFS:BASE	None	1	-	-27866	-
			Assortativity	2 vs. 1	1.17 ± 0.02	-25702	χ2 = 2166, df = 1, p < 0.001
			Density	3 vs. 1	-0.86 ± 0.31	-27861	χ2 = 7.203, df = 1, p < 0.01
			Magnitude	4 vs. 1	0.17 ± 0.04	-27855	χ2 = 13.515, df = 1, p < 0.001

Table A10 GLMM results assessing the relationship between the standardised variation in fertilisation success (I_P) and assortativity (r).

Simulation type	Full model	Competitive mechanism	Parameter removed	Model number & comparison	Parameter estimate ± SE	AIC	LRT
Probabilistic	Standardised variance in paternity share (I_P) vs. Assortativity	WFA:BASE	None	1	-	42963	-
			Assortativity	2 vs. 1	0.09 ± 0.13	42961	$\chi 2 = 0.43$, df = 1, p = 0.5
	Fixed effects: Density + Assortativity + *n* females + Magnitude		Density	3 vs. 1	-2.85 ± 1.7	42963	$\chi 2 = 2.733$, df = 1, p = 0.1
	Random effects: Original network		Magnitude	4 vs. 1	1.38 ± 0.26	42984	$\chi 2 = 22.815$, df = 1, p < 0.001
			n females	5 vs. 1	-0.3 ± 0.006	42983	$\chi 2 = 22.023$, df = 1, p < 0.001
		WFS:BASE	None	1	-	-28842	-
			Assortativity	2 vs. 1	0.08 ± 0.01	-28794	$\chi 2 = 50.662$, df = 1, p < 0.001
			Density	3 vs. 1	-0.42 ± 0.06	-28811	$\chi 2 = 33.486$, df = 1, p < 0.001
			Magnitude	4 vs. 1	0.02 ± 0.01	-28838	$\chi 2 = 5.783$, df = 1, p < 0.05
			n females	5 vs. 1	-0.001 ± 0.0003	-28829	$\chi 2 = 15.369$, df = 1, p < 0.001
Fixed	Standardised variance in paternity share (I_P) vs. Assortativity	WFA:BASE	None	1	-	48236	-
			Assortativity	2 vs. 1	1.93 ± 0.32	48270	$\chi 2 = 36.119$, df = 1, p < 0.001
	Fixed effects: Density + Assortativity + Magnitude		Density	3 vs. 1	0.26 ± 2.14	48234	$\chi 2 = 0.014$, df = 1, p = 0.9
	Random effects: Original network		Magnitude	4 vs. 1	1.27 ± 0.3	48250	$\chi 2 = 15.34$, df = 1, p < 0.001
		WFS:BASE	None	1	-	-213.374	-
			Assortativity	2 vs. 1	0.8 ± 0.06	-48.159	$\chi 2 = 167.22$, df = 1, p < 0.001
			Density	3 vs. 1	-0.69 ± 0.4	-212.44	$\chi 2 = 2.9342$, df = 1, p = 0.09
			Magnitude	4 vs. 1	0.19 ± 0.06	-205.31	$\chi 2 = 10.06$, df = 1, p < 0.005

APPENDIX§3

Table A11 Model results assessing the relationship between the opportunity for sexual selection (I_s) and nestedness (NODF) and assortativity (r) for probabilistic simulations. Both nestedness and assortativity are scaled by subtracting the mean and dividing by their respective standard deviations so that slopes are comparable.

Full model	Parameter	Parameter estimate ± SE	*t*
Opportunity for sexual selection (I_s) vs. Nestedness Fixed effects: Density + Nestedness + Magnitude + *n* females, Random effects: Original network	Scaled Nestedness (NODF)	0.19 ± 0.005	36.06
Opportunity for sexual selection (I_s) vs. Assortativity Fixed effects: Density + Assortativity + Magnitude + *n* females, Random effects: Original network	Scaled Assortativity	0.02 ± 0.003	7.580

CHAPTER 6. THE STRUCTURE OF SEXUAL NETWORKS IN RED JUNGLEFOWL (*GALLUS GALLUS*) AND ITS IMPLICATIONS FOR SEXUAL SELECTION

Grant C. McDonald[1], Lewis Spurgin[2], Eleanor Fairfield[3], David Richardson[3] and Tommaso Pizzari[1]

[1]*Edward Grey Institute, Department of Zoology, University of Oxford, Oxford, UK.*
[2]*Faculty of Mathematics and Natural Sciences, University of Gronigen, Gronigen Netherlands.*
[3]*School of Biological Sciences, University of East Anglia, Norwich, UK.*

ABSTRACT

The mating patterns of a population are the result of a complex network of sexual interactions. The structure of these interactions will have strong implications for the strength of sexual selection on males. This complex structure can be broken down into three main axes, i) variation in male mating success, ii) female promiscuity (i.e. the population level of polyandry), and iii) the relationship between male mating success and the promiscuity of his mates. In this study, we employ an experimental approach using Red junglefowl (*Gallus gallus*) to determine how all these three parameters combine to shape the operation of both pre- and post-copulatory episodes of sexual selection. We analyse patterns of variation at multiple scales: within groups over time, and between groups. We confirm predictions that polyandry should decrease the overall opportunity for sexual selection and increase the relative importance of postcopulatory episodes of sexual selection. Furthermore, we expand on previous work to show that for a given level of polyandry, the mating patterns of groups, in terms of the relationship between male mating success and that of his partners can act to limit the decrease in the potential strength of sexual selection and simultaneously increase the relative strength of precopulatory sexual selection. We argue that this component of mating systems may be an important yet unappreciated modulator of the effects of polyandry on the operation of sexual selection.

INTRODUCTION

Sexual selection is the selective force that operates on variation in reproductive success attributable to intrasexual competition for access to the gametes of the opposite sex (Andersson 1994). This intrasexual competition can occur at two

stages, before mating (precopulatory sexual selection) and after mating (postcopulatory sexual selection) (Eberhard 2009; Parker and Birkhead 2013). Precopulatory sexual selection on males operates on variation in the product of the number of unique mating partners that males attain (*M*) and the average fecundity of those partners (*N*) (i.e. the total number of ova with which males have access to fertilisation) (Darwin 1871; Webster et al. 1995). Postcopulatory sexual selection on males operates on variation in the proportion of those ova that male fertilise (*P*) and can occur when females mate with more than one male (polyandry) (Parker 1970; Childress and Hartl 1972; Thornhill 1983; Webster et al. 1995). Precopulatory sexual selection therefore operates on traits that confer mating advantage and increase partner fecundity, whereas postcopulatory sexual selection favours those traits that increase fertilisation success (Birkhead and Pizzari 2002). Both pre- and post-copulatory sexual selection are strong modulators of evolutionary processes including reproductive strategies, secondary sexual trait elaboration and speciation (Andersson 1994; Birkhead and Møller 1998; Simmons 2001; Birkhead and Pizzari 2002; Seddon et al. 2008; Parker and Pizzari 2010; Kraaijeveld et al. 2011).

The relative roles of pre- and post-copulatory sexual selection largely depend on the mating patterns of a population (mating systems) (Bateman 1948; Emlen and Oring 1977; Shuster and Wade 2003; Shuster 2009; Collet et al. 2012). There are three key axes along which mating patterns can vary with implications for sexual selection in males: i) variation in male mating success (i.e. the mating skew), ii) the pattern of female sharing across males (i.e. polyandry), and iii) the relationship between male mating success and the mating success of his partners ("mating structure") (McDonald and Pizzari In press, Chapter 5). Despite their potential importance in modulating sexual selection, only recently has an appreciation for the

role of these three axes begun to develop (Collet et al. 2012; Kvarnemo and Simmons 2013). More recently, McDonald & Pizzari (Chapter 5) used a simulation approach to generate predictions for how both polyandry and mating structure are expected to modify sexual selection. These results show that polyandry can either increase or decrease total sexual selection depending on both, the promiscuity of males and mating structure. However, the collective role of these mechanisms in sexual selection remains to be empirically investigated (Wade and Shuster 2005).

The mating patterns of a population are the result of a complex network of sexual interactions ("the mating topology"), which not only defines the variation in mating success, and the population level of polyandry but also simultaneously defines the postcopulatory competitive landscape. Over the past ten years there has been an explosion of research in evolutionary ecology utilizing network analysis (Krause et al. In Press, 2007, 2009; Croft et al. 2008; Wey et al. 2008; Whitehead 2008; Sih et al. 2009). This array of tools now provides researchers with a quantitative vocabulary to measure variance in the structure of mating networks and the resulting effects on sexual selection. Such tools include individual-level metrics that quantify the potential intensity of sperm competition for each individual as well as population-level metrics that quantify the covariance between the mating success of multiple individuals (Sih et al. 2009; McDonald et al. 2013). In this study, we use quantitative sexual selection techniques in combination with network tools to understand how the structure of the mating network shapes variance in male reproductive success and the operation of sexual selection. Specifically, we address the potential for polyandry and mating structure to modulate both pre- and post-copulatory selection and total sexual selection. Using an experimental approach across replicate groups of red junglefowl (*Gallus gallus*), we firstly i) measure the operation of sexual selection on males and

decompose variance in male reproductive success into its component parts. Secondly, we explore the role of polyandry and mating structure in determining the operation of sexual selection both ii) within groups over time, and iii) across groups. To assess mating structure we quantify the relationship between male and female mating patterns using two network measures, degree assortativity and nestedness. Both measures represent alternative ways to measure the relationship between male and female promiscuity (*M*) at the population level. For the across-group analysis we also quantify weighted nestedness which quantifies the relationship between male and female *M* taking into account the number of copulations between individuals (McDonald and Pizzari In press; Almeida-Neto and Ulrich 2011). Finally, we move beyond group-level characterisation of network properties and iv) measure sexual selection on two different aspects of male mating behaviour, remating rate (i.e. mean number of copulations per female) and exclusivity, a recently proposed network measure that quantifies how exclusive a male's mating access is with a sexual partner (Sih et al. 2009), and explore how polyandry and mating structure modify the relative roles of all these male mating behaviours.

METHODS

STUDY POPULATION & EXPERIMENTAL DESIGN

We use replicate semi-natural groups of Red junglefowl (*Gallus gallus*), a species that lives in small social groups (2-28 individuals) (Collias and Collias 1996). All individuals used in this study originate from a semi-natural population of red junglefowl kept at the University of Oxford field station (refer to previous work that describes this population e.g. Collet et al. 2012, 2014). All experimental work was

conducted between the months April-October across three breeding seasons between 2011 and 2013. We used 12 male-female mating groups in total. Each experimental group consisted of 10 adult males and 12 adult females held in an enclosure approximately 100m^2. Due to limitations on available males, 13 males were reused across different male groups. Because of similar constraints on the number of females available, we assembled 6 groups of 12 females each. Each female group was matched with more than one group of males (in a similar pattern the that described in figure 1) but females were not reused across female groups. Birds were fed *ad libitum* using a mixture of grower pellets and fresh seeds at ratio of 2:1. Food was replenished everyday in the morning before birds came down from roosting and water was replaced every 2 days at the same time.

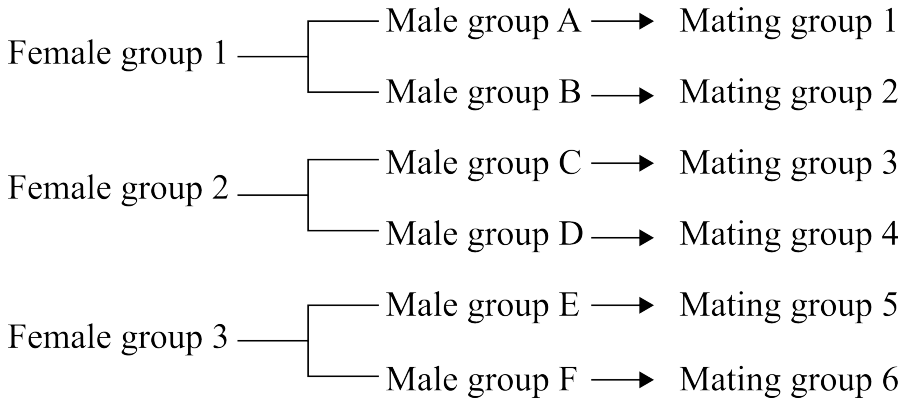


Figure 1. Diagram showing how replicate female groups were used repeatedly across unique male groups to create individual mating groups.

DATA COLLECTION

Before each trial males and females were housed in single sex enclosures, females for a minimum 14 days to ensure sperm depletion (Etches 1996). Males were allowed into the experimental enclosure for 3 days prior to the release of females to allow male dominance hierarchies to form and stabilize. On the morning of the first day of each trail all females joined the males in the experimental enclosure. Behavioural observations were made everyday for 6 hours between 0500-0800hr in

the morning and in the evening between 1800-2100hr GMT for a total of 60 hours per group, totalling 720 hours of behavioural observations. We used *ad libitum* sampling technique as all individuals could be observed at once (Altmann 1974). We recorded all male-male interactions including pecks, chases, fights, waltzes and avoidances (Johnsen et al. 2001). A male was considered a subdominant in interactions if he retreated one body length of the other male (Johnsen et al. 2001; Froman et al. 2002; Wilson et al. 2009). We recorded all copulation attempts by males on females and all copulation events were recorded. Copulations were considered successful when cloacal contact was observed or when the male tail was lowered over the female cloaca and cloacal contact was assumed to occur. All eggs laid by females were collected during the 10 days of experimentation and the 11th day a trial was complete, totalling 468 eggs. All eggs were incubated artificially for 5-9 days before taking tissue samples. Embryo tissue samples were subsequently stored in 100% ethanol at -20° C until later parentage assignment.

MOLECULAR METHODS AND ANALYSES

For all groups we ignore all eggs from day one of each trial as eggs laid on the first day of a trial are unlikely to be fertilized on the same day (Etches 1996), and instead only consider parentage data from days 2-10 of the trial and all eggs laid on the 11th day after the trial was complete. DNA was extracted following a standard ammonium acetate precipitation protocol (Nicholls et al. 2000). All samples were genotyped at 15 variable microsatellite loci arranged into 2 multiplex reactions, using primers designed to amplify in domestic chicken (Crooijmans et al. 1996, 1997; Gibbs et al. 1997; Hanotte et al. 1997; Dawson et al. 1998; Groenen et al. 2000) or across a wide range of bird species (Dawson et al. 2010). PCRs were carried out in 2 µl

volumes, using the reagents and methods described in Kenta et al (2008). Cycling conditions for both multiplexes were as follows: an initial heating step of 95˚C for 15 minutes was followed by 35 cycles of 94˚C for 30 seconds, 56˚C for 90 seconds and 72˚C for 60 seconds. A final extension step of 60˚C for 30 minutes completed the reaction. PCR products were diluted by 1 in 150, and fragments were separated on an ABI 3730 capillary sequencer. Allele sizes were assessed against ROX 500 size standard using the software GeneMapper version 3.1 (Applied Biosystems).

Null allele frequencies for each locus were estimated using CERVUS version 3.0.1 (Kalinowski et al. 2007). Molecular parentage analyses were carried out in CERVUS, using the approaches outlined in Dean et al. (2010). Because of the larger number of potential parents in our experimental design compared to previous experiments, we could not assign parentage to all offspring with 95% confidence (see results). Subsequent analyses using parentage data were therefore carried out including only individuals that could be assigned parentage with 95% confidence. None of the 15 microsatellite loci had high null allele frequencies (highest = 0.03), so all were used for parentage analyses.

DATA ANALYSIS

We first combined paternity data with behavioural data on male mating success. If a male sired offspring with a female but this pair was never observed copulating, we added this female to the males mating success and assumed that this pair copulated once in analyses of remating rates. All statistical analysis was carried out using R statistical software (R Core Team 2014).

i) The operation of sexual selection

Before assessing the effects of mating system parameters on sexual selection, we quantified the potential for sexual selection to operate on males across groups, by calculating standardised variance in male reproductive success, and standardized variance in its pre- and post-copulatory constituents as described in table 1 (Crow 1958; Arnold and Duvall 1994; Webster et al. 1995). To demonstrate the importance of both pre- and post-copulatory episodes across all groups, we then measured multivariate selection gradients by regressing *T* over *M*, *N* and *P*. These models included female group ID and male ID as random effects to control for pseudoreplication and group ID to allow variation in mean *T* across replicate groups. Significance tests were carried out by removing one parameter at a time and models were compared using likelihood ratio tests (LRTs).

Table 1. Formula definitions for measures of sexual selection.

Selection Coefficient	Formula
Opportunity for Selection (I_T)	$Var(T)/\bar{T}^2$
Opportunity for Sexual Selection (I_S)	$Var(M)/\bar{M}^2$
Mean standardised variance in mean partner fecundity (I_N)	$Var(N)/\bar{N}^2$
Standardised variance in mating success (*M*)	$\frac{\bar{N}^2\bar{P}^2Var(M)}{\bar{T}^2}$
Standardised variance in partner fecundity (*N*)	$\frac{\bar{M}^2\bar{P}^2Var(N)}{\bar{T}^2}$
Standardised variance in fertilization success (*P*)	$\frac{\bar{M}^2\bar{N}^2Var(P)}{\bar{T}^2}$
Standardised Covariance between mating success (*M*) and fertilization success (*P*)	$\frac{2\bar{M}\bar{P}\bar{N}^2Cov(M,P)}{\bar{T}^2}$
Standardised Covariance between mating success (*M*) and partner fecundity (*N*)	$\frac{2\bar{M}\bar{N}\bar{P}^2Cov(M,N)}{\bar{T}^2}$
Standardised Covariance between partner fecundity (*N*) and fertilisation success (*P*)	$\frac{2\bar{N}\bar{P}\bar{M}^2Cov(N,P)}{\bar{T}^2}$

ii) Within-group polyandry and mating structure

Female fowl can store viable sperm within their sperm storage tubules for a median period of three weeks (Etches 1996). Such prolonged sperm storage means that over successive days, polyandry can have a cumulative effect on postcopulatory sexual selection on males. To investigate this temporal effect, we subdivided a trial into five 2-day blocks and calculated the cumulative average mating success of males and females over successive blocks (i.e. days 1-2, 1-4, 1-6, 1-8, 1-10), which generated a range of increasing levels of polyandry within groups across the 10 days of each trial. We then calculated non-cumulative average *T* and *N* for each two-day block (i.e. separately for days 1-2, 3-4, 5-6, 7-8 and 9-10). We calculated *T* and *N* in this way rather than cumulatively across time, as mean *T* influences the opportunity for selection and as such a cumulative analysis may result in a conflation between the effects of increasing polyandry and increasing mean *T*. This would make it difficult to draw conclusions about whether potential decreases in I_T are driven by polyandry *per se* or simply increasing group productivity (Downhower et al. 1987). We paired cumulative measures of male mating success, *M,* calculated as above for cumulative days with *T* and *N* for the same block (e.g. *M* for days 1-2 with *T* and *N* for days 1-2; *M* for days 1-4 with *T* and *N* from days 2-4 and so on). We then calculated non-cumulative *P* for all males in each two-day block. For each block of days in each group we constructed mating networks, with males and females as nodes and edges connecting male-female pairs that were observed copulating and/or produced offspring together. We then calculated average group polyandry and two measures of mating structure, nestedness (NODF) and degree assortativity (*r*) for each 2-day block. Both network measures quantify the relationship between a male's *M* and that

of his partner(s). Assortativity (r) is measured as the Pearson correlation coefficient between a male's degree (M) and the mean of his partner's degree minus one, and so r ranges from 1 to -1. Nestedness was calculated using one of the most widely used methods named NODF (nestedness measure based on overlap and decreasing fills, Almeida-Neto et al. 2008), which measures the extent to which those nodes with many connections tend to be connected to nodes with fewer mating partners. This method is based on the rules of percentage overlap and decreasing fill between pairs of rows and columns and thus quantifies extent to which the mating patterns of individuals with low degree form subsets of the mating partners of those individuals with a higher degree (McDonald & Pizzari, *in press*, Chapter 4). NODF measures the nestedness of the whole network ranging from 100 (perfect nestedness) to zero (no nestedness). We used linear mixed effect models to assess the effect of these parameters on changes in within group measures of sexual selection. Before doing so, we quantified the relationship between NODF and assortativity with polyandry and between NODF and assortativity. NODF was highly correlated with polyandry (Appendix§1 Figure A1). This strong correlation resulted in high multicolinearity in models that included both polyandry and NODF, resulting in high variance inflation factors (VIFs), reducing precision in significance tests of correlated parameters. Attempts to scale parameters did not reduce the multicolinearity and so all models testing the effect of polyandry and mating structure on measures of sexual selection for within-group analyses included only polyandry and assortativity as our measure of mating structure. Previous studies (Chapter 5) revealed curvature in the relationship between polyandry and measures of sexual selection and visual inspection of results confirmed this, so all models assessing changes in measures of sexual selection with polyandry included a linear polyandry term, a quadratic polyandry term and

assortativity as fixed effects. Each model also included female group and group ID to control for pseudoreplication. Significance tests were carried out by removing one parameter at a time and likelihood ratio tests (LRTs). We conducted the above models for the following measures of sexual selection: I_T, standardized variances in *M*, *N, P* and all covariances (Webster et al. 1995) as well as standardized Bateman gradients (*β'ss*) calculated for each group independently, by firstly standardizing both *T* and *M* by their respective means, as Jones (2009). Finally, we because of the number of independent models used to assess the effects of mating patterns on selection measures; we used Bonferroni correction of *p*-values by dividing p-values by the number of models. However, Bonferroni corrections are very conservative, and may increase type II errors, we therefore present both corrected and uncorrected results (Whitlock and Schluter 2008).

To assess the whether components vary in the size of their contribution to the opportunity for selection we also carried out each model for the percentage contributions of *M*, *N, P* and all covariances. Because of higher infertility in eggs laid at the beginning of experiments, as females may be more sperm limited due to fewer copulations (Etches 1996), the first day block (days 1-2) also tended to have lower mean *T* and so we carried out all analyses in this section with and without the first 2-days block. Results did not change qualitatively and so we present the analysis including all five 2-day blocks. For these models we also used Bonferroni corrections and present results both corrected and uncorrected results.

Finally, we qualitatively compare the results presented here with quantitative predictions produced through simulations by McDonald and Pizzari (Chapter 5). McDonald and Pizzari generated predictions for increasing polyandry in similarly-sized groups (10 males and 10 females) across a range of sperm competitive

mechanisms. The simulations we have chosen for direct comparison are generated from polygynous groups with a winner fertilise all sperm competitive mechanism (WFA), where females only produce one ovum and so if a male is successful in postcopulatory competition he fertilises the single ovum and all other sperm competitors achieve zero paternity with that female. This is the most appropriate comparison with our data as female junglefowl only produce approximately 1-2 eggs every two days and so is most similar to the levels of fecundity in our 2-day blocks (Hocking 2009). Furthermore, in these simulations males possess phenotypic traits that can influence the probability of fertilisation success, which is well documented in fowl (Pizzari et al. 2002). For original results see Appendix§2 fig. A2, and fig. 4 in Chapter 5.

iii) Across-group polyandry and mating structure

To assess the effect of variation in polyandry and mating structure on the operation of sexual selection across groups we calculated *T*, *M*, *N* and *P* for all males in all groups across all ten days, resulting in a single value per measurement. For each group we calculated average group polyandry and fitted GLMs with the selection measure as the response and polyandry as the only explanatory variable to assess the effects of polyandry overall and for comparison with previous work (Collet et al. 2012, Chapter 5). Visual inspection did not reveal strong curvature in results and so polyandry was fitted only as a linear predictor. We then calculated three measures of mating structure: nestedness (NODF) and Assortativity (*r*) (as above) and weighted nestedness (WNODF). Weighted NODF is an extension of NODF that takes into account the number of repeat copulations (Almeida-Neto and Ulrich 2011). Specifically, we calculated row-wise weighted nestedness (WNODFrow), this measure simultaneously quantifies two aspects of mating structure, i) the tendency for

the mating partners of males with lower *M* to be a reduced subset of the mating partners of males with higher *M* and ii) the tendency for the remating rate of males with low *M* to be reduced relative to males with higher *M*. WNODFrow ranges from 0-100, with high WNODFrow suggesting strong row-wise weighted nestedness and would be expected to increase variation in reproductive success as high *M* males also have a higher remating rate across those females.

For all three coefficients we assessed the effect on four measures that have been examined in previous simulation studies of mating structure; I_T, standardized variance in *M*, standardized variance in *P*, and standardized univariate Bateman gradients (*β'ss*) (Chapter 5). To do this we fitted the models including NODF and assortativity as explanatory variables controlling for polyandry. All models also included female group as a fixed effect to control for pseudoreplication. These were added as fixed effects rather than random effects due the small sample size because LRTs for small samples are anti-conservative. Across groups, the relationship between polyandry and NODF was weaker than within groups, although still suggestive of multicolinearity ($\sqrt{VIFs}\sim 2$). We thus carried out the analysis including all three variables and an additional analysis excluding NODF and only including assortativity and polyandry. Results did not change qualitatively and so we present results from models including all three explanatory variables. Finally, we fitted GLMs for WNODFrow against I_T, standardized variance in *M* and *P* as well as standardized univariate Bateman gradients (*β'ss*) with average group polyandry as a covariate. Again, we checked for multicolinearity between predictors for models containing WNODFrow which may inflate errors around predictors but we found no strong evidence for multicolinearity. For all models we also used Bonferroni corrections and present both corrected and uncorrected results.

iv) Sexual selection on male mating patterns

We focused on three descriptors of male mating patterns: male mating success (*M*), male average remating rate per female, and male exclusivity. Male remating rate is calculated as the average number of copulations across all females mated. Exclusivity (*e*) is a network metric that describes the exclusivity a male has with a female mating partner in the network calculated as the reciprocal of partners degree (*d*) i.e. if a female mates with 3 males, any one of those male's exclusivity is 1/3 (Sih et al. 2009). For each male that mated we calculated his mean partner exclusivity as:

$$\bar{e_i} = \sum_{k=1}^{n} e_k \, / n$$

where *n* is the number of *k* females with whom the i_{th} focal male mated.

We investigated the effect of polyandry and network structure on these measures of male mating patterns by confirming whether both measures were under postcopulatory sexual selection across all groups using *P* as a response variable and including remating rate and exclusivity as explanatory variables. This model was a GLMM with binomial errors and included as random effects male ID, female group ID and group ID nested within female group to control for pseudoreplication. For each group, we then calculated linear postocpulaotry selection gradients on both exclusivity and remating rate in the same way individually for each group and calculated the total contribution of each trait to standardized variation in *P* using the approach proposed by Moorad and Wade (2013) for *T*. We then assessed the effect of polyandry on the strength of postcopulatory selection as measures (i.e. partial phenotypic selection gradients and the total contribution to *P* for each trait) using GLMs with polyandry as a fixed effect and female group as a covariate to control for pseudoreplication.

RESULTS

1) THE OPERATION OF SEXUAL SELECTION

There was high variation and right-tailed skew in male reproductive success *T* across the 12 replicate groups (fig. 2a). Variation in male mating success (*M*) and mean partner fecundity (*N*) were relatively low, indicating a large role for postcopulatory sexual selection in contributing to variation in *T* (fig. 2b-d). This was confirmed by the fact that standardized variance in *P* contributed the most to variance in reproductive success (fig. 3).

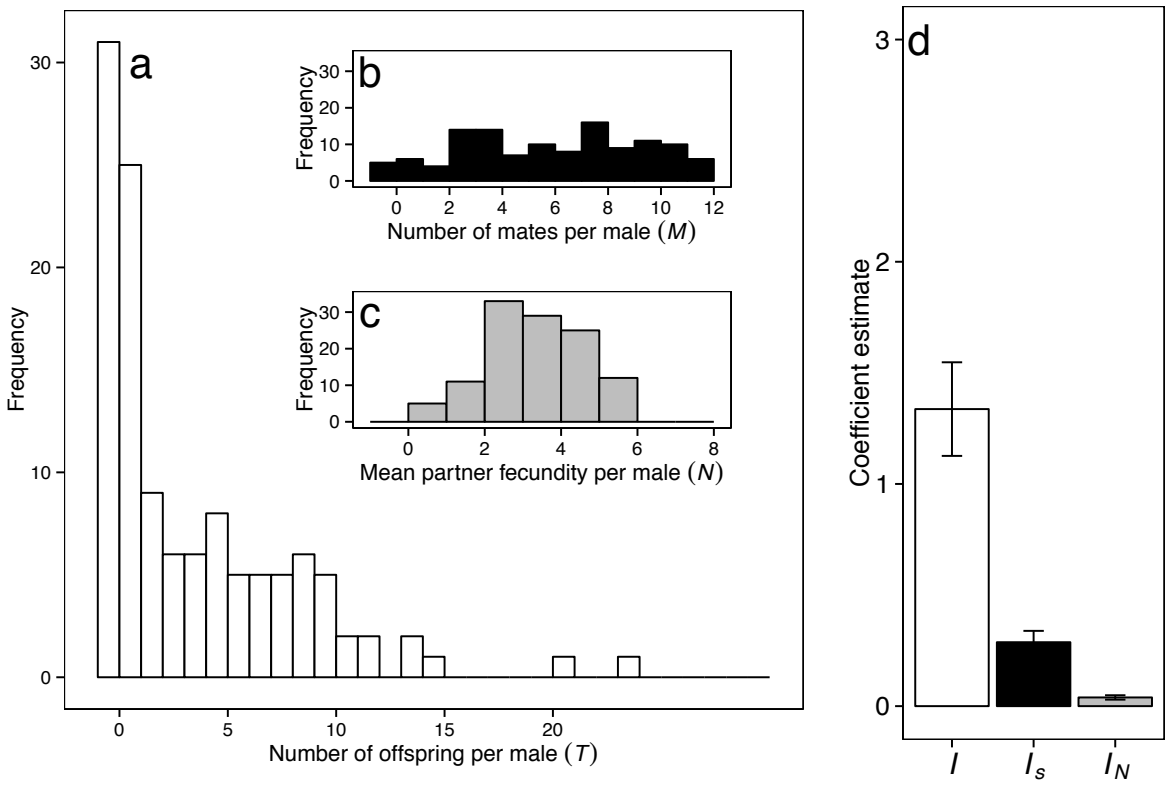


Figure 2. Panels a-c show frequency distribution of male reproductive success (*T*), mating success (*M*) and mean partner fecundity (*N*) respectively, for data pooled across all 12 groups. Data are binned into bars with a width = 1. Panel d shows mean opportunity for selection, mean opportunity for sexual selection and mean standardized variance in mean partner fecundity ±1SE.

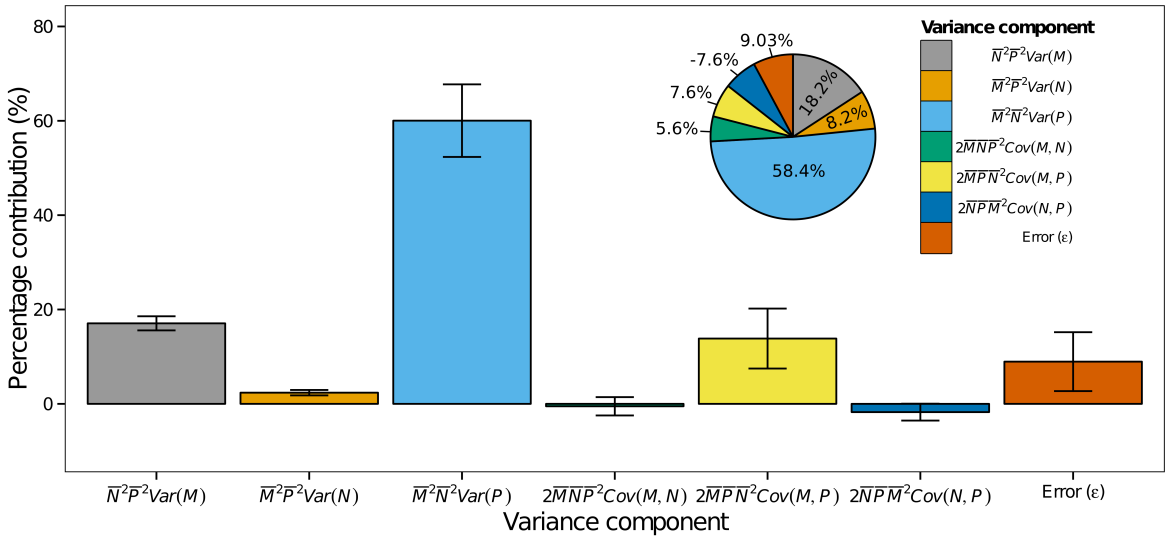


Figure 3. Bars represent mean percentage contribution of standardized fitness components ±1SE. Pie charts show percentage contribution of standardized fitness components for data pooled across all 12 groups. See table 1 for formulae definitions.

To demonstrate the importance of both pre- and post-copulatory fitness components in our study groups, we calculated multivariate selection gradients. These results confirm positive directional selection operating through *M*, *N* and *P*. (Table 2, fig. 4).

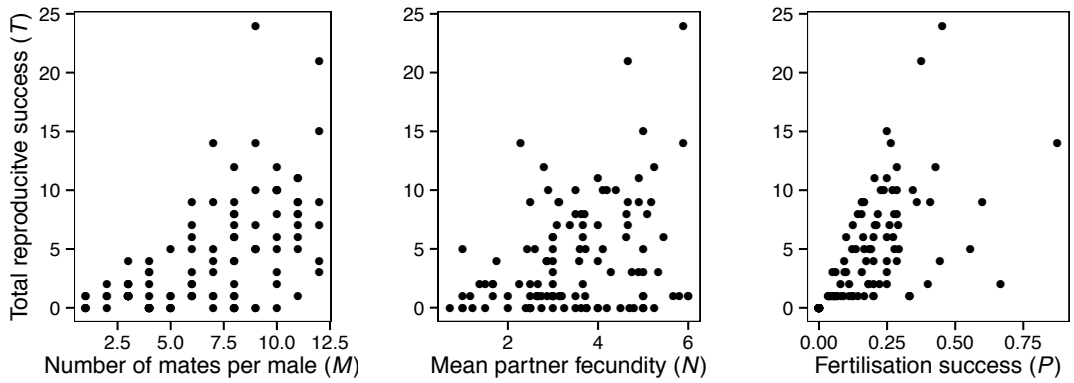


Figure 4. Plots show relationship between total reproductive success (*T*) and pre- and post-copulatory components (*M*, *N* and *P*) used to calculate multivariate selection gradients.

Table 2. Model results for multivariate sexual selection gradients.

Full model	Parameter removed	Model No & Comparison	Estimate	SE	LRT	AIC
$T \sim M + N + P +$ (Female Group/Group) + (Male)	None	1	-	-		398.4
	M	1 vs. 2	0.204	0.02037	$\chi2$=88.913, df=1, p<0.001	485.32
Random Effects: n(obs)=115, n(Males) = 103, n(Groups) =12, n(Female Groups) = 6	N	1 vs. 3	0.334	0.0556	$\chi2$=28.707, df=1, p<0.001	425.11
	P	1 vs. 4	4.105	0.3121	$\chi2$=131.2, df=1, p<0.001	527.6
var(Males) = 0, var(Groups) =0.02, var(Female Groups) = 0 (Poisson)						

II) WITHIN-GROUP POLYANDRY AND MATING STRUCTURE

Replicate groups differed markedly in both absolute levels of average polyandry and the rate at which cumulative polyandry increased over time (fig. 5).

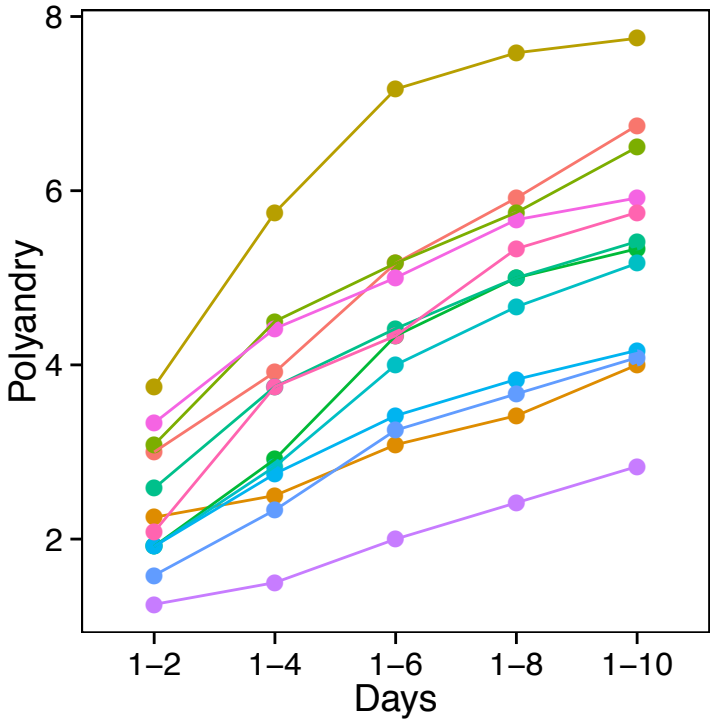


Figure 5. Cumulative increase in average polyandry for all 12 groups across the 10 days of each trial. Colours represent different groups.

Within-group analyses demonstrated an initial sharp decelerating decline in I_T with increasing polyandry (fig. 6). This decrease was largely due to a reduction in variance in mating success (*M*), mean partner fecundity (*N*), the covariance between the two (*Cov(M,N)*), and a decrease in the covariance between mating success and paternity share *Cov(M,P)* (fig. 6, table 3). The reduced variance in precopulatory components of sexual selection (i.e. *M* and *N*) was also confirmed by flatter standardised Bateman gradients (β'_{ss}) (fig. 6, table 3). In opposition to this, we found an increase in the variance in paternity share, i.e the opportunity for postcopulatory sexual selection, I_P (fig. 6, table 3).

Analyses testing the effect of polyandry on the relative contribution of *M*, *N* and *P* to total variation in male reproductive success (I_T) revealed that despite the reduced variance in *M* there was overall no significant change in the percentage contribution of *M* (fig. 7, table 4), partly driven by the slower rate of decline in variation in *M* compared to I_T. On the other hand, the contribution of *N* to I_T, and that of its covariance with *M* both declined significantly with increasing polyandry. The contribution of postcopulatory sexual selection to I_T instead significantly increased with polyandry, confirming the role of polyandry in increasing the relative importance of postcopulatory sexual selection (fig. 7, table 4). In figure 8, we qualitatively compare the results presented here with quantitative predictions produced through simulations by McDonald and Pizzari (Chapter 5). Our empirical results from the present study are in general agreement with the effect of polyandry predicted by simulations for populations with winner fertilise all sperm competition (WFA) and high male promiscuity, suggesting similar mechanisms underpin variation in reproductive success in male red junglefowl across the two day blocks in which *P* was calculated. In the original work by McDonald and Pizzari (Chapter 5) there were two

variations of these simulations where males possess a postcopulatory trait that negatively or positively predicts paternity share. We find greatest similarity with those simulations where male postcopulatory traits negatively covary with mating success, as these simulations also identify a decrease in standardised Bateman gradients with increasing polyandry. The decline in *M* in this study was not as marked as predicted by simulations suggesting that at low levels of polyandry junglefowl groups do not demonstrate the same very high levels of mating skew as in simulations, although part of this difference may be due to a difference in the absolute distribution of polyandry in empirical groups. This is because polyandry is never as low as 1 male per female in empirical groups (minimum polyandry > 2.5), whereas in simulations polyandry goes as low as 1 (i.e. zero polyandry) and it is at this level that standardized variance in *M* is at its highest in simulations. The original simulation figure is presented in Appendix§2 figure A2.

Our results also revealed the potential for mating structure, as measured by assortativity (*r*), to modulate the effect of polyandry on the operation of sexual selection. Decreasing values of assortativity describe a tendency for networks to become more disassortative, and therefore demonstrate a negative covariance between a male's mating success and the mating success of his mating partners. We found a negative relationship between assortativity and the opportunity for selection (I_T). This negative relationship indicates that, as network become more disassortative ($Cov(M_{male}, M_{female} < 0)$), there is an associated increase in I_T. As networks become more disassortative there was an increase in the variance in *M* and in the covariance between *M* and *P*. Furthermore, increasing disassortativity decreased the percentage contribution of *P* to I_T but simultaneously increased the contribution of the covariance between *M* and *P* and the covariance between M and N. Overall, we found no

significant effect of polyandry assortativity or NODF on standardised covariances between *N* and *P* (*Cov(N,P)*) or the percentage contribution *Cov(N,P)* to I_T (Appendix§2 Table A1). Suggesting that network structure does not modify the relationship between a male's fertilization success and the average fecundity of his partners.

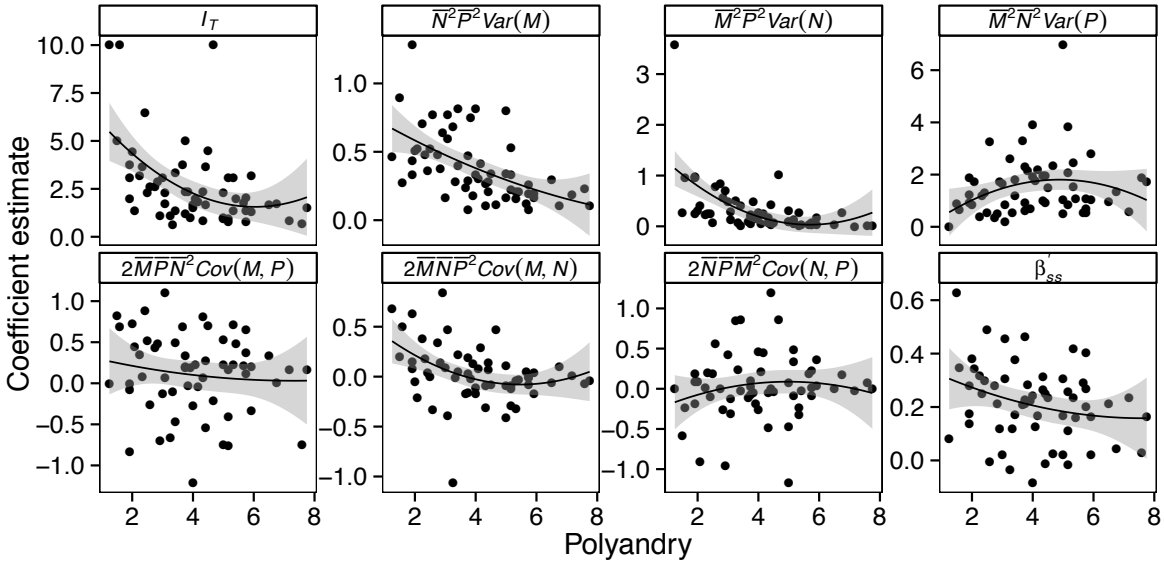


Figure 6. Plots show the effect of average group polyandry on the opportunity for selection (I_T) and standardized variances in *M, N, P* and all covariances, and individual group univariate Bateman gradients (β*'ss*). Curves show quadratic relationships and shaded areas represent 95% confidence intervals. See table 1 for formulae definitions.

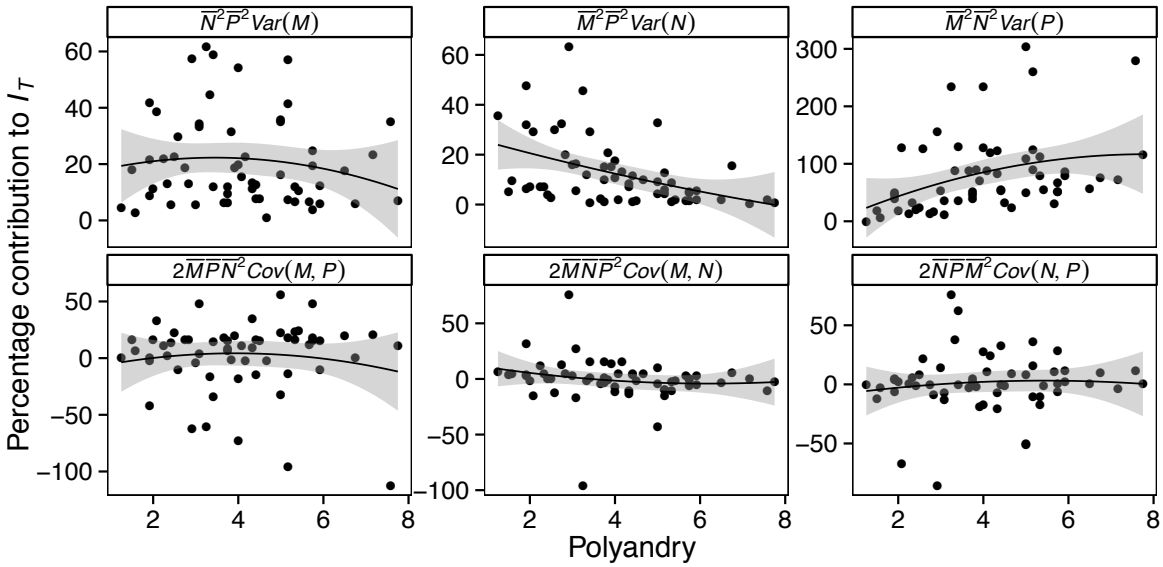


Figure 7. Plots show the effect of average group polyandry on the percentage contribution of standardized variances in *M, N, P* and all covariances to the opportunity for selection (I_T). Curves show quadratic relationships and shaded areas represent 95% confidence intervals. See table 1 for formulae definitions.

Table 3. Model results assessing the effect of polyandry and assortativity (*r*) on the opportunity for selection (I_T), standardized variance in M, N, P, Cov(M,P), Cov(M,N) and standardised univariate Bateman gradients (*β'ss)*. * significant after Bonferroni correction.

Response	Coefficient Removed	Model & Comparison	Estimate	SE	LRT	AIC
Opportunity for	None	1				248.440
Selection (I_T)	Polyandry^2	1vs2	0.165	0.084	$\chi2$=3.7881,df=1,p=0.052	250.230
	Poyandry	2vs3	-1.976	0.726	$\chi2$=11.304,df=1,p<0.001*	259.540
	Assorativity	1vs4	-3.164	1.582	$\chi2$=3.2508,df=1,p=0.071	249.700
Standardised variance in	None	1				-16.073
mating success (*M*)	Polyandry^2	1vs2	0.009	0.009	$\chi2$=0.8947,df=1,p>0.3	-17.178
	Poyandry	2vs3	-0.165	0.079	$\chi2$=19.586,df=1,p<0.001*	0.408
	Assorativity	1vs4	-0.296	0.195	$\chi2$=2.4737,df=1,p>0.1	-15.599
Standardised variance in	None	1				76.120
partner fecundity (*N*)	Polyandry^2	1vs2	0.058	0.019	$\chi2$=8.9136,df=1,p<0.005*	83.033
	Poyandry	2vs3	-0.643	0.169	$\chi2$=13.36,df=1,p<0.001*	94.393
	Assorativity	1vs4	0.286	0.355	$\chi2$=0.7841,df=1,p>0.3	74.904
Standardised variance in	None	1				182.780
fertilization success (*P*)	Polyandry^2	1vs2	-0.076	0.048	$\chi2$=2.6161,df=1,p>0.1	183.400
	Poyandry	2vs3	0.855	0.418	$\chi2$=3.9107,df=1,p<0.05	185.310
	Assorativity	1vs4	0.481	1.013	$\chi2$=0.3096,df=1,p>0.5	181.090
Standardised covariance	None	1				71.946
between mating success	Polyandry^2	1vs2	-0.003	0.019	$\chi2$=0.0259,df=1,p>0.8	69.972
(*M*) and fertilization	Poyandry	2vs3	-0.053	0.166	$\chi2$=3.7994,df=1,p=0.051	71.772
success (*P*) *Cov(M,P)*	Assorativity	1vs4	-1.820	0.412	$\chi2$=17.932,df=1,p<0.001*	87.878
Standardised covariance	None	1				20.324
between mating success	Polyandry^2	1vs2	0.027	0.012	$\chi2$=4.6604,df=1,p<0.05	23.573
(*M*) and partner	Poyandry	2vs3	-0.283	0.105	$\chi2$=5.2483,df=1,p<0.05	28.233
fecundity (*N*) *Cov(M,N)*	Assorativity	1vs4	0.462	0.218	$\chi2$=4.6237,df=1,p<0.05	22.948
Standardised univariate	None	1				-70.761
Bateman Gradient (*β'ss*)	Polyandry^2	1vs2	0.003	0.006	$\chi2$=0.2519,df=1,p>0.6	-72.509
	Poyandry	2vs3	-0.052	0.048	$\chi2$=4.4659,df=1,p<0.05	-70.043
	Assorativity	1vs4	-0.155	0.123	$\chi2$=1.572,df=1,p>0.2	-71.189

Table 4. Model results assessing the effect of polyandry and assortativity (*r*) on the percentage contribution of components of male reproductive success to the opportunity for selection (I_T). * significant after Bonferroni correction.

Response	Coefficient Removed	Model & Comparison	Estimate	SE	LRT	AIC
Percentage contribution standardised variance in mating success (*M*)	None	1				505.77
	Polyandry^2	1vs2	-0.586	0.741	$\chi2$=0.714,df=1,p>0.3	504.48
	Poyandry	2vs3	3.982	6.490	$\chi2$=0.564,df=1,p>0.4	503.05
	Assorativity	1vs4	0.785	13.623	$\chi2$=0.0001,df=1,p>0.9	503.77
Percentage contribution standardised variance in partner fecundity (*N*)	None	1				464.11
	Polyandry^2	1vs2	0.306	0.518	$\chi2$=0.373,df=1,p>0.5	462.49
	Poyandry	2vs3	-5.855	4.533	$\chi2$=10.903,df=1,p<0.001*	471.39
	Assorativity	1vs4	28.985	9.377	$\chi2$=9.173,df=1,p<0.005*	471.29
Percentage contribution standardised variance in fertilization success (*P*)	None	1				661.89
	Polyandry^2	1vs2	-1.374	2.767	$\chi2$=0.264,df=1,p>0.6	660.16
	Poyandry	2vs3	29.466	24.225	$\chi2$=11.573,df=1,p<0.001*	669.73
	Assorativity	1vs4	131.767	50.117	$\chi2$=6.925,df=1,p<0.01*	666.82
Percentage contribution of the standardised covariance between mating success (*M*) and fertilization success (*P*) *Cov(M,P)*	None	1				578.33
	Polyandry^2	1vs2	-1.361	1.390	$\chi2$=1.128,df=1,p>0.2	577.46
	Poyandry	2vs3	9.156	12.163	$\chi2$=0.808,df=1,p>0.3	576.27
	Assorativity	1vs4	-79.937	27.072	$\chi2$=8.675,df=1,p<0.01*	585.01
Percentage contribution of the standardised covariance between mating success (*M*) and partner fecundity (*N*) *Cov(M,N)*	None	1				524.22
	Polyandry^2	1vs2	0.757	0.865	$\chi2$=0.808,df=1,p>0.3	523.02
	Poyandry	2vs3	-7.970	7.550	$\chi2$=0.909,df=1,p>0.3	521.93
	Assorativity	1vs4	36.099	15.835	$\chi2$=4.777,df=1,p<0.05	526.99

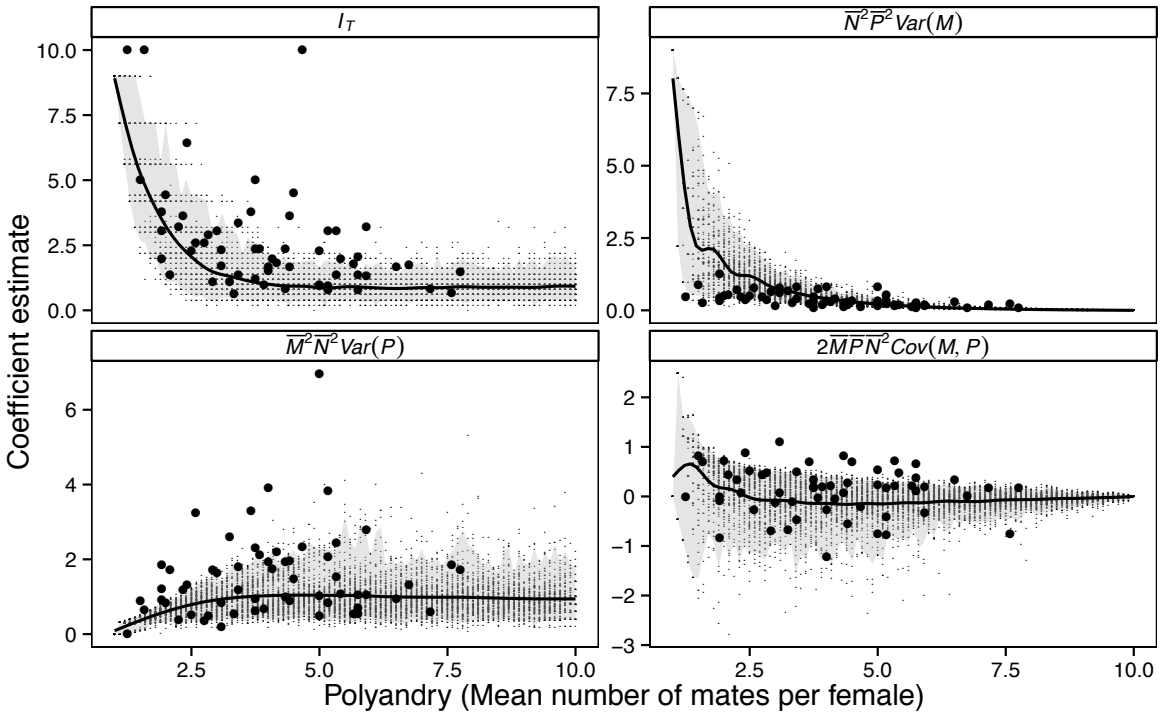


Figure 8. Plots show simulation results for the effect of polyandry on the opportunity for selection (I_T), standardized variance in *M*, *P* and *Cov(M,P)* from WFA models presented in McDonald & Pizzari (Chapter 5). In simulated populations one male mated with every female in the population at polyandry = 1. Simulations were then carried out increasing polyandry by adding randomly one copulation between a male and a female until every male mated every female (polyandry = 10). For each increment in polyandry, we generated 100 independent populations and show: total variation in reproductive success (I_T), standardised variance components in mating success (I_M) and paternity share (I_P) and the standardised covariance between *M* and *P* (*Cov(M,P)*) as calculated in table 1. Each male possessed a trait *z* (normally distributed with a mean of five and standard deviation of one) which covaries positively with *P* and negatively with *M*. Females lay only one egg and a male's probability of fertilizing a female's only egg is a function of his *z* size relative to the sum of the *z* of the other males to mate with the same female (i.e. winner fertilises all, WFA). Small points represent 9000 simulated mating populations, shaded areas represent upper and lower quartiles of simulations, smooth fitted lines are fitted from a general additive model (knots = 4) to simulated data. Large points are empirical data from 12 mating groups presented in figure 5 of this chapter.

III) ACROSS-GROUP POLYANDRY AND MATING STRUCTURE

Across groups, relationships between polyandry and measures of selection were qualitatively similar to the within-group analysis. We found a strong reduction in the potential for sexual selection in groups with higher levels of polyandry (fig. 9, table 5). Again, we also found a reduction in the standardised variation in *M* with increasing polyandry that was marginally non-significant before Bonferroni correction (Table 5). The percentage contribution of *M* did not change with polyandry (Table 5), however by removing one group with the lowest level of polyandry, we found a marginally non-significant negative relationship between polyandry and the percentage contribution of *M* to variation in reproductive success before Bonferroni correction (t=-2.29, $d.f.$=4, p=0.08) (fig. 9 shows all

data including the lowest polyandry group). At the same time, polyandry was strongly positively related with the contribution of *P* to total variation in reproductive success (fig. 9, table 6). Overall, networks tended to be negatively assorted, meaning that males with high mating success tended to mate with females with low mating success and vice versa (fig. 10). However, models testing the effect of mating structure (NODF, assortativity, WNODF row) did not reveal a significant effect on measures of sexual selection across groups (table 7 & 8). Suggesting that when data are pooled across all 10 days, high levels of polyandry and variation between groups obscures the effects of mating assortativity.

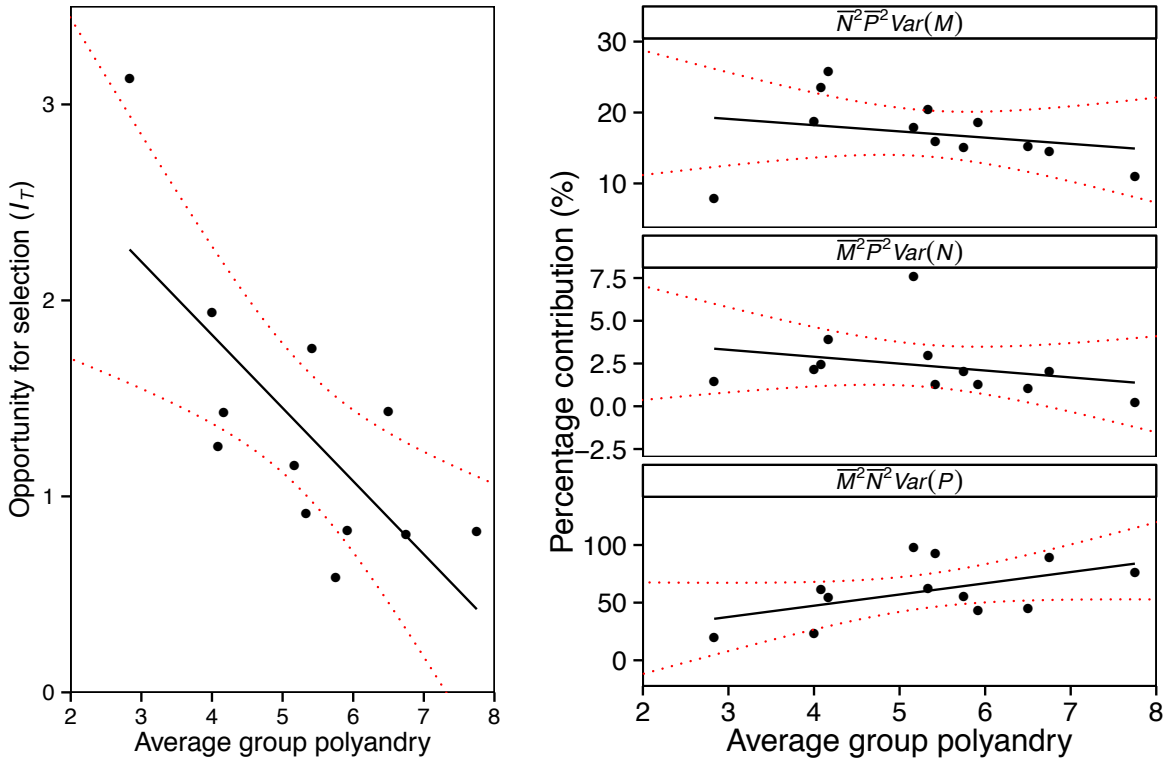


Figure 9. Panels show the relationship between polyandry and the opportunity for selection (I_T) as well as the percentage contribution of standardised variance in *M*, *N* and *P*.

Table 5. Model results assessing the effect of polyandry on the opportunity for selection (I_T), standardized variance in *M, N, P*, all their covariances and standardized univariate Bateman gradients (β*'ss*). All models included female group as a fixed effect. *significant after Bonferroni correction.

Response	Explanatory variables	Estimate	SE	*df*	*t*	p-value
I_T	Polyandry + Female group ID	-0.501	0.118	5	-4.258	0.008
Standardised variance in *M*		-0.053	0.023	5	-2.276	0.072
Standardised variance in *N*		-0.008	0.007	5	-1.122	0.313
Standardised variance in *P*		0.057	0.118	5	0.487	0.647
Standardised variance in *Cov(M,P)*		-0.073	0.084	5	-0.869	0.424
Standardised variance in *Cov(M,N)*		-0.027	0.013	5	-1.989	0.103
Standardised variance in *Cov(N,P)*		-0.017	0.031	5	-0.552	0.605
Standardised univariate Bateman gradient (β'_{ss})		-0.182	0.085	5	-2.139	0.085

Table 6. Model results assessing the effect of polyandry on the percentage contribution of standardized variance in *M, N, P* and all covariances to the opportunity for selection (I_T). All models included female group as a fixed effect. *significant after Bonferroni correction.

Response	Explanatory variables	Estimate	SE	*df*	*t*	p-value
Percentage contribution *M*	Polyandry + Female group ID	0.319	1.779	5	0.179	0.865
Percentage contribution *N*		0.042	0.608	5	0.069	0.948
Percentage contribution *P*		16.928	4.881	5	3.468	0.018
Percentage contribution *Cov(M,P)*		-0.944	7.497	5	-0.126	0.905
Percentage contribution *Cov(M,N)*		-0.926	1.152	5	-0.804	0.458
Percentage contribution *Cov(N,P)*		-0.954	2.408	5	-0.396	0.708

Table 7. Model results assessing the effect of polyandry and mating structure (NODF and assortativity) on the opportunity for selection (I_T), standardized variance in *M, P*, *Cov(M,P)* and standardized univariate Bateman gradients ($\beta'ss$). All models included female group as a fixed effect. *significant after Bonferroni correction.

Response	Explanatory variables	Estimate	SE	df	*t*	p-value
I_T	NODF	-0.009	0.023	3	-0.38	0.729
	Polyandry	-0.387	0.217		-1.785	0.172
	Assortativity	-2.219	3.868		-0.574	0.606
Standardised variance in *M*	NODF	0.001	0.003	3	0.292	0.789
	Polyandry	-0.087	0.028		-3.101	0.053
	Assortativity	1.102	0.501		2.202	0.115
Standardised variance in *P*	NODF	-0.011	0.023	3	-0.465	0.673
	Polyandry	0.063	0.218		0.288	0.792
	Assortativity	2.367	3.885		0.609	0.585
Standardised variance in *Cov(M,P)*	NODF	-0.003	0.014	3	-0.226	0.836
	Polyandry	0.027	0.134		0.2	0.855
	Assortativity	-3.04	2.39		-1.272	0.293
Standardised univariate Bateman gradient (β'_{ss})	NODF	0.006	0.017	3	0.33	0.763
	Polyandry	-0.245	0.163		-1.501	0.23
	Assortativity	1.04	2.907	3	0.358	0.744

Table 8. Model results assessing the effect of weighted row nestedness (WNODFrow) on the opportunity for selection (I_T) , standardized variance in *M, P* , Cov(M,P) and standardized univariate Bateman gradients ($\beta'ss$). All models included female group as a fixed effect. *significant after Bonferroni correction.

Response	Explanatory variables	Estimate	SE	df	*t*	p-value

I_T	WNODFrow	0.012	0.022	4	0.561	0.605
	Polyandry	-0.56	0.165		-3.399	0.027
M	WNODFrow	0.004	0.004	4	0.945	0.398
	Polyandry	-0.071	0.03		-2.335	0.08
P	WNODFrow	0.017	0.021	4	0.819	0.459
	Polyandry	-0.026	0.159		-0.163	0.879
Cov(M,P)	WNODFrow	-0.003	0.006	4	-0.434	0.686
	Polyandry	-0.005	0.045		-0.111	0.917
β'ss	WNODFrow	0.006	0.016	4	0.398	0.711
	Polyandry	-0.213	0.121		-1.754	0.154

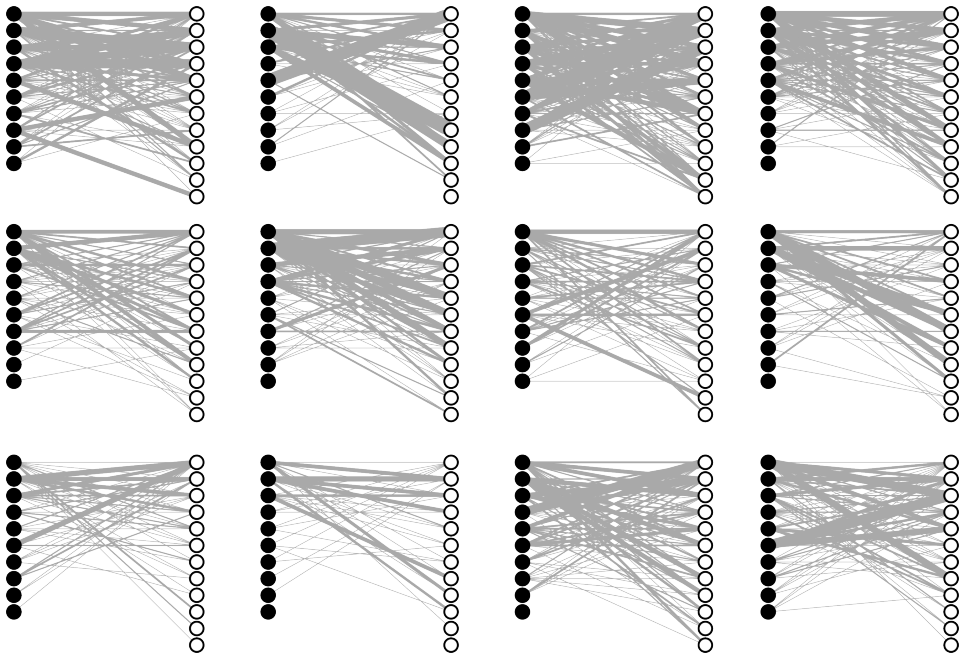


Figure 10. Network visualization of all mating groups: black nodes are males and white nodes are females. Edges represent copulations and edge thickness the number of repeat copulations. Males and females are ordered from top to bottom by decreasing mating success (i.e. number of partners).

IV) SEXUAL SELECTION ON MALE MATING PATTERNS

We identified significant positive directional postcopulatory sexual selection on both male remating rate and mean partner exclusivity (fig. 11 & 12, table 9)

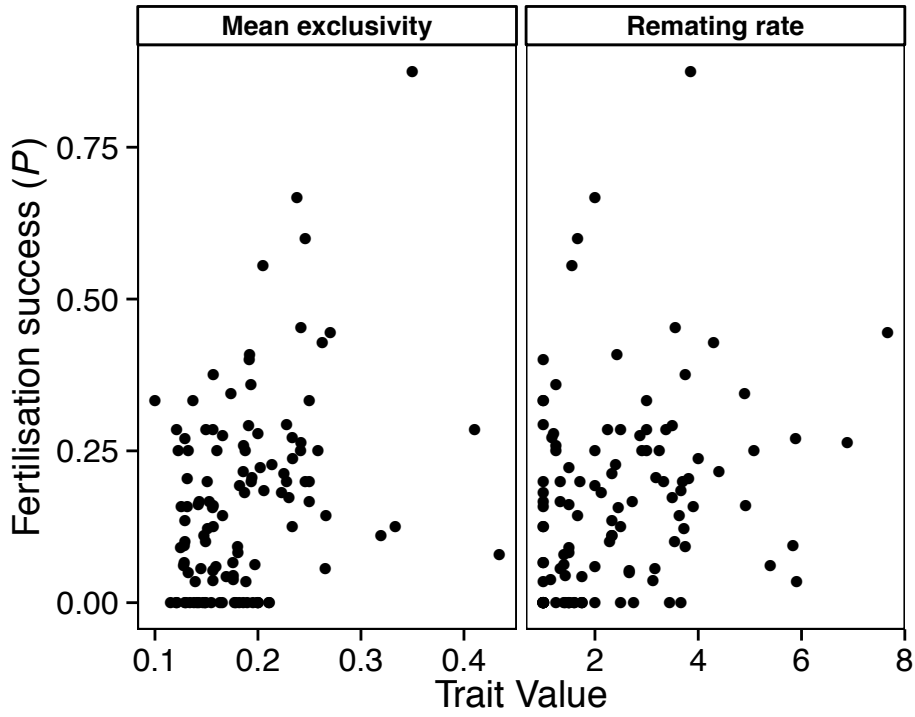


Figure 11. Panels show the relationship between the fertilization success (*P*) of males and male mating patterns; mean exclusivity and remating rates across all 12 groups.

Table 9. Model results for postcopulatory sexual selection on male remating rate and mean partner exclusivity.

Full model	Parameter removed	Model No & Comparison	Estimate	SE	LRT	AIC
P ~ Remating rate + exclusivity	-	1	-	-	-	505.62
Random: n(obs)=115, n(Males) = 103,n(Groups) =12, n(Female Groups) = 6 var(Males) = 0.648, var(Groups) =1.96e-9, var(Female Groups) = 1.28e-9 (Binomial)	Remating rate	1 vs. 2	0.2165	0.0696	$\chi 2$=9.447, df= 1, p< 0.005	513.06
	Mean exclusivity	1 vs. 3	6.7945	1.6491	$\chi 2$=15.807, df=1, p<0.001	519.42

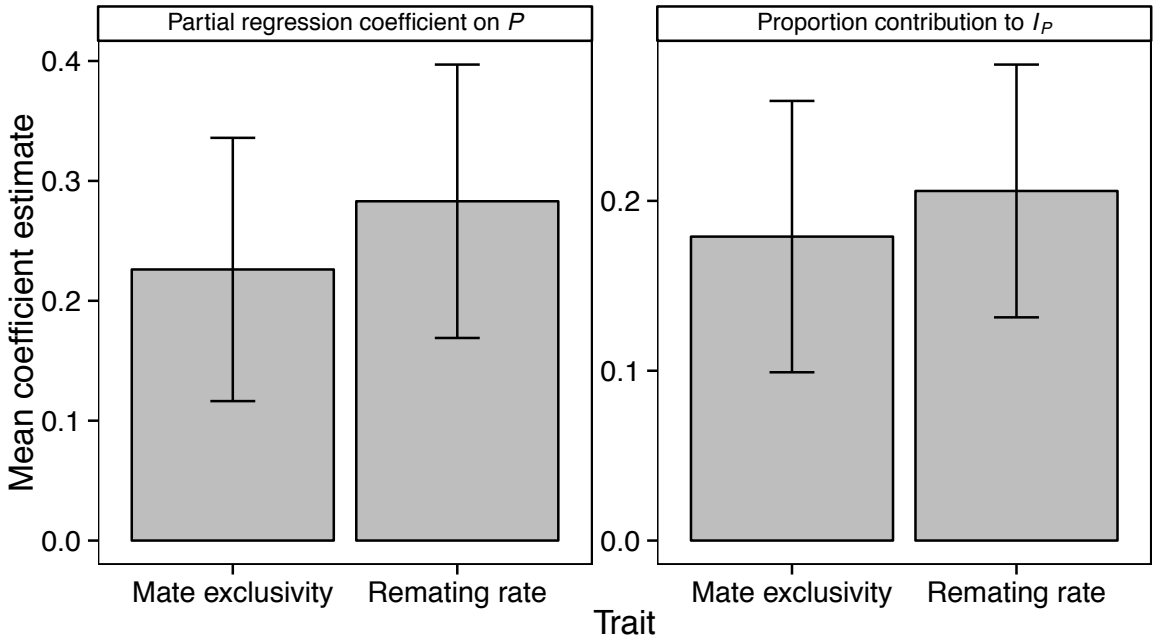


Figure 12. Plots show mean partial regression coefficients for standardised selection on mate exclusivity and remating rate against male fertilization success (*P*) and mean contribution to the opportunity for postcopulatory sexual selection I_P as calculated in Table 1. Error bars represent ± 1SE.

We found no significant effect of polyandry on standardized selection gradients. However, there was a marginally non-significant increase in the contribution of remating rate to variance in male reproductive success, after removing the group with the lowest value of polyandry (fig. 13, table 10). The relationship between polyandry and selection on mean exclusivity revealed a more nuanced pattern, revealing a quadratic relationship such that selection on mean partner exclusivity tended to be strongest at intermediate levels of polyandry.

Table 10. Model results for the effect of polyandry on postcopulatory sexual selection on male remating rate and mean partner exclusivity.

Response	Explanatory variables	Estimate	SE	*df*	*t*	p-value
Partial standardized selection gradient on mean partner exclusivity	Polyandry	2.175	0.995	4	2.186	0.0941
	Polyandry^2	-0.221	0.103		-2.15	0.098
Proportion contribution mean partner exclusivity	Polyandry	-0.097	0.126	5	-0.77	0.484
Partial standardized selection gradient on remating rate	Polyandry	-0.123	0.124	5	-0.995	0.365
Proportion contribution remating rate (excluding group with lowest polyandry)	Polyandry	0.092	0.041	5	2.266	0.0861

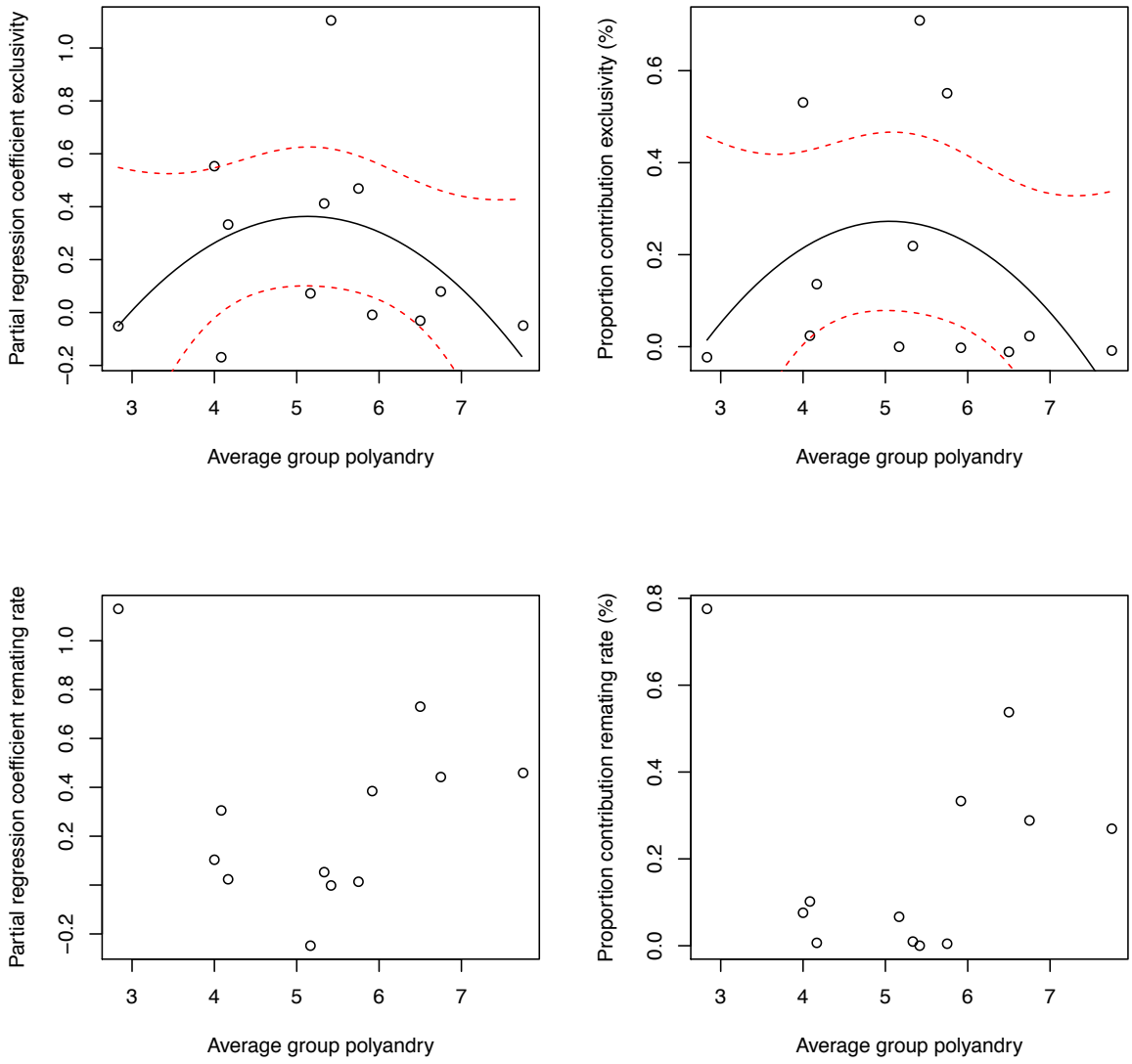


Figure 13. Top row shows the relationship between polyandry and the strength of selection on male average exclusivity and the contribution of exclusivity to standardised variance in paternity share. Bottom row shows the relationship between polyandry and the strength of selection on male average remating rate and the contribution of remating rate to standardised variance in paternity share. Smooth black lines represent fitted quadratic models and dotted red lines represent 95% confidence intervals.

DISCUSSION

In this study we used an experimental approach across replicate promiscuous groups of Red junglefowl (*Gallus gallus*) to explore the role of polyandry and mating structure in determining the operation of sexual selection both within groups over time and between

groups. Overall, our results not only reveal similar patterns to that of previous work in junglefowl (Collet et al. 2012) but also both expand on and clarify this work.

POLYANDRY

We show that an increase in cumulative polyandry within groups over time results in an overall decrease in the potential strength for total sexual selection as measured by the opportunity for selection (I_T). This reduction is largely driven by decreases in standardised variance in *M*, *N* and the covariance between the two. This is because increasing polyandry tends to homogenise mating success across males as the mating table becomes increasingly filled. This also drives a reduction in variance in *N* as males increasingly share the same females. We also show that strength of precopulatory sexual selection on mating success as measured by Bateman gradients also decreases. Because Bateman gradients are calculated as *Cov(M,T)/Var(M)*, this reduction must be due to a change in the relationship between mating success and reproductive success, as otherwise we would expect Bateman gradients to increase with decreasing *M*. Our results suggest the reduction in Bateman gradients is due to the combined effect of decreases in both *Cov(M,P)* and *Cov(M,N)* reducing *Cov(M,T)* at a faster rate than it reduces variance in *M*, as the covariances between mating success, average partner and paternity share (i.e. *Cov(M,P)* and *Cov(M,N)*) will strongly determine *Cov(M,T)*.

This decrease in the variance of precopulatory components of sexual selection is contrasted by a strong increase in the relative importance of postcopulatory process in determining variation in reproductive success. Recent theoretical work has provided quantitative predictions for the expected changes in variance components across fitness components with polyandry (McDonald & Pizzari in press, Chapter 5). Our empirical results present a reasonable fit with the predictions of theoretical simulations investigating changes in sexual selection under increasing polyandry in originally polygynous populations.

Qualitative comparisons with the predictions from simulations provide scope to infer the potential mechanisms of both sperm competition and trait distributions in this system. The strongest similarity between the data presented here and simulated predictions regards simulations where females produced only 1 egg and sperm competition follows a winner fertilise all patter (WFA). In this study, female fecundity is relatively low, much lower than for example, many invertebrate systems. Therefore, males successful in fertilising ova will most likely fertilise a very large proportion of a female's clutch, similar to WFA. McDonald & Pizzari (in press, Chapter 5) provide results for three variations of this WFA simulation, one where the probability of fertilisation is equal across males that mated with a female, and two where males possess a postcopulatory trait (z) that covaries positively with fertilisation probability but either positively or negatively with M. Only in cases where z is negatively correlated with M do Bateman gradients decrease across ranges of polyandry, as observed in this study. This is because initially, at low levels of polyandry, males with high M face little postcopulatory competition and so M predicts T well. As polyandry increases however, those males relatively poor in precopulatory competition increase their mating success and in doing so increasingly steal ova from those males that are more successful in precopulatory competition. In Red junglefowl, the relationship between fertilisation success and mating success is however more complex. Many traits may positively covary with mating success, such as male social status, which not only confers privileged mating access to females but also influences paternity share through cryptic female choice for socially dominant males (Pizzari and Birkhead 2000; Pizzari et al. 2002; Collet et al. 2014). However, several studies have shown that high social status may negatively correlate with traits that positively influence sperm competitiveness both in junglefowl (Pizzari et al. 2002, 2007) and across other species (Preston et al. 2001; Navara et al. 2012). Such negative covariances may be driven by trade-offs in investment in precopulatory competition relative to postcopulatory

competition (Parker 1998; Parker et al. 2013). Although we did not measure sperm traits in this study, previous studies in jungelfowl have demonstrated a reduction in phenotypic sexual selection on male social status as the relative importance of pre- and post-copulatory sexual selection reverses, this decrease may be in part accentuated by a potential negative correlation between the expression of pre- and post-copulatory competitive traits (Collet et al. 2012). Finally, empirical results do show some differences from previous simulated results, for instance the decline in variance in *M* is of a lower magnitude than that predicted by our simulations. This is potentially because polyandry in the junglefowl groups never got as low as in the simulations or because at low levels of polyandry, junglefowl populations are somewhat more monogamous than the simulated populations which were characterised by extreme polygyny (i.e. high male mating skew).

MATING STRUCTURE

Beyond confirming the previous expectations our results provide the first empirical demonstration that mating structure, as measured by the covariance between a male's mating success and the mating success of his partners, can modify variation in sexual selection in agreement with predictions (McDonald and Pizzari In press; Sih et al. 2009; McDonald et al. 2013, McDonald & Pizzari, Chapter 5). We find that as predicted, the more disassortative a mating network the more variation in male reproductive success. This is because males with high *M* also tend to have high exclusivity with females partners and therefore high *P* across their mates. This increases both variance in male reproductive success and precopulatory sexual selection by driving the covariance between *M* and *T*. These results therefore suggest that for any given level of polyandry, mating structure is an additional parameter of mating systems that can act either with or against the operation of polyandry, tempering or accentuating the effect of polyandry on variation in reproductive success and the strength of

precopulatory sexual selection. Classically, mating systems have been classified and explored on the basis of male and/or female promiscuity (i.e. mating success, *M*) in isolation. The present study goes beyond both formal and verbal treatments of the potential role of the within-population covariance between male and female *M* in shaping sexual selection (Shuster and Wade 2003; Wade and Shuster 2005; Sih et al. 2009), providing an empirical demonstration of the influence of such covariance on the operation of sexual selection. Mating assortativity therefore constitutes a third key facet of mating systems that may drive variation in the operation of sexual selection across populations even when polyandry remains consistent across populations.

Our results across groups broadly agree with results within, however we fail to demonstrate significant effects of mating structure on the operation of selection at this level. This may be driven by the very high levels of polyandry saturating mating tables, providing less scope for network structure to vary, thereby limiting its effect on sexual selection and our ability to detect this effect. For example, consider a mating network where 50% of the possible mating pairs are realised versus a mating network where 90% of the possible mating pairs are realised. Potential variation in mating structure for the more polyandrous network is more limited, reducing the potential for variation in mating structure across groups while simultaneously reducing any possible effect of mating structure itself on sexual selection. The demonstration of a negative covariance between male and female mating success, and its effects on sexual selection, also raises interesting question of how such mating patterns may further drive the evolution of mating systems. For example, if females suffer costs of mating with extravagant/attractive males with high *M*, antagonistic sexual selection may favour the evolution of reduced promiscuity for females who prefer extravagant males, further strengthening the negative mating covariance and feeding back to increase precopulatory sexual selection on males (Holland and Rice 1998). More simply, Shuster and Wade (2003)

suggest that such a pattern may be caused by a Fisherian process, driving linkage between male and female promiscuity. In our study, the covariance between *M* and *N* is reduced as mating networks become more negatively assorted, which may create conditions conducive to the latter mechanism, if this is driven by harmful effects of males on females (for potential harm caused in fowl see Pizzari 2001; Galliard et al. 2005). The operation of these mechanisms will of course depend on the extent to which the mating success of both sexes are heritable, rather than one sex in isolation and the potential for linkage between these traits. For example, studies have demonstrated that female propensity for extra-pair mating may evolve as a correlated response to increased selection on male extra-pair mating (Forstmeier et al. 2011), however other studies have cautioned at the generality of this relationship (Reid et al. 2014).

Our results suggest that polyandry increases the relative importance of postcopulatory sexual selection and so we may expect polyandry to increase the importance of mating behaviours involved in postcopulatory competition. We found that remating rate and more exclusive mating access with females positively predicted fertilisation success for males. Both these traits represent alternative ways to defend paternity share, either through increasing representation of sperm by delivering more ejaculates or via mating with females that represent lower sperm competition intensity/mate guarding (although in fowl remating can simultaneously increase exclusivity as mounting by males reduces female propensity to remate (Løvlie et al. 2005)). Despite this, we only report tentative evidence that increasing polyandry modulates the selective importance of these traits, such that remating rate tends to explain more variation in paternity share as polyandry increases.

Finally, the results presented in this study were facilitated by highly detailed behavioural observations. Investigating the generality of the results presented here in other systems presents a non-trivial challenge, as more natural and even other experimental

systems may not lend such detailed knowledge of copulation patterns. Despite highly detailed behavioural data, this study was only able to investigate a relatively short period of reproductive competition relative to the potential reproductive lifespan of Red junglefowl (Collias and Collias 1996). Although the main aim of this study was to demonstrate the potential for polyandry and mating structure to modify the operation of sexual selection, it will be important for further studies to determine how such patterns contribute to lifetime reproductive success in other systems. For example, mating structure may increase the opportunity for sexual selection over a particular period of time, however if males frequently exchange network position over multiple reproductive episodes, this may limit the potential for mating structure to increase the potential variation in male lifetime reproductive success.

CONCLUSION

Taken together, our results demonstrate that mating structure in combination with polyandry can modify the operation of sexual selection on males. We confirm predictions that polyandry should decrease the overall opportunity for sexual selection by reducing variation in mating success across males but simultaneously increase the importance of postcopulatory sexual selection. We further show that mating network structure can act to limit this potential reduction in the variance in male reproductive success, by modifying the postcopulatory competitive landscape, and demonstrate the utility of population-level sexual network measures in quantifying mating structure. We argue that this component of mating systems may be an important modulator of the effects of polyandry on the operation of sexual selection.

APPENDIX§1

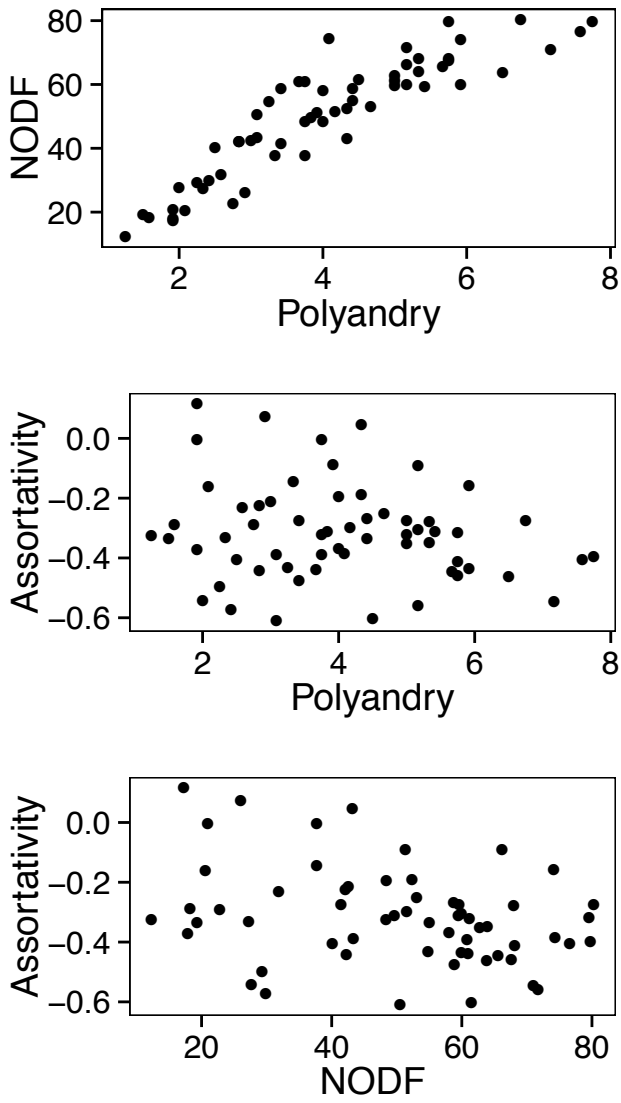


Figure A1 Plots show relationship between polyandry, assortativity (*r*) and nestedness (NODF) for all groups across cumulative days from the within-group analysis.

APPENDIX§2

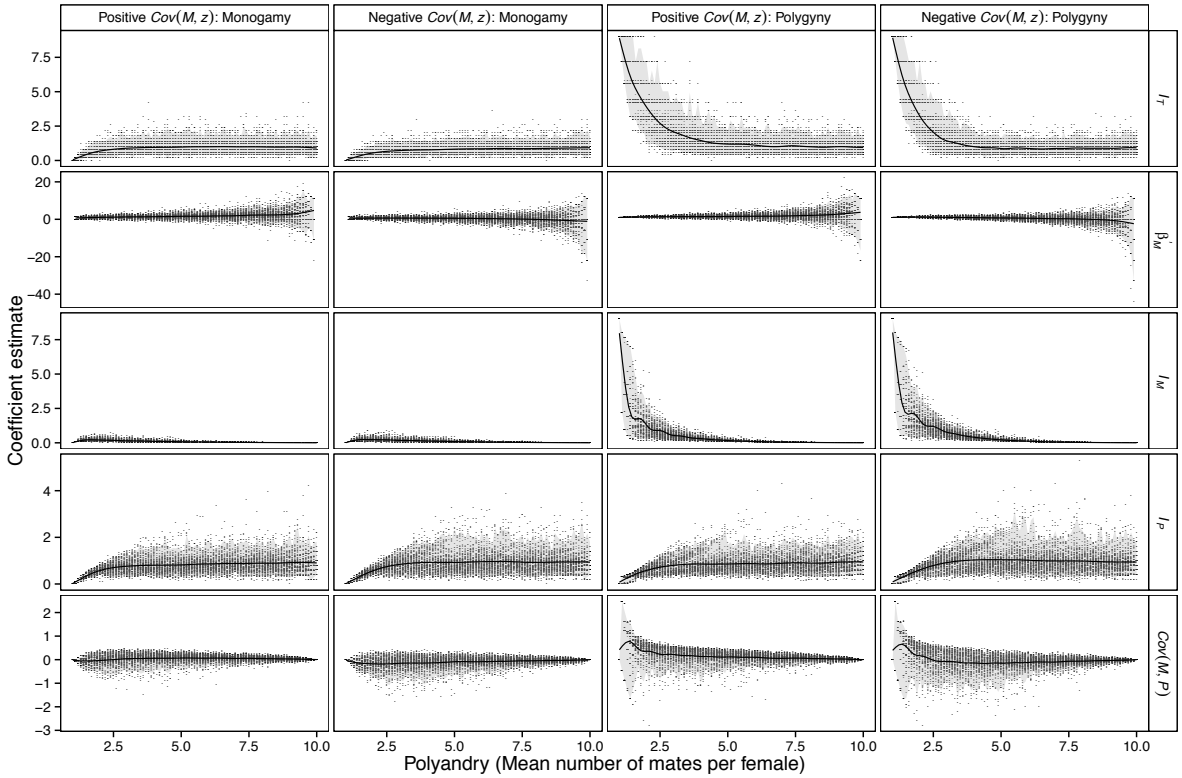


Figure A2. Simulation testing the effect of polyandry on standardised variance in male reproductive success. Each panel shows the results 9,000 mating populations. In monogamous populations each male mated with one female, such that all males and females had one mating partner at polyandry = 1. In polygynous populations one male mated with every female in the population at polyandry = 1. Simulations were then carried out by increasing polyandry by adding randomly one copulation between a male and a female until every male mated every female (polyandry = 10). For each increment in polyandry, we generated 100 independent populations and show: total variation in reproductive success (I_T), standardised variance components in mating success (I_M) and paternity share (I_P) and standardised univariate bateman gradients (β_M). Each panel shows a smooth fitted line fitted from a general additive model and shaded areas represent interquartile ranges. We consider a scenario where a male trait *z* (normally distributed with a mean of five and standard deviation of one) covaries positively with *P* and *M*, and where females lay only one egg. A male's probability of fertilizing a female's only egg is a function of his *z* size relative to the sum of the *z* of the other males to mate with the same female (i.e. winner fertilises all, WFA).

Table A1 Model results assessing the effect of polyandry and assortativity (*r*) on standardised *Cov(M,P)* and its contribution to the opportunity for selection.

Response	Coefficient Removed	Model & Comparison	Estimate	SE	LRT	AIC
Standardised	None	1				73.390
covariance	Polyandry^2	1vs2	-0.019	0.019	$\chi2$=0.4101,df=1,p>0.5	72.451
between *N*	Poyandry	2vs3	0.187	0.169	$\chi2$=1.0613,df=1,p>0.3	70.861
and *P*,						
Cov(N,P)	Assorativity	1vs4	-0.087	0.380	$\chi2$=0.0862,df=1,p>0.7	71.476
Percentage	None	1				556.84
contribution	Polyandry^2	1vs2	-0.761	1.161	$\chi2$=0.435,df=1,p>0.5	555.28
Cov(N,P)	Poyandry	2vs3	7.123	10.160	$\chi2$=0.077,df=1,p>0.7	553.36
	Assorativity	1vs4	-23.644	23.134	$\chi2$=1.234,df=1,p>0.2	556.08

CHAPTER 7. POSTCOPULATORY SEXUAL SELECTION AND THE ROLE OF COMPETITIVE ENIRONMENT IN RED JUNGLEFOWL (*GALLUS GALLUS*)

Grant C. McDonald[1], Lewis Spurgin[2], Eleanor Fairfield[3], David Richardson[3] and Tommaso Pizzari[1]

[1]*Edward Grey Institute, Department of Zoology, University of Oxford, Oxford, UK.*
[2]*Faculty of Mathematics and Natural Sciences, University of Gronigen, Gronigen Netherlands.*
[3]*School of Biological Sciences, University of East Anglia, Norwich, UK.*

ABSTRACT

Heterogeneity in the abiotic environment is expected to influence the evolution of phenotypic characters. It is now accepted that variation in the social environment experienced by individuals can also shape the evolution of quantitative characters. Estimating the contribution of the social environment to phenotypic evolution is challenging because it relies on the estimation of functionally relevant competitive groups across individuals. Here we use a recently proposed method based on sexual networks, to estimate the sperm competitive environment of male Red junglefowl (*Gallus gallus*), and its effects on selection on male competitive traits using multilevel selection analyses. Using this method we demonstrate the role that the social competitive environment may play in shaping selection on male competitive traits. Furthermore, we extend previous methods and show by increasing the resolution of analyses, to calculate a unique sperm competitive environment for every ova released by a given female. We show that increasing the detail of analyses may provide more sensitive approach, increasing accuracy in identifying predictors of male reproductive success.

INTRODUCTION

One of the key developments in evolutionary biology research to arise from the modern synthesis over eight decades ago was to progress the study of selection as a topic in its own right (Fisher 1930). Since that time understanding the strength and form of selection in nature has been a key goal of evolutionary biology (Brodie et al.

1995). Sexual selection research has particularly embraced this approach aiming to understand those traits that confer competitive advantage and the ecological and demographic variables that shape the value of this advantage (Grafen 1987). In 1983, Lande and Arnold provided quantitative techniques to measure both the strength and direction of phenotypic selection operating on competitive traits. This methodology initiated an explosion of research in the decades to follow that has greatly improved our understanding of the traits affecting fitness in non-sexual contexts as well as in both pre- and post-copulatory intrasexual competition (Andersson 1994; Kingsolver et al. 2001; Shuster and Wade 2003; Kingsolver and Pfennig 2007).

Selection operates on variance in relative fitness which is measured at the level of the popualtion (Lande and Arnold 1983). Classical selection techniques therefore measure selection at the level of the popualtion, and when combined with information on heritabilty and genetic variances and covariances allow predictions to made regarding phenotypic evolution (Price 1970). However, by estimating selection at the populaiton level, these techniques ignore the role of population structure in potentially reducing or accentuating population-level estimates of selection. When populations are structured in subgroups, e.g. via population viscosity or preferential interactions between similar/disimilar phenoptypes, selection on a trait at the local scale will not generate phenotpic change at the global scale unless it results in covariance between the trait and fitness at population-level (Robertson 1968; Wallace 1975; Wade 1985; Goodnight et al. 1992; McDonald et al. 2013). Therefore, ignoring population structure when comparing patterns of selection across populations, may lead to erronous interpretations (McDonald et al. 2013, McDonald & Pizzari, in press, chapter 4). For example, consider two populations, each with similar directional selection on traits but populations differ in structure. The population-level covariance

between trait and fitness may be obscured in the structured population but remain in the unstructured popualtion (McDonald et al. 2013). Comparing population-level selection measures would lead to the erronous conclusion that the trait was important in one population but not the other. Furthermore, by increasing or decreasing the variance between competitor traits, heterogeneity in the social environment created by population competitive structure is expected be important in both accentuating or slowing the evolution of phenotypic traits (Wolf et al. 1999; Wolf 2003; Eldakar et al. 2010; Formica et al. 2011). To overcome the confounding effects of population structure when measuring selection and to estimate the contribution of the social environment to total selection operating on phenotypic characters, several methods have been developed that explicitly estimate the effect of an individual's competitive environment on individual relative fitness (Nunney 1985; Heisler and Damuth 1987; Wolf et al. 1999; Okasha 2004). In doing so, these approaches allow the estimation of selection acting at the local scale both on an indiviual's trait and the phenoptypes of the individuals with which a focal competes. This provides estimates of the realtionship between a focal individuals trait and fitness holding constant the competitive environment and vice versa, revealing patterns in selection potentially obscured by population structure. Despite the potential for socio-competitive structure to influence phenotypic evolution (Weinig et al. 2007; Eldakar et al. 2010; Oh and Badyaev 2010; Aronsen et al. 2013; Saltz 2013; Wacker et al. 2013), relatively few studies have attempted to incorporate the socio-competitive environment in estimates of phenotypic selection compared to the wealth of population-level selection studies (Gontard-Danek and Møller 1999; Jennions et al. 2001; Kingsolver et al. 2001; Hunt et al. 2009; Shuster et al. 2013) and this may in part be due to the empirical challenge of identifying the local competitive group of a focal individual with meaningful

accuracy (Formica et al. 2010). More recentely however, Mcdonald et al (2013) presented a quantitative method to identify an individuals competitive group from detailed behavioural data using a conceptial framework that is built on networks of competitive interactions between individuals. Across the studies that have explored the role of competitive structure, results have revealed that structure in the competitive environment can lead to a limiting or accentuating effect on phenotypic selection on individual traits (e.g. Weinig et al. 2007; Formica et al. 2011).

In this study, we use empirical data from semi-natural populations of Red junglefowl, *Gallus gallus*, to measure sexual selection considering both population- and local-levels, using the multilevel selection methodology developed by McDonald et al. (2013). Red junglefowl typically live in sexually promiscuous groups, which offer opportunity for postcopulatory sexual selection on males through sperm competition and cryptic female choice (Pizzari et al. 2002; Collet et al. 2012). We focus on postcopulatory episodes of selection on three male traits, namely male social status, male age and male remating rate. All traits have been implicated in determining male fertilization success in the fowl (Pizzari et al. 2002; Dean et al. 2010; Collet et al. 2012). We predict male social status and remating rate to increase a male's share in paternity *P*. This is because male social status not only provides privileged access mating opportunities but has also been shown to influence the pattern of female cryptic choice, as females preferentially eject the sperm of relatively subdominant males (Pizzari and Birkhead 2000; Pizzari et al. 2002; Dean et al. 2011). Remating rate is expected to increase paternity share by increasing the relative representation of a males ejaculate (Collet et al. 2012). Male age on the other hand, is predicted to have a potentially negative effect on male fertilization success due an age dependent decline in male reproductive traits beyond a certain age, i.e. reproductive

senescence. This decline is predicted to occur due strong selection in previous selective episodes reducing the apparent competitiveness of individuals in later episodes (Williams 1957). Reproductive senescence has been documented in feral fowl populations. Individual males suffer a decline in several traits pertinent to postcopulatory competition, including the probability of ejaculation, the number of sperm inseminated and sperm quality (Dean et al. 2010; Cornwallis et al. 2014). Firstly, we i) assess postcopulatory selection at the population level (global selection) to estimate phenotypic selection on all three traits. In such groups the fertilisation success (*P*), defined as the proportion of ova fertilized across all the females mated by the focal male, may be a function not only of his own phenotype, e.g. social status, but also the phenotypes of his local competitors, e.g. the males that mate with the same females. We therefore then use ii) a multilevel selection approach based on mating network information, as suggested by McDonald et al. (2013) to measure the way in which the phenotype of local competitors influences selection. This approach treats each batch of eggs (i.e. clutch) as individual competitive events assuming that if two males mate a female they compete to fertilise all her ova. However, such assumptions may result in a coarse measure of competitor phenotypes as females may produce several eggs before a new competitor mates that female and so males may only compete over a select subset of the clutch of a female. Finally, to address this limitation, iii) we show how multilevel methods can be further expanded to increase the level of resolution at which selection acts. To do so we use a temporal approach where for each egg a female lays we calculate the unique competitive environment and reanalyze multilevel selection using this method.

METHODS

STUDY POPULATION & EXPERIMENTAL DESIGN

We use replicate semi-natural groups of Red junglefowl (*Gallus gallus*), a species that lives in small social groups (2-28 individuals) (Collias and Collias 1996). All individuals used in this study originate from a semi-natural population of Red junglefowl kept at the University of Oxford field station (refer to previous work that describes this population e.g. Collet et al. 2012, 2014). All experimental work was conducted between the months April-October across three breeding seasons between 2011-2013. We used 12 male-female mating groups in total. Each group consisted of 10 males and 12 females held in an enclosure approximately 100m^2. Due to limitations on available individuals 13 males were reused across different male groups. In total there were 6 female groups that were reused with more than one group of males (in a similar pattern described in figure. 1) but females were not reused across female groups. Birds were fed *ad libitum* using a mixture of grower pellets and fresh seeds at ratio of 2:1. Food was replenished everyday in the morning before birds came down from roosting and water was replaced every 2 days at the same time.

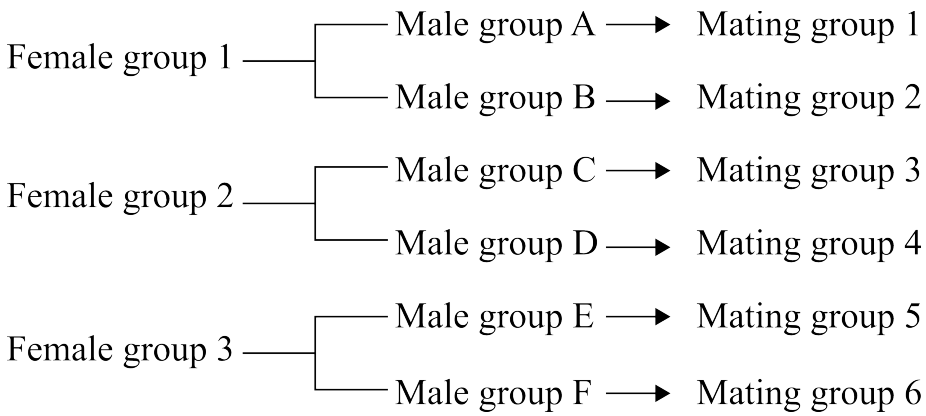


Figure 1. Diagram showing how replicate female groups were used repeatedly across unique male groups to create the individual mating groups.

DATA COLLECTION

Before each trial males and females were housed in single sex enclosures. Male groups were released into the experimental enclosure 3 days prior to the release of females to allow male dominance hierarchies to form and stabilize. All females were isolated in single sex enclosures before trials for a minimum 14 days to ensure sperm depletion from previous matings.

On the morning of the first day of each trail all females were entered into an arena along with males. Behavioural observations were made everyday for 6 hours between 0500-0800hr in the morning and in the evening between 1800-2100hr GMT for a total of 10 days per group, resulting in 60 hours of observations per group, totaling 720 hours of behavioural observations. We used *ad libitum* sampling technique as all individuals could be observed at once (Altmann 1974). We recorded all male-male interactions including pecks, chases, fights, waltzes and avoidances (Johnsen et al. 2001). A male was considered a subdominant if in interactions he retreated at least one body length from the other male. We recorded all copulation attempts by males and all copulation events were recorded. Copulations were considered successful when cloacal contact was observed or when the males' tail was lowered over the female's cloaca and contact was assumed to occur. All eggs were collected during the 10 days of experimentation and for the 14 days after experimentation, totalling 468 eggs. Eggs were weighed to the nearest gram and then incubated for between 5-9 days and then tissue samples were subsequently stored in 100% ethanol at -20C until later parentage assignment.

MOLECULAR METHODS AND ANALYSES

For all groups we ignore all eggs from day one of each trial as eggs laid on the first day of a trial are unlikely to be fertilized on the same day, and instead only consider parentage data from days 2-10 of the trial and all eggs laid on the 11th day after the trial was complete. DNA was extracted following a standard ammonium acetate precipitation protocol (Nicholls et al. 2000). All samples were genotyped at 15 variable microsatellite loci arranged into 2 multiplex reactions, using primers designed to amplify in domestic chicken (Crooijmans et al. 1996, 1997; Gibbs et al. 1997; Hanotte et al. 1997; Dawson et al. 1998; Groenen et al. 2000) or across a wide range of bird species (Dawson et al. 2010). PCRs were carried out in 2 μl volumes, using the reagents and methods described in Kenta et al (2008). Cycling conditions for both multiplexes were as follows: an initial heating step of 95˚C for 15 minutes was followed by 35 cycles of 94˚C for 30 seconds, 56˚C for 90 seconds and 72˚C for 60 seconds. A final extension step of 60˚C for 30 minutes completed the reaction. PCR products were diluted by 1 in 150, and fragments were separated on an ABI 3730 capillary sequencer. Allele sizes were assessed against ROX 500 size standard using the software GeneMapper version 3.1 (Applied Biosystems).

Null allele frequencies for each locus were estimated using CERVUS version 3.0.1 (Kalinowski et al. 2007). Molecular parentage analyses were carried out in CERVUS, using the approaches outlined in Dean et al. (2010). Because of the larger number of potential parents in our experimental design compared to previous experiments, we could not assign parentage to all offspring with 95% confidence Subsequent analyses using parentage data were therefore carried out including only individuals that could be assigned parentage with 95% confidence. None of the 15

microsatellite loci had high null allele frequencies (highest = 0.03), so all were used for parentage analyses.

DATA ANALYSIS

We combined paternity data with behavioural data on male mating success. If a male sired offspring with a female but this pair was never observed copulating, we added this female to the male's mating success and assumed that this pair copulated once in analyses that utilize the number of repeated copulations between individual males and females. To determine male social status we first calculated David's score (David 1987) within each group as our measure of male social status including all male-male interactions including pecks, chases, fights, waltzes and avoidances. David's score is most appropriate when data sets have many interactions and high asymmetry in interaction strength between individuals (Gammell et al. 2003; de Vries et al. 2006). Because we aimed to determine the effect of male social status on male mating success, competitive interactions over females and mating opportunities were not used to assess male dominance. In all analyses we focus on male fertilisation success and so all males who did not mate (n=5) were excluded from the mating data set but not for calculations of male social status. Throughout GLMM analyses below we included Male ID, Female ID and Group ID as random effects to control for psuedoreplication where appropriate. All statistical analysis was carried out using R statistical software (R Core Team 2014).

i) Global analysis

We first measured population level selection using classical selection techniques (Lande and Arnold 1983) on all three male traits. To do so, we fitted

binomial GLMMs with male fertilization success *P* as the response variable and male social status, male age (in years, ranging from 1-6 years) and male average remating rate calculated as the mean number of copulations of males across all his female mates. Because there is potential for these traits to be correlated with each other, which may reduce the accuracy of estimates of selection, we tested for multicolinearity between these predictors by calculating variance inflation factors for models including all three traits. Variance inflation factors were low ($\sqrt{VIFs} < 2$) demonstrating traits were not highly correlated across the data set. For global analyses we also ran models with Female group ID as a fixed effect rather than a random effect and results did not qualitatively change, see appendix§1 table A1.

ii) Multilevel analysis

We first assessed the extent of population structure by calculating the covariance between the trait of the focal male and the mean traits of his local competitors in all groups. To explore whether this structure is more or less than what is expected under random mating, we took male and female empirical mating networks and randomly shuffled these matrices according to two simulations for both, male status and male age separately. One simulation shuffles matrices holding constant the number of connections (Probabilistic) and the other shuffles matrices holding constant both the number of connections and the variance in mating success cross males and females and is thus more restrictive (Fixed). We iterated this 1000 times for both probabilistic and fixed models for each group and compared observed covariances with simulated distributions. We did not use this simulation approach for male remating rate as it may produce male mating patterns that are less biologically plausible, i.e. produce networks where males have very high remating rates across

vary large number of females, positive remating rates for males that do not mate and/or decimal remating rates for males that mate one female.

We measured multilevel selection by calculating weighted competitive group traits for all males. To do so we used the network approach presented in McDonald et al. (2013). For all groups we first constructed mating networks where male and females were nodes, and edges between males and females represent mating. From these networks we then calculated male-male sperm competitive networks. These were constructed such that if two males both mated with the same female they shared a competitive edge. Edges were given value such that if males both copulated with the same three females, they shared a competitive edge with a weight of three. For each male, we then calculated the mean of his competitor's social status, age and remating rate, each trait weighted by the value of the competitive tie between males. This method describes the neighbor method of calculating group trait in multilevel selection, where the trait value of the focal individual is not included in the competitive group trait (Nunney 1985; Okasha 2004). We fitted binomial GLMMs with male fertilization success (*P*) as the response variable and focal male social status, focal male age, focal male average remating as well as weighted average competitor traits for all three male traits. In this analysis a total of 5 females mated with only one male. In such situations there is no logical value to calculate group trait for females and so they were excluded from the data set. Due to this, it is most appropriate to also remove these eggs from the calculation of male *P* from the males with whom they mated. We ran both analyses with including eggs from these females and without and we found no qualitative differences. We present models excluding these eggs in the main results and models including these in appendix§2 table A2.

iii) Temporal (i.e. per egg) multilevel analysis

For each egg we identified a unique competitive environment that included all males that had mated with the female at least once prior to 24:00hours the night before the day that egg was laid as copulations on the day an egg is laid are unlikely to result in fertilization (Howarth 1971; Birkhead and Møller 1993; Etches 1996). For all these males we calculated their focal traits (i.e. their social status, age, and "fertilization potential"). A male's fertilization potential (FP) was calculated as the sum of the copulations delivered to that female before the day that egg was laid. Female fowl can store sperm for up to 14 days in sperm storage tubules (SSTs) (Etches 1996). Because sperm are lost from a female's SSTs since the time of insemination (Wishart 1987; Birkhead et al. 1995; Hocking 2009) the probability of fertilisation by a given ejaculate decreases over time (Pizzari et al. 2008). We therefore also attempted to individually discount each copulation as a function of the time elapsed from when the male copulated with the female to the time in which the egg was laid. We assumed a constant rate of $(1.28)\text{x}10^{-2}$ decline of sperm per hour as estimated in previous literature i.e. for the i_{th} ejaculate, $FP_i = 1\times e^{-1.28\times10^{-1}\times t}$ (Lessells and Birkhead 1990). However, AIC comparisons across models indicated that discounting did not provide a better explanation and so we present results from models where FP is simply calculated as the sum of copulations between a male and a female before an egg was laid. We also excluded from this analysis any egg for which females were not observed copulating at least once before the egg was laid. Furthermore, if a female laid an egg that was sired by a male that was not observed copulating with the female before that egg was laid, we removed that egg and all subsequent eggs laid by that female to reduce uncertainty over when that male mated the focal female. All final models therefore included a binary response variable describing whether a male fertilized the focal egg or not as the response variable,

focal male age, social status and FP as well as competitor mean age, social status and the total sum of all competitor males' FPs. We used the total sum of competitor FPs in this case as the aim was to quantify the total intensity of potential sperm competition for a given ovum, treating all ejaculates equally. If for example a focal male was to compete with one competitor who delivered 11 ejaculates, his competitor FP would equal 11. Now, consider that a second competitor of the focal male mated with that female only once, now the focal male's FP would be 5.5. We feel that such an approach based on total sum rather than mean more meaningfully captures the intensity of sperm competition faced by the focal male. Our models therefore bears conceptual similarity the sperm crowding model of Shuster and Wade (2003) that quantifies the number of competing sperm for every focal sperm inseminated, but estimates separately the effects of focal and competitor investment. In some respects, all analyses assume selection is constant through time. In an attempt to confirm this, for each group we split datasets into two-day blocks, i.e. days 1-2, 3-4, 5-6, 7-8 and 9-10. For each 2-day block we calculated a male's total relative reproductive success (*T*) and measured standardized selection gradients on male social status, age and remating rate. We then ran separate models for each trait with increasing time as the explanatory variable to assess patterns of change in selection in these traits over time.

RESULTS

i) Global analysis

Global selection analyses identified significant positive postcopulatory sexual selection on average remating rate and a negative relationship between a male's *P* and his age (fig. 2, table 1). Male social status on the other hand, was not related to *P* (fig. 2, table 1).

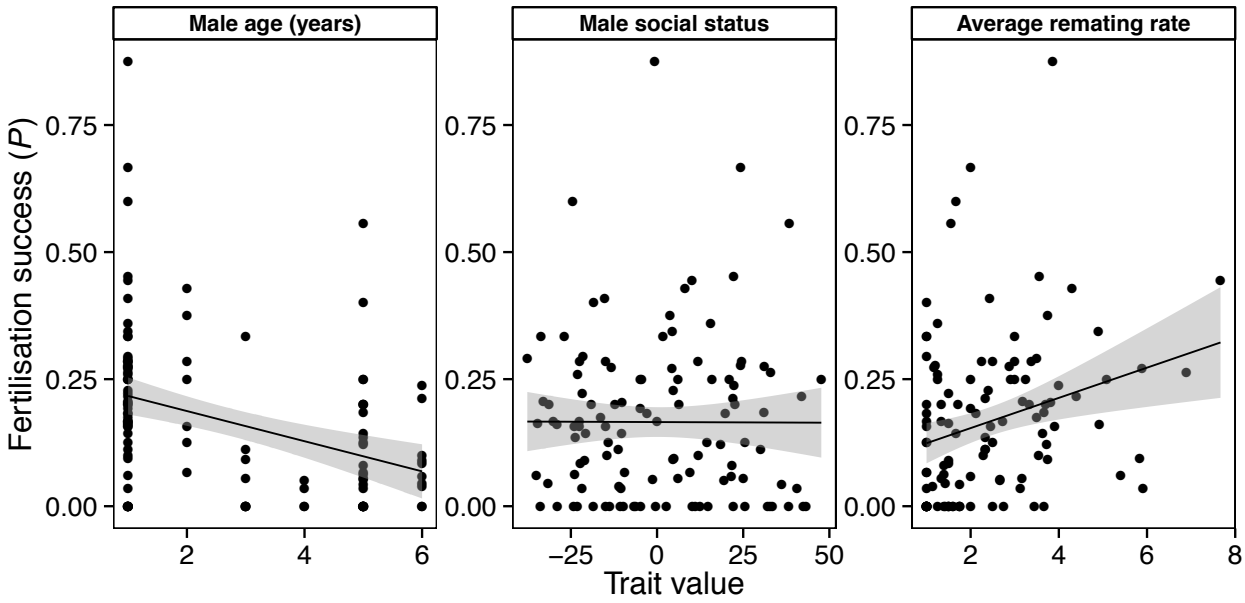


Figure 2. Plots showing the relationship between male age, male social status and male average remating rate against the proportion of eggs fertilized for all males across al groups. Lines represent linear models and shaded areas 95% confidence intervals.

Table 1. GLMM results for global selection analysis of male phenotypic traits. Significant p-values represented in bold.

Full model	Parameter removed	Estimate	SE	Model No. and Comparison	LRT	AIC
P ~ Male social status + Male age + Average remating rate + (Female Group ID/ Group ID) + (Male ID) n(obs)=115, n(Female Group ID) =6, n(Group ID)= 12, n(Male ID)=103 var(Female Group ID) =0, var(Group ID)= 0.032, var(Male ID)=0.571	-	-	-	1		505.56
	Social Status	0.003	0.005	2vs1	$\chi2$=0.492, df=1, p>0.4	504.06
	Age	-0.273	0.063	3vs1	$\chi2$=16.916, df=1, **p<0.001**	520.48
	Remating rate	0.165	0.075	4vs1	$\chi2$=4.829, df=1, **p<0.05**	508.39

ii) Multilevel selection analysis

Replicate mating groups tended to show a negative covariance between focal trait and competitor trait for all three phenotypic traits (fig. 3). This suggests that the ejaculate of males of high social status tended to compete preferentially with the ejaculates of males of low social status (fig. 3). Similarly, older males tended to share more females with younger males, and males with high remating rate tended to compete with males who copulated less frequently with their partners (fig. 3). We next used simulations based on these empirical results to test whether such

covariances deviated from expectations under random mating interactions for male age and male social status.

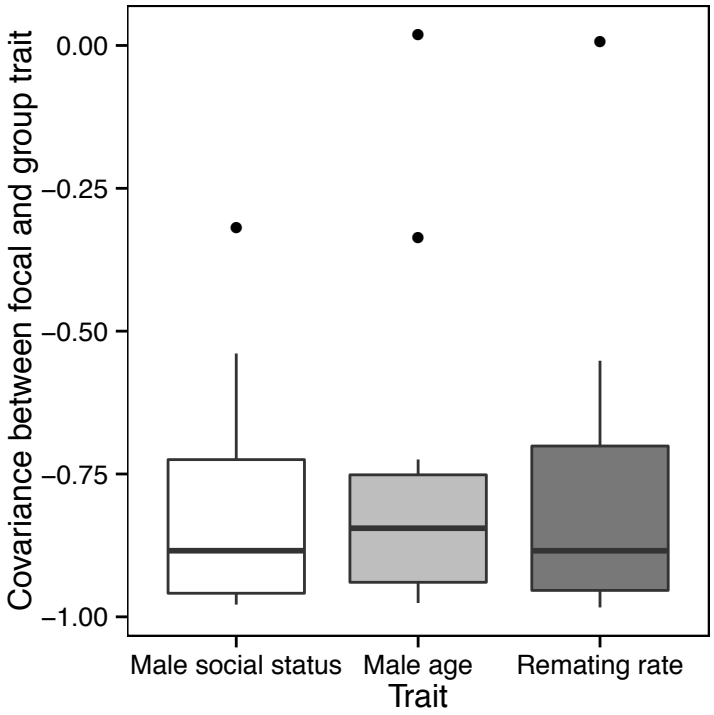


Figure 3. Boxplots show covariances between focal male traits and the average trait value of his competitors for all 12 mating groups, for male social status and male age.

Simulations indicated that negative covariances between focal and competitor traits for both male status and male age, were not more or less than expected from random mating interactions (fig. 4). This suggests that the observed covariances may not require the operation of special social mechanisms governing the relationship between competitor traits (i.e. preferential competition).

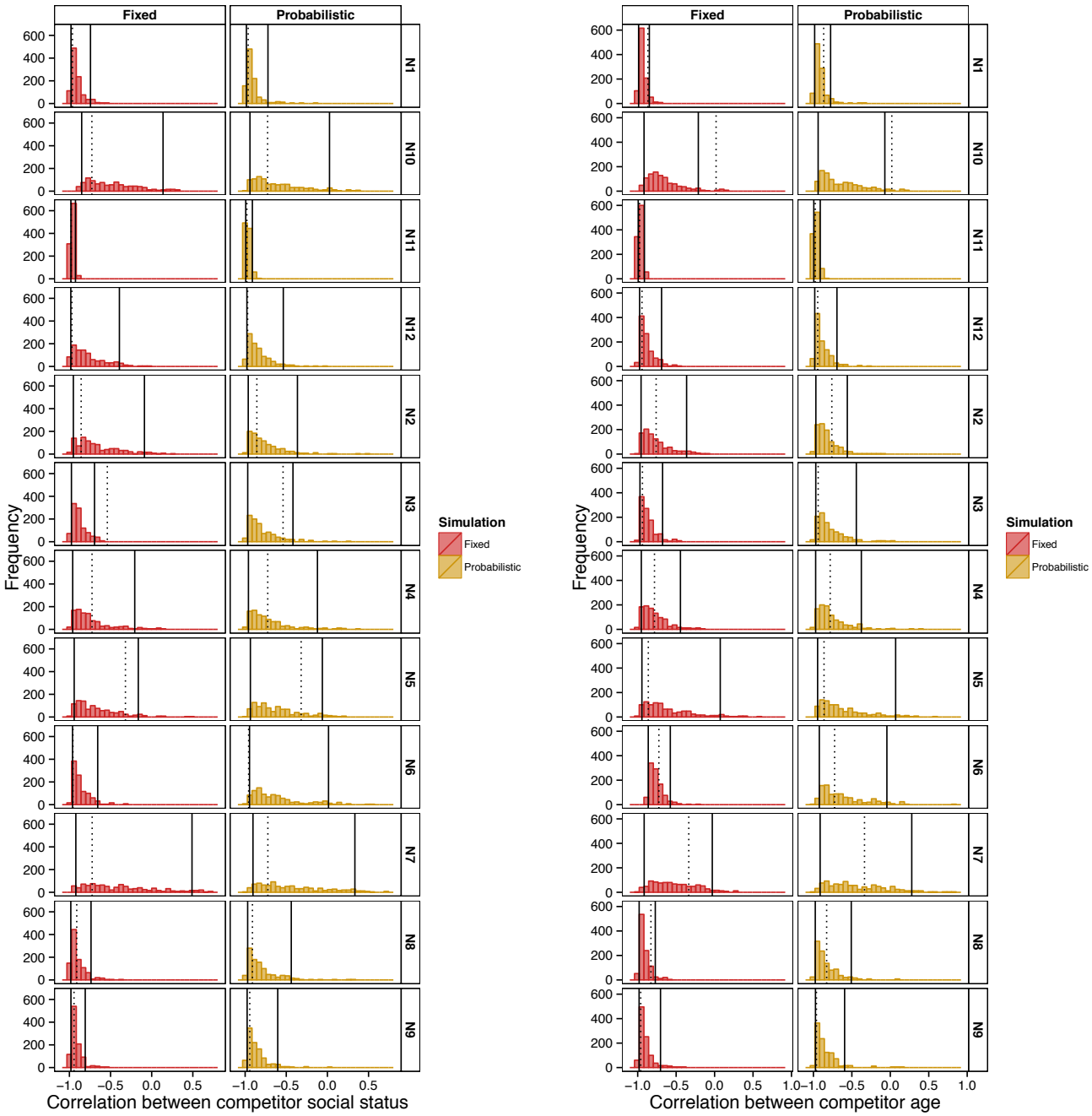


Figure 4. Plots show simulated distributions of covariances between focal male traits and the average trait of his sperm competitors for male age and male social status based on two alternative simulation techniques: probabilistic and fixed. Fixed simulations hold constant variation in male mating success whereas probabilistic simulations do not. Area between solid black lines includes 90% of simulated data values. Dotted black lines represent empirical covariances for traits from all 12 mating groups. Plots demonstrate that empirical covariances rarely exceed that expected under random models of male competition.

The model measuring multilevel selection identified directional selection on male focal traits in accordance with global analyses, i.e. positive postcopulatory sexual selection on remating rate and negative selection on male age (fig. 5, table 2). In accordance with this, we also estimated significant negative selection on

competitor remating rate suggesting that engaging in sperm competition with males who remate frequently with their mating partners tends to reduce a focal male's fertilization success (fig. 5, table 2). We did not however find significant effects of either competitor age or competitor social status (table 2). Model results including exclusive eggs are presented in appendix§2 table A2.

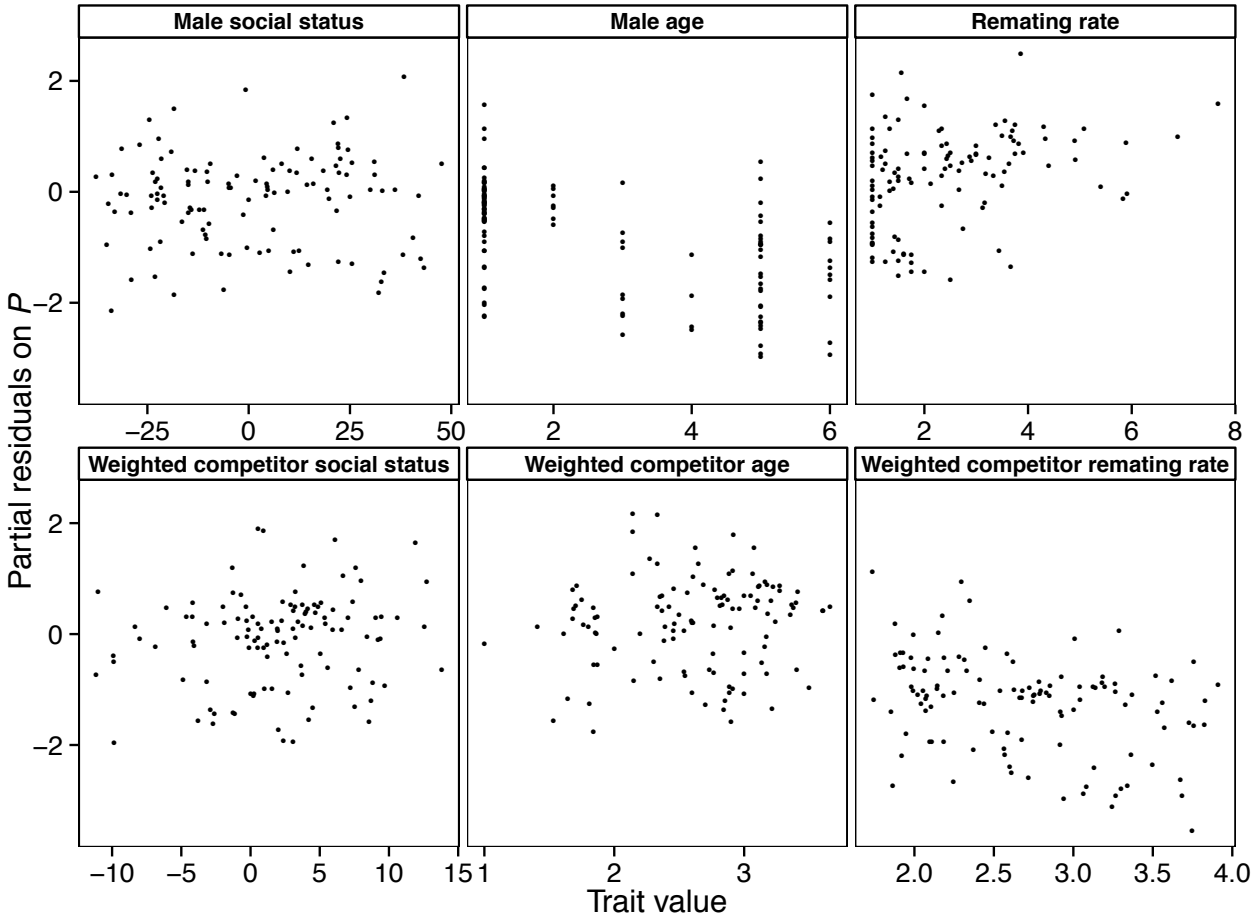


Figure 5. Plots show focal male traits and the average trait value of his competitors for all males in all groups for male social status, male age and male average remating rate against partial residuals estimated form GLMMs.

Table 2. GLMM results for multilevel selection analysis of male focal and average competitor phenotypic traits. Significant p-values represented in bold.

Full model	Parameter removed	Estimate	SE	Model No. and Comparison	LRT	AIC
P ~ Male social status + Male age + Average remating rate + Competitor social status + Competitor age + Competitor + remating rate + (Female Group ID/ Group ID) + (Male ID)	-	-	-	1	-	500.74
	Competitor Social Status	0.019	0.028	2 vs. 1	$\chi2$=0.467, df=1, p>0.4	499.21
	Competitor Age	0.129	0.210	3 vs. 1	$\chi2$=0.374, df=1, p>0.5	499.12
n(obs)=115, n(Female Group ID) =6, n(Group ID)= 12, n(Male ID)=103	Competitor Remating rate	-0.413	0.174	4 vs. 1	$\chi2$=5.569, df=1, **p<0.05**	504.31
	Social Status	0.005	0.005	5 vs. 1	$\chi2$=0.692, df=1, p>0.4	499.43
var(Female Group ID) =2.1e-08, var(Group ID)= 5.6e-07 var(Male ID)=0.517 (Binomial)	Age	-0.269	0.063	6 vs. 1	$\chi2$=17.871, df=1, p<0.001	514.61
	Remating rate	0.164	0.070	7 vs. 1	$\chi2$=5.613, df=1, **p<0.05**	504.35

iii) Temporal (i.e. per egg) multilevel selection analysis

Using a temporal multilevel selection analysis that allows the estimation of male competitive group for each individual egg, we found that the number of times a male mates with a female before an egg is laid positively predicted his chances of fertilizing this egg, and that the more copulations the female received from other males negatively predicted the focal male's chances of fertilization (fig. 6, table 3). Similarly, male age negatively predicted male fertilization success (table 3). In contrast to the general multilevel selection analysis presented above, the per egg temporal analysis detected a marginally non-significant effect of average competitor age, such that competing with on average older males increased the fertilization success of the focal male (table 3). Two factors could explain this discrepancy in the influence of competitors' age between the general multilevel analysis and the per egg temporal multilevel analysis. First, the per egg temporal approach is more sensitive

due to finer detail of the analysis. Second, the per egg temporal analysis is based on a subset of the data, which may result in an over representation of groups with more negative selection on competitor male age. To disentangle these possibilities, we conducted a post-hoc analysis comparing the standardized selection gradients of individual groups from non-temporal multilevel selection analyses against their representation in the data set. We found no significant evidence of a relationship between increasing representation in the data set and the slope of selection on mean competitor male age, suggesting that the influence of competitors' age detected by the per egg temporal analysis was not due to over representation of particular groups (Appendix§3 figure A1).

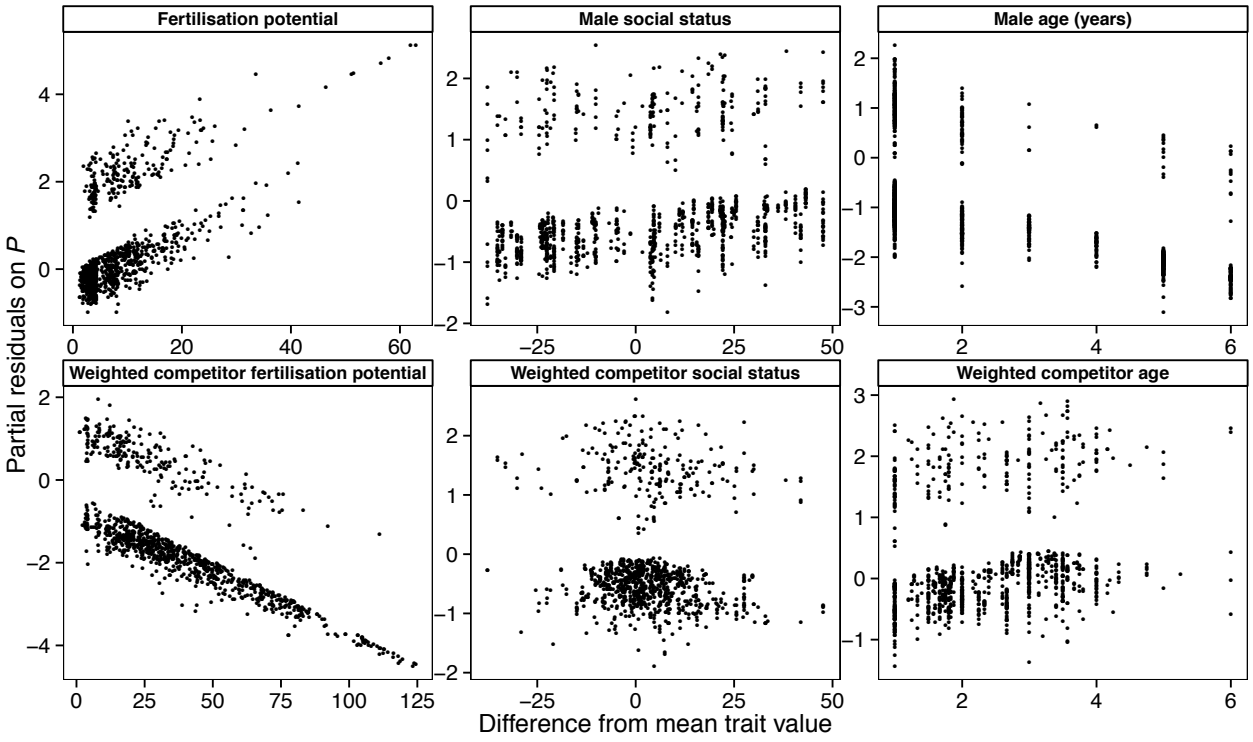


Figure 6. Plots show focal male traits and the average trait value of his competitors for all males across all groups for male social status, male age and male average remating rate against partial residuals estimated from temporal analyses GLMMs.

Table 3. GLMM results for temporal multilevel selection analysis of male focal and competitor phenotypic traits. Significant p-values represented in bold.

Full model	Parameter removed	Estimate	SE	Model No. and Comparison	LRT	AIC
Probability of fertilisation ~	-	-	-	1	-	927.62
Male social status + Male age + Male fertilization potential + Competitor social status +	Competitor Social Status	-0.003	0.002	2 vs. 1	$\chi2$=0.157, df=1, p>0.6	925.77
Competitor age + Competitor fertilization potential + (Female Group ID/ Group ID/Egg ID) +	Competitor Age	0.170	0.002	3 vs. 1	$\chi2$=3.039, df=1, p=0.08	928.66
(Male ID)	Competitor fertilization	-0.114	0.002	4 vs. 1	$\chi2$=68.622, df=1,	994.24
n(obs)=1037, n(Female Group	potential				**p<0.001**	
ID) =6, n(Group ID)= 12, n(Egg	Social Status	0.008	0.002	5 vs. 1	$\chi2$=1.748, df=1, p>0.1	927.37
ID)=222, n(Male ID)=91						
var(Female Group ID) =1.2e-8,	Age	-0.357	0.002	6 vs. 1	$\chi2$=19.78, df=1,	945.4
var(Group ID)= 3.4e-8, var(Egg ID)=3.6e-7, var(Male ID)=0.478					**p<0.001**	
	Fertilization	0.257	0.002	7 vs. 1	$\chi2$=48.06, df=1,	973.68
(Binomial)	potential				**p<0.001**	

Finally, models assessing changes in selection on phenotypic traits over time revealed no consistent changes in selection on these traits (table 4).

Table 4. Linear model results for changes in standardized partial selection gradients on male phenotypic traits across time.

Response	Explanatory variables	Estimate	SE	*df*	*t*	p-value
Male social status	Intercept	0.238	0.207	3	1.148	-
	Time	-0.011	0.062		-0.173	0.873
Male age	Intercept	-0.627	0.143	3	-4.392	-
	Time	0.06	0.043		1.385	0.26
Remating rate	Intercept	0.004	0.104	3	0.038	-
	Time	0.003	0.031		0.099	0.927

DISCUSSION

This study aimed to measure sexual selection on three male phenotypic traits at three different levels of resolution in an attempt to assess the relative utility of including population competitive structure in measures of sexual selection. We focused on postcopulatory sexual selection on three male phenotypic traits in replicate

populations of Red junglefowl, all of which have strong predictions regarding the pattern of selection expected.

Using a population level (global) analysis, the study found that male age negatively predicted male fertilization success whereas male remating rate (the average number of copulations per female partner) positively increased a male's fertilization success. Both these results fit well with previous published work (Dean et al. 2010; Cornwallis et al. 2014). In our groups, the ages of males ranged from 1 to 6 years old, previous work has shown that older males senescence in a variety of sexual traits pertinent to fertilization success including the probability of transferring ejaculates as well as sperm traits themselves that are known to predict fertilization probability (Dean et al. 2010; Cornwallis et al. 2014). Remating rate is expected to positively predict fertilization success as a mechanisms for males to both increase the representation of their ejaculates within females and therefore defend paternity, but also potentially reduce the probability of these females remating with other males (Løvlie et al. 2005; Parker and Pizzari 2010). Interestingly however, we found no effect of male social status, a trait known to strongly affect precopulatory success, but also important in postcopulatory processes due to cryptic female choice (CFC) for the ejaculates of relatively more socially dominant males (Dean et al. 2011). This potential difference may be due to the larger group sizes used in our experiments reducing the potential for CFC to accurately bias paternity towards the most dominant males. This could be driven by firstly the overall high level of promiscuity in our groups and the potential for structure of social hierarchies to vary with group size becoming more egalitarian (Estevez et al. 2003), potentially reducing female ability to determine the relative dominance of males.

Importantly, controlling for competitive structure using multilevel selection methods confirmed qualitatively similar selection patterns on male focal traits, but also revealed strong opposing selection on the average remating rate of a males competitors. This result suggests that males are not only selected to increase their representation in sperm competition, but should preferentially compete with those males who remate with partners less often. Previous work has shown that males may modify their competitive environment to maximize their relative competitive success in both natural and experimental populations. For instance, in populations of the house finch, *Haemorhous mexicanus*, Oh and Badyaev (2010) found that males increase relative attractiveness by associating with relatively less ornamented males. Experimentally, Gasparini et al. (2013) showed that male guppies, *Poecilia reticulata*, choose social contexts that increase their relative attractiveness. In the context of sperm competition, a subset of males with privileged access to females may preferentially mate with those females who are less promiscuous which may drive the negative covariance between a male's remating rate and the remating rate of his competitors as found tin this study. Although such active choice has been demonstrated in some species (Dosen and Montgomerie 2004), this pattern may be driven simply by a covariance between those traits that confer greater mating success and those traits that allow males to exclude other males from copulating with his partners, i.e. social status. However, the results of the present study find no evidence that postcopualtory selection (through the combined potential effects of sperm competition and cryptic female choice) promotes male social status in relatively large social groups under semi-natural conditions, either including the eggs of completely exclusive females or excluding these eggs.

In general, we found negative covariances between all focal and competitor traits. This negative covariance between focal and competitor traits is expected to increase the strength of selection on focal traits by competing males of low competitive value with males of high competitive value, increasing the variance in relative reproductive success (Wolf et al. 1999; Formica et al. 2011). Accordingly, when we compare the slopes of significant predictors of fertilisation success from global selection analyses, i.e. remating rate and male age, with those slopes on focal traits from multilevel analyses we see slightly steeper slopes on those characters for global estimates compared to multilevel estimates. This suggests that the negative covariance between competitors contributes to the positive directional selection of focal characters. However, these differences in slopes are small and we should be cautious of over interpretation. This weak difference may in part be due to how the negative covariance between focal and competitor traits are driven in this system. Results testing the significance of trait correlations compared to randomization of the network suggest that these covariances are not more than we would expect from random mating with respect to trait values. As such, covariances may be passively driven and although overall they may appear relatively strong, very high levels of polyandry may reduce the effect the covariance between male traits in sperm competition. The data sets used here may therefore not be well suited to exploring the role of competitive structure, as structure is somewhat limited by high levels of polyandry, however this analysis provides support for the potential information gained by conducting such analyses, for understanding the contribution of population structure to phenotypic evolution.

Our more detailed temporal analysis investigating variation in the fertilization of individual eggs, reveals the potential for such detailed approach to provide further

information on the potential roles of traits in shaping reproductive success. This analysis similarly shows that male fertilization success can be predicted by the number of copulations with a female in combination with his age and that mating with promiscuous females negatively predicts fertilization success. In the temporal analysis however, our indicator of remating rate for focal and competitor males has a slightly different interpretation compared to that used in the general multilevel selection analyses, as the temporal analysis actually measures the number of copulations rather than the average remating rate of males. Using this approach revealed a marginally significant effect of competitor male age, demonstrating potential information gained by increasing the resolution of analyses. The general multilevel analyses across females assumes that males compete for all ova in a batch, even though due to time differences in copulations males may only compete for the say, last two ova rather than all ova in the batch. Taking this into account via per egg analyses may therefore provide a less coarse measure of a male's competitive environment increasing accuracy in identifying predictors of reproductive success.

Overall, our results reveal directional postcopulatory sexual selection on male traits and the potential for variation in the sperm competitive environment to affect reproductive success of males. Importantly, these results refer to a relatively short competitive window compared to the potential reproductive lifespan of Red junglefowl and concern only selection on paternity share (*P*). Predicting trait evolution and how competitive structure contributes towards this will require firstly more comprehensive measures of reproductive success (i.e. lifetime reproductive success) and knowledge of how precopulatory selection patterns reinforce or impose the strength and direction of postcopulatory selection.

CONCLUSION

Our multilevel selection analyses demonstrate how the socio-competitive environment may contribute to the evolution of male mating behaviour and the potential for selection to favour traits that allow males to modify their sperm competitive environment. Furthermore, we show that using a more fine–scaled approach that quantifies a unique competitive environment for males for each ova for which they compete, can provide a more accurate representation of male competitive environment qualitatively changing our interpretation of the aspects of social environment that affect male reproductive success. The analyses presented in this study rely on highly detailed behavioural data (McDonald et al. 2013), as well as knowledge of the fertilisable period for particular ova produced by individual females. This requirement may limit the facility of these analyses in systems in which i) the availability of copulation data are limited and ii) the fertilisable period for individual/independent batches of ova is not known. For example in some broadcast spawners it may be challenging to delimit whether the gametes of particular in individuals have been mixed and are therefore in direct competition. Similarly, in some species both the timing of ovulation and sperm storage mechanisms may be less well known, precluding analyses of individual ova. Despite these limitations there are a large number of potential systems where such analyses may be fruitful, for example many invertebrate species allow highly detailed knowledge of copulation behaviour in the laboratory (e.g. Pélissié et al. 2014). Similarly some vertebrate models such as the external fertilising European bitterling, *Rhodeus amarus*, provide the potential for both knowledge on when females expose eggs to sperm and which males spawn across a given set of eggs (Reichard et al. 2009). Finally, several more natural systems provide cases where detailed information on the timing of copulation can be collected

and the fertile period of females are well characterised (e.g. Preston et al. 2003), providing exciting scope to explore these methods across other systems.

APPENDIX§1

Table A1 GLMM results for global selection analysis of male phenotypic traits including female group ID as a fixed effect. Significant p-values represented in bold.

Full model	Parameter removed	Estimate	SE	Model No. and Comparison	LRT	AIC
P ~ Male social status + Male age + Average remating rate + Female Group ID +(Group ID) + (Male ID)	-	-	-	1		503.12
	Social Status	0.003	0.005	2vs1	$\chi2$=0.489, df=1, p>0.4	501.61
	Age	-0.287	0.061	3vs1	$\chi2$=20.524, df=1, **p<0.001**	521.64
n(obs)=115, n(Female Group ID) =6, n(Group ID)= 12, n(Male ID)=103 var(Group ID)= 1e-6, var(Male ID)=0.501	Remating rate	0.189	0.072	4vs1	$\chi2$=6.98, df=1, **p<0.01**	508.1
(Binomial)						

APPENDIX§2

Table A2 GLMM results for multilevel selection analysis of male focal and average competitor phenotypic traits. Results include eggs of exclusive females in calculations of *P*. Significant p-values represented in bold.

Full model	Parameter removed	Estimate	SE	Model No. and Comparison	LRT	AIC
P ~ Male social status + Male age + Average remating rate + Competitor social status + Competitor age + Competitor + remating rate + (Female Group ID/ Group ID) + (Male ID)	-	-	-	1		501.85
	Competitor Social Status	0.020	0.028	2 vs. 1	$\chi2$=0.51, df=1, p>0.4	500.36
	Competitor Age	0.120	0.211	3 vs. 1	$\chi2$=0.324, df=1, p>0.5	500.17
n(obs)=115, n(Female Group ID) =6, n(Group ID)= 12, n(Male ID)=103	Competitor Remating rate	-0.446	0.175	4 vs. 1	$\chi2$=6.492, df=1, **p<0.05**	506.34
	Social Status	0.005	0.005	5 vs. 1	$\chi2$=0.872, df=1, p>0.3	500.72
var(Female Group ID) =0, var(Group ID)= 1.7e-09 var(Male ID)=0.518	Age	-0.263	0.063	6 vs. 1	$\chi2$=16.274, df=1, **p<0.001**	516.12
(Binomial)	Remating rate	0.166	0.070	7 vs. 1	$\chi2$=5.687, df=1, **p<0.05**	505.53

APPENDIX§3

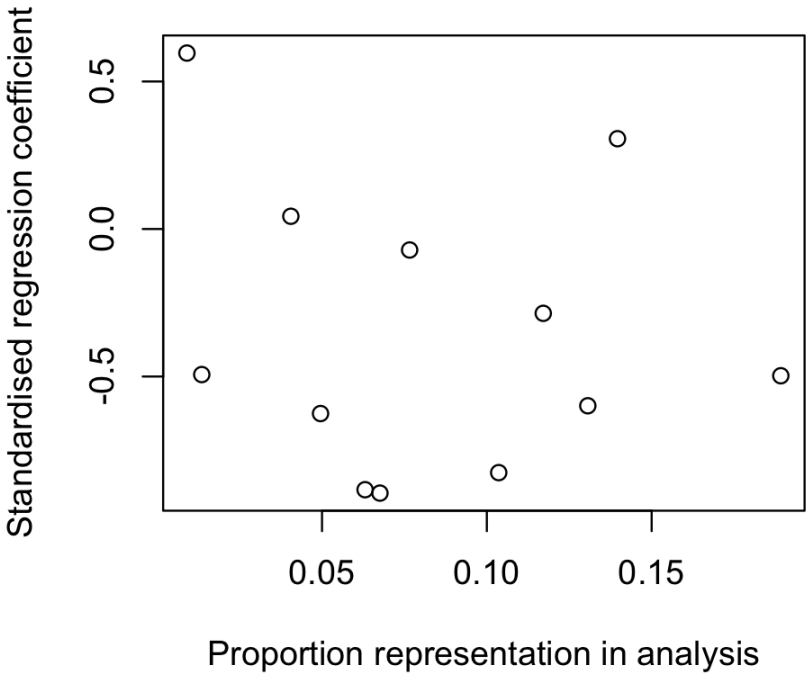


Figure A1 Standardized regression coefficients of male fertilisation success on competitor male age against the representation of those groups in temporal multilevel selection analysis.

CHAPTER 8. ENVIRONMENTAL VARIATION AND ITS IMPLICATIONS FOR MATING SYSTEMS, SEXUAL SELECTION AND FEMALE FECUNDITY IN RED JUNGLEFOWL (*GALLUS GALLLUS*)

Grant C. McDonald[1], Lewis Spurgin[2], Eleanor Fairfield[3], David Richardson[3] and Tommaso Pizzari[1]

[1]*Edward Grey Institute, Department of Zoology, University of Oxford, Oxford, UK.*
[2]*Faculty of Mathematics and Natural Sciences, University of Gronigen, Gronigen Netherlands.*
[3]*School of Biological Sciences, University of East Anglia, Norwich, UK.*

ABSTRACT

Changes in the abiotic environment are predicted to modulate the pattern of intersexual interactions and the mating systems of populations. These changes are in turn expected to affect the operation of sexual selection. In this study we test the effects of two environmental manipulations, resource distribution and the structural complexity of environments on the patterns of inter- and intra-sexual interactions in replicate groups of Red junglefowl (*Gallus gallus*). We assess the effect of these changes on group mating systems, sexual selection on males and female fecundity. We find that both resource distribution and environmental complexity modify the pattern of intersexual interactions but only environmental complexity has an impact on both mating system and the strength of phenotypic sexual selection on males. Finally, we find that the structural complexity of environments may have implications for female fecundity and group productivity, mediated by changes in male harassment of females.

INTRODUCTION

The mating system of a population describes the pattern of mating behaviour across individuals within that population (Klug 2001; Shuster and Wade 2003; Shuster 2009). Research has highlighted an immense variety in animal mating systems between and within species, ranging from strict monogamy to high levels of both male and female promiscuity (Emlen and Oring 1977; Thornhill and Alcock

1983; Clutton-Brock 1989; Andersson 1994; Shuster and Wade 2003; Shuster 2009). Because mating systems describe the distribution of mates across individuals, they also largely determine variation in reproductive success and are therefore expected to be intimately linked to the operation of sexual selection within populations (Emlen and Oring 1977; Shuster and Wade 2003). Explaining such variety in mating systems and the resulting consequences for the operation of sexual selection is a fundamental challenge in evolutionary biology and key to our understanding of how sexual selection shapes the evolutionary trajectories of populations.

Typically, the reproductive success of males is expected to be more variable than that of females, which is largely driven by the fundamental advantage of males in terms of the rate of gametogenesis (Bateman 1948; Andersson 1994; Shuster and Wade 2003). This provides opportunity for a subset of males to monopolise multiple reproductive opportunities and therefore attain a higher reproductive rate than of females (Kokko et al. 2012). This increased potential reproductive rate of a subset of males stimulates competition among males for the monopoly of reproductive opportunities with females. In this context females and their ova constitute the reproductive resources to monopolised by males. Theory predicts that if local ecology varies the potential for males to monopolise females, this will influence mating systems. Such changes in mating system will in turn alter intrasexual variation in mating success and sexual selection. Although these predictions are not new, experimental tests of how local ecological conditions shape sexual selection have taken a back seat to more direct treatments of the social environment, i.e. operational and adult sex ratios, and density (Kvarnemo and Ahnesjo 1996; Mills et al. 2007; Head et al. 2008; de Jong et al. 2009; Rios Moura and Peixoto 2013; Wacker et al. 2013).

Among attempts to explain variation in sexual selection and mating systems, one dominant theme is that spatial and temporal variation in ecological factors may shape local patterns in sexual interactions within populations (Crook 1965; Lack 1968; Orians 1969; Emlen and Oring 1977; Reynolds et al. 1993; Endler and Houde 1995; Reynolds 1996; Clark et al. 1997; Kelly et al. 1999; Reichard et al. 2009; Mück et al. 2013). Two such fundamental ecological variables include resource distribution and the complexity of the environment (Emlen and Oring 1977; Borgia 1979; Reichard et al. 2009; Tomkins et al. 2011; Candolin and Wong 2012; Candolin and Vlieger 2013; Cogliati et al. 2014). Building on previous work that related the social organisation of species to ecological conditions (Crook 1965; Lack 1968), Emlen and Oring (1977) introduced more formal predictions based on how the distribution of resources essential to reproduction may affect mating systems and the operation of selection on males. This theory predicts that the spatiotemporal distribution of resources that females require for reproduction (e.g. food, nesting sites) dictates the spatiotemporal distribution of reproductively active females. This in turn governs the distribution of males in space and time and the degree to which they can monopolise or share females. For instance, when resources are evenly distributed throughout space and/or in time, the ability of one or a few males to defend those resources and the associated mating opportunities associated is reduced. This should reduce variation in male reproductive success and sexual selection on traits associated with defending such resources. Alternatively, when resources are more clumped in space and/or time, a proportion of individual males may control access to those resources and the reproductive opportunities they confer, increasing variation in male reproductive success. Environmental complexity, instead, is not explicitly related to resources essential to reproduction. This theory focuses on how the structural

complexity of the environment may change the nature of socio-sexual interactions that in turn may affect mating system dynamics. For example, whether the environment is open or thick with vegetation may alter the mating patterns e.g. creating ecological opportunity for extra-pair copulations (Mays and Ritchison 2004; Tryjanowski et al. 2007) and/or modifying the reproductive benefits of alternative strategies such as sneaking (or dominance) (Candolin and Vlieger 2013). Both theories predict that ecological conditions change the pattern of sexual interactions, which then translate into differences in the pattern of sexual selection at the population level.

Experimental studies that explore the influence of the environment on mating systems and sexual selection, often consider the effect of only one ecological parameter, limiting generalization of the relative roles of different ecological factors i.e. environmental complexity and resource distribution. Furthermore, one often-overlooked facet regards how changes in mating patterns, and sexual interactions more generally, may have implications beyond changes in the strength and direction of selection on males. For instance, a wealth of studies have shown that male behaviour can reduce female fecundity when males harm females, creating a conflict over mating rates between the sexes (Galliard et al. 2005; Wigby and Chapman 2005; Darden et al. 2009). If changes in mating system are mediated by changes in male-female interactions, such as male harassment of females, we may also expect local ecology to impact female fecundity, potentially reducing group productivity (Eldakar et al. 2009; Eldakar and Gallup 2011; Holman and Kokko 2013).

In this study, we aim to address some of these shortcomings by investigating changes in mating systems and resulting sexual selection in replicate populations of Red junglefowl (*Gallus gallus*) as a response to experimental manipulations of both

resource distribution and environmental complexity. Specifically, we test the effect of changes in resource distribution and environmental complexity on: i) measures of both pre- and post-copulatory sexual selection on males and phenotypic selection on male social status (a trait previously well established as a target for sexual selection in male fowl (Pizzari and Birkhead 2000; Pizzari 2001; Pizzari et al. 2002; Collet et al. 2012). ii) We then investigate changes in mating system, precopulatory intersexual interactions and intrasexual interactions between males, associated with experimental treatments. From a male perspective, the key parameter determining mating system is the mating patterns of females, i.e. whether females are monogamous or polyandrous and with which males they mate. We therefore investigate changes in mating system through variation in polyandry across ecological treatments as potential drivers of variation in sexual selection in males. Finally, we iii) explore how our experimental treatments may affect female fecundity and whether this may impact group productivity. We do so by assessing the impact of experimental treatments on two female reproductive traits, fecundity and average egg mass.

METHODS

STUDY POPULATION

We used replicate semi-natural groups of Red junglefowl, a species that lives in small social groups (2-28 individuals) (Collias and Collias 1996). All individuals used in this study originate from a semi-natural population of red junglefowl kept at the University of Oxford field station (refer to previous work that describes this population e.g. Collet et al. 2012, 2014). All experimental work was conducted between the months April-October across three breeding seasons between 2011-2013.

EXPERIMENTAL DESIGN

Resource Distribution Experiment

We used 12 mating groups in total. Each group consisted of 10 males and 12 females held in an outdoor enclosure of approximately 100m^2 provided with two. Due to limitations on available individual males, 13 males were reused across different male groups. In total we used 6 unique groups of 12 females that were reused with more than one group of males, in a similar pattern as described in figure 1, but individual females were not reused across female groups. In each trial a mating group was studied for 10 days, and were provided with either clumped or distributed resources for 5 days then a further 5 days with the alternate treatment (fig. 2a). Birds were fed *ad libitum* using a mixture of grower pellets and fresh seeds at ratio of 2:1. In the clumped resources treatment all food was placed in one feeder and the other feeder was left empty, in distributed resources treatment the same amount of food was split equally between both feeders to manipulate food distribution keeping total food abundance constant across treatments. Along side food we placed water containers for birds to drink. For clumped resources we only filled the water container beside the single feeder in use, whereas in the distributed resource treatment a water container was filled beside each of the two feeders in use. Food was replenished every day in the morning before birds came down from roosting and water was replaced every 2 days at the same time. The order with which mating groups received either distributed or clumped resources was randomized and balanced such that an equal number of groups started or ended with clumped resources. Similarly, corner of the enclosures that contained food and water was randomized each trial.

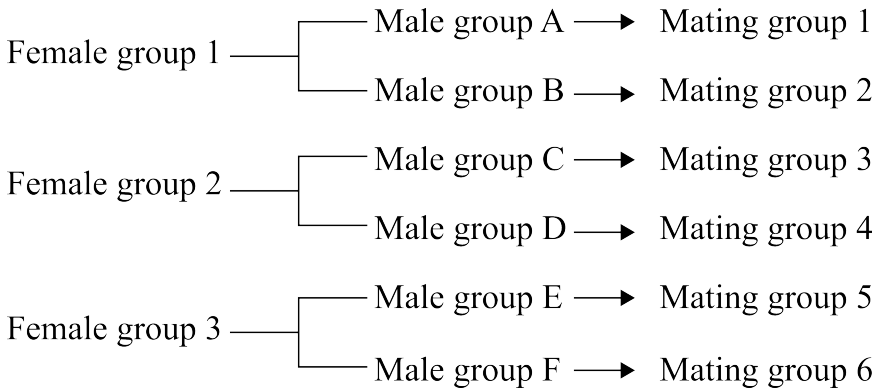


Figure 1. Diagram showing how replicate female groups were used repeatedly across unique male groups to create the individual mating groups used in resource distribution and environmental complexity experiments

Environmental Complexity Experiment

We used 8 mating groups in total, each consisting of 10 males and 12 females held in an outdoor enclosure approximately 100m^2. Only one male was reused across different male groups due to limitations on the availability of birds. Female groups were reused, in a similar pattern as described above for the resource distribution experiments and 5 females were reused across female groups. Each group was provided with either simple or complex environmental conditions for 5 days then a further 5 days with the alternate treatment (fig 2b). We simulated environmental complexity by placing wooden panels across the enclosure. In the complex treatment we used filled panels that were covered with 2 layers of 70% shade netting. In the simple environment treatment we used sham panels that were left without netting, such that birds could freely move through the centre of the panels. Birds were fed using a mixture of grower pellets and fresh seeds at ratio of 2:1 split equally across two feeders placed in opposite corners of the enclosure. Along side food we placed water containers for birds to drink as above. The order with which mating groups received either simple or complex environments was randomized and balanced such that an equal number of groups started or ended with the complex environmental treatment. The corner of the enclosures that contained food and water was randomized each trial.

DATA COLLECTION

Before each trial males and females were housed in single-sex enclosures. Male groups were allowed into the experimental enclosure 3 days prior to the release of females to allow dominance hierarchies to form and stabilize in the absence of females. All females were isolated in single sex enclosures before trials for a minimum 14 days to ensure sperm depletion from previous mating (Etches 1996). On the morning of the first day of each trail all females joined the males in the experimental enclosure. Behavioural observations were made every day for 6 hours between 0500-0800hr in the morning and in the evening between 1800-2100hr GMT for a total of 60 hours per group, totalling 1200 hours of behavioural observations across both experiments. We used *ad libitum* sampling as all individuals could be observed at once (Altmann 1974). We recorded all male-male interactions including pecks, chases, waltzes fights and avoidances (Johnsen et al. 2001). A male was considered a subdominant in interactions if he retreated at least one body length from the other male (Johnsen et al. 2001; Froman et al. 2002; Wilson et al. 2009). Because we aimed to determine the effect of social status on male mating success, competitive interactions over females and mating opportunities were not used to assess male dominance. We recorded all copulation attempts by males and all copulation events. Copulations were considered successful when cloacal contact was observed or when the males' tail was lowered over the female's cloaca and contact was assumed to occur. Furthermore, for all copulation attempts we scored resistance to a male, from 1-6, with 6 representing the highest level of resistance as follows: 1 = the female crouches in front of the male and solicits a copulation, with no resistance displayed; 2 = the male approaches the female from behind with raised hackles, and the female

responds by crouching; 3 = the male approaches the female, and the female walks away; 4 = the male grabs the female, the female passively accepts the copulation; 5 = the male runs after and grabs the female, and the female initially resists before accepting the copulation; and 6 = the female resists the whole copulation. This rank is not independent of male interest and female resistance must but interpreted in light of the covariance with male behaviour i.e. more resistance from a female may mean more male sexual interest. Furthermore, for each copulation event we recorded whether another male interrupted the focal male's copulation attempt and whether the copulation was successful or not. An interruption was defined as whether a competitor male either; 1) approached within one body length after the onset of the copulation event, 2) pecked, kicked or otherwise tried to physically disrupt the copulation attempt (Pizzari 2001). All eggs laid by females were collected during the 10 days of experimentation and for the 14 days after experimentation, totalling 844 eggs. All eggs were weighed to the nearest gram and then incubated for between 5-9 days before tissue samples were taken and subsequently stored in 100% ethanol at -20C until later parentage assignment.

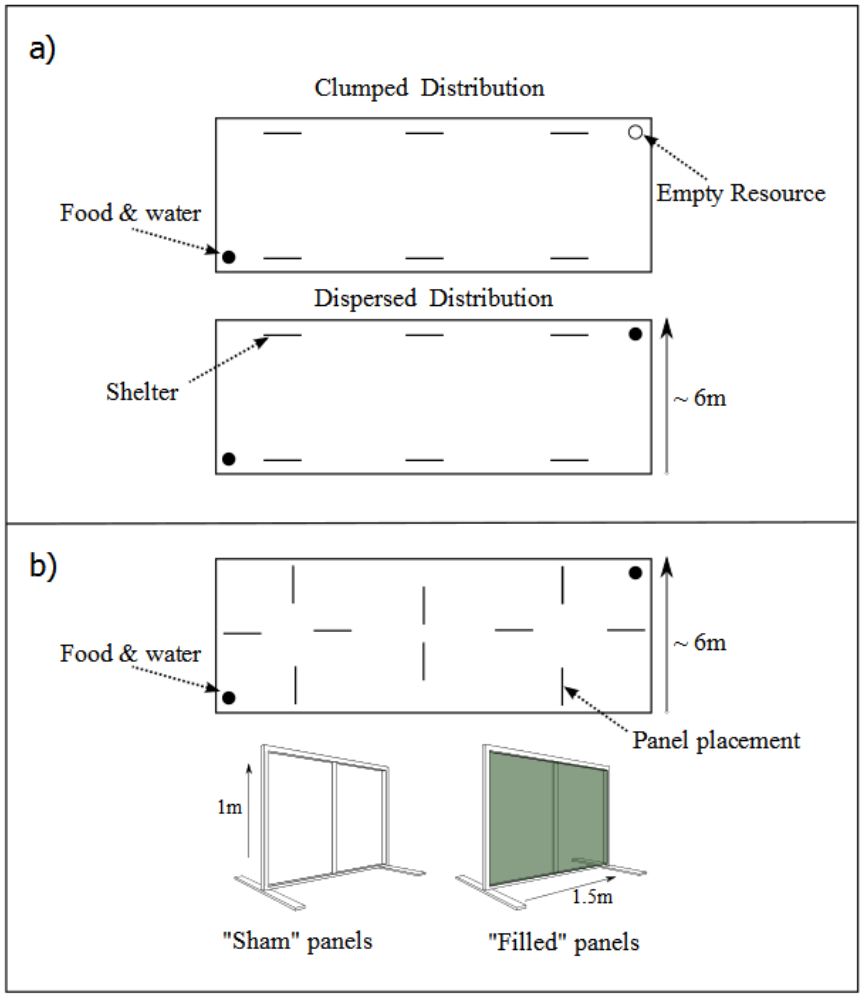


Figure 2(a) Experimental enclosure design for resource distribution experiment treatments (b) Experimental enclosure design for environmental complexity experiments, in the simple treatment all panels were "sham" panels and in the complex treatment all panels were "filled" panels.

MOLECULAR METHODS AND ANALYSES

For all groups we ignored all eggs from day one of each trial as eggs laid on the first day of a trial are unlikely to be fertilized on the same day (Howarth 1971; Birkhead and Møller 1993; Etches 1996), and instead only consider parentage data from days 2-10 of the trial and all eggs laid on the 11th day after the trial was complete. DNA was extracted following a standard ammonium acetate precipitation protocol (Nicholls et al. 2000). All samples were genotyped at 15 variable microsatellite loci arranged into 2 multiplex reactions, using primers designed to

amplify in domestic chicken (Crooijmans et al. 1996, 1997; Gibbs et al. 1997; Hanotte et al. 1997; Dawson et al. 1998; Groenen et al. 2000) or across a wide range of bird species (Dawson et al. 2010). PCRs were carried out in 2 µl volumes, using the reagents and methods described in Kenta et al (2008). Cycling conditions for both multiplexes were as follows: an initial heating step of 95˚C for 15 minutes was followed by 35 cycles of 94˚C for 30 seconds, 56˚C for 90 seconds and 72˚C for 60 seconds. A final extension step of 60˚C for 30 minutes completed the reaction. PCR products were diluted by 1 in 150, and fragments were separated on an ABI 3730 capillary sequencer. Allele sizes were assessed against ROX 500 size standard using the software GeneMapper version 3.1 (Applied Biosystems).

Null allele frequencies for each locus were estimated using CERVUS version 3.0.1 (Kalinowski et al. 2007). Molecular parentage analyses were carried out in CERVUS, using the approaches outlined in Dean et al. (2010). Because of the larger number of potential parents in our experimental design compared to previous experiments, we could not assign parentage to all offspring with 95% confidence. Subsequent analyses using parentage data were therefore carried out including only individuals that could be assigned parentage with 95% confidence. None of the 15 microsatellite loci had high null allele frequencies (highest = 0.03), so all were used for parentage analyses.

DATA ANALYSIS

We tested changes in competitive structure via two main axes, the mating system (i.e. polyandry) and male aggressive interactions, associated with both resource distribution and structural complexity treatments. We assess how these parameters may be linked to changes in sexual selection and female fecundity. In this

study we measure selection at the population level (i.e. at the level of the entire mating group) because the aim of this chapter was to assess how differing ecological environments affect patterns of selection at the level of the population, which is also the level at which theory predicts we should detect variation in the patterns of selection. However, manipulations may affect within-group patterns of intrasexual interactions which may not be identified using population-level methods (for further details see chapters 3-6, McDonald and Pizzari In press; McDonald et al. 2013). However, holding polyandry constant, preliminary analysis suggested that patterns of mating network structure or male-male competitive network structure did not vary across treatments (e.g. see Appendix§1 and results fig.7 and table 19), although future work should further explore how ecological manipulations may affect competitive structure beyond polyandry including mating network structure (see chapter 4 - 6) and the relationship between individual traits and the traits of their competitors (see chapter 3, 4 & 7). To changes in selection and intra- and intersexual interactions across treatments we used a combination of general linear models (GLMs) and generalised linear mixed models (GLMMs). In some mating groups a female had to be removed due to illness or death. In these groups a new female was replaced immediately and we have excluded the data for the female that was present for the least number of days. Females are able to store sperm for up to 14 days after a copulation potentially resulting in carry over effects across treatments from the first 5 days on the next 5 days on patterns of paternity (Etches 1996). Therefore, all analyses that include paternity data in comparisons between groups use only the first 5 days of behaviour and days 2-6 of parentage e.g. for the resource distribution experiment there are 6 groups exposed to the distributed resources treatment and 6 which were exposed to the clumped resources treatment for the first 5 days. We combined

paternity data with behavioural data such that if males were not observed mating with a female but sired an egg of that female, we included that female in the male's mating success (see Collet et al. 2012). For analyses that require no paternity data (i.e. number of copulation attempts by males or male-male interactions) we use the full 10 days of each experiment and use paired comparisons across treatments.

Where appropriate we used female group ID, mating group ID, male ID and female ID as random effects for to control for pseudoreplication of groups and individuals. For comparisons of group-level metrics sample were small (n = 12 or 8) and which may limit statistical power. Instead of providing a posthoc power analysis we provide confidence intervals for parameters in these cases (Colegrave and Ruxton 2003). Furthermore, in such cases we used GLMs controlling for pseudoreplication as a fixed effect rather than random effects in GLMMs due to the anticonservative nature of likelihood ratio tests (LRTs) for small sample sizes (Bolker et al. 2009). We therefore present the more conservative GLM results in the main text. However, we also present GLMM results in appendices because GLMs might reduce the predictive power as fixed effects require more degrees of freedom. For all GLMMs we use LRTs to test the significance of fixed effects in mixed models. When appropriate we accounted for overdispersion in GLMMs by including an individual level random effect (Elston et al. 2001). We used poisson, binomial or gaussian distributions in models as appropriate. In some cases we improved model fit by scaling explanatory variables by subtracting mean and dividing by 1 standard deviation. Finally, for analyses including indices that run from zero to one, e.g. modularity (see methods below), we arcsine square root transformed response variables to ensure assumptions of normality were met.

i) Sexual Selection on Males

To assess the maximum potential strength of sexual selection operating on males we first calculated a variety of variance-based selection measures for all groups in both treatments in resource distribution and environmental complexity experiments including the opportunity for selection (standardized variance in reproductive success, I_T) and the opportunity for sexual selection (standardized variance in mating success, I_s) (Crow 1958; Arnold and Duvall 1994) as well as the contribution of: mating success (*M*), mean partner fecundity (*N*), total fertilisation success (*P*), and their respective covariances to the total variation in reproductive success (Webster et al. 1995) (Table 1).

We compared the slope of selection through both pre- and post-copulatory components *M*, *N* and *P* with multivariate sexual selection gradients (Collet et al. 2012). Firstly, we tested whether *M*, *N* and *P* positively and significantly predicted reproductive success with GLMMs in both experiments including all three as fixed effects and experimental treatment as a covariate (e.g. for the resource distribution experiment, whether groups were exposed to distributed or clumped). We then tested whether experimental treatments increased or decreased the respective slopes by including all interactions between *M*, *N* and *P* with treatment. To compare phenotypic selection on male social status we first calculated David's score (David 1987) within each group as our measure of male social status. David's score is most appropriate when data sets have many interactions and high asymmetry in interaction strength between individuals (Gammell et al. 2003; de Vries et al. 2006). We separately calculated male social hierarchies for each treatment for each group in both experiments. We then tested for changes in patterns of selection on male social status through total reproductive success as well as through individual components *M*, *N* and

P for both experiments across treatments using GLMMs. These models included social status, treatment and the interaction between the two as fixed effects.

Table 1. Selection coefficient parameters and formulas.

Selection Coefficient	Formula
Opportunity for Selection (I_T)	$Var(T)/\bar{T}^2$
Opportunity for Sexual Selection (I_S)	$Var(M)/\bar{M}^2$
Standardised percentage contribution of mating success (*M*)	$\left(\frac{\bar{N}^2\bar{P}^2Var(M)}{\bar{T}^2}/I_T\right)*100$
Standardised percentage contribution of partner fecundity (*N*)	$\left(\frac{\bar{M}^2\bar{P}^2Var(N)}{\bar{T}^2}/I_T\right)*100$
Standardised percentage contribution of fertilization success (*P*)	$\left(\frac{\bar{M}^2\bar{N}^2Var(P)}{\bar{T}^2}/I_T\right)*100$
Standardised percentage contribution of the Covariance between mating success (*M*) and fertilization success (*P*)	$\left(\frac{2\bar{M}\bar{P}\bar{N}^2Cov(M,P)}{\bar{T}^2}/I_T\right)*100$
Standardised percentage contribution of the Covariance between mating success (*M*) and partner fecundity (*N*)	$\left(\frac{2\bar{M}\bar{N}\bar{P}^2Cov(M,N)}{\bar{T}^2}/I_T\right)*100$
Standardised percentage contribution of the Covariance between partner fecundity (*N*) and fertilisation success (*P*)	$\left(\frac{2\bar{N}\bar{P}\bar{M}^2Cov(N,P)}{\bar{T}^2}/I_T\right)*100$

ii) Mating system and inter- and intra-sexual interactions

We investigated changes in mating system associated with experimental treatments of either resource distribution or environmental complexity by comparing the average polyandry from groups in the first 5 days of a trial. We did this, using GLMMs with individual females as data points rather than group level averages of polyandry. This allowed us to control for the pseudoreplication of females used across replicate groups as group level analyses ignore the replication of individual females.

To assess the impact of experimental treatments on intersexual interactions that may mediate both changes in mating system and the potential female fecundity, we compared several metrics across treatments, including the number of males attempting to mate with a female, the number of mating attempts received by individual females, the levels of resistance expressed by females and the frequency with which copulation attempts were interrupted by competing males. To assess the effects of treatments on the number of male mating attempts, we used two

complementary approaches. Firstly, we simply compared the mean number of attempts that females received across all males that attempted to mate with them. Our second approach compared the number of copulation attempts across all possible male-female combinations between treatments in both experiments. This latter approach allowed us to also assess the relationship between the number of mating attempts of a male with individual females and the male's social status across treatments, and thus address the following general question: does the environment influence the relationship between male social status and the number of per female copulation attempts? We predict that if the relationship between male social status and number of attempts changes (i.e. less dominant males attempt more often) this may vary the pattern of sexual selection on male mating success across treatments. We further compared the level of female resistance across treatments and the relationship between female resistance and male social status. To assess whether copulation attempts were more or less likely to be disrupted by competitor males we first compared the probability that an attempted copulation (successful or not) received an interruption from a competitor male. These models included the social status of the focal male attempting to copulate and the interaction between his social status and experimental treatment. This enabled us to ask whether males of a given social status were more or less likely to have competitors attempt to interrupt their copulation attempts and whether this relationship varied across treatments as such effects may mediate changes on selection of male social status as well as changes in mating patterns more generally. We also confirmed separately whether interruptions attempts reduce the probability of copulation success.

We compared male-male interactions across treatments by calculating the linearity and the steepness of social hierarchies. Any changes in selection may be

mediated not only by changes in the relationship between male social status and fitness (i.e. reproductive success) on a constant, unchanging social hierarchy, but also by changes in the shape of social hierarchies themselves. The linearity of a social hierarchy describes the extent to which dominance relationships are transitive, that is hierarchies are more linear when dominance relationships can be ordered such that when A dominates B and B dominates C, then A should also dominate C. The steepness of hierarchies refers to the size of the differences between adjacently ranked individuals in their overall success in winning dominance encounters (de Vries et al. 2006). Hierarchies are steeper when the difference in dominance score is large between individuals adjacent in the hierarchy and shallow when dominant individuals differ little in their dominance scoring relative to adjacent individuals. Together, these measures quantify the extent to which social hierarchies are egalitarian or despotic (van Shaik 1987; de Vries et al. 2006). We compared both of these group-level measures in paired comparisons across all groups between treatments for both experiments. To analyse male-male aggressive interactions we concentrated on pecks, chases, waltzes and the outcome of fights. We then compared the density of male interaction networks, which describes the number of pairs of males that aggressively interact out of the total number of possible pairs. We then carried out a similar analysis that allowed us to control for male ID by using GLMMs that compared the number of males with which focal males acted aggressively (node degree) and also the total number of aggressive interactions expressed by males (node strength) across treatments.

ii) Female fecundity

We assessed the potential effects of changes in mating patterns and intersexual interactions on female reproductive output with GLMMs, which compared female

fecundity and mean egg mass between treatments for both experiments. We separately used GLMMs to assess whether any changes in female reproductive output may be explained by the total number of mating attempts received by females and female average resistance across data pooled across all ten days. We also used GLMMs to assess whether the slopes of female fecundity on mean female resistance and total number of mating attempts received by females differed between treatments for both experiments. To do this we fitted models for both experiments with female fecundity as the response variable and tested the interaction between treatments and either the number of mating attempts or mean female resistance respectively. Finally, the mating patterns of populations are also predicted to affects the dynamics and spread of sexually transmitted infections (STIs) which may impact on female fecundity and the health of individuals in the population as a whole (Salathe and Jones 2010; Griffin and Nunn 2012; Ashby and Gupta 2013). We tested whether treatments affected changes in mating structure pertinent to the dynamics of STI spread. However, we found no significant effects and these results and methods are presented in Appendix§1 table A1.

RESULTS

SEXUAL SELECTION ON MALES

For both the resource distribution and environmental complexity experiments, the maximum potential strength of sexual selection (Opportunity for selection, I_T) did not differ significantly between treatments using a GLM or GLMM modelling approach (fig. 3, Table 2, Appendix§1 Table A2). Similarly, standardized variation in mating success (Opportunity for sexual selection, I_s) did not differ between treatments

using the GLM approach (Table 3). However, GLMMs did reveal a non-significant tendency for I_s to be lower in the simple environment relative to the complex environment in the environmental complexity experiment (Appendix§2 Table A2).

Table 2. GLM results comparing Opportunities for selection (I_T) across treatments for both resource distribution and structural complexity experiments. In all cases female group was added as a fixed factor to control for pseudoreplication of female groups across replicates.

Experiment	Model	Parameter	Estimate	SE	*T*	*df*	p-value	95% CI
Resource	I_T ~ Treatment + Female Group ID	Dispersed	2.54	0.825				L= 0.924, U= 4.156
		Clumped	1.935	0.927	-0.652	5	>0.5	L=0.319, U=3.551
Complexity	I_T ~ Treatment + Female Group ID	Complex	1.67	0.642				L= 0.412, U= 2.927
		Simple	0.894	0.51	-0.133	3	>0.9	L=-1.067, U=0.932

Table 3. GLM results comparing Opportunities for sexual selection (I_s) across treatments for both resource distribution and structural complexity experiments. In all cases female group was added as a fixed factor to control for pseudoreplication of female groups across replicates.

Experiment	Model	Parameter	Estimate	SE	*T*	*df*	p-value	95% CI
Resource	I_s ~ Treatment + Female Group ID	Dispersed	0.567	0.259				L= 0.059, U= 1.074
		Clumped	0.579	0.291	0.043	5	>0.9	L= -0.559, U= 0.584
Complexity	I_s ~ Treatment + Female Group ID	Complex	0.8	0.291				L=0.229, U= 1.37
		Simple	0.348	0.231	-1.953	3	>0.1	L=-0.905, U= 0.002

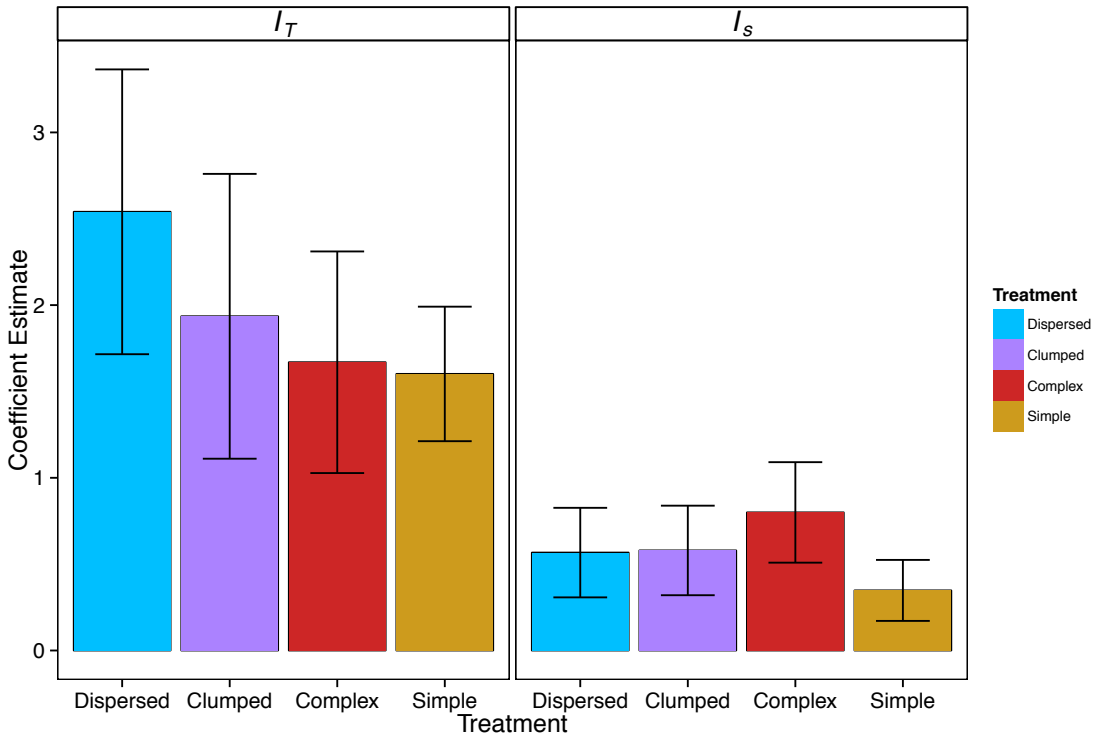


Figure 3. Opportunity for Selection (I_T) and Opportunity for Sexual Selection (I_s) in the resource distribution and environmental complexity experiments. Bars represent means ±1SE estimated from linear models.

Across all groups and all treatments, standardized variation in fertilization success (P) contributed the most to the total variation in reproductive success with mating success being the next largest contributor. However, bar charts in figure 4 reveal some differences between resources treatments in the resource distribution experiment: percentage contribution P was much larger in clumped resources, and $Cov(M,P)$ and $Cov(N,P)$ were negative in clumped resources. However, boxplots suggest that these differences are due to some influential groups rather than consistent differences (fig. 4). Similarly, for environmental complexity experiments bar charts suggest that percentage contribution of M was much larger in complex environment (fig. 4). Again however, boxplots reveal that differences in the mean contribution of different fitness components are due to some influential groups rather than consistent

differences. Consistent with this, neither GLMs or GLMMs detect significant differences across the contribution of fitness components *M*, *N* and *P* (Table 4, Appendix§2 Table A3). Similarly, GLMs failed to detect significant differences in the magnitude of covariances in reproductive components (*M*, *N*, *P*) between experimental treatments, with the possible exception of the covariance between *M* and *P* in (*Cov*(*M*,*P*)), which showed a non-significant tendency to be weaker in the clumped resources treatment (Table 5). The GLMM approach detected significant differences in *Cov(N,P)* between the two resource distribution treatments, and in *Cov(M,N)* between environmental complexity treatments (Appendix§2 Table A4).

Table 4. GLM results for the percentage contribution for the fitness components *M*, *N* and *P* to the opportunities for selection across resource distribution and structural complexity treatments. All models controlled for replication of female groups by adding female group as a fixed factor.

Experiment	Fitness Component	Explanatory variables	Parameter	Estimate	SE	*t*	*df*	p-value	95% CI
Resource	Percentage contribution of *M*	Treatment + Female Group ID	Dispersed	30.692	12.046				L= 7.082, U=54.302
			Clumped	41.501	13.539	0.798	5	>0.4	L= -15.727, U= 37.345
Complexity			Complex	42.539	25.837				L= -8.101, U=93.179
			Simple	14.646	20.533	-1.358	3	>0.2	L= -68.138, U=12.351
Resource	Percentage contribution of *N*	Treatment + Female Group ID	Dispersed	6.944	4.202				L= -1.292, U=15.181
			Clumped	14.473	4.723	0.798	5	>0.4	L= -1.728, U= 16.786
Complexity			Complex	-5.171	8.1				L= -21.047, U=10.703
			Simple	1.017	6.437	0.961	3	>0.4	L= -6.428, U=18.805
Resource	Percentage contribution of *P*	Treatment + Female Group ID	Dispersed	87.615	45.162				L= -0.901, U=176.132
			Clumped	152.789	45.162	1.284	5	>0.2	L= -34.310, U= 164.658
Complexity			Complex	-5.171	50.489				L= -104.095, U= 93.819
			Simple	37.286	30.64	1.058	5	>0.3	L= -36.186, U=121.101

Table 5. GLM results for the percentage contribution of covariances between the fitness components *M*, *N* and *P* to the opportunities for selection across resource distribution and structural complexity treatments. All models controlled for replication of female groups by adding female group as a fixed factor.

Experiment	Covariance between fitness components	Explanatory variables	Parameter	Estimate	SE	t	df	p-value	95% CI
Resource	*Cov(M,N)*	Treatment + Female Group ID	Dispersed	5.248	6.222				L= -6.946, U=17.442
			Clumped	19.243	6.222	2.002	5	0.102	L= 7.049, U=31.437
Complexity			Complex	9.305	6.343				L= -3.128, U= 21.737
			Simple	-0.398	3.85	-1.925	3	0.15	L= -7.944, U= 7.148
Resource	*Cov(M,P)*	Treatment + Female Group ID	Dispersed	-11.416	18.48				L= -47.641, U= 24.809
			Clumped	-56.955	18.48	-2.192	5	0.08	L= -93.18, U= -20.73
Complexity			Complex	16.092	41.923				L= -66.076, U= 98.259
			Simple	26.555	25.446	0.314	3	0.774	L= -23.319, U= 76.429
Resource	*Cov(N,P)*	Treatment + Female Group ID	Dispersed	-15.012	10.77				L= -36.125, U= 6.104
			Clumped	-36.783	10.77	-1.798	5	0.132	L= -57.9, U= -15.668
Complexity			Complex	3.562	9.089				L= -14.251, U= 21.376
			Simple	0.726	5.517	-0.393	3	0.721	L= -10.087, U= 11.538

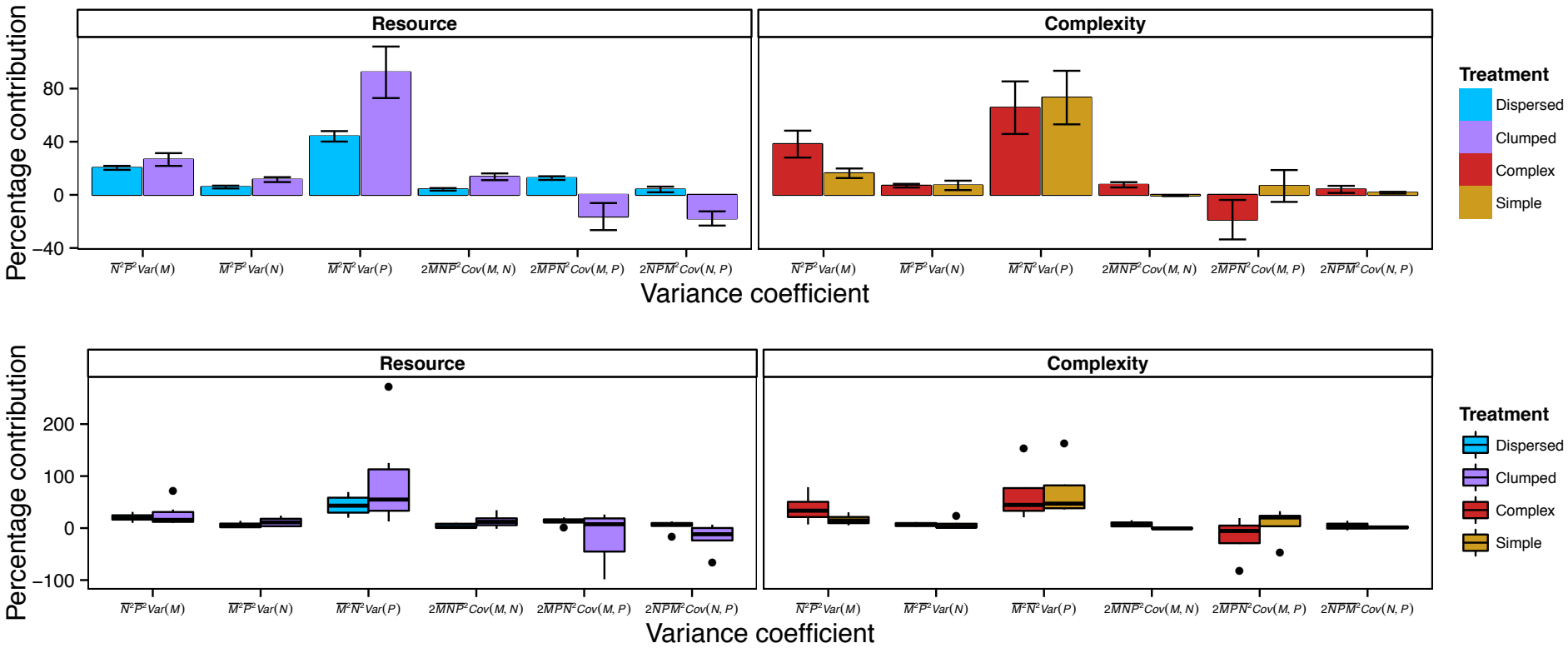


Figure 4. Top row shows barplots for percentage contribution of *M*, *N*, *P* and covariances to total variation in reproductive success (I_T), means ±1SE. Bottom row shows the same data as top row but using boxplots to display the percentage contribution of *M*, *N*, *P* and covariances to total variation in reproductive success (I_T).

Before assessing the effect of treatments on sexual selection gradients, we first calculated multivariate sexual selection gradients for both experiments, controlling for experimental treatments to demonstrate the importance of *M*, *N* and *P* in determining reproductive success. For both resource distribution and environmental complexity experiments all male reproductive success components (*M*, *N* and *P*) significantly and positively predicted male reproductive success (Table 6). We then tested the effect of experimental treatments on the slope of selection through all three components (*M*, *N* and *P*), by adding the interaction between experimental treatment and *M*, *N* and *P* in multivariate selection gradients models. For both resource distribution and environmental complexity experiments there were no significant interactions between treatments and multivariate selection gradients, demonstrating that neither experiment influenced the strength of sexual selection acting through *M, N* or *P* (Table 7). Results for univariate Bateman gradients were qualitatively similar and are presented in Appendix§2 Table A5).

Table 6. GLMM results for multivariate selection gradients for resource distribution and environmental complexity experiments, testing the effects of *M*, *N* and *P* on male total reproductive success (*T*) controlling for experimental treatment.

Experiment	Full model	Dropped terms	Estimate	SE	Model no. and comparisons	AIC	Likelihood ratio test
Resource	$T \sim M + N + P +$ Treatment random:(Female Group/Group) + (Male ID) *n*(obs.) = 109, *n*(Female Group) = 6, *n*(Group) = 12, *n*(Male ID)=98. var(Female Group) = 0, var(Female Group/Group) = 1.75E-02, var(Male ID)=6.40E-09 (Poisson)				1	269.33	
		P	3.097	0.309	1 vs. 2	357.31	$\chi2$=89.97, df=1, p<0.001
		N	0.752	0.123	1 vs. 3	296.77	$\chi2$= 29.44, df=1, p<0.001
		M	0.227	0.026	1 vs. 4	341.45	$\chi2$= 74.128, df=1, p<0.001
		Treatment (Clumped)	-0.018	0.141	1 vs. 5	267.34	$\chi2$=0.016, df=1, p>0.8
Complexity	$T \sim M + N + P +$ Treatment random:(Female Group/Group) + (Male ID) n(obs.) = 73 n(Female Group) = 4, n(Group) = 8. var(Female Group) = 0, var(Female Group/Group) =0 (Poisson)				1	196.04	
		P	3.376	0.327	1 vs. 2	294.05	$\chi2$=100.0, df=1, p<0.001
		N	0.551	0.164	1 vs. 3	204.34	$\chi2$= 10.305, df=1, p< 0.005
		M	0.247	0.031	1 vs. 4	263.43	$\chi2$=69.389, df=1, p<0.001
		Treatment (Simple)	0.003	0.162	1 vs. 5	194.04	$\chi2$=4e-04, df=1, p>0.9

*Interaction
/ Nested factors

Table 7. Model results for multivariate selection gradients for resource distribution and environmental complexity experiments, testing the effects of experimental treatments on the slopes of *M*, *N* and *P* on male total reproductive success (*T*).

Experiment	Full model	Dropped terms	Estimate	SE	Model no. and comparisons	AIC	Likelihood ratio test
Resource	$T \sim M + N + P +$ Treatment + Treatment**M* + Treatment**N* + Treatment**P* random: (Female Group/Group) + (Male ID) n(obs.) = 109 n(Female Group) = 6, n(Group) = 12, n(Male ID)=98. var(Female Group) = 0 , var(Female Group/Group) = 1.75E-02, var(Male ID)=6.40E-09 (Poisson)	-	-	-	1	271.89	-
		P * Treatment (Clumped)	-0.461	0.711	1 vs. 2	270.32	$\chi2$= 0.43, df=1, p>0.5
		N * Treatment (Clumped)	-0.348	0.319	1 vs. 3	271.14	$\chi2$= 1.247, df=1, p>0.2
		M * Treatment (Clumped)	0.096	0.059	1 vs. 4	272.74	$\chi2$=2.853, df=1, p=0.09
Complexity	$T \sim M + N + P +$ Treatment + Treatment**M* + Treatment**N* + Treatment**P* random: (Female Group/Group) n(obs.) = 73 n(Female Group) = 4, n(Group) = 8. var(Female Group) = 0 , var(Female Group/Group) =0 (Poisson)	-	-		1	198.92	
		P * Treatment (Simple)	0.426	0.679	1 vs. 2	197.32	$\chi2$= 0.393, df=1, p>0.5
		N * Treatment (Simple)	0.522	0.333	1 vs. 3	199.34	$\chi2$= 2.422, df=1, p>0.1
		M * Treatment (Simple)	0.066	0.067	1 vs. 4	197.9	$\chi2$= 0.972, df=1, p>0.3

* Interaction
/ Nested factors

We calculated several phenotypic selection gradients on male social status, including total sexual selection using total reproductive success (*T*) as the response and separately for precopulatory selection components *M* and *N* and postcopulatory sexual selection through *P* (Tables 8-11). For the resource distribution experiment there were no significant changes in total sexual selection on male social status and male social status did not significantly predict total reproductive success (Table 8).

For the environmental complexity experiments, selection on male social status did not significantly predict male reproductive success, however there was a non-significant tendency for total sexual selection on male social status to be weaker in simple environments compared to complex environments (fig. 5, Table 9). Similarly, there was a non-significant tendency for male social status to positively predict male mating success in both resource distribution and environmental complexity experiments (Table 9). The experimental manipulation of resource distribution did not change this potential relationship (Table 9). Manipulations of environmental complexity on the other hand, revealed that simple environments had significantly lower mean mating success and marginally non-significant weaker selection on male social status (fig. 5, Table 9). We did not detect significant directional selection on male social status through both *N* or *P* or any interaction with treatment for either resource distribution or environmental complexity experiments (Tables 10 & 11). Finally, we also compared the slopes of sexual selection on male remating rate (average number of copulations per female mating partner). We found no significant effect of treatment on average male remating rate in the resource distribution experiment, however in the environmental complexity experiment we detected a marginally non-significant change in the relationship between male status and average remating rate, such that the positive relationship between male social status and remating rate became weaker in simple environments ($\chi 2=3.38$, *d.f.*=68,1, p=0.066; full results presented in appendix§2 table A6).

Table 8. Model results for resource distribution and environmental complexity experiments, testing the effects of experimental treatments on the slopes of selection on male social status on male total reproductive success (*T*).

Experiment	Full model	Dropped terms	Estimate	SE	Model no. and comparisons	AIC	Likelihood ratio test
Resource	Reproductive Success (*T*) vs. Status & Treatment random: (Female Group/Group) + (Male ID) n(obs.) = 120, n(Female Group) = 6, n(Group) = 12, n(Male ID)=107 . var(Female Group) = 2.23E-02 , var(Female Group/Group) = 9.93E-08, var(Male ID)=1.08 (Poisson)				1	444.98	
		Status * Treatment (Clumped)	-0.0003	0.013	1 vs. 2	442.98	$\chi2$=5e-04, df=1, p>0.9
		Treatment (Clumped)	0.0702	0.254	2 vs. 3	441.06	$\chi2$=0.076, df=1, p>0.7
		Status	0.006	0.01	2 vs. 4	441.83	$\chi2$= 0.852, df=1, p>0.3
Complexity	Reproductive Success (*T*) vs. Status & Treatment random: (Female Group/Group) + dummy n(obs.) = 80, n(Female Group) = 4, n(Group) = 8. var(Female Group) = 0, var(Female Group/Group) = 0 (Poisson)				1	330.48	
		Status * Treatment (Simple)	-0.0287	0.015	1 vs. 2	332.3	$\chi2$= 3.817, df=1, p=0.05
		Treatment (Simple)	-0.063	0.306	2 vs. 3	441.06	$\chi2$=0.021, df=1, p>0.8
		Status	0.011	0.011	2 vs. 4	330.53	$\chi2$=0.236, df=1, p>0.6

* Interaction
/ Nested factors
dummy =Individual level random effect to account for overdispersion

Table 9. Model results for resource distribution and environmental complexity experiments, testing the effects of experimental treatments on the slopes of selection on male social status on male mating success (*M*).

Experiment	Full model	Dropped terms	Estimate	SE	Model no. and comparisons	AIC	Likelihood ratio test
Resource	Mating Success (*M*) vs.				1	612.63	
	Status & Treatment random:(Female Group/Group) +	Status * Treatment (Clumped)	-0.004	0.006	1 vs. 2	611.05	$\chi2$=0.418, df=1, p>0.5
	(Male ID)	Treatment (Clumped)	-0.005	0.191	2 vs. 3	609.06	$\chi2$= 0.004, df=1, p>0.9
	n(obs.) = 120, n(Female Group) = 6, n(Group) = 12, n(Male ID)=107 . var(Female Group) = 3.83E-08 , var(Female Group/Group) = 6.52E-02, var(Male ID)=2.23E-01 (Poisson)	Status	0.008	0.004	2 vs. 4	612.8	$\chi2$= 3.748, df=1, p=0.05
Complexity	Mating Success (*M*) vs.				1	426.65	
	Status & Treatment random: (Female Group/Group) +	Status * Treatment (Simple)	-0.013	0.007	1 vs. 2	428.49	$\chi2$=3.835, df=1, p=0.05
	dummy	Treatment (Simple)	0.353	0.142	2 vs. 3	431.92	$\chi2$=5.431, df=1, p<0.05
	n(obs.) = 80, n(Female Group) = 4, n(Group) = 8. var(Female Group) = 2.89E-10 , var(Female Group/Group) = 0 (Poisson)	Status	0.01	0.005	2 vs. 4	426.79	$\chi2$=0.305, df=1, p>0.5

* Interaction
/ Nested factors
dummy =Individual level random effect to account for overdispersion

Table 10. Model results for resource distribution and environmental complexity experiments, testing the effects of experimental treatments on the slopes of selection on male social status through mean partner fecundity (*N*).

Experiment	Full model	Dropped terms	Estimate	SE	Model no. and comparisons	AIC	Likelihood ratio test
Resource	Partner Fecundity (*N*) vs. Status & Treatment random:(Female Group/Group) + (Male ID) n(obs.) = 109 n(Female Group) = 6, n(Group) = 12, n(Male ID)=98. var(Female Group) = 0.16108431 , var(Female Group/Group) = 0.121, var(Male ID)=0.013 (Gaussian)				1	218.68	
		Status * Treatment (Clumped)	0.001	0.005	1 vs. 2	216.75	$\chi2$= 0.067, df=1, p>0.7
		Treatment (Clumped)	-0.044	0.246	2 vs. 3	214.78	$\chi2$= 0.03, df=1, p>0.8
		Status	0.001	0.004	2 vs. 4	215.07	$\chi2$= 0.32, df=1, p>0.5
Complexity	Partner Fecundity (*N*) vs. Status & Treatment random: (Female Group/Group) n(obs.) = 73 n(Female Group) = 4, n(Group) = 8. var(Female Group) = 0 , var(Female Group/Group) = 0.097 (Gaussian)				1	172.98	
		Status * Treatment (Simple)	0.002	0.008	1 vs. 2	171.08	$\chi2$= 0.1, df=1, p>0.7
		Treatment (Simple)	-0.16	0.27	2 vs. 3	169.44	$\chi2$=0.36, df=1, p>0.5
		Status	-0.003	0.006	2 vs. 4	169.3	$\chi2$= 0.217, df=1, p>0.6

* Interaction
/ Nested factors

Table 11 Model results for resource distribution and environmental complexity experiments, testing the effects of experimental treatments on the slopes of selection on male social status through male fertilisation success (*P*).

Experiment	Full model	Dropped terms	Estimate	SE	Model no. and comparisons	AIC	Likelihood ratio test
Resource	Fertilisation Success (*P*) vs. Status & Treatment random:(Female Group/Group) + (Male ID)				1	350.74	
		Status * Treatment (Clumped)	0.004	0.011	1 vs. 2	348.86	$\chi2$= 0.122, df=1, p>0.7
	n(obs.) = 109 n(Female Group) = 6, n(Group) = 12, n(Male ID)=98.	Treatment (Clumped)	0.115	0.227	2 vs. 3	347.15	$\chi2$= 0.294, df=1, p>0.5
	var(Female Group) = 0 , var(Female Group/Group) = 0, var(Male ID)=0.472 (Binomial)	Status	-0.002	0.008	2 vs. 4	346.86	$\chi2$=0.005, df=1, p>0.9
Complexity	Fertilisation Success (*P*) vs. Status & Treatment random: (Female Group/Group)				1	277.85	
		Status * Treatment (Simple)	-0.021	0.015	1 vs. 2	277.83	$\chi2$= 1.982, df=1, p>0.1
	n(obs.) = 73 n(Female Group) = 4, n(Group) = 8.	Treatment (Simple)	-0.415	0.319	2 vs. 3	277.47	$\chi2$= 1.647, df=1, p>0.1
	var(Female Group) = 0 , var(Female Group/Group) =2.37E-11, (Binomial)	Status	0.003	0.011	2 vs. 4	277.12	$\chi2$= 1.298, df=1, p>0.2

* Interaction
/ Nested factors

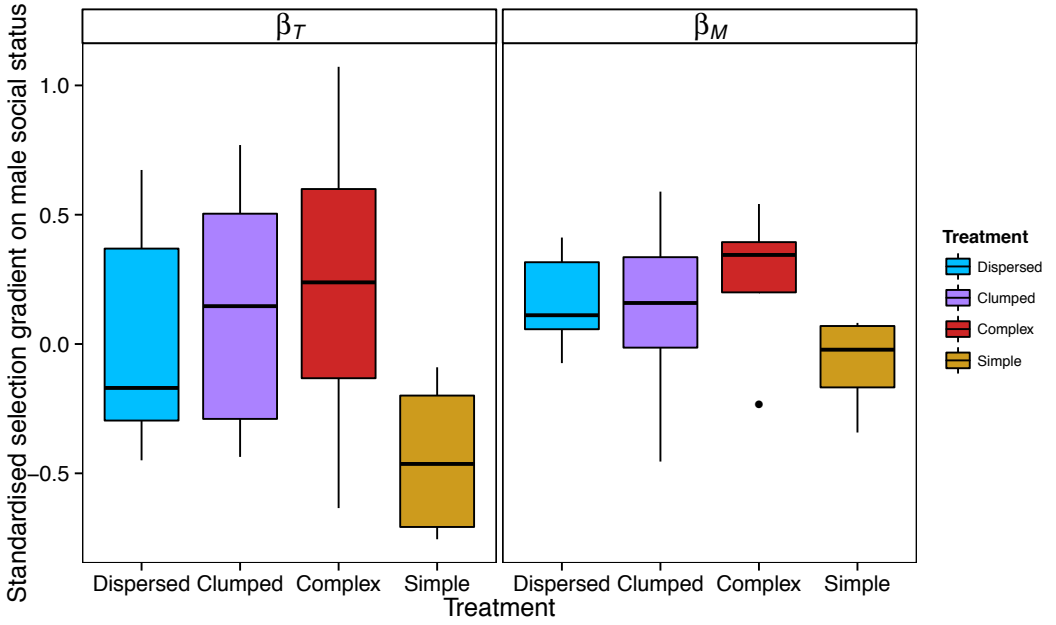


Figure 5. Boxplots show standardized phenotypic selection gradients calculated for male social status on total reproductive success (β_T) and mating success (β_M).

MATING SYSTEM AND INTER- AND INTRA-SEXUAL INTERACTIONS

In the resource distribution experiment, average polyandry was higher in the clumped resources treatment, however this difference was not significant (fig. 6, Table 12). Manipulations of the environmental complexity did however show a significantly higher average polyandry in the simple environment treatment compared with the complex environment treatment (fig. 6, Table 12).

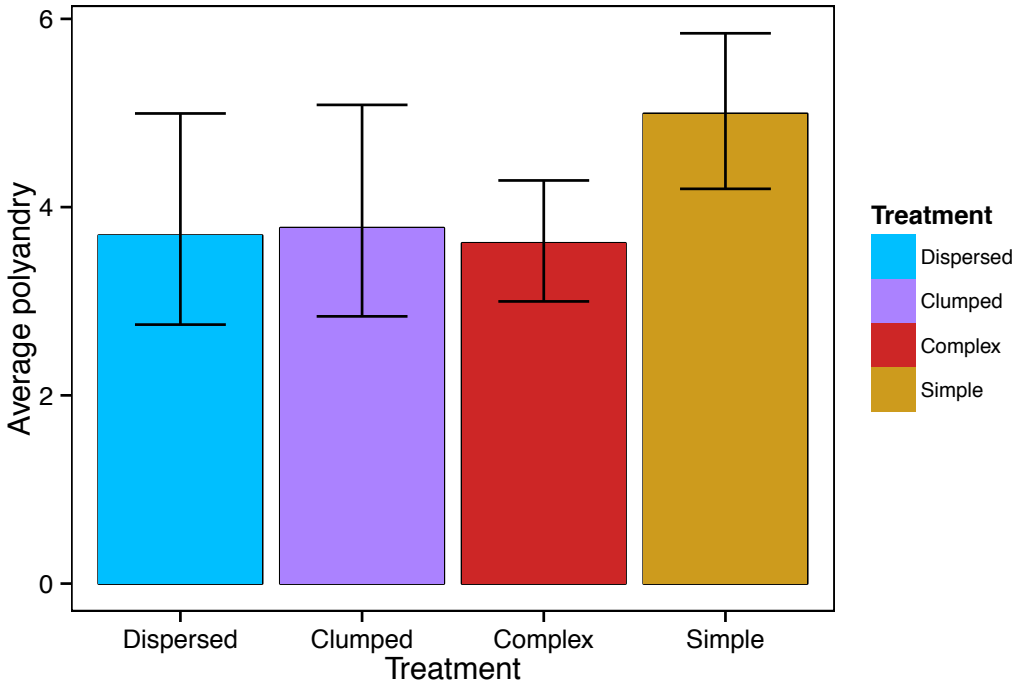


Figure 6. Effect of treatments of resource distribution and environmental complexity experiments on average polyandry in replicate groups (mean number of mates per female ±95% CIs).

We investigated potential changes in male-female sexual interactions that may mediate female mating patterns across treatments. To do so, we compared the total number of unique males that attempted to mate each female. There was no difference in the number of males that attempted to mate with individual females across treatments in neither the resource distribution nor the environmental complexity experiment (Appendix§2 Table A7). We assessed the effect of treatments on the

number of male copulation attempts with two complementary approaches. Firstly, we compared the mean number of attempts that females received across all males that attempted to mate with them. These results showed that the number of attempts did not change across resource distribution treatments but that females received significantly more mating attempts in simple rather than complex environments (Table 13). Our second approach compared the number of copulation attempts across all possible male-female pairs across treatments, and the relationship between copulation attempts and male social status. In the resource distribution experiment, male social status positively predicted the number of copulation attempts and males attempted to copulate with females more often when resources were clumped rather than dispersed, although this second result was marginally non-significant (Table 14). In the environmental complexity experiment males attempted to copulate significantly more in the simple compared to the complex environment treatment, but there was no effect of male social status. To investigate whether changes in mating attempts may be associated with changes in mating patterns and female polyandry, we tested the effect of number of mating attempts on male mating success across data pooled over all 10 days of each experiment for each group. Results demonstrate that the number of attempts positively and significantly predicted the mating success of males (Appendix§2 Table A8).

Table 12. GLMM results for resource distribution and environmental complexity experiments, testing the effects of experimental treatments on group polyandry.

Experiment	Full model	Dropped terms	Estimate	SE	Model no. and comparisons	AIC	Likelihood ratio test
Resource	Polyandry vs. Treatment random:(Female Group/Group) n(obs.) = 144, n(Female Group) = 6, n(Group) = 12 . var(Female Group) =0 , var(Female Group/Group) =0.082 (Poisson)				1	621.817	
		Treatment (Clumped)	0.021	0.187	1 vs. 2	619.83	$\chi2$=0.013, df=1, p>0.9
Complexity	Polyandry vs. Treatment random: (Female Group/Group) + (Female ID) n(obs.) = 96, n(Female Group) = 4, n(Group) = 8, n(Female ID)=47. var(Female Group) = 0 , var(Female Group/Group) = 0.001, var(Female ID)=0.018 (Poisson)				1	415.069	
		Treatment (Simple)	0.322	0.105	1 vs. 2	419.278	$\chi2$= 6.21, df=1, p<0.05

* Interaction
/ Nested factors

Table 13. GLMM results for resource distribution and environmental complexity experiments, testing the effects of experimental treatments on the mean number of copulation attempts experience by females.

Experiment	Full model	Dropped terms	Estimate	SE	Model no. and comparisons	AIC	Likelihood ratio test
Resource	Mean number mating attempts ~ Treatment random: (Female group ID/Group ID/Female ID)	-	-	-	1	1249.193	-
		Treatment (Clumped)	0.243	0.193	1 vs. 2	1248.789	$\chi 2$= 1.596, df=1, p>0.2
	n(obs.) = 288, n(Female Group) = 6, n(Group) = 12, n(Female ID) = 144.	-	-	-	-	-	-
	var(Female Group) = 1.6108303 , var(Female Group/Group) = 0.886, var(Female Group/Group /Female ID)=1.592 (Poisson)						
Complexity	Mean number mating attempts ~ Treatment random: (Female group ID/Group ID) + (Female ID)	-	-	-	1	782.605	-
		Treatment (Simple)	0.439	0.219	1 vs. 2	784.568	$\chi 2$=3.96, df=1, p<0.05
	n(obs.) = 191 n(Female Group) = 4, n(Group) = 8, n(Female ID) = 47.						
	var(Female Group) =0.147, var(Female Group/Group) = 0.108, var(Female ID)=1.883 (Poisson)						

* Interaction
/ Nested factor

Table 14. GLMM results for resource distribution and environmental complexity experiments, testing the effects of experimental treatments on the mean number of copulation attempts experience by females across all males.

Experiment	Full model	Dropped terms	Estimate	SE	Model no. and comparisons	AIC	Likelihood ratio test
Resource	Number mating attempts ~ Status (scaled) + Treatment random: (Female group ID/Group ID/Female ID) + (Male ID) + (dummy)	-	-	-	1	12800	-
		Status * Treatment (Clumped)	-0.08	0.035	1 vs. 2	12804	$\chi2$= 5.138, df=1, p<0.05
		Treatment (Clumped)	0.072	0.035	2 vs. 3	12805	$\chi2$=3.56, df=1, p=0.059
	n(obs.) = 2856, n(Female Group) = 6, n(Group) = 12, n(Male ID)=98, n(Female ID) = 144.	Status	0.219	0.053	2 vs. 4	12814	$\chi2$= 11.898,| df=1, p<0.001
		-	-	-	-	-	-
	var(Female Group) = 1.27E-06 , var(Female Group/Group) = 2.59E-01, var(Male ID)=5.95E-01, var(Female ID)=3.41E-05						
	(Poisson)						
Complexity	Number mating attempts ~ Status (scaled) + Treatment random: (Female group ID/Group ID/ Male ID) + (Female ID) + (dummy)	-	-	-	1	8285.2	-
		Status * Treatment (Simple)	0.022	0.039	1 vs. 2	8283.5	$\chi2$=0.305, df=1, p>0.5
		Treatment (Simple)	0.159	0.04	2 vs. 3	8296.7	$\chi2$=15.156, df=1, p<0.001
	n(obs.) = 1875 n(Female Group) = 4, n(Group) = 8, n(Male ID)=79, n(Female ID) = 47.	Status	0.014	0.08	2 vs. 4	8281.6	$\chi2$= 0.109, df=1, p>0.7
		-	-	-	-	-	-
	var(Female Group) =0.023, var(Female Group/Group) = 0.016 var(Female Group/Group /Male ID)=0.566, var(Female ID)=0.211						
	(Poisson)						

* Interaction
/ Nested factors
dummy =Individual level random effect to account for overdispersion

We also investigated whether male-female interactions differed qualitatively across treatments for both experiments. To do so, we first assessed the level of resistance expressed by females and estimated the effect of resource distribution and environmental complexity treatments on the level of female resistance. We

investigated whether male social status affected the level of female resistance and whether the shape of the relationship between male social status and female resistance changed with experimental treatments. In the resource distribution experiment, we found that females on average demonstrated more resistance in the clumped resource treatment and higher-ranking males experienced lower resistance (Table 15). There was no significant interaction between treatment and male status in resource experiments. For the environmental complexity experiment there was no difference in the mean level of female resistance across treatments but again higher-ranking males encountered significantly lower resistance (Table 15). We also identified a significant interaction between male social status and female resistance in the environmental complexity experiment: the negative relationship between male social status and female resistance became stronger in the simple, rather than in the complex environment treatment, suggesting that an increment in social status resulted in a greater reduction in female resistance in simpler environments.

Table 15. GLMM results assessing the effect of male social status and experimental treatment on female resistance for resource distribution and experimental complexity experiments,

Experiment	Full model	Dropped terms	Estimate	SE	Model no. and comparisons	AIC	Likelihood ratio test
Resource	Resistance Score ~ Status + Treatment random: (Female group ID/Group ID/Female ID) + (Male ID) n(obs.) = 2123, n(Female Group) = 6, n(Group) = 12, n(Male ID)=107, n(Female ID) = 144. var(Female Group) = 0.031, var(Female Group/Group) = 8.3E-15, var(Male ID)=0.012 var(Female group ID/Group ID/Female ID)=0.051 (Poisson)	-	-	-	1	5981.4	-
		Status * Treatment (Clumped)	-4.70E-05	0.002	1 vs. 2	5979.4	$\chi2$= 3e-04, df=1, p>0.9
		Treatment (Clumped)	-0.003	0.041	2 vs. 3	5977.4	$\chi2$=0.007, df=1, p>0.9
		Status	-0.009	0.002	2 vs. 4	6000.0	$\chi2$= 22.58, df=1, p<0.001
Complexity	Resistance Score ~ Status + Treatment random: (Female group ID/Group ID/Male ID) + (Female ID) n(obs.) = 1458, n(Female Group) = 4, n(Group) = 8, n(Male ID)=79, n(Female ID) = 47. var(Female Group) =1.31E-13 , var(Female Group/Group) = 0.016, var(Female Group ID/Group ID/Male ID)=0.174, var(Female ID)=0.039 (Poisson)	-	-	-	1	4023.6	-
		Status * Treatment (Simple)	-0.005	0.002	1 vs. 2	4025.5	$\chi2$= 3.878, df=1, p<0.05
		Treatment (Simple)	0.054	0.048	2 vs. 3	4024.6	$\chi2$=1.088, df=1, p>0.2
		Status	-0.009	0.003	2 vs. 4	4041.4	$\chi2$= 17.866, , df=1, p<0.001

* Interaction
/ Nested factors

We next assessed how experimental treatments may affect direct male-male interactions. Firstly, we assessed whether treatments influenced the probability that a competitor male attempted to interrupt any copulation attempt by the focal male, as this may drive changes in mating system and polyandry. There was a significant effect of treatment on the probability that a competitor male attempted to interrupt a copulation attempt of a focal male for both resource distribution and environmental

complexity experiments, such that clumped resources and simple environment treatments, both increased the risk of copulation disruption. This is important as attempted copulation interruptions reduce the probability of copulation success (Appendix§2 Table A9).

Table 16. GLMM results assessing the effect of male social status and experimental treatment on the probability that a copulation attempt receives an attempted interruption by a rival male for resource distribution and experimental complexity experiments.

Experiment	Full model	Dropped terms	Estimate	SE	Model no. and comparisons	AIC	Likelihood ratio test
Resource	Probability of interruption ~ Status * Treatment random: (Female group ID/Group ID/Female ID) + (Male ID) n(obs.) = 10895 n(Female Group) = 6, n(Group) = 12, n(Female ID) = 144, (Male ID) = 107 var(Female Group) = 0 , var(Female Group/Group) = 0.1052407, var(Female Group/Group/Female ID)=0.11 (Male ID) =0.123 (Binomial)	-	-	-	1	10969.0	-
		Status * Treatment (Clumped)	-0.002	0.002	1 vs. 2	10968.0	$\chi2$=0.981, df=1, p>0.3
		Treatment (Clumped)	0.227	0.05	2 vs. 3	10987.0	$\chi2$=20.214, df=1, p<0.001
		Status	-0.001	0.003	2 vs. 4	10968.0	$\chi2$=1.758, df=1, p>0.1
Complexity	Probability of interruption ~ Status * Treatment random: (Female group ID/Group ID/Male ID) + (Female ID) n(obs.) = 6667, n(Female Group) = 4, n(Group) = 8, n(Female ID) = 47, Male ID = 79 var(Female Group) = 0.016, var(Female Group/Group) = 0.014, (Female Group/Group/Male ID)=0.136, var(Female ID)=0.181 (Binomial)	-	-	-	1	8263.8	-
		Status * Treatment (Simple)	-0.007	0.003	1 vs. 2	8268.3	$\chi2$= 6.447, df=1, p<0.05
		Treatment (Simple)	0.141	0.055	2 vs. 3	8272.3	$\chi2$= 6.056, df=1, p<0.05
		Status	0.001	0.003	2 vs. 4	8267.7	$\chi2$=1.449, df=1, p>0.2

* Interaction
/ Nested factors

For intrasexual interactions, we first assessed potential changes in the structure of male-male social hierarchies by comparing both the linearity and the steepness of male social hierarchies across treatments for both experiments because such changes in social structure may mediate changes in selection on status. Male social hierarchies did not vary in either steepness or linearity across treatments in both resource distribution and environmental complexity experiments (Table 17 & 18).

Table 17. GLMM results for assessing the effect of treatment on the linearity of male social hierarchies for both resource distribution and environmental complexity experiments.

Experiment	Full model	Dropped terms	Estimate	SE	Model no. and comparisons	AIC	Likelihood ratio test
Resource	Linearity ~ Treatment + Female Group ID random: (Group ID) n(obs.) = 24, n(Group) = 12 var(Group) =0.02 (Gaussian)	Treatment (Clumped)	0.06	0.046	1 1 vs. 2	-10.748 -11.072	$\chi2$=1.676, df=1, p>0.1
Complexity	Linearity ~ Treatment + Female Group ID random: + (Group ID) n(obs.) = 16 n(Female Group) = 4, n(Group) = 8 var(Group) = 0.042 (Gaussian)	Treatment (Simple)	-0.019	0.0424	1 1 vs. 2	-5.937 -7.719	$\chi2$=0.218, df=1, p>0.6

Table 18. GLMM results for assessing the effect of treatment on the steepness of male social hierarchies for both resource distribution and environmental complexity experiments.

Experiment	Full model	Dropped terms	Estimate	SE	Model no. and comparisons	AIC	Likelihood ratio test
Resource	Steepness ~ Treatment + Female Group ID random: + (Group ID) n(obs.) = 24, n(Group) = 12 var(Group) =0.005 (Gaussian)				1	-29.116	
		Treatment (Clumped)	0.036	0.037	1 vs. 2	-30.127	$\chi2$=0.989, df=1, p>0.3
Complexity	Steepness ~ Treatment + Female Group ID random: (Group ID) n(obs.) = 16, n(Group) = 8 var(Group) = 0.017 (Gaussian)				1	-22.516	
		Treatment (Simple)	0.014	0.024	1 vs. 2	-24.145	$\chi2$=0.371 df=1, p>0.5

We then compared the frequency of aggressive interactions between males across treatments for both experiments. The overall density of male-male interaction networks did not vary significantly with treatments in either the resource distribution or the environmental complexity experiment, suggesting the number of males interacting aggressively with each other did not change across treatments for both resource and environmental complexity experiments. We then used a more detailed analysis to compare both the number males with which individuals displayed aggression and the total number of aggressive interactions expressed between those males. We found no significant differences in the number of aggressive interactants or the total number of aggressive behaviours displayed across males. Figure 7 shows two typical male-male interaction networks from the two experiments.

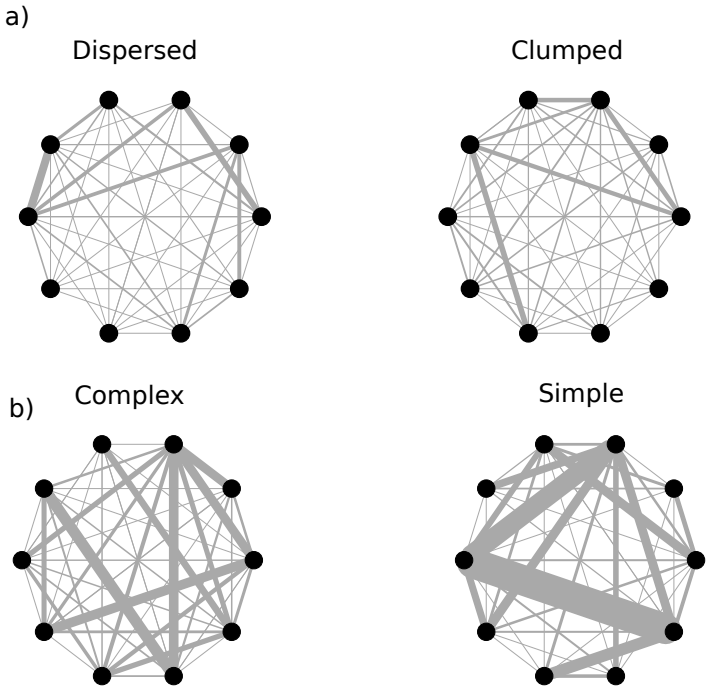


Figure 7. Example male-male aggressive interaction networks for (a) resource distribution experiments and (b) environmental complexity experiments. Within each experiment the same group of males is shown in both treatments. Black nodes represent males and edge thickness is proportional to the summed number of aggressive interactions between males.

Table 19. GLMM results assessing the effect of treatment on the density of male-male aggressive interaction networks for both resource distribution and environmental complexity experiments.

Experiment	Full model	Dropped terms	Estimate	SE	Model no. and comparisons	AIC	Likelihood ratio test
Resource	Density ~ Treatment + Female Group ID random: (Group ID) n(obs.) = 24, n(Group) = 12 var(Group) =0.002 (Gaussian)				1	-71.841	
		Treatment (Clumped)	0.006	0.012	1 vs. 2	-73.59	$\chi2$=0.251, df=1, p>0.6
Complexity	Density ~ Treatment + Female Group ID random: (Group ID) n(obs.) = 16, n(Group) = 8 var(Group) = 0.001 (Gaussian)				1	-45.983	
		Treatment (Simple)	-0.02	0.018	1 vs. 2	-46.714	$\chi2$=1.269, df=1, p>0.2

Table 20. GLMM results assessing the effect of treatments on the number of males with which individual males acted aggressively (degree) and the total number of aggressive behaviours across those males (strength) both resource distribution and environmental complexity experiments.

Experiment	Response	Full model	Dropped terms	Estimate	SE	Model no. and comparisons	AIC	Likelihood ratio test
Resource	Degree	Treatment random: (Female group/Group) + (Male ID) n(obs.) = 240, n(Female Group)=6, n(Group) = 12 , n(Male ID)=107 var(Female Group)=0, var(Female Group/Group) = 0 , var(Male ID)=0.309 (Poisson)	 Treatment (Clumped)	 0.013	 0.061	1 1 vs. 2	1082.312 1080.359	 $\chi2$=0.047, df=1, p>0.8
Complexity		Treatment random: (Female group/Group) + (Male ID) n(obs.) = 160, n(Female Group)=4,(Female Group/Group) = 8 , n(Male ID)=79 var(Female Group)=0, var(Female Group/Group) = 0, var(Male ID)=0.381 (Poisson)	- Treatment (Simple)	 -0.047	 0.075	1 1 vs. 2	724.183 722.556	 $\chi2$=0.373, df=1, p>0.5 -
Resource	Strength	Treatment random: (Female Group/Group) + (Male ID) + (dummy) n(obs.) = 240, n(Female Group)=6, n(Group) = 12 , n(Male ID)=107 var(Female Group)=2.95E-10, var(Female Group/Group) = 0.31 , var(Male ID)=1.81 (Poisson)	- Treatment (Clumped)	 0.057	 0.098	1 1 vs. 2	2235.281 2233.611	 $\chi2$=0.33, df=1, p>0.5
Complexity		Treatment random: (Female Group/Group) + (Male ID) + (dummy) n(obs.) = 160, n(Female Group)=4, n(Group) = 8 , n(Male ID)=79 var(Female Group)=0, var(Female Group/Group) = 0 , var(Male ID)=2.2352 (Poisson)	 Treatment (Simple)	 0.063	 0.083	1 1 vs. 2	1405.868 1404.435	 $\chi2$=0.567, df=1, p>0.4

/ Nested factors
dummy =Individual level random effect to account for overdispersion

FEMALE FECUNDITY

To assess the potential effects of changes in environmental conditions we first investigated how fecundity may relate to intersexual interactions separately for data from each experiment pooled across treatments. We found that females that received more attempts had higher fecundity and this relationship was significant for groups in both resource distribution and environmental complexity experiments. Furthermore, there was a marginally non-significant tendency for those females presenting the highest resistance to copulations to have lower fecundity in both the resource distribution and the environmental complexity experiment (Table 21). We also found a tendency for female fecundity to decrease in clumped resources and in simple environments (Table 22, Figure 8), however there was no evidence that either environmental manipulation changed the relationship between female fecundity and the number of mating attempts or mean female resistance (Appendix§2 table A10) or an interaction between treatment. Similarly, we found no significant effect of treatment for either experiment on the average mass of eggs across females (Table 23, Figure 8).

Table 21. GLMM results demonstrating the relationship between female fecundity and the total number of mating attempts females received and the average resistance expressed by females to copulation attempts across pooled data from all 10 days of trials for both resource distribution and environmental complexity experiments.

Experiment	Full model	Dropped terms	Estimate	SE	Model no. and comparisons	AIC	Likelihood ratio test
Resource	Fecundity ~ Total mating attempts (scaled) + Mean resistance random: (Female group ID/Group ID/Female ID)				1	622.55	
		Total mating attempts	0.223	0.067	1 vs. 2	623.73	$\chi2$=10.621, df=1, p<0.005
	n(obs.) = 144, n(Female Group) = 6, n(Group) = 12, n(Female ID) = 144. var(Female Group) = 0.029, var(Female Group ID/ Group ID) = 0.015 var(Female Group/Group/Female ID)=0.105 (Poisson)	Mean resistance	-0.249	0.137	1 vs. 3	631.17	$\chi2$=3.181, df=1, p= 0.074
Complexity	Fecundity ~ Total mating attempts (scaled) + Mean resistance random: (Female group ID/Group ID) + (Female ID)				1	423.64	
		Total mating attempts	0.183	0.063	1 vs. 2	424.76	$\chi2$=7.979, df=1, p<0.005
	n(obs.) = 96 n(Female Group ID) = 4, n(Group ID) = 8, n(Female ID) = 47. var(Female Group) =0 , var(Female Group/Group) = 0, var(Female ID)=0.071 (Poisson)	Mean resistance	-0.242	0.139	1 vs. 3	429.62	$\chi2$= 3.121, df=1, p= 0.077

/ Nested factors

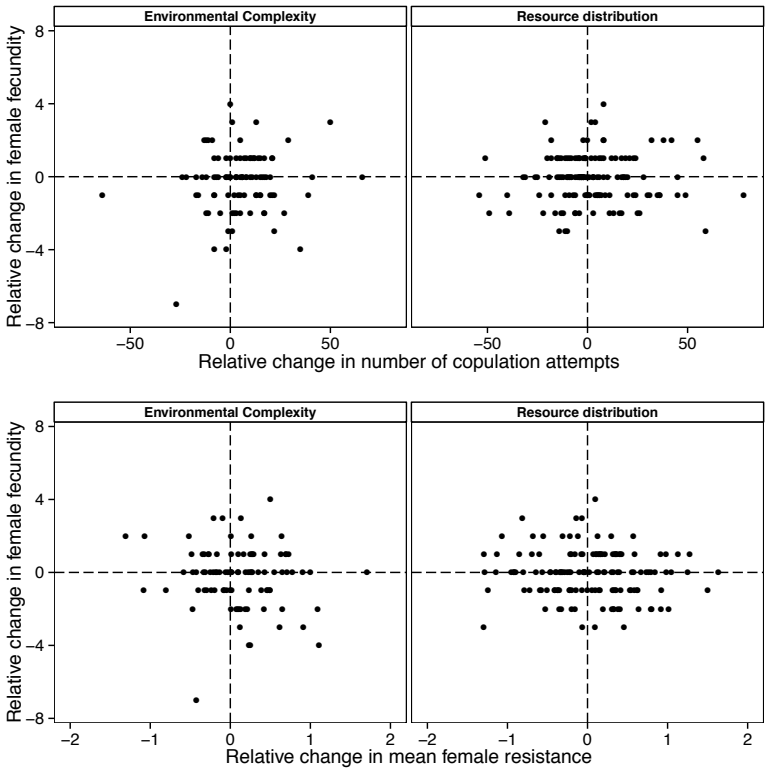


Figure 8 Panels show the difference in fecundity between treatments in resource experiments and complexity experiments against relative changes in the total number of mating attempts females received and the average resistance expressed by females to copulation attempts across pooled data from all 10 days of trials. For resource distribution plots relative changes are calculated as clumped minus distributed and for environmental complexity experiments as simple minus complex.

Table 22. GLMM results demonstrating the effect of treatments on female fecundity for both resource distribution and environmental complexity experiments.

Experiment	Full model	Dropped terms	Estimate	SE	Model no. and comparisons	AIC	Likelihood ratio test
Resource	Fecundity ~ Treatment random: (Female group ID/Group ID/Female ID) n(obs.) = 288, n(Female Group) = 6, n(Group) = 12, n(Female Group Female ID) = 0.118 var(Female Group) = 0.073 var(Female Group/Group) = 0.033, var(Female Group/Group/Female ID)=0.105 (Poisson)	Treatment (Clumped)	-0.06	0.091	1 1 vs. 2	915.305 913.724	$\chi 2$=0.419, df=1, p>0.5
Complexity	Fecundity ~ Treatment random: (Female group ID/Group ID) + (Female ID) n(obs.) = 192 n(Female Group) = 4, n(Group) = 8, n(Female ID) = 47. var(Female Group) =0 , var(Female Group/Group) = 0, var(Female ID)=0.09 (Poisson)	Treatment (Simple)	-0.132	0.102	1 1 vs. 2	656.928 656.578	$\chi 2$=1.65, df=1, p>0.1

/ Nested factors

Table 23. GLMM results demonstrating the effect of treatments on female egg mass for both resource distribution and environmental complexity experiments.

Experiment	Full model	Dropped terms	Estimate	SE	Model no. and comparisons	AIC	Likelihood ratio test
Resource	Egg Mass ~ Treatment random: (Female group ID/Group ID/Female ID) n(obs.) = 468 n(Female Group) = 6, n(Group) = 12, n(Female ID) = 125 var(Female Group) = 0 , var(Female Group/Group) = 0, var(Female Group/Group/Female ID)=8.056 (Gaussian)				1	2438.28	
		Treatment (Clumped)	0.222	0.26	1 vs. 2	2437.01	$\chi 2$=0.725, df=1, p>0.3
Complexity	Egg Mass ~ Treatment random: (Female group ID/Group ID) + (Female ID) n(obs.) = 377, n(Female Group) = 4, n(Group) = 8, n(Female ID) = 43. var(Female Group) =0.546 var(Female Group/Group) = 0.06, var(Female ID)=5.819 (Gaussian)				1	1803.35	
		Treatment (Simple)	-0.398	0.247	1 vs. 2	1803.95	$\chi 2$=2.603, df=1, p>0.1

/ Nested factors

DISCUSSION

In this study we used an experimental approach to examine the potential for two key ecological parameters, resource distribution and environmental complexity, to modify the operation of sexual selection on males as well as mating patterns, intersexual interactions and male-male interactions. Finally, we sought to quantify the potential effect of both ecological factors on female fecundity. Overall, our results indicate that both ecological parameters have the potential to vary intersexual interactions but suggest only the effect of environmental complexity treatments were strong enough to vary mating patterns and the operation of selection on males.

RESOURCE DISTRIBUTION

Theory predicts that when resources essential to reproduction are defendable in space and/or time, this provides opportunity for a limited subset of individuals to control access to these resources and the reproductive opportunities they confer (Emlen and Oring 1977). Specifically, with respect to sexual selection, theory predicts greater variance in male reproductive success and selection on those traits that confer monopoly when those resources are defendable. Predictions implicitly assume an increase of the importance of precopulatory sexual selection, as males that control resources will gain increased mating rights to those females associated with resources. In our experiments the extent of female polyandry did not change with resource distribution suggesting that monopolisation of females did not vary with treatments. In agreement with this, we found no effect of resource distribution on the magnitude or the relative importance of fitness components as contributors to total variation in reproductive success. Furthermore, our results show that the slopes of Bateman gradients and the strength of phenotypic selection on male social status did not change across

treatments, demonstrating that the absolute strength of sexual selection through both pre- and post-copulatory components was unaffected. Interestingly however, we did find significant changes in the patterns of intersexual interactions. Both the number of attempts that females received increased in clumped resources compared with dispersed resources and the positive relationship between status and the number of copulation attempts became flatter in clumped resources. Demonstrating that the number of copulation attempts increased across all males, however this did not result in an increase in polyandry. These potential differences may be driven by greater proximity of birds in larger groups when feeding in one location in clumped resources as previous work has showed that an increase in male density can increase the level of male-female harassment (Rowe 1992; Gosden and Svensson 2009). We also found that the number of copulation interruptions increased in clumped resources. This could be explained by clumped resources increasing proximity and/or chances of interacting with conspecifics increasing the probability of interruptions. Such effects have been demonstrated in the European bitterling, *Rhodeus amarus*, where the availability of spawning sites modified local male densities and high local male densities decreased the number of successful spawning attempts due to more courtship interruptions (Reichard et al. 2004). This difference in interruption rate may in part explain why changes in mating attempts do not translate into changes in mating patterns and sexual selection, as increases in interruptions may cancel out the respective increase in number of copulations attempts.

Our results therefore do not confirm the role of resource distribution in shaping the strength of sexual selection in Red junglefowl. This is in conflict with previous work in other species demonstrating variation in sexual selection with resource distribution (Twiss et al. 2007; Reichard et al. 2009; Mück et al. 2013). Two potential reasons for this lack of effect include the resource used and the spatial scale over which resources were distributed. In junglefowl food resources are a relevant component of courtship, demonstrated by male

courtship feeding of females (Stokes 1971; Evans and Evans 1999; Pizzari 2003). However, courtship feeding usually involves animal prey and novel food novel items (Stokes 1971) and so the common and abundant feed used in this experiment may be unlikely to play a similar role. Furthermore, the foraging range of junglefowl flocks is typically larger in nature than that allowed by the experimental enclosure of our study, which may have not been large enough to sufficiently replicate dispersed resources in this species (Collias and Collias 1967).

ENVIRONMENTAL COMPLEXITY

By changing the environment in which sexual interactions occur, environmental complexity is expected to modify the pattern of sexual interactions themselves, which may in turn be reflected in changes in sexual selection (Edenbrow et al. 2011; Candolin and Wong 2012; Candolin and Vlieger 2013). Our results show that environmental complexity significantly alters the pattern of male-female sexual interactions but not direct male-male aggressive interactions. We show that in simple environments female junglefowl are significantly more polyandrous than in complex environments. Furthermore in simple environments females receive more copulation attempts than in complex environments and this effect is stronger than in resource distribution experiments, and this may be the of driver of the increase in female polyandry. Despite the increase in polyandry we do not however find an increase in the relative contribution of *M* and *P*, although we did detect a marginally non-significant increase in remating rates of males. This may be because overall levels in polyandry were high in both environments and the magnitude in changes in variation in *M* and *P* with further increases in polyandry are expected to be reduced for already high levels of polyandry (McDonald and Pizzari, Chapter 5). We do however find that sexual selection male social status, a trait that provides exclusive access to mating in junglefowl (Collet et al. 2012), reduces in simple environments and this also leads to a moderate reduction in total

sexual selection on male social status. Similarly, we find that manipulations of environmental complexity affect the number of copulation interruptions, such that in simple environments copulation attempts are more likely to have a rival attempt to disrupt them, but this increase is not enough to prevent an increase in female promiscuity.

The stronger selection on male social status in complex environments may be mediated by female choice. In some species more structurally complex environments may change female behaviour, either by providing seclusion for females to express mating preferences, minimising the risk of responses from social partners or by reducing the effectiveness of male mate guarding (Westneat and Sherman 1997; Mays and Ritchison 2004; Tryjanowski et al. 2007). Female fowl can solicit copulations from males and are more likely to do so in scenarios where the potential for male harassment is reduced (Løvlie and Pizzari 2007). Taken in combination with female preference for socially dominant males (Pizzari and Birkhead 2000), this may increase selection on male social status in complex environments. However, if this was the driver we may expect resistance also to be lower in complex environments (Løvlie and Pizzari 2007). Instead we find that dominant males face proportionally less resistance in simple compared with complex environments, which may be expected to increase selection on status in simple environments. However, this level of resistance is also linked to the strength of male interest in copulating with females and may be confounded by more frequent but less intense copulation attempts in simple environments. The increase in polyandry is perhaps more likely explained by the increase copulation attempts overall. This potential increase in female harassment may also explain the decrease in female resistance towards more social dominant males in complex environments if harassment drives convenience polyandry (Rowe 1992). Along with the increase in harassment we also find a reduction in egg mass and female fecundity in simple environments. Male harassment can affect females by reducing feeding rates, energy

expenditure and via physical damage (Magurran and Seghers 1994; Galliard et al. 2005; Eldakar et al. 2009; Eldakar and Gallup 2011), potentially reducing group productivity. Furthermore, harassment may disrupt the ability of females to express social preferences and/or increase female-female aggression (Darden et al. 2009). However, in our experiment we actually find a positive relationship between male copulation attempts and female fecundity, but this is most likely may be driven by male choice for more fecund females (Collet et al. 2014). Furthermore, effects of harassment on female fecundity may increase over longer periods and may be underestimated in this study as experiments only last for a relatively short period. Studies should therefore seek to measure such fecundity costs of longer more potentially relevant time scales.

CONCLUSIONS

Overall, these results suggest that both resource distribution and environmental complexity may shape the pattern of male-female interactions but that the magnitude of these effects are much stronger for variation in environmental complexity., However, effects on mating systems and female fecundity were limited, which may be in part due to a lower power to detect differences across groups due to limited number of replicate groups in this estudy. Despite this we found that the complexity of environments may drive changes in female polyandry and phenotypic sexual selection on males. Our also results also suggest that such changes in intersexual interactions mediated by environmental structure, could lead to reduced female fecundity. Such effects may have implications for group productivity (Eldakar and Gallup 2011; Carazo et al. 2014) and conservation, if anthropogenic change shapes the structural complexity of environments (i.e. deforestation) (Tuomainen and Candolin 2011). Future studies investigating such effects should extend the period of analysis across multiple breeding seasons and if possible include measures of lifetime reproductive

success for both males and females, which was not possible in this study. This would facilitate a more robust test of the effects of manipulations on female fecundity and the consistency of manipulations in modifying patterns of selection across multiple selective episodes.

APPENDIX§1

Table A1. We assessed the potential for both resource and environmental complexity experiments to modify the structure of networks and change the potential transmission dynamics of sexually transmitted infections. To do this we a measure of modularity (Q) that measures the extent to which networks are can be split in to subcomponents composed of groups of individuals that are more connected to each other than they are to other members of the network (Newman 2006). Mating networks that are more modular may differ in the dynamics of infection spread, for example simulations studies suggest that modularity may slow the spread of parasites and reduce the peak prevalence of epidemics (Salathe and Jones 2010; Griffin and Nunn 2012). As a heuristic tool, typically modularity (Q) values of >0.3 are thought to represent meaningful modularity, whereas networks below this level are more homogenous without strong structure (Newman 2004). Using GLMs we found no significant differences in network modularity between treatments. Table shows GLM results demonstrating the effect of treatments on mating network modularity for both resource distribution and environmental complexity experiments

Experiment	Model	Parameter	Estimate	SE	*t*	*df*	p-value	95% CI
Resource	Modularity ~ Treatment + Female Group ID	Dispersed	0.388	0.136				L= 0.122, U= 0.654
		Clumped	0.412	0.136	0.153	5	>0.8	L=-0.275, U=0.322
Complexity	Modularity ~ Treatment + Female Group ID	Complex	0.217	0.134				L= -0.046, U= 0.480
		Simple	0.112	0.081	-0.984	3	>0.3	L= -0.314, U= 0.104

APPENDIX§2

Table A2 Results from GLMMs assessing the effect of treatments on the Opportunity for selection (I_T) and the Opportunity for sexual selection (I_s) for both resource distribution and environmental complexity experiments.

Experiment	Response	Fixed and Random effects	Parameter	Estimate	SE	Likelihood ratio test
Resource	Opprtunity for selection (I_T)	Fixed:Treatment, Random Effect: femalegroupID (n=8,var=0)	Dispersed	1.882	0.431	
			Clumped	1.545	0.609	$\chi2$=0.302, df=1, p>0.3
Complexity		Fixed: Treatment, Random Effect: femalegroupID (n=4,var=0.232)	Complex	1.927	0.353	
			Simple	1.73	0.395	$\chi2$=0.243, df=1, p>0.6
Resource	Opportunity for sexual selection (I_s)	Fixed: Treatment, Random Effect: femalegroupID (n=8,var=0)	Dispersed	0.46	0.135	
			Clumped	0.498	0.191	$\chi2$=0.04, df=1, p>0.8
Complexity		Fixed: Treatment, Random Effect: femalegroupID (n=4,var=0)	Complex	0.592	0.102	
			Simple	0.285	0.145	$\chi2$=3.58, df=1, p=0.058

Table A3 Results from GLMMs assessing the effect of treatments on the percentage contribution of *M*, *N* and *P* to the Opportunity for selection (I_T) for both resource distribution and environmental complexity experiments.

Experiment	Response	Model	Parameter	Estimate	SE	Likelihood ratio test
Resource	Percentage contribution M	Fixed: Treatment, Random Effect: femalegroupID (n=8,var=2.253)	Dispersed	20.25	6.641	
			Clumped	26.51	9.338	$\chi2$=0.438, df=1, p>0.5
Complexity		Fixed: Treatment, Random Effect: femalegroupID (n=4,var=0)	Complex	38.176	9.881	
			Simple	16.227	13.974	$\chi2$=2.15, df=1, p>0.1
Resource	Percentage contribution N	Fixed: Treatment, Random Effect: femalegroupID (n=8,var=6.633)	Dispersed	5.195	2.9	
			Clumped	11.186	3.749	$\chi2$=1.947, df=1, p>0.1
Complexity		Fixed: Treatment, Random Effect: femalegroupID (n=4,var=13.166)	Complex	5.279	3.638	
			Simple	7.64	4.621	$\chi2$=0.162, df=1, p>0.6
Resource	Percentage contribution P	Fixed: Treatment, Random Effect: femalegroupID (n=8,var=333.574)	Dispersed	41.545	26.655	
			Clumped	90.237	35.754	$\chi2$=1.684, df=1, p>0.1
Complexity		Fixed: Treatment, Random Effect: femalegroupID (n=4,var=1303.459)	Complex	46.878	26.814	
			Simple	76.474	29.977	$\chi2$=0.727, df=1, p>0.3

Table A4 Results from GLMMs assessing the effect of treatments on the percentage contribution of standardised covariances between *M*, *N* and *P* to the Opportunity for selection (I_T) for both resource distribution and environmental complexity experiments.

Experiment	Response	Fixed and Random effects	Parameter	Estimate	SE	Likelihood ratio test
Resource	Percentage contribution *Cov(M,N)*	Fixed: Treatment, Random Effect: femalegroupID (n=6,var0)	Dispersed	4.14	3.571	$\chi2$=3.07, df=1, p=0.08
			Clumped	13.589	3.571	
Complexity		Fixed: Treatment, Random Effect: femalegroupID (n=4,var0)	Complex	7.568	1.861	$\chi2$=6.247, df=1, p<0.05
			Simple	-0.528	1.861	
Resource	Percentage contribution *Cov(M,P)*	Fixed: Treatment, Random Effect: femalegroupID (n=6,var=333.656)	Dispersed	20.266	14.26	$\chi2$=2.908, df=1, p=0.088
			Clumped	-12.783	13.82	
Complexity		Fixed: Treatment, Random Effect: femalegroupID (n=4,var=322.969)	Complex	-11.756	18.195	$\chi2$=0.57, df=1, p>0.4
			Simple	6.638	18.367	
Resource	Percentage contribution *Cov(N,P)*	Fixed: Treatment, Random Effect: femalegroupID (n=6,var=106.728)	Dispersed	6.044	7.839	$\chi2$=4.478, df=1, p<0.05
			Clumped	-15.413	7.589	
Complexity		Fixed: Treatment, Random Effect: femalegroupID (n=4,var=0)	Complex	4.1	2.544	$\chi2$=0.467, df=1, p>0.4
			Simple	1.606	2.544	

Table A5 Results from GLMMs quantifying univariate Bateman gradients (β_{ss}) and the effect of treatments on the slope of β_{ss} for both resource distribution and environmental complexity experiments.

Experiment	Full model	Dropped terms	Estimate	SE	Model no. and comparisons	AIC	Likelihood ratio test
Resource	Reproductive Success vs. Mating Success & Treatment Full model, fixed term: $T \sim M$ * Treatment random:(Female Group/Group) + (Male ID) n(obs.) = 120, n(Female Group) = 6, n(Group) = 12, n(Male ID)=107 . var(Female Group) =0, var(Female Group/Group) = 0.023 var(Male ID)=0.263 (Poisson)				1	373.95	
		M * Treatment (Clumped)	0.061	0.066	1 vs. 2	372.81	$\chi2$= 0.863, df=1, p>0.3
		Treatment (Clumped)	-0.314	0.475	2 vs. 3	370.97	$\chi2$=0.161, df=1, p=0.689
		M	0.269	0.047	2 vs. 4	441.83	$\chi2$=71.019, df=1, p<0.001
Complexity	Reproductive Success vs. Mating Success & Treatment Full model, fixed term: $T \sim M$ * Treatment random: (Female Group/Group) + (dummy) n(obs.) = 80, n(Female Group) = 4, n(Group) = 8. var(Female Group) = 0 , var(Female Group/Group) = 0 (Poisson)				1	298.92	
		M * Treatment (Simple)	-0.037	0.094	1 vs. 2	297.07	$\chi2$= 0.155, df=1, p>0.6
		Treatment (Simple)	-0.223	0.666	2 vs. 3	297.9	$\chi2$=2.822, df=1, p= 0.09
		M	0.293	0.062	2 vs. 4	330.53	$\chi2$=35.456 df=1, p<0.001

Table A6 GLMMs results for the effect of treatment and male social status on male average remating for both resource distribution and environmental complexity experiments.

Experiment	Full model	Dropped terms	Estimate	SE	Model no. and comparisons	AIC	Likelihood ratio test
Resource Distribution	-	None			1	238.27	
-	Average remating rate ~ Dominance + Manipulation + Dominance * Manipulation + (Female group/Group) + (MaleID)	Status * Treatment (Clumped)	-0.009	0.006	2 vs. 1	238.41	$\chi2$=2.139, df=1, p>0.1
-	-	Treatment (Clumped)	0.056	0.123	3 vs2	236.65	$\chi2$=0.238, df=1, p>0.6
-	n(obs) =109, n(Group ID) = 12, n(femalegroupID) =6, n(MaleID) = 98	Status	0.006	0.005	4 vs 2	238.41	$\chi2$=0.151, df=1, p>0.6
Environmental Complexity	-	None			1	137.35	
-	Average remating rate ~ Dominance + Manipulation + Dominance * Manipulation + (Female group/Group)	Status * Treatment (Simple)	-0.012	0.007	2 vs. 1	138.73	$\chi2$=3.376, df=1, p=0.066
-	-	Treatment (Simple)	0.202	0.144	3 vs2	139.01	$\chi2$=2.281, df=1, p>0.1
-	n(obs) =73, n(Group ID) = 8, n(femalegroupID) =4	Status	0.011	0.005	4 vs 2	138.04	$\chi2$=1.319, df=1, p>0.2

Table A7 Results for GLMMS assessing the affect of treatment on the number of males attempting to copulate females for resource distribution and environmental complexity experiments.

Experiment	Full model	Dropped terms	Estimate	SE	Model no. and comparisons	AIC	Likelihood ratio test
Resource	Number males attempting ~ Treatment random: (Female Group ID/Group ID/Female ID) n(obs.) = 288, n(FemaleGroup) = 6, n(Group) = 12, n(FemaleID) = 144. var(FemaleGroup) = 0 , var(FemaleGroup/Group) = 0.025 var(FemaleID)=0 (Poisson)				1	1247.235	
		Treatment (Clumped)	0.001	0.043	1 vs 2	1245.236	$\chi2$= 0.0004, df=1, p>0.9
Complexity	Number males attempting ~ Treatment random: (Female Group ID/groupID) + (Female ID) n(obs.) = 191 n(FemaleGroup) = 4, n(Group) = 8, nn(FemaleID) = 47. var(FemaleGroup) =2.36E-04, var(FemaleGroup/Group) =0.007, var(FemaleID)=1.62E-08 (Poisson)				1	836.093	-
		Treatment (Simple)	0.04	0.052	1 vs 2	834.674	$\chi2$=0.581, df=1, p>0.4

/ Nested factor

Table A8 Results for GLMMS assessing the relationship between the mean number of copulation attempts of males across all females they attempted to mate and male mating success.

Experiment	Full model	Dropped terms	Estimate	SE	Model no. and comparisons	AIC	Likelihood ratio test
Resource	Mating Success ~ Mean number of attempts random: (femalegroupID/groupID/ FemaleID) + (Male ID) + (dummy) n(obs.) = 120 n(Female Group) = 6, n(Group) = 12, (Male ID) = 107 var(Female Group) = 2.84E-03 , var(Female Group/Group) = 1.65E-07, var(Male ID)=1.01E-02 (Poisson)	Mean number of attempts	0.063	0.007	1 1 vs. 2	578.042 644.576	- $\chi2$=68.534, df=1, p<0.001
Complexity	Mating Success ~ Mean number of attempts random: (femalegroupID/groupID/ FemaleID) + (Male ID) + (dummy) n(obs.) = 80 n(Female Group) = 4, n(Group) = 8. var(Female Group) =0, var(Female Group/Group) =0.02 (Poisson)	Mean number of attempts	0.088	0.01	1 1 vs. 2	416.819 484.512	 $\chi2$=69.693, df=1, p<0.001

/ Nested factor
dummy =Individual level random effect to account for overdispersion

Table A9 Model results for assessing the effect of whether a copulation attempt was interrupted by another male on the probability that the copulation attempt was completed successfully for pooled data across all 10 days for both resource distribution and environmental complexity experiments.

Experiment	Full model	Dropped terms	Estimate	SE	Model no. and comparisons	AIC	Likelihood ratio test
Resource	Probability of mating ~ Interrupted random: (femalegroupID/groupID/ FemaleID) + (Male ID) n(obs.) = 10895 n(Female Group) = 6, n(Group) = 12, n(FemaleID) = 144, n(Male ID) = 107 var(Female Group) = 0 , var(Female Group/Group) = 0.105, var(Female Group/Group/Female ID)=0.110, var(Male ID) =0.123 (Binomial)				1	9664.498	
		Not Interrupted	-2.008	0.108	1 vs. 2	10055.326	$\chi 2$=392.829, df=1, p<0.001
		Interrupted	-0.832	0.113			
Complexity	Probability of interruption ~ Status * Treatment random: (femalegroupID/groupID/ Male ID) + (Female ID) n(obs.) = 6667, n(Female Group) = 4, n(Group) = 8, n(Female ID) = 47, n(Male ID) = 79 var(Female Group) = 0.016, var(Female Group/Group) = 0.014, (Female Group/Group/Male ID)=0.136, var(Female ID)=0.181 (Binomial)				1	6476.107	
		Not Interrupted	-1.791	0.118	1 vs. 2	6581.312	$\chi 2$= 107.205, df=1, p<0.001
		Interrupted	-1.1078	0.121			

/ Nested factor

Table A10 GLMM model results testing whether the slope of female fecundity on mean female resistance and mean number of copulation attempts received by females differed across treatment in both resource distribution and environmental complexity experiments.

Experiment	Interaction term	Estimate	SE	$\chi 2$	p-vlue
Resource Distribution	Treatment*Mean number of attempts	-0.003	0.037	0.001	>0.9
	Treatment*Mean female resistance	-0.008	0.210	0.005	>0.9
Environmental Complexity	Treatment*Mean number of attempts	-0.056	0.047	0.74	>0.3
	Treatment*Mean female resistance	0.186	0.214	1.43	>0.2

CHAPTER 9. DISCUSSION

In thesis I set out to further our understanding of two broad questions, 1) why it is that sexual selection favours the evolution of particular traits and 2) why do the patterns of sexual selection on such traits differ between groups and populations? Specifically, I focused on the role that variation in intrasexual competitive structure, the non-random distribution of socio-competitive environments across individuals, plays in shaping variation in the operation of sexual selection both within and across populations. To achieve this, I used a combination of empirical and theoretical tools. This complimentary approach allowed me to generate predictions and develop methodologies as well as test these ideas in a more realistic biological setting. Across both these approaches one common theme was the use of sexual networks as descriptors of the sexual behaviour of populations. Throughout, this served as both a conceptual aid and a quantitative tool to explore the role of competitive structure in determining sexual selection. Below follows a brief summary of the main findings of this thesis.

MAIN FINDINGS

Chapter 2 began by detailing the complex relationship between population competitive structure and the operation of sexual selection. It introduced the role of the 'softness' of sexual selection, the scale at which selection operates, and the key modulators of this property of selection in structured populations. For example, how inter-group differences in competitive structure, in combination with sexual selection on males, can drive differences in the magnitude of sexual conflict between groups. This conflict in turn may impact on the productivity of groups, and act to harden

sexual selection, varying population-level patterns of sexual selection. Overall, this chapter illustrates how population subdivision in combination with dynamism in the softness of sexual selection may have broad implications for a variety of fundamental evolutionary processes, including mutation load and the maintenance of variation in fitness-related traits.

Building on this, chapters 3 and 4 developed quantitative methods, based on network techniques to explore, how variation in competitive structure can shape variation in the strength of pre- and post-copulatory episodes of sexual selection on phenotypic traits both between and within populations. In doing so, these chapters, more formally introduce female multiple mating (polyandry) as a modulator of competitive structure. The role of polyandry was further explored in chapters 5 and 6. Firstly, chapter 5 used a simulation approach to generate predictions of how variation in the extent of polyandry is expected to shape the relative roles of pre- and post-copulatory sexual selection, demonstrating a complex relationship that depends on both male promiscuity and sperm competitive mechanisms. This chapter also used quantitative network tools developed in chapter 4 to assess the effect of non-random variation in sperm competitive intensity (mating structure) within a population. In doing so, this chapter demonstrated that such mating structure can act in opposition to the effects of polyandry adding a further layer of complexity to differences between populations in the operation of pre- and post-copulatory sexual selection. With quantitative predictions regarding the role of polyandry and mating structure in hand, Chapter 6 moved on to test these predictions in the empirical model system Red junglefowl under experimental settings. This work largely confirmed predictions generated in chapter 5 under more realistic biological conditions.

Chapter 7 further developed the quantitative methods presented in chapter 3 to explore the role of competitive structure in shaping phenotypic sexual selection on competitive traits in male Red junglefowl. This chapter again used polyandry as a platform in which to study the postcopulatory competitive landscape experienced by males. In doing so, this chapter demonstrated that a male's sperm competitive environment can predict his fertilisation success and that competitive structure can influence sexual selection on male phenotypic traits. Furthermore, this chapter extended the methods developed in chapter 3 so as to increase the resolution at which male competitive environments are determined. In doing so, this analysis may provide a more sensitive approach, improving our interpretation of the important facets of competitive structure that drive social selection on male traits.

Finally, Chapter 8 explored the potential for environmental conditions to influence intergroup differences in competitive structure and how this may translate into variation in the operation of sexual selection. We used replicate groups of Red junglefowl and an experimental approach to manipulate two ecological aspects of the environment, resource distribution and the structural complexity of the environment. This study found that variation in both ecological factors (i.e. whether resources are clumped or distributed in space, and whether environments are structurally complex or simple) can shape the patterns of male-female interactions. However, only variation in environmental complexity varied the mating system by increasing polyandry in simple environments. This change in mating system was related to changes the strength of sexual selection on male phenotypic traits. Finally, this study also provided support for a potential reduction in female fecundity in simpler environments. This final point brings the thesis full circle to themes discussed in chapter 2 and expands on them by suggesting the potential ecological drivers of

variation among groups in competitive structure that not only vary the strength and direction of sexual selection, but also the softness of sexual selection through variation in group productivity

Overall this thesis describes a fundamental role of variation in competitive structure both within and between populations to shape intra- and inter-population patterns of sexual selection. In doing so it highlighted three routes through which competitive structure can vary including i) population structure, ii) polyandry, and iii) non-random variation in the intensity of competition. Taken together however, this work has demonstrated that all these routes are intertwined and fully understanding the role of one parameter, requires knowledge of the others.

THE DETERMINANTS OF COMPETITIVE STRUCTURE: A HOLISTIC PERSPECTIVE

Throughout this thesis one dominant theme to emerge is the role that female behaviour has in shaping the competitive landscape that individual males face. This is because, in structured populations, whether male intrasexual competition is local or global will largely rely on female movement between these groups of males (Chapter 2-4). Furthermore, the structure of the postcopulatory environment will rely on the pattern of polyandry across and within such groups of males. Therefore, the patterns of female movement and polyandry can both generate and homogenise pre- and post-copulatory competitive structure, whether males show strong or weak spatial aggregation (Chapter 7). However, the effect of polyandry is not limited to postcopulatory sexual selection, polyandry can also vary the importance of precopulatory competition by increasing or decreasing the importance of

precopulatory components of sexual selection (Chapters 5 & 6). Furthermore, polyandry also provides opportunity for non-random variation in the sperm competitive environment (Chapter 4-6), which can also act to shape the strength of precopulatory sexual selection. This potential is not only provided by polyandry, but simultaneously modulated by polyandry, because the extent of polyandry in any population also limits the scope for potential variation competitive structure within a population (fig. 1). This perspective shows that population structure, polyandry and non-random variation in competitive structure are intimately linked, and a thoughtful study of one route to intrasexual competitive structure will require knowledge of all three.

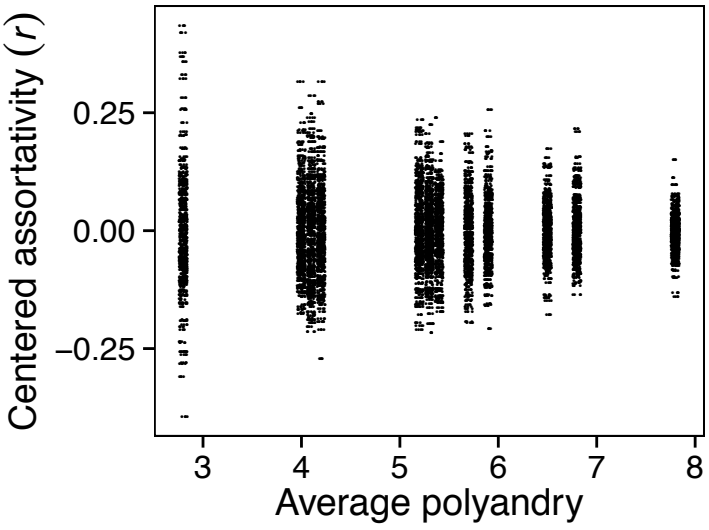


Figure 1. This figure shows randomisations of the empirical mating networks of 12 Red junglefowl groups as used in Chapter 6. For each group, we generated 1000 randomisations of the empirical network, holding the constant average polyandry. For each random randomisation, we calculated assortativity (*r*): the correlation between a male's mating success and the mean mating success of his partners. The plot shows that although polyandry first provides the potential for competitive structure, increasing levels of polyandry act to limit the potential variation in competitive structure by saturating the mating table.

STUDY LIMITATIONS AND CONSTRAINTS

Throughout this thesis we have used and developed a variety of novel quantitative network techniques or applied more well-known techniques in novel contexts. As with any quantitative technique these methods have limitations in both the context in which they can be successfully employed and the quality of data required for implementation. Furthermore, as with all empirical work, some aspects of our experimental research using the model system Red junglefowl (*Gallus gallus*) were limited by a number of practical constraints. In this section we highlight the some of the main limitations of the methods used in this thesis more broadly and the constraints imposed by the experimental model system in this thesis.

MATING STRUCTURE AND POLYANDRY

In chapters 5 and 6 we first apply quantitative techniques to measure mating structure of groups in terms of the covariance between male mating success and the mating success of his partners. In chapter 5 we use a simulation approach that provides idealised conditions in which we have complete knowledge of the mating patterns of males and females. In chapter 6 we then apply these methods to data collected from experimental groups of Red junglefowl (*Gallus gallus*). This model system also allows the collection of highly detailed copulation data as birds can be observed continuously, in close proximity and individuals are uniquely identifiable. However, many natural systems and even other experimental systems may not provide such comprehensive behavioural data. For example, in many systems copulations between males and females may not be observable directly but instead

inferred from genetic parentage assignment (Collet et al. 2014; Taylor et al. 2014). Determining male and female mating patterns in this way underestimate male and female promiscuity, and because the likelihood of inferring multiple sires increases with female clutch size, systematically bias estimates of promiscuity upwards for more fecund females (Collet et al. 2014). We suggest that estimating mating structure using genetic parentage alone as a means of determining male-female mating networks may reduce the interpretability of results and may not accurately characterize the distribution of sperm competitive environments across males. Instead, such genetic mating network structure may better represent the results of sperm competition rather than the competitive landscape itself. This may result in under- or overestimating the disassortativity or nestedness of a given mating network. This problem may be particularly exacerbated when average female clutch size is low e.g. when female clutch size is restricted to one egg, genetic parentage assignment removes completely the potential for female polyandry and any level of mating structure even when females are behaviourally polyandrous.

Similarly, when behavioural data does not accurately capture actual mating patterns, missing data between male and female pairs may result an underestimation of female polyandry and an erroneous mating network structure. Such effects may be particularly strong when unobserved matings are grouped across males of a given mating success e.g. because males with low mating success follow a particular reproductive tactic that is consistently less observable. The potential to for such issues to generate noise in measures of mating structure may be particularly large when mating networks are sparse when small variations in network structure may cause large variation in network measures (see section above). We therefore advise that the use of such methods requires detailed behavioural data on copulations, or genetic

parentage assignment only in those cases where genetic data is strongly representative of behavioural mating patterns. One potentially future research goal will be to assess the affect of missing data on the accuracy of estimates of network structure. This could be achieved by conducting a sensitivity analysis on either simulated or empirical data, where the researcher resamples their empirical networks assuming different sampling regimes. This would allow comparisons of simulated sampling regimes to empirical networks to estimate the effect of missing data on network measures.

The data requirements discussed above, although restrictive, are not insurmountable and there is great potential in some natural systems where detailed behavioural data sets along with genetic parentage are available including both vertebrate and invertebrate system (e.g. Preston et al. 2005; Rodríguez-Muñoz et al. 2010). Furthermore, a variety of experimental model systems provide scope to explore these methods in more controlled laboratory or semi-natural conditions (e.g. Branson et al. 2009; Reichard et al. 2009; Pélissié et al. 2014).

MULTILEVEL SELECTION AND DELINEATING COMPETITIVE GROUPS

In chapters 3, 4 and 7 we develop and then apply network methods to determine individual competitive environments. These methods can be used to define competitive groups for any episode of competition, including both pre- and post-copulatory intrasexual competition. Individual competitive groups arise as emergent properties based on knowledge of individual competitive interactions and do not require individuals to be arbitrarily designated into *a priori* groups. These methods therefore require detailed behavioural data of competitive interactions. As such these methods may not be applicable to all systems or to all selective episodes within a

particular system. For example, in chapter 7 we focus on postcopulatory sexual selection where defining what constitutes a competitive interaction may be more straightforward in this system, i.e. copulations are easily observed and copulations can largely be assumed to place two individuals in postcopulatory competition, although this also requires assumptions regarding sperm transfer and information about sperm storage. In other systems it may be difficult to observe copulations per say but it may be possible to observe direct male-male precopulatory competition e.g. in direct pairwise contest competition in butterflies or cactus bugs (Kemp and Wiklund 2001; Procter et al. 2012), fighting contests in horned beetles (Eberhard 1979) or intersexual competition via presence of males at the same lek (Höglund and Alatalo 1995). In other systems where competitive interactions are largely indirect, e.g. mediated by long range chemical signalling, then defining whether individuals are in competition and over which batches of ova, may be more difficult. In such cases it may be more suitable to define competitive group using other methods, such home range estimates (Formica et al. 2010, 2011).

Finally, it is important to note that in many cases, not all individuals may compete in observable interactions but may be excluded prior to rounds of selection, e.g. some males may never engage in direct contests but may be excluded from this stage of competition due to a prior round of more indirect competition. Therefore, in all cases when measuring selection and inferring the potential for sexual selection to modify trait distributions across generations it is important to interpret these measures in light of the trait distributions measured and individuals that may have already been excluded prior to the current round of competition (Hadfield 2008; Klug et al. 2010). A particularly useful approach may be to combine multilevel selection measures with population-level global selection analyses that includes those individuals that may

have already been excluded prior to the round of competition measured within the multilevel selection framework.

MODEL SYSTEM LIMITATIONS

Predicting the evolutionary trajectory of a trait requires knowledge of both the relationship between trait and fitness at the population level and the additive genetic variance in the trait (Falconer and Mackay 1996). For one breeding season we can therefore measure reproductive success and selection gradients, to predict the change in trait mean in the new generation. Importantly, these results refer only to the breeding season measured which may be a relatively short competitive window compared to the potential reproductive lifespan of the species. When individuals face life-history trade-offs or selection varies in direction or strength across or within breeding seasons then predictions for trait evolution based on one time unit may not be met due to opposing selection at a later stage (e.g. Preston et al. 2001). In our empirical model system Red junglefowl, we were not able to measure the lifetime reproductive success of individuals and therefore selection estimates refer only to one relatively short selective episode. This limits our ability to predict evolutionary change in traits in this system. However, the main goal of this thesis was to understand the circumstances in which variation in competitive structure may modify the strength and direction of selection rather than predicting evolutionary change *per se*. Future studies that seek to predict how changes in sexual selection due to competitive structure relate to rates of evolutionary change in quantitative traits should aim to measure reproductive success across multiple breeding seasons or across the entire life span of individuals (i.e. lifetime reproductive success). Similarly, in some chapters we investigate specifically one window of sexual selection, i.e.

postcopulatory sexual selection in chapter 7. However, predicting population level change in traits will depend on how precopulatory episodes of sexual selection oppose or reinforce postcopulatory sexual selection (see Hunt et al. 2009 for a similar discussion regarding inter and intrasexual episodes of sexual seleciton).

CONCLUSION

This thesis has demonstrated that studying variation in the structure of competitive environments is a useful approach to understand variation in sexual selection, principally on males. Presumably this approach and the techniques utilized, could be fruitful in many research areas including how competitive structure shapes both social and sexual selection on females and how variation in the structure of relatedness similarly shapes the evolution of reproductive strategies.

REFERENCES

Abramson, G., C. A. Trejo Soto, and L. Oña. 2011. The role of asymmetric interactions on the effect of habitat destruction in mutualistic networks. PLoS ONE 6:e21028.

Agrawal, A. F. 2010. Ecological determinants of mutation load and inbreeding depression in subdivided populations. Am. Nat. 176:111–22.

Agrawal, A. F., E. D. Brodie, and M. J. Wade. 2001. On indirect genetic effects in structured populations. Am. Nat. 158:308–323.

Alcock, J. 1994. Postinsemination associations between males and females in insects - the mate-guarding hypothesis. Annu. Rev. Entomol. 39:1–21.

Alemu, S. W., P. Bijma, S. H. Møller, L. Janss, and P. Berg. 2014. Indirect genetic effects contribute substantially to heritable variation in aggression-related traits in group-housed mink (*Neovison vison*). Genet. Sel. Evol. 46:30.

Almbro, M., and L. W. Simmons. 2014. Sexual selection can remove an experimentally induced mutation load. Evolution 68:295–300.

Almeida-Neto, M., P. Guimarães, P. R. Guimarães, R. D. Loyola, and W. Ulrich. 2008. A consistent metric for nestedness analysis in ecological systems: reconciling concept and measurement. Oikos 117:1227–1239.

Almeida-Neto, M., and W. Ulrich. 2011. A straightforward computational approach for measuring nestedness using quantitative matrices. Env. Model Softw 26:173–178.

Alonzo, S. H., and R. R. Warner. 2000. Allocation to mate guarding or increased sperm production in a mediterranean Wrasse. Am. Nat. 156:266–275.

Altmann, J. 1974. Observational study of behavior: sampling methods. Behaviour 49:227–267.

Andersson, M. 1994. Sexual selection. Princeton University Press, Princeton, NJ.

Andrade, M. C. B., and M. M. Kasumovic. 2005. Terminal investment strategies and male mate choice: extreme tests of Bateman. Integr. Comp. Biol. 45:838–47.

Aplin, L. M., D. R. Farine, J. Morand-Ferron, and B. C. Sheldon. 2012. Social networks predict patch discovery in a wild population of songbirds. Proc. R. Soc. B Biol. Sci. 279:4199–4205.

Araújo, M. S., P. R. Guimarães, R. Svanbäck, A. Pinheiro, P. Guimarães, S. F. dos Reis, and D. I. Bolnick. 2008. Network analysis reveals contrasting effects of intraspecific competition on individual vs. population diets. Ecology 89:1981–1993.

Arnold, S. J. 1994. Bateman's principles and the measurement of sexual selection in plants and animals. Am. Nat. 144:S126–S149.

Arnold, S. J., and D. Duvall. 1994. Animal mating systems: a synthesis based on selection theory. Am. Nat. 143:317–348.

Arnold, S., and M. Wade. 1984. On the measurement of natural and sexual selection: theory. Evolution 38:709–719.

Arnqvist, G. 1992. Spatial variation in selective regimes: sexual selection in the Water strider, *Gerris odontogaster*. Evolution 46:914–929.

Arnqvist, G., and T. Nilsson. 2000. The evolution of polyandry: multiple mating and female fitness in insects. Anim. Behav. 60:145–164.

Aronsen, T., A. Berglund, K. B. Mobley, I. I. Ratikainen, and G. Rosenqvist. 2013. Sex ratio and density affect sexual selection in a sex-role reversed fish. Evolution 67:3243–3257.

Ashby, B., and S. Gupta. 2013. Sexually transmitted infections in polygamous mating systems. Philos. Trans. R. Soc. B Biol. Sci. 368:1–11.

Bailey, N. W., and A. J. Moore. 2012. Runaway sexual selection without genetic correlations: social environments and flexible mate choice initiate and enhance the Fisher process. Evolution 66:2674–2684.

Bailey, N. W., and M. Zuk. 2012. Socially flexible female choice differs among populations of the Pacific field cricket: geographical variation in the interaction coefficient psi (Ψ). Proc. R. Soc. B Biol. Sci. 279:3589–96.

Bascompte, J., and P. Jordano. 2007. Plant-animal mutualistic networks: the architecture of biodiversity. Annu. Rev. Ecol. Evol. Syst. 38:567–593.

Bascompte, J., P. Jordano, C. J. Melián, and J. M. Olesen. 2003. The nested assembly of plant–animal mutualistic networks. Proc. Natl. Acad. Sci. U. S. A. 100:9383–9387.

Bateman, A. J. 1948. Intra-sexual selection in *Drosophila*. Heredity 2:349–368.

Bell, J. M., J. D. Karron, and R. J. Mitchell. 2005. Interspecific competition for pollination lowers seed production and outcrossing in *Mimulus ringens*. Ecology 86:762–771.

Benton, T. G., and M. R. Evans. 1998. Mate choice using correlation: measuring the effect of female sampling behaviour. Behav. Ecol. Sociobiol. 44:91–98.

Bertram, S. M., and R. Gorelick. 2009. Quantifying and comparing mating systems using normalized mutual entropy. Anim. Behav. 77:201–206.

Birkhead, T. R., and A. P. Møller. 1998. Sperm competition and sexual selection. Academic Press.

Birkhead, T. R., and A. P. Møller. 1993. Why do male birds stop copulating while their partners are still fertile? Anim. Behav. 45:105–118.

Birkhead, T. R., and T. Pizzari. 2002. Postcopulatory sexual selection. Nat. Rev. Genet. 3:262–73.

Birkhead, T. R., G. J. Wishart, and J. D. Biggins. 1995. Sperm precedence in the Domestic fowl. Proc. R. Soc. Lond. B Biol. Sci. 261:285–292.

Bolker, B. M., M. E. Brooks, C. J. Clark, S. W. Geange, J. R. Poulsen, M. H. H. Stevens, and J. S. S. White. 2009. Generalized linear mixed models: a practical guide for ecology and evolution. Trends Ecol. Evol. 24:127–135.

Borgia, G. 1979. Sexual selection and the evolution of mating sysems. Pp. 19–80 *in* M. S. Blum and N. A. Blum, eds. Sexual selection and reproductive competition in insects. Academic Press.

Boughman, J. W. 2001. Divergent sexual selection enhances reproductive isolation in sticklebacks. Nature 411:944–8.

Branson, K., A. a Robie, J. Bender, P. Perona, and M. H. Dickinson. 2009. High-throughput ethomics in large groups of *Drosophila*. Nat. Methods 6:451–7.

Brodie, E. D., A. Moore, and F. Janzen. 1995. Visualizing and quantifying natural selection. Trends Ecol. Evol. 10:313–318.

Brown, B. J., R. J. Mitchell, and S. A. Graham. 2002. Competition for pollination between an invasive species (Purple loosestrife) and a native congener. Ecology 83:2328–2336.

Burdfield-Steel, E. R., and D. M. Shuker. 2011. Reproductive interference. Curr. Biol. 21:R450–R451.

Candolin, U., and L. Vlieger. 2013. Estimating the dynamics of sexual selection in changing environments. Evol. Biol. 40:589–600.

Candolin, U., and B. B. M. Wong. 2012. Behavioural responses to a changing world: mechanisms and consequences. Oxford University Press, Oxford.

Carazo, P., C. K. W. Tan, F. Allen, S. Wigby, and T. Pizzari. 2014. Within-group male relatedness reduces harm to females in *Drosophila*. Nature 505:672–675.

Caruso, C. M. 2000. Competition for pollination influences selection on floral traits of Ipomopsis aggregata. Evolution 54:1546–1557.

Chapman, T., G. Arnqvist, J. Bangham, and L. Rowe. 2003. Sexual conflict. Trends Ecol. Evol. 18:41–47.

Charlesworth, D., and J. H. Willis. 2009. The genetics of inbreeding depression. Nat. Rev. Genet. 10:783–796.

Childress, D., and D. L. Hartl. 1972. Sperm preference in *Drosophila melanogaster*. Genetics 71:417–427.

Christley, R. M., G. L. Pinchbeck, R. G. Bowers, D. Clancy, N. P. French, R. Bennett, and J. Turner. 2005. Infection in social networks: using network analysis to identify high-risk individuals. Am. J. Epidemiol. 162:1024–1031.

Clark, D. C., S. J. DeBano, and A. J. Moore. 1997. The influence of environmental quality on sexual selection in *Nauphoeta cinerea* (Dictyoptera: Blaberidae). Behav. Ecol. 8:46–53.

Clutton-Brock, T. H. 1989. Review Lecture: Mammalian Mating Systems. Proc. R. Soc. B Biol. Sci. 236:339–372.

Clutton-Brock, T. H., and G. A. Parker. 1992. Potential reproductive rates and the operation of sexual selection. Q. Rev. Biol. 67:437–456.

Cogliati, K. M., A. F. Mistakidis, J. R. Marentette, A. Lau, B. M. Bolker, B. D. Neff, and S. Balshine. 2014. Comparing population level sexual selection in a species with alternative reproductive tactics. Behav. Ecol. 1524–1533.

Colegrave, N., and G. D. Ruxton. 2003. Confidence intervals are a more useful complement to nonsignificant tests than are power calculations. Behav. Ecol. 14:446–447.

Collet, J. M., R. F. Dean, K. Worley, D. S. Richardson, and T. Pizzari. 2014. The measure and significance of Bateman's principles. Proc. R. Soc. B Biol. Sci. 281:20132973.

Collet, J., D. S. Richardson, K. Worley, and T. Pizzari. 2012. Sexual selection and the differential effect of polyandry. Proc. Natl. Acad. Sci. U. S. A. 109:8641–5.

Collias, N. ., E. C. Collias, D. Hunsaker, and L. Minning. 1966. Locality fixation, mobility and social organization within an unconfined population of red jungle fowl. Anim. Behav. 14:550–9.

Collias, N. E., and E. C. Collias. 1967. A field study of the Red junglefowl in north-central India. The Condor 69:360–386.

Collias, N. E., and E. C. Collias. 1996. Social organization of a red junglefowl, *Gallus gallus*, population related to evolution theory. Anim. Behav. 51:1337–1354.

Connor, E. F., and D. Simberloff. 1979. The assembly of species communities: chance or competition? Ecology 60:1132–1140.

Corner, L. A. L., D. U. Pfeiffer, and R. S. Morris. 2003. Social-network analysis of Mycobacterium bovis transmission among captive brushtail possums (*Trichosurus vulpecula*). Prev. Vet. Med. 59:147–167.

Cornwallis, C. K., Rebecca Dean, and T. Pizzari. 2014. Sex-specific patterns of aging in sexual ornaments and gametes. Am. Nat. 184:E66–E78.

Cornwallis, C. K., and T. Uller. 2010. Towards an evolutionary ecology of sexual traits. Trends Ecol. Evol. 25:145–52.

Coyne, J. A., and H. A. Orr. 2004. Speciation. Sinauer Associates, Sunderland, Mass.

Crepet, W. L., and K. J. Niklas. 2009. Darwin's second "abominable mystery": Why are there so many angiosperm species? Am. J. Bot. 96:366–381.

Croft, D., R. James, and J. Krause. 2008. Exploring animal social networks. Princeton University Press, Princeton, NJ.

Croft, D. P., R. James, A. J. W. Ward, M. S. Botham, D. Mawdsley, and J. Krause. 2005. Assortative interactions and social networks in fish. Oecologia 143:211–219.

Crooijmans, R. P., R. J. Dijkhof, J. J. van der Poel, and M. A. Groenen. 1997. New microsatellite markers in chicken optimized for automated fluorescent genotyping. Anim. Genet. 28:427–437.

Crooijmans, R. P., P. A. van Oers, J. A. Strijk, J. J. van der Poel, and M. A. Groenen. 1996. Preliminary linkage map of the chicken (*Gallus gallus domesticus*) genome based on microsatellite markers: 77 new markers mapped. Poult. Sci. 75:746–754.

Crook, J. H. 1965. The adaptive significance of avian social organisations. Symp Zool Soc Lond 14:181–218.

Crow, J. F. 1958. Some possibilities for measuring selection intensities in man. Hum. Biol. 30:1–13.

Crow, J. F., and M. Kimura. 2009. An introduction to population genetics theory. Blackburn Press.

Darden, S. K., R. James, I. W. Ramnarine, and D. P. Croft. 2009. Social implications of the battle of the sexes: sexual harassment disrupts female sociality and social recognition. Proc. R. Soc. B Biol. Sci. 276:2651–2656.

Darwin, C. 1859. On the Origin of Species. John Murray, London, UK.

Darwin, C. 1871. The descent of man and selection in relation to sex. John Murray, London, UK.

David, H. A. 1987. Ranking from unbalanced paired-comparison data. Biometrika 74:432–436.

Dawson, D. A., G. J. Horsburgh, C. Küpper, I. R. K. Stewart, A. D. Ball, K. L. Durrant, B. Hansson, I. Bacon, S. Bird, A. Klein, A. P. Krupa, J.-W. Lee, D. Martín-Gálvez, M. Simeoni, G. Smith, L. G. Spurgin, and T. Burke. 2010. New methods to identify conserved microsatellite loci and develop primer sets of high cross-species utility - as demonstrated for birds. Mol. Ecol. Resour. 10:475–494.

Dawson, D., S. McConnell, A. Wardle, M. Gibbs, and T. Burke. 1998. Characterization and mapping of 15 novel chicken microsatellite loci. Anim. Genet. 29:159–160.

Dean, R., C. K. Cornwallis, H. Løvlie, K. Worley, D. S. Richardson, and T. Pizzari. 2010. Male reproductive senescence causes potential for sexual conflict over mating. Curr. Biol. 20:1192–1196.

Dean, R., S. Nakagawa, and T. Pizzari. 2011. The risk and intensity of sperm ejection in female birds. Am. Nat. 178:343–354.

De Jong, K., S. Wacker, T. Amundsen, and E. Forsgren. 2009. Do operational sex ratio and density affect mating behaviour? An experiment on the two-spotted goby. Anim. Behav. 78:1229–1238.

De, P., A. E. Singh, T. Wong, W. Yacoub, and A. M. Jolly. 2004. Sexual network analysis of a gonorrhoea outbreak. Sex. Transm. Infect. 80:280–285.

De Vries, H., J. M. G. Stevens, and H. Vervaecke. 2006. Measuring and testing the steepness of dominance hierarchies. Anim. Behav. 71:585–592.

Doherty, P. F., G. Sorci, J. A. Royle, J. E. Hines, J. D. Nichols, and T. Boulinier. 2003. Sexual selection affects local extinction and turnover in bird communities. Proc. Natl. Acad. Sci. U. S. A. 100:5858–62.

Donohue, K. 2004. Density-dependent multilevel selection in the Great lakes sea rocket. Ecology 85:180–191.

Donohue, K. 2003. The influence of neighbor relatedness on multilevel selection in the Great Lakes sea rocket. Am. Nat. 162:77–92.

Dorus, S., P. D. Evans, G. J. Wyckoff, S. S. Choi, and B. T. Lahn. 2004. Rate of molecular evolution of the seminal protein gene SEMG2 correlates with levels of female promiscuity. Nat. Genet. 36:1326–9.

Dosen, L. D., and R. Montgomerie. 2004. Mate preferences by male guppies (*Poecilia reticulata*) in relation to the risk of sperm competition. Behav. Ecol. Sociobiol. 55:266–271.

Downhower, J. F., L. S. Blumer, and L. Brown. 1987. Opportunity for selection: an appropriate measure for evaluating variation in the potential for selection? Evolution 41:1395–1400.

Duncan, S. I., S. E. Riechert, B. M. Fitzpatrick, and J. A. Fordyce. 2010. Relatedness and genetic structure in a socially polymorphic population of the spider *Anelosimus studiosus*. Mol. Ecol. 19:810–818.

Dziminski, M. A., J. D. Roberts, M. Beveridge, and L. W. Simmons. 2010. Among-population covariation between sperm competition and ejaculate expenditure in frogs. Behav. Ecol. 21:322–328.

Eberhard, W. 1996. Female control: sexual selection by cryptic female choice. Princeton University Press.

Eberhard, W. 1979. The function of horns in Podischnus agenor (Dynastinae) and other beetles. Pp. 231–258 *in* M. Blum and N. Blum, eds. Sexual selection and reproductive competition in insects. Academic Press, New York, NY.

Eberhard, W. G. 2009. Postcopulatory sexual selection: Darwin's omission and its consequences. Proc. Natl. Acad. Sci. U. S. A. 106:10025–10032.

Edenbrow, M., S. K. Darden, I. W. Ramnarine, J. P. Evans, R. James, and D. P. Croft. 2011. Environmental effects on social interaction networks and male reproductive behaviour in guppies, *Poecilia reticulata*. Anim. Behav. 81:551–558.

Eldakar, O. T., M. J. Dlugos, J. W. Pepper, and D. S. Wilson. 2009a. Population structure mediates sexual conflict in Water striders. Science 326:816–816.

Eldakar, O. T., M. J. Dlugos, R. S. Wilcox, and D. S. Wilson. 2009b. Aggressive mating as a tragedy of the commons in the water strider *Aquarius remigis*. Behav. Ecol. Sociobiol. 64:25–33.

Eldakar, O. T., and A. C. Gallup. 2011. The group-level consequences of sexual conflict in multigroup populations. PLoS ONE 6:e26451.

Eldakar, O. T., D. S. Wilson, M. J. Dlugos, and J. W. Pepper. 2010. The role of multilevel selection in the evolution of sexual conflict in the water strider *Aquarius remigis*. Evolution 64:3183–3189.

Elston, D. A., R. Moss, T. Boulinier, C. Arrowsmith, and X. Lambin. 2001. Analysis of aggregation, a worked example: numbers of ticks on red grouse chicks. Parasitology 122:563–569.

Emlen, S., and L. Oring. 1977. Ecology, sexual selection and the evolution of mating systems. Science 197:215–223.

Endler, J. A., and A. E. Houde. 1995. Geographic variation in female preferences for male traits in *Poecilia reticulata*. Evolution 49:456–468.

Estevez, I., L. J. Keeling, and R. C. Newberry. 2003. Decreasing aggression with increasing group size in young domestic fowl. Appl. Anim. Behav. Sci. 84:213–218.

Etches, R. J. 1996. Reproduction in poultry. First edition. CABI, Wallingford, Oxon.

Evans, C. S., and L. Evans. 1999. Chicken food calls are functionally referential. Anim. Behav. 58:307–319.

Falconer, D. S., and T. F. C. Mackay. 1996. Introduction to quantitative genetics. 4 edition. Benjamin Cummings, Essex, UK.

Fang, Q., and S.-Q. Huang. 2013. A directed network analysis of heterospecific pollen transfer in a biodiverse community. Ecology 94:1176–1185.

Fichtenberg, C. M., S. Q. Muth, B. Brown, N. S. Padian, T. A. Glass, and J. M. Ellen. 2009. Sexual network position and risk of sexually transmitted infections. Sex. Transm. Infect. 85:493–498.

Fisher, R. A. 1930. The genetical theory of natural selection. Oxford University Press, Oxford, UK.

Flanagan, R. J., R. J. Mitchell, D. Knutowski, and J. D. Karron. 2009. Interspecific pollinator movements reduce pollen deposition and seed production in *Mimulus ringens* (Phrymaceae). Am. J. Bot. 96:809–815.

Formica, V. A., M. E. Augat, M. E. Barnard, R. E. Butterfield, C. W. Wood, and E. D. Brodie. 2010. Using home range estimates to construct social networks for species with indirect behavioral interactions. Behav. Ecol. Sociobiol. 64:1199–1208.

Formica, V. A., J. W. McGlothlin, C. W. Wood, M. E. Augat, R. E. Butterfield, M. E. Barnard, and E. D. Brodie. 2011. Phenotypic assortment mediates the effect of social selection in a wild beetle population. Evolution 65:2771–2781.

Formica, V. A., and E. M. Tuttle. 2009. Examining the social landscapes of alternative reproductive strategies. J. Evol. Biol. 22:2395–2408.

Formica, V. A., C. W. Wood, W. B. Larsen, R. E. Butterfield, M. E. Augat, H. Y. Hougen, and E. D. Brodie 3rd. 2012. Fitness consequences of social network position in a wild population of forked fungus beetles (*Bolitotherus cornutus*). J. Evol. Biol. 25:130–137.

Forstmeier, W., K. Martin, E. Bolund, H. Schielzeth, and B. Kempenaers. 2011. Female extrapair mating behavior can evolve via indirect selection on males. Proc. Natl. Acad. Sci. U. S. A. 108:10608–10613.

Fortuna, M. A., C. García, P. R. Guimarães, and J. Bascompte. 2008. Spatial mating networks in insect-pollinated plants. Ecol. Lett. 11:490–498.

Fortuna, M. A., D. B. Stouffer, J. M. Olesen, P. Jordano, D. Mouillot, B. R. Krasnov, R. Poulin, and J. Bascompte. 2010. Nestedness versus modularity in ecological networks: two sides of the same coin? J. Anim. Ecol. 79:811–817.

Freeman-Gallant, C. R., N. T. Wheelwright, K. E. Meiklejohn, S. L. States, and S. V. Sollecito. 2005. Little effect of extrapair paternity on the opportunity for sexual selection in Savannah sparrows (*Passerculus sandwichensis*). Evolution 59:422–430.

Froman, D. P., T. Pizzari, A. J. Feltmann, H. Castillo-Juarez, and T. R. Birkhead. 2002. Sperm mobility: mechanisms of fertilizing efficiency, genetic variation and phenotypic relationship with male status in the domestic fowl, *Gallus gallus domesticus*. Proc. R. Soc. B Biol. Sci. 269:607–612.

Gabriel, W., R. Bürger, and M. Lynch. 1991. Population extinction by mutational load and demographic stochasticity. Pp. 49–59 *in* P. A. Seitz and P. V. Loeschcke, eds. Species conservation: a population-biological approach. Birkhäuser Basel.

Gage, M. J. G. 1995. Continuous variation in reproductive strategy as an adaptive response to population density in the moth *Plodia interpunctella*. Proc. R. Soc. B Biol. Sci. 261:25–30.

Galeano, J., J. M. Pastor, and J. M. Iriondo. 2009. Weighted-interaction nestedness estimator (WINE): A new estimator to calculate over frequency matrices. Env. Model Softw 24:1342–1346.

Gale, D. 1957. A theorem on flows in networks. Pac. J. Math. 7:1073–1082.

Galen, C., and T. Gregory. 1989. Interspecific pollen transfer as a mechanism of competition: Consequences of foreign pollen contamination for seed set in the alpine wildflower, *Polemonium viscosum*. Oecologia 81:120–123.

Galliard, J.-F. L., P. S. Fitze, R. Ferrière, and J. Clobert. 2005. Sex ratio bias, male aggression, and population collapse in lizards. Proc. Natl. Acad. Sci. U. S. A. 102:18231–18236.

Gammell, M. P., H. de Vries, D. J. Jennings, C. M. Carlin, and T. J. Hayden. 2003. David's score: a more appropriate dominance ranking method than Clutton-Brock et al.'s index. Anim. Behav. 66:601–605.

García-Navas, V., E. S. Ferrer, J. Bueno-Enciso, R. Barrientos, J. J. Sanz, and J. Ortego. 2014. Extrapair paternity in Mediterranean blue tits: socioecological factors and the opportunity for sexual selection. Behav. Ecol. 25:228–238.

Gardner, A., S. A. West, and G. Wild. 2011. The genetical theory of kin selection. J. Evol. Biol. 24:1020–1043.

Gasparini, C., G. Serena, and A. Pilastro. 2013. Do unattractive friends make you look better? Context-dependent male mating preferences in the guppy. Proc. R. Soc. B Biol. Sci. 280:1–6.

Gibbs, M., D. A. Dawson, C. McCamley, A. F. Wardle, J. A. Armour, and T. Burke. 1997. Chicken microsatellite markers isolated from libraries enriched for simple tandem repeats. Anim. Genet. 28:401–417.

Girvan, M., and M. E. J. Newman. 2002. Community structure in social and biological networks. Proc. Natl. Acad. Sci. U. S. A. 99:7821–7826.

Godfrey, S. S., C. M. Bull, R. James, and K. Murray. 2009. Network structure and parasite transmission in a group living lizard, the gidgee skink, *Egernia stokesii*. Behav. Ecol. Sociobiol. 63:1045–1056.

Gontard-Danek, M. C., and A. P. Møller. 1999. The strength of sexual selection: a meta-analysis of bird studies. Behav. Ecol. 10:476–486.

Gonzalez, A., B. Rayfield, and Z. Lindo. 2011. The disentangled bank: How loss of habitat fragments and disassembles ecological networks. Am. J. Bot. 98:503–516.

Goodnight, C. J., J. M. Schwartz, and L. Stevens. 1992. Contextual analysis of models of group selection, soft selection, hard selection , and the evolution of altruism. Am. Nat. 140:743–761.

Gosden, T. P., and E. I. Svensson. 2009. Density-dependent male mating harassment, female resistance, and male mimicry. Am. Nat. 173:709–721.

Gosden, T. P., and E. I. Svensson. 2008. Spatial and temporal dynamics in a sexual selection mosaic. Evolution 62:845–856.

Gotelli, N. J. 2000. Null model analysis of species co-occurence patters. Ecology 81:2606–2621.

Grafen, A. 1987. Measuring sexual selection: why bother. Pp. 221–233. *in* Sexual selection: testing the alternatives. Wiley, Chichester.

Granovetter, M. 1973. The strength of weak ties. Am. J. Sociol. 78:2063–2069.

Griffin, R. H., and C. L. Nunn. 2012. Community structure and the spread of infectious disease in primate social networks. Evol. Ecol. 26:779–800.

Groenen, M. A. M., H. H. Cheng, N. Bumstead, B. F. Benkel, W. E. Briles, T. Burke, D. W. Burt, L. B. Crittenden, J. Dodgson, J. Hillel, S. Lamont, A. P. de Leon, M. Soller, H. Takahashi, and A. Vignal. 2000. A consensus linkage map of the chicken genome. Genome Res. 10:137–147.

Gröning, J., and A. Hochkirch. 2008. Reproductive interference between animal species. Q. Rev. Biol. 83:257–282.

Gupta, S., R. M. Anderson, and R. M. May. 1989. Networks of sexual contacts: implications for the pattern of spread of HIV. AIDS 3.

Guttridge, T. L., S. H. Gruber, J. Krause, and D. W. Sims. 2010. Novel acoustic technology for studying free-ranging shark social behaviour by recording individuals' interactions. PloS One 5:e9324.

Hadfield, J. D. 2008. Estimating evolutionary parameters when viability selection is operating. Proc. R. Soc. B Biol. Sci. 275:723–734.

Hamilton, W. D. 1971. Selection of selfish and altruistic behavior in some extreme models. Pp. 57–91 *in* Man and beast: comparative social behavior. Smithsonian Institution Press, Washington, D.C.

Hamilton, W. D. 1964a. The genetical evolution of social behaviour. I. J. Theor. Biol. 7:1–16.

Hamilton, W. D. 1964b. The genetical evolution of social behaviour. II. J. Theor. Biol. 7:17–52.

Hanotte, O., A. Pugh, C. Maücher, D. Dawson, and T. Burke. 1997. Nine novel chicken microsatellite loci and their utility in other Galliformes. Anim. Genet. 28:311–313.

Head, M. L., A. K. Lindholm, and R. Brooks. 2008. Operational sex ratio and density do not affect directional selection on male sexual ornaments and behavior. Evolution 62:135–144.

Heisler, I., and J. Damuth. 1987. A method for analyzing selection in hierarchically structured populations. Am. Nat. 130:582–602.

Higashi, M., G. Takimoto, and N. Yamamura. 1999. Sympatric speciation by sexual selection. Nature 402:523–526.

Higgins, K., and M. Lynch. 2001. Metapopulation extinction caused by mutation accumulation. Proc. Natl. Acad. Sci. U. S. A. 98:2928–2933.

Hocking, P. M. 2009. Biology of Breeding Poultry. CABI.

Ho, E. K. H., and A. F. Agrawal. 2012. The effects of competition on the strength and softness of selection. J. Evol. Biol. 25:2537–46.

Höglund, J., and R. V. Alatalo. 1995. Leks. Princeton University Press, Princeton, N.J.

Holland, B., and W. R. Rice. 1998. Chase-away sexual selection: antagonistic seduction versus resistance. Evolution 52:1–7.

Hollis, B., and D. Houle. 2011. Populations with elevated mutation load do not benefit from the operation of sexual selection. J. Evol. Biol. 24:1918–1926.

Holman, L., and H. Kokko. 2013. The consequences of polyandry for population viability, extinction risk and conservation. Philos. Trans. R. Soc. B Biol. Sci. 368:20120053.

Howarth, B. 1971. Transport of spermatozoa in the reproductive tract of turkey hens. Poult. Sci. 50:84–89.

Hunt, J., C. J. Breuker, J. a Sadowski, and A. J. Moore. 2009. Male-male competition, female mate choice and their interaction: determining total sexual selection. J. Evol. Biol. 22:13–26.

James, A., J. W. Pitchford, and M. J. Plank. 2012. Disentangling nestedness from models of ecological complexity. Nature 487:227–230.

Jennions, M. D., A. P. Moller, and M. Petrie. 2001. Sexually selected traits and adult survival: a meta-analysis. Q. Rev. Biol. 76:3–36.

Johannesen, J., and Y. Lubin. 2001. Evidence for kin-structured group founding and limited juvenile dispersal in the sub-social spider *Stegodyphus lineatus* (Araneae, Eresidae). J. Arachnol. 29:413–422.

Johnsen, T. S., M. Zuk, and E. A. Fessler. 2001. Social dominance, male behaviour and mating in mixed-sex flocks of red Junglefowl. Behaviour 138:1–18.

Jones, A. G. 2009. On the opportunity for sexual selection, the Bateman gradient and the maximum intensity of sexual selection. Evolution 63:1673–84.

Jones, A. G., and N. L. Ratterman. 2009. Mate choice and sexual selection: what have we learned since Darwin? Proc. Natl. Acad. Sci. U. S. A. 106 Suppl:10001–8.

Jones, A. G., D. Walker, C. Kvarnemo, K. Lindström, and J. C. Avise. 2001. How cuckoldry can decrease the opportunity for sexual selection: data and theory from a

genetic parentage analysis of the sand goby, *Pomatoschistus minutus*. Proc. Natl. Acad. Sci. U. S. A. 98:9151–9156.

Joppa, L. N., and S. Pimm. 2010. On nestedness in ecological networks. 12:35–46.

Kalinowski, S. T., M. L. Taper, and T. C. Marshall. 2007. Revising how the computer program CERVUS accommodates genotyping error increases success in paternity assignment. Mol. Ecol. 16:1099–1106.

Kasumovic, M. M., M. J. Bruce, M. C. B. Andrade, and M. E. Herberstein. 2008. Spatial and temporal demographic variation drives within-season fluctuations in sexual selection. Evolution 62:2316–2325.

Kay, K. M., and R. D. Sargent. 2009. The role of animal pollination in plant speciation: Integrating ecology, geography, and genetics. Annu. Rev. Ecol. Evol. Syst. 40:637–656.

Kelly, C. D., J.-G. J. Godin, and J. M. Wright. 1999. Geographical Variation in Multiple Paternity within Natural Populations of the Guppy (*Poecilia reticulata*). Proc. R. Soc. B Biol. Sci. 266:2403–2408.

Kemp, D. J., and C. Wiklund. 2001. Fighting without weaponry: a review of male-male contest competition in butterflies. Behav. Ecol. Sociobiol. 49:429–442.

Kenta, T., J. Gratten, N. S. Haigh, G. N. Hinten, J. Slate, R. K. Butlin, and T. Burke. 2008. Multiplex SNP-SCALE: a cost-effective medium-throughput single nucleotide polymorphism genotyping method. Mol. Ecol. Resour. 8:1230–1238.

Kingsolver, J. G., H. E. Hoekstra, J. M. Hoekstra, D. Berrigan, S. N. Vignieri, C. E. Hill, a Hoang, P. Gibert, and P. Beerli. 2001. The strength of phenotypic selection in natural populations. Am. Nat. 157:245–61.

Kingsolver, J. G., and D. W. Pfennig. 2007. Patterns and power of phenotypic selection in nature. Bioscience 57:561–572.

Kirkpatrick, M., and M. J. Ryan. 1991. The evolution of mating preferences and the paradox of the lek. Nature 350:33–38.

Klug, H. 2001. Animal Mating Systems. eLS, doi: 10.1002/9780470015902.a0022553.

Klug, H., K. Lindström, and H. Kokko. 2010. Who to include in measures of sexual selection is no trivial matter. Ecol. Lett. 13:1094–102.

Koenig, M., C. Tessone, and Y. Zenou. 2014. Nestedness in networks: a theoretical model and some applications. Theor. Econ. 9:695–752.

Kokko, H., H. Klug, and M. D. Jennions. 2012. Unifying cornerstones of sexual selection: operational sex ratio, Bateman gradient and the scope for competitive investment. Ecol. Lett. 15:1340–51.

Kondrashov, A. S., and M. Shpak. 1998. On the origin of species by means of assortative mating. Proc. R. Soc. B Biol. Sci. 265:2273–2278.

Kozak, G. M., A. B. Rudolph, B. L. Colon, and R. C. Fuller. 2012. Postzygotic isolation evolves before prezygotic isolation between fresh and saltwater populations of the Rainwater killifish, *Lucania parva*. Int. J. Evol. Biol. 2012.

Kraaijeveld, K., F. J. L. Kraaijeveld-Smit, and M. E. Maan. 2011. Sexual selection and speciation: the comparative evidence revisited. Biol. Rev. 86:367–377.

Krakauer, A. H. 2005. Kin selection and cooperative courtship in wild turkeys. Nature 434:69–72.

Krause, J., D. P. Croft, and R. James. 2007. Social network theory in the behavioural sciences: potential applications. Behav. Ecol. Sociobiol. 62:15–27.

Krause, J., R. James, D. Franks, and D. Croft (eds). In Press. Animal Social Networks. Oxford University Press.

Krause, J., D. Lusseau, and R. James. 2009. Animal social networks: an introduction. Behav. Ecol. Sociobiol. 63:967–973.

Krause, J., A. D. M. Wilson, and D. P. Croft. 2011. New technology facilitates the study of social networks. Trends Ecol. Evol. 26:5–6.

Krupa, J. J., and A. Sih. 1993. Experimental studies on water strider mating dynamics: spatial variation in density and sex ratio. Behav. Ecol. Sociobiol. 33:107–120.

Kvarnemo, C., and I. Ahnesjo. 1996. The dynamics of operational sex ratios and competition for mates. Trends Ecol. Evol. 11:404–408.

Kvarnemo, C., and L. W. Simmons. 2013. Polyandry as a mediator of sexual selection before and after mating Polyandry as a mediator of sexual selection before and after mating. Philos. Trans. R. Soc. B Biol. Sci. 368:1–16.

Lack, D. 1968. Ecological adaptations for breeding in birds. 1st ed edition. Chapman and Hall, London.

Laffafian, A., J. D. King, and A. F. Agrawal. 2010. Variation in the strength and softness of selection on deleterious mutations. Evolution 64:3232–3241.

Laiolo, P., and J. R. Obeso. 2012. Multilevel selection and neighbourhood effects from individual to metapopulation in a wild passerine. PloS One 7:e38526.

Lande, R., and S. J. Arnold. 1983. The measurement of selection on correlated characters. Evolution 37:1210–1226.

Lawrence, J. M. 2013. Sea Urchins: Biology and Ecology. Academic Press.

Lesobre, L., F. Lacroix, E. Le Nuz, Y. Hingrat, T. Chalah, and M. S. Jaime. 2010. Absence of male reproductive skew, along with high frequency of polyandry and

conspecific brood parasitism in the lekking Houbara bustard *Chlamydotis undulata undulata*. J. Avian Biol. 41:117–127.

Lessells, C. M., and T. R. Birkhead. 1990. Mechanisms of sperm competition in birds: mathematical models. Behav. Ecol. Sociobiol. 27:325–337.

Levitan, D. R., and C. Petersen. 1995. Sperm limitation in the sea. Trends Ecol. Evol. 10:228–231.

Liljeros, F., C. R. Edling, L. A. N. Amaral, H. E. Stanley, and Y. Åberg. 2001. The web of human sexual contacts. Nature 411:907–908.

Løvlie, H., C. K. Cornwallis, and T. Pizzari. 2005. Male mounting alone reduces female promiscuity in the fowl. Curr. Biol. 15:1222–1227.

Løvlie, H., and T. Pizzari. 2007. Sex in the morning or in the evening? Females adjust daily mating patterns to the intensity of sexual harassment. Am. Nat. 170:E1–E13.

Lusseau, D., and M. E. J. Newman. 2004. Identifying the role that animals play in their social networks. Proc. R. Soc. B Biol. Sci. 271:S477–S481.

Lusseau, D., B. Wilson, P. S. Hammond, K. Grellier, J. W. Durban, K. M. Parsons, T. R. Barton, and P. M. Thompson. 2006. Quantifying the influence of sociality on population structure in bottlenose dolphins. J. Anim. Ecol. 75:14–24.

Lyon, B. E., and R. Montgomerie. 2012. Sexual selection is a form of social selection. Philisophical Trans. R. Soc. B 367:2266–2273.

Magurran, A. E., and B. H. Seghers. 1994. A cost of sexual harassment in the Guppy, *Poecilia reticulata*. Proc. R. Soc. B Biol. Sci. 258:89–92.

Marsh, M. K., M. R. Hutchings, S. R. McLeod, and P. C. L. White. 2010. Spatial and temporal heterogeneities in the contact behaviour of rabbits. Behav. Ecol. Sociobiol. 65:183–195.

May, R. M. 2013. Networks and webs in ecosystems and financial systems. Philos. Trans. R. Soc. Math. Phys. Eng. Sci. 371:1–8.

May, R. M., S. A. Levin, and G. Sugihara. 2008. Complex systems: ecology for bankers. Nature 451:893–895.

Mays, H. L., and G. Ritchison. 2004. The effect of vegetation density on male mate guarding and extra-territorial forays in the yellow-breasted chat (*Icteria virens*). Naturwissenschaften 91:195–198.

McDonald, D. B. 2007. Predicting fate from early connectivity in a social network. Proc. Natl. Acad. Sci. U. S. A. 104:10910–10914.

McDonald, G. C., R. James, J. Krause, and T. Pizzari. 2013. Sexual networks: measuring sexual selection in structured, polyandrous populations. Philos. Trans. R. Soc. B Biol. Sci. 368:1–10.

McDonald, G. C., and T. Pizzari. In press. Mating behaviour: sexual networks and sexual selection. *in* Animal social networks: Perspectives and challenges. Oxford University Press, Oxford.

McNamara, K. B., R. L. Brown, M. A. Elgar, and T. M. Jones. 2008. Paternity costs from polyandry compensated by increased fecundity in the hide beetle. Behav. Ecol. 19:433–440.

Mills, S. C., A. Grapputo, E. Koskela, and T. Mappes. 2007. Quantitative measure of sexual selection with respect to the operational sex ratio: a comparison of selection indices. Proc. R. Soc. B Biol. Sci. 274:143–150.

Mitchell-Olds, T., and R. G. Shaw. 1987. Regression analysis of natural selection: statistical inference and biological interpretation. Evolution 41:1149–1161.

Mitchell, R. J., R. E. Irwin, R. J. Flanagan, and J. D. Karron. 2009. Ecology and evolution of plant-pollinator interactions. Ann. Bot. 103:1355–1363.

Moccia, M. D., A. Widmer, and S. Cozzolino. 2007. The strength of reproductive isolation in two hybridizing food-deceptive orchid species. Mol. Ecol. 16:2855–2866.

Moorad, J., and M. J. Wade. 2013. Selection gradients, the opportunity for selection, and the coefficient of determination. Am. Nat. 181:291–300.

Moore, A. J., E. D. Brodie III, and J. B. Wolf. 1997. Interacting phenotypes and the evolutionary process: I. Direct and indirect genetic effects of social interactions. Evolution 51:1352–1362.

Moore, J. C., and J. R. Pannell. 2011. Sexual selection in plants. Curr. Biol. 21:176–182.

Morris, R. J., O. T. Lewis, and H. C. J. Godfray. 2004. Experimental evidence for apparent competition in a tropical forest food web. Nature 428:310–3.

Muchhala, N., and J. D. Thomson. 2012. Interspecific competition in pollination systems: costs to male fitness via pollen misplacement. Funct. Ecol. 26:476–482.

Mück, I., S. Wacker, L. C. Myhre, and T. Amundsen. 2013. Nest distribution affects behaviour and mating success in a marine fish. Behav. Ecol. Sociobiol. 67:609–619.

Muniz, D. G., P. R. Guimaraes, B. A. Buzatto, and G. Machado. 2014. A sexual network approach to sperm competition in a species with alternative mating tactics. Behav. Ecol., doi: 10.1093/beheco/aru166.

Navara, K. J., E. M. Anderson, and M. L. Edwards. 2012. Comb size and color relate to sperm quality: a test of the phenotype-linked fertility hypothesis. Behav. Ecol. 23:1036–1041.

Newman, M. 2010. Networks: An Introduction. OUP Oxford.

Newman, M. E. J. 2002. Assortative mixing in networks. Phys Rev Lett 89:208701.

Newman, M. E. J. 2004. Fast algorithm for detecting community structure in networks. Phys. Rev. E 69:066133.

Newman, M. E. J. 2003. Mixing patterns in networks. Phys. Rev. E 67:026126.

Newman, M. E. J. 2006. Modularity and community structure in networks. Proc. Natl. Acad. Sci. U. S. A. 103:8577–82.

Nicholls, J. A., M. C. Double, D. M. Rowell, and R. D. Magrath. 2000. The evolution of cooperative and pair breeding in Thornbills acanthiza (Pardalotidae). J. Avian Biol. 31:165–176.

Nunney, L. 1985. Group selection altruism and structured-deme models. Am. Nat. 126:212–230.

O'Hara, R. K., and A. R. Blaustein. 1985. *Rana cascadae* tadpoles aggregate with siblings: an experimental field study. Oecologia 67:44–51.

Oh, K. P., and A. V. Badyaev. 2010. Structure of social networks in a passerine bird: consequences for sexual selection and the evolution of mating strategies. Am. Nat. 176:E80–9.

Ohtsuki, H., C. Hauert, E. Lieberman, and M. a Nowak. 2006. A simple rule for the evolution of cooperation on graphs and social networks. Nature 441:502–5.

Okasha, S. 2004a. Multilevel selection and the partitioning of covariance: a comparison of three approaches. Evolution 58:486–494.

Okasha, S. 2004b. Multi-level selection, covariance and contextual analysis. Br. J. Philos. Sci. 55:481–504.

Olesen, J. M., J. Bascompte, Y. L. Dupont, and P. Jordano. 2007. The modularity of pollination networks. Proc. Natl. Acad. Sci. U. S. A. 104:19891–19896.

Olesen, J. M., and P. Jordano. 2002. Geographic patterns in plant-pollinator mutualistic networks. Ecology 83:2416–2424.

Ollerton, J., R. Winfree, and S. Tarrant. 2011. How many flowering plants are pollinated by animals? Oikos 120:321–326.

Opsahl, T., and P. Panzarasa. 2009. Clustering in weighted networks. Soc. Netw. 31:155–163.

Orians, G. H. 1969. On the evolution of mating systems in birds and mammals. Am. Nat. 103:589–603.

Pannell, J. R., and A.-M. Labouche. 2013. The incidence and selection of multiple mating in plants. Philos. Trans. R. Soc. B Biol. Sci. 368:1–11.

Parker, G. A. 1970. Sperm competition and its evolutionary consequences in insects. Biol. Rev. 45:525–567.

Parker, G. A. 1998. Sperm competition and the evolution of ejaculates: towards a theory base. Pp. 3–54 *in* Sperm Competition and Sexual Selection.

Parker, G. A., and T. R. Birkhead. 2013. Polyandry: the history of a revolution. Philos. Trans. R. Soc. B Biol. Sci. 368:1–13.

Parker, G. A., C. M. Lessells, and L. W. Simmons. 2013. Sperm competition games: a general model for precopulatory male–male competition. Evolution 67:95–109.

Parker, G. A., and L. Partridge. 1998. Sexual conflict and speciation. Philos. Trans. R. Soc. B Biol. Sci. 353:261–274.

Parker, G. A., and T. Pizzari. 2010. Sperm competition and ejaculate economics. Biol. Rev. 85:897–934.

Pélissié, B., P. Jarne, V. Sarda, and P. David. 2014. Disentangling precopulatory and postcopulatory sexual selection in polyandrous species. Evolution 68:1320–1331.

Petrie, M., and G. Roberts. 2007. Sexual selection and the evolution of evolvability. Heredity 98:198–205.

Pizzari, T. 2003. Food, vigilance, and sperm: the role of male direct benefits in the evolution of female preference in a polygamous bird. Behav. Ecol. 14:593–601.

Pizzari, T. 2001. Indirect partner choice through manipulation of male behaviour by female fowl, *Gallus gallus domesticus*. Proc. R. Soc. B Biol. Sci. 268:181–186.

Pizzari, T., and T. R. Birkhead. 2000. Female feral fowl eject sperm of subdominant males. Nature 405:787–789.

Pizzari, T., C. K. Cornwallis, and D. P. Froman. 2007. Social competitiveness associated with rapid fluctuations in sperm quality in male fowl. Proc. R. Soc. B Biol. Sci. 274:853–860.

Pizzari, T., D. P. Froman, and T. R. Birkhead. 2002. Pre- and post-insemination episodes of sexual selection in the fowl, *Gallus g. domesticus*. Heredity 88:112–116.

Pizzari, T., and A. Gardner. 2012. The sociobiology of sex: inclusive fitness consequences of inter-sexual interactions. Philos. Trans. R. Soc. B Biol. Sci. 367:2314–2323.

Pizzari, T., and N. Wedell. 2013. The polyandry revolution. Philos. Trans. R. Soc. B Biol. Sci. 368:20120041.

Pizzari, T., K. Worley, T. Burke, and D. P. Froman. 2008. Sperm competition dynamics: ejaculate fertilising efficiency changes differentially with time. BMC Evol. Biol. 8:332.

Plesnar, A., M. Konior, and J. Radwan. 2011. The role of sexual selection in purging the genome of induced mutations in the bulb mite (*Rizoglyphus robini*). Evol. Ecol. Res. 13:1–8.

Preston, B. T., I. R. Stevenson, J. M. Pemberton, D. W. Coltman, and K. Wilson. 2005. Male mate choice influences female promiscuity in Soay sheep. Proc. R. Soc. B Biol. Sci. 272:365–73.

Preston, B. T., I. R. Stevenson, J. M. Pemberton, D. W. Coltman, and K. Wilson. 2003a. Overt and covert competition in a promiscuous mammal: the importance of weaponry and testes size to male reproductive success. Proc. R. Soc. B Biol. Sci. 270:633–640.

Preston, B. T., I. R. Stevenson, J. M. Pemberton, and K. Wilson. 2001. Dominant rams lose out by sperm depletion. Nature 409:681–682.

Preston, B. T., I. R. Stevenson, and K. Wilson. 2003b. Soay rams target reproductive activity towards promiscuous females' optimal insemination period. Proc. R. Soc. B Biol. Sci. 270:2073–2078.

Price, G. R. 1970. Selection and covariance. Nature 227:520–21.

Price, T., M. Kirkpatrick, and S. J. Arnold. 1988. Directional selection and the evolution of breeding date in birds. Science 240:798–799.

Procter, D. S., A. J. Moore, and C. W. Miller. 2012. The form of sexual selection arising from male-male competition depends on the presence of females in the social environment. J. Evol. Biol. 25:803–812.

Psorakis, I., S. J. Roberts, I. Rezek, and B. C. Sheldon. 2012. Inferring social network structure in ecological systems from spatio-temporal data streams. J. R. Soc. Interface R. Soc. 9:3055–66.

Queller, D. C. 1992. Does population viscosity promote kin selection? Trends Ecol. Evol. 7:322–324.

Radwan, J. 2004. Effectiveness of sexual selection in removing mutations induced with ionizing radiation. Ecol. Lett. 7:1149–1154.

Radwan, J., J. Unrug, K. Snigórska, and K. Gawrońska. 2004. Effectiveness of sexual selection in preventing fitness deterioration in bulb mite populations under relaxed natural selection. J. Evol. Biol. 17:94–99.

Rankin, D. J., K. Bargum, and H. Kokko. 2007. The tragedy of the commons in evolutionary biology. Trends Ecol. Evol. 22:643–651.

R Core Team. 2014. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.

Reichard, M., P. Jurajda, and C. Smith. 2004. Male-male interference competition decreases spawning rate in the European bitterling (*Rhodeus sericeus*). Behav. Ecol. Sociobiol. 56:34–41.

Reichard, M., M. Ondracková, A. Bryjová, C. Smith, and J. Bryja. 2009. Breeding resource distribution affects selection gradients on male phenotypic traits:

experimental study on lifetime reproductive success in the bitterling fish (*Rhodeus amarus*). Evolution 63:377–390.

Reid, J. M., P. Arcese, L. F. Keller, and S. Losdat. 2014. Female and male genetic effects on offspring paternity: additive genetic (co)variances in female extra-pair reproduction and male paternity success in Song sparrows (*Melospiza melodia*). Evolution 68:2357–2370.

Reid, M., and J. Stamps. 1997. Female mate choice tactics in a resource-based mating system: field tests of alternative models. Am. Nat. 150:98–121.

Reynolds, J. D. 1996. Animal breeding systems. Trends Ecol. Evol. 11:68–72.

Reynolds, J. D., M. D. Gross, and M. J. Coombs. 1993. Environmental conditions and male morphology determine alternative mating behaviour in Trinidadian guppies. Anim. Behav. 45:145–152.

Reynolds, R. G., and B. M. Fitzpatrick. 2007. Assortative mating in Poison-dart frogs based on an ecologically important trait. Evolution 61:2253–2259.

Rezende, E. L., J. E. Lavabre, P. R. Guimarães, P. Jordano, and J. Bascompte. 2007. Non-random coextinctions in phylogenetically structured mutualistic networks. Nature 448:925–928.

Rios Moura, R., and P. E. C. Peixoto. 2013. The effect of operational sex ratio on the opportunity for sexual selection: a meta-analysis. Anim. Behav. 86:675–683.

Robertson, A. 1968. The spectrum of genetic variation. Pp. 5–16 *in* R. C. Lewontin, ed. Population biology and evolution. Syracuse University Press, Syracuse, NY.

Rodríguez-Muñoz, R., A. Bretman, J. Slate, C. A. Walling, and T. Tregenza. 2010. Natural and sexual selection in a wild insect population. Science 328:1269–72.

Rowe, L. 1992. Convenience polyandry in a water strider: foraging conflicts and female control of copulation frequency and guarding duration. Anim. Behav. 44:189–202.

Rubenstein, D., and R. Wrangham (eds). 1987. Ecological aspects of social evolution: birds and mammals. Princeton University Press, Princeton, NJ.

Rutz, C., Z. T. Burns, R. James, S. M. H. Ismar, J. Burt, B. Otis, J. Bowen, and J. J. H. St Clair. 2012. Automated mapping of social networks in wild birds. Curr. Biol. 22:R669–71.

Ryder, T. B., D. B. McDonald, J. G. Blake, P. G. Parker, and B. A. Loiselle. 2008. Social networks in the lek-mating wire-tailed manakin (*Pipra filicauda*). Proc. R. Soc. B Biol. Sci. 275:1367–1374.

Saavedra, S., and D. B. Stouffer. 2013. "Disentangling nestedness" disentangled. Nature 500:E1–E2.

Saavedra, S., D. B. Stouffer, B. Uzzi, and J. Bascompte. 2011. Strong contributors to network persistence are the most vulnerable to extinction. Nature 478:233–235.

Salathe, M., and J. H. Jones. 2010. Dynamics and control of diseases in networks with community structure. PLoS Comput Biol 6:e1000736.

Saltz, J. B. 2013. Genetic composition of social groups influences male aggressive behaviour and fitness in natural genotypes of *Drosophila melanogaster*. Proc. R. Soc. B Biol. Sci. 280:20131926.

Santos, F. C., J. M. Pacheco, and T. Lenaerts. 2006a. Cooperation prevails when individuals adjust their social ties. PLoS Comput. Biol. 2:e140.

Santos, F. C., J. M. Pacheco, and T. Lenaerts. 2006b. Evolutionary dynamics of social dilemmas in structured heterogeneous populations. Proc. Natl. Acad. Sci. U. S. A. 103:3490–4.

Sargent, R. D., and D. D. Ackerly. 2008. Plant–pollinator interactions and the assembly of plant communities. Trends Ecol. Evol. 23:123–130.

Schlicht, E., and B. Kempenaers. 2013. Effects of social and extra-pair mating on sexual selection in Blue tits (*cyanistes Caeruleus*). Evolution 67:1420–1434.

Schmid, B. V., and M. Kretzschmar. 2012. Determinants of sexual network structure and their impact on cumulative network measures. PLoS Comput Biol 8:e1002470.

Scott, J. 2000. Social network analysis. Sage Publications, London, UK.

Seddon, N., C. A. Botero, J. A. Tobias, P. O. Dunn, H. E. A. MacGregor, D. R. Rubenstein, J. A. C. Uy, J. T. Weir, L. A. Whittingham, and R. J. Safran. 2013. Sexual selection accelerates signal evolution during speciation in birds. Proc. R. Soc. B Biol. Sci. 280:20131065.

Seddon, N., R. M. Merrill, and J. A. Tobias. 2008. Sexually selected traits predict patterns of species richness in a diverse clade of suboscine birds. Am. Nat. 171:620–31.

Shuster, S. M. 2009. Sexual selection and mating systems. Proc. Natl. Acad. Sci. U. S. A. 106:10009–10016.

Shuster, S. M., W. R. Briggs, and P. A. Dennis. 2013. How multiple mating by females affects sexual selection. Philos. Trans. R. Soc. B Biol. Sci. 368:20120046.

Shuster, S., and M. J. Wade. 2003. Mating systems and strategies. Princeton University Press, Princeton, NJ.

Siepielski, A. M., J. D. DiBattista, J. A. Evans, and S. M. Carlson. 2011. Differences in the temporal dynamics of phenotypic selection among fitness components in the wild. Proc. R. Soc. B Biol. Sci. 278:1572–1580.

Sih, A., S. F. Hanser, and K. a. McHugh. 2009. Social network theory: new insights and issues for behavioral ecologists. Behav. Ecol. Sociobiol. 63:975–988.

Simmons, L. W. 2001. Sperm competition and its evolutionary consequences in the insects. Princeton University Press.

Skogsmyr, I., and Å. Lankinen. 2002. Sexual selection: an evolutionary force in plants? Biol. Rev. 77:537–562.

Snowberg, L. K., and D. I. Bolnick. 2008. Assortative mating by diet in a phenotypically unimodal but ecologically variable population of stickleback. Am. Nat. 172:733–739.

Squires, Z. E., B. B. M. Wong, M. D. Norman, and D. Stuart-Fox. 2012. Multiple fitness benefits of polyandry in a cephalopod. PLoS ONE 7:e37074.

Staniczenko, P. P. A., J. C. Kopp, and S. Allesina. 2013. The ghost of nestedness in ecological networks. Nat. Commun. 4:1391.

Stevens, L., C. J. Goodnight, and S. Kalisz. 1995. Multilevel selection in natural populations of *Impatiens capensis*. Am. Nat. 145:513–526.

Stokes, A. W. 1971. Parental and courtship feeding in Red junglefowl. The Auk 88:21–29.

Sugihara, G., and H. Ye. 2009. Complex systems: cooperative network dynamics. Nature 458:979–980.

Taylor, M. L., T. A. R. Price, and N. Wedell. 2014. Polyandry in nature: a global analysis. Trends Ecol. Evol. 29:376–383.

Taylor, P. D. 1992a. Altruism in viscous populations - an inclusive fitness model. Evol. Ecol. 6:352–356.

Taylor, P. D. 1992b. Inclusive fitness in a homogeneous environment. Proc. R. Soc. B Biol. Sci. 249:299–302.

Thornhill, R. 1983. Cryptic female choice and its implications in the scorpionfly. Am. Nat. 122:765–788.

Thornhill, R., and J. Alcock. 1983. The evolution of insect mating systems. Harvard University Press, Cambridge, MA.

Tomkins, J. L., W. N. Hazel, M. A. Penrose, J. W. Radwan, and N. R. LeBas. 2011. Habitat complexity drives experimental evolution of a conditionally expressed secondary sexual trait. Curr. Biol. 21:569–573.

Traveset, A., and D. M. Richardson. 2006. Biological invasions as disruptors of plant reproductive mutualisms. Trends Ecol. Evol. 21:208–216.

Tryjanowski, P., M. Antczak, and M. Hromada. 2007. More secluded places for extra-pair copulations in the Great grey shrike *Lanius excubitor*. Behaviour 144:23–31.

Tsuji, K. 1995. Reproductive conflicts and levels of selection in the Ant *Pristomyrmex pungens*: contextual analysis and partitioning of covariance. Am. Nat. 146:586–607.

Tuomainen, U., and U. Candolin. 2011. Behavioural responses to human-induced environmental change. Biol. Rev. 86:640–657.

Twiss, S. D., C. Thomas, V. Poland, J. A. Graves, and P. Pomeroy. 2007. The impact of climatic variation on the opportunity for sexual selection. Biol. Lett. 3:12–15.

Ulrich, W., M. Almeida-Neto, and N. J. Gotelli. 2009. A consumer's guide to nestedness analysis. Oikos 118:3–17.

Van Shaik, C. P. 1987. The ecology of social relationships amongst female primates. Pp. 195–218 *in* Comparative socioecology of mammals and man. Blackwell Scientific, Oxford.

Wacker, S., K. Mobley, E. Forsgren, L. C. Myhre, K. de Jong, and T. Amundsen. 2013. Operational sex ratio but not density affects sexual selection in a fish. Evolution 67:1937–1949.

Wade, M. J. 1985. Soft selection, hard selection, kin selection, and group selection. Am. Nat. 125:61–73.

Wade, M. J., C. J. Goodnight, and L. Stevens. 1999. Design and interpretation of experimental studies of interdemic selection : a reply to Getty. Am. Nat. 154:599–603.

Wade, M. J., and S. M. Shuster. 2005. Don't throw Bateman out with the bathwater! Integr. Comp. Biol. 45:945–951.

Wallace, B. 1975. Hard and soft selection revisited. Evolution 29:465–473.

Wallace, B. 1968. Polymorphism, population size, and genetic load. Pp. 87–108 *in* L. R.C., ed. Population biology and evolution. Syracuse University Press, Syracuse, NY.

Ward, A., R. I. Holbrook, J. Krause, and P. Hart. 2005. Social recognition in sticklebacks: the role of direct experience and habitat cues. Behav. Ecol. Sociobiol. 57:575–583.

Ward, A. J. W., P. J. B. Hart, and J. Krause. 2004. The effects of habitat- and diet-based cues on association preferences in three-spined sticklebacks. Behav. Ecol. 15:925–929.

Watts, D. J., and S. H. Strogatz. 1998. Collective dynamics of "small-world" networks. Nature 393:440–442.

Webster, M. S., S. Pruett-Jones, D. F. Westneat, and S. J. Arnold. 1995. Measuring the effects of pairing success, extra-pair copulations and mate quality on the opportunity for sexual selection. Evolution 49:1147–1157.

Webster, M. S., K. A. Tarvin, E. M. Tuttle, and S. Pruett-Jones. 2007. Promiscuity drives sexual selection in a socially monogamous bird. Evolution 61:2205–2211.

Wedell, N., M. J. G. Gage, and G. A. Parker. 2002. Sperm competition, male prudence and sperm-limited females. Trends Ecol. Evol. 17:313–320.

Weimann, G. 1983. The strength of weak conversational ties in the flow of information and influence. Soc. Netw. 5:245–267.

Weinig, C., J. a Johnston, C. G. Willis, and J. N. Maloof. 2007. Antagonistic multilevel selection on size and architecture in variable density settings. Evolution 61:58–67.

Westneat, D. F., and P. W. Sherman. 1997. Density and extra-pair fertilizations in birds: a comparative analysis. Behav. Ecol. Sociobiol. 41:205–215.

West, S. A., I. Pen, and A. S. Griffin. 2002. Cooperation and competition between relatives. Science 296:72–75.

Wey, T., D. T. Blumstein, W. Shen, and F. Jordán. 2008. Social network analysis of animal behaviour: a promising tool for the study of sociality. Anim. Behav. 75:333–344.

Whitehead, H. 2008. Analyzing animal societies: quantitative methods for vertebrate social analysis. University of Chicago Press.

Whitlock, M. C. 2002. Selection, load and inbreeding depression in a large metapopulation. Genetics 160:1191–202.

Whitlock, M. C., and A. F. Agrawal. 2009. Purging the genome with sexual selection: reducing mutation load through selection on males. Evolution 63:569–582.

Whitlock, M., and D. Schluter. 2008. The analysis of biological data. Roberts & Company Publishers, Greenwood Village, Colo.

Whittingham, L. A., and J. T. Lifjeld. 1995. Extra-pair fertilizations increase the opportunity for sexual selection in the monogamous House martin *Delichon urbica*. J. Avian Biol. 26:283–288.

Wigby, S., and T. Chapman. 2005. Sex peptide causes mating costs in female *Drosophila melanogaster*. Curr. Biol. 15:316–321.

Williams, G. C. 1957. Pleiotropy, natural selection, and the evolution of senescence. Evolution 11:398–411.

Willson, M. F. 1994. Sexual selection in plants: perspective and overview. Am. Nat. 144:13–39.

Wilson, D. R., X. J. Nelson, and C. S. Evans. 2009. Seizing the opportunity: subordinate male fowl respond rapidly to variation in social context. Ethology 115:996–1004.

Wilson, D. S. 1975. A theory of group selection. Proc. Natl. Acad. Sci. U. S. A. 72:143–6.

Wishart, G. J. 1987. Regulation of the length of the fertile period in the domestic fowl by numbers of oviducal spermatozoa, as reflected by those trapped in laid eggs. J. Reprod. Fertil. 80:493–498.

Wolf, J. B. 2003. Genetic architecture and evolutionary constraint when the environment contains genes. Proc. Natl. Acad. Sci. U. S. A. 100:4655–4660.

Wolf, J. B., E. D. Brodie III, J. M. Cheverud, A. J. Moore, and M. J. Wade. 1998. Evolutionary consequences of indirect genetic effects. Trends Ecol. Evol. 13:64–69.

Wolf, J. B., W. E. Harris, and N. J. Royle. 2008. The capture of heritable variation for genetic quality through social competition. Genetica 134:89–97.

Wolf, J., E. Brodie, and A. Moore. 1999. Interacting phenotypes and the evolutionary process. II. Selection resulting from social interactions. Am. Nat. 153:254–266.

Wolf, J. B. W., D. Mawdsley, F. Trillmich, and R. James. 2007. Social structure in a colonial mammal: unravelling hidden structural layers and their foundations by network analysis. Anim. Behav. 74:1293–1302.

Wolf, J. B. W., and F. Trillmich. 2008. Kin in space: social viscosity in a spatially and genetically substructured network. Proc. R. Soc. B Biol. Sci. 275:2063–2069.

Zahradnik, T. D., M. a. Lemay, and E. G. Boulding. 2008. Choosy males in a littorinid gastropod: male *Littorina subrotundata* prefer large and virgin females. J. Molluscan Stud. 74:245–251.