

# 1 Sexual selection in socially-structured, polyandrous populations:

## 2 Some insights from the fowl

3 Tommaso Pizzari\*<sup>1</sup>, Grant C. McDonald\*

4 \*Edward Grey Institute, Department of Zoology, University of Oxford, Oxford OX1 3SZ, UK  
5 \*corresponding author: [tommaso.pizzari@zoo.ox.ac.uk](mailto:tommaso.pizzari@zoo.ox.ac.uk)  
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## Abstract

Sexual selection is widely recognised as the evolutionary agent driving male exaggeration and strategies of intrasexual competition over reproductive opportunities. Two advances have characterised the development of our understanding of sexual selection in recent years. The first was the realisation that sexual selection can extend to postcopulatory episodes whenever females mate with multiple males (polyandry). The second concerns the operation of sexual selection in structured population in light of increasing evidence that populations are often non-randomly assembled. Populations of domestic fowl (*Gallus domesticus*) and red junglefowl (*Gallus gallus*), which are typically socially structured and polyandrous, have offered a convenient vertebrate model system to study patterns and mechanisms of sexual selection, providing a helpful counterpoint to studies of socially monogamous systems. Here, I review our understanding of the way sexual selection operates in these populations, with emphasis on recent work focusing on the inter-related implications of polyandry and social structure.

*“In the same manner as man can improve the breed of his game-cocks by the selection of those birds which are victorious in the cockpit, so it appears that the strongest and most vigorous males, or those provided with the best weapons, have prevailed under nature, and have led to the improvement of the natural breed or species.”*

*Darwin, C. R. (1871) The Descent of Man and Selection in Relation to Sex, p. 259.*

## 1. Introduction

Galliform birds in general, and the fowl in particular, have inspired the study of sexual selection since Darwin first proposed this evolutionary process. The over-arching aim of this article is to review how studies of fowl populations have contributed to the development of our understanding of sexual selection. Below, I: i) provide a brief introduction to sexual selection theory, ii) illustrate the way in which the relatively recent realisation of widespread polyandry, and considerations of population structure are drastically changing our understanding of the process of sexual selection, and iii) briefly introduce the fowl model system.

## 1.1 Sexual selection: a brief synopsis

The term ‘sexual selection’ refers to a selective process that acts upon variance in reproductive success among members of the same sex and population, which arises exclusively from intrasexual competition over access to mating and fertilisation opportunities (Darwin 1871; Andersson 1994). Sexual selection therefore represents a social subset of Darwinian natural selection.

The reason for why sexual selection is often stronger in males than in females was formalised later through Bateman (1948) and Trivers (1972), in terms of the asymmetry in investment in gamete size by males and females (anisogamy). The implication of anisogamy is that because sperm outnumber ova (typically by orders of magnitude), male reproductive success is strongly limited by competition with other males over access to ova, while females do not normally need to compete among themselves to fertilise their eggs. This numerical asymmetry modulates sex-specific patterns of selection. Male reproductive success can be approximated as a function of the number of females with whom a male was able to spawn/mate successfully (mating success). The univariate regression of male reproductive success over mating success therefore is predicted to generate a positive relationship with a slope (“Bateman gradient”) equal to the average female clutch size of the species/population. The Bateman gradient captures the strength of sexual selection on male mating success. Given the abundance of sperm, a single mating is often expected to fertilise the entire clutch of eggs, so females are generally not expected to increase their reproductive success by mating more than once (or with more than one partner). The female Bateman gradient has therefore been assumed to asymptote shortly after one mating (i.e. equal to the constant represented by average clutch size). Expectations for sex-specific Bateman gradients were originally based on the results of Bateman’s pioneering study of *Drosophila melanogaster* (Bateman 1948). While these patterns have attracted considerable discussion (Gowaty et al. 2012), more recent studies have now provided robust empirical evidence that in most species the male Bateman gradient is generally steeper than the female (Janike et al. 2016), although female Bateman gradients are often positive rather than flat (see below).

The notion that anisogamy underpins stronger intrasexual competition in males than females also helped make evolutionary sense of Darwin's original observation that females are typically "coy", often "endeavoring for a long time to escape from the male" (Darwin 1871). Evolutionary sex roles are emergent properties of the theoretical framework laid out by Darwin (1871), Bateman (1948) and Trivers (1972) (the "Darwin-Bateman paradigm", Dewsbury 2005). This however, does not mean that intrasexual competition is necessarily negligible in females and mate choice negligible in males. Rather, the paradigm provides a first-basis expectation for the relative importance of these processes between the sexes (Parker & Pizzari 2015). Darwin (1871) originally distinguished between two episodes of sexual selection. One acting on male ability to directly outcompete other males over access to reproductive opportunities (intra-sexual selection), which would account for the evolution of traits such as armaments and weapons, and another inter-sexual episode, driven by the differential response of females to the phenotype of different male suitors, which Darwin posited would lead to the evolution of traits such as ornaments and courtship displays.

Darwin's view of sexual selection was historically met with mixed response. While the idea of direct competition among males was accepted quite readily, the notion that females could play a selective role by actively discriminating among males was originally met with scepticism. Early theoretical efforts considered scope for potential genetic benefits driving the evolution of female preferences (Andersson 1994; Kokko et al. 2006). More recent studies have explored the possibility of non-adaptive female preferences (e.g. "sensory drive", Endler & Basolo 1998; "sensory traps", Christy 1995), or altogether maladaptive mechanisms ("Chase-away", Holland & Rice 1998), driven by divergences in the fitness interests of prospective reproductive partners (i.e. 'sexual conflict'; Parker 1979; 2006). A common property of these models is that they predict directional sexual selection on male genotypes. While consistent patterns of strong directional selection are required to explain the evolution of exaggeration, theory would predict that, by eroding genetic variance in the selected male ornament, the process would gradually limit scope for mate choice and the ability of a population to further evolve in response to sexual selection. A plethora of

hypotheses have been proposed to resolve this conundrum, known as the “lek paradox” (Borgia 1979), through environmental and genetic mechanisms that act to maintain additive genetic variance in the face of sexual selection. One category of such hypotheses is based on differential compatibilities of parental genotypes, which predict that the reproductive success of different male genotypes is influenced by the complementarity or similarity of the female genotypes. This process would buffer directional sexual selection and maintain variation in male traits by generating non-directional (e.g. balancing) patterns of selection, acting on the interaction between the genotypes of reproductive partners and promoting certain genetic combinations, rather than a specific genotype *per se*.

## 1.2 The polyandry revolution

Although mechanisms of gametic competition and selection long preceded the evolution of competition among males and discrimination by females, the evolutionary significance of gametic interactions was intuited only long after Darwin’s intuition of sexual selection. The catalyst was the realisation of polyandry in internally fertilising species. In 1970, Geoff Parker recognised that polyandry in insect species could prolong intrasexual selection to after mating by forcing the ejaculates of different males to vie for the fertilisation of the same ova (Parker 1970; Simmons 2001), a process that became known as sperm competition. Similarly, early observations of mating and post-mating behaviour in female insects introduced the notion that intersexual selection may also extend beyond mating through processes that would enable females to systematically bias the outcome of sperm competition, a process known as cryptic female choice (Childress & Hartl 1974; Thornhill 1982; Eberhard 1996; Firman et al. 2017). The last 30 years have witnessed an explosion of the study of polyandry and a growing realisation that some degrees of polyandry (sometimes extremely high) are typical in many sexually-reproducing taxa (Taylor et al. 2014).

The realisation of widespread polyandry is rapidly revolutionising our understanding of the operation of sexual selection on males. The main implication is that polyandry introduces a new source of competitive variation in male reproductive success in the form of

variation in paternity share of the eggs of a female. This variation generates intra- and inter-sexual episodes of sexual selection, which arise after mating and before fertilisation (i.e. sperm competition and cryptic female choice, respectively). The study of sexual selection after mating (postcopulatory sexual selection) has traditionally focused on a number of interrelated themes: i) the drivers of polyandry, ii) mechanisms of sperm competition, iii) the traits influencing paternity share under sperm competition, iv) male strategies to respond to sperm competition, including strategic ejaculate allocation, v) the demonstration of cryptic female choice (Simmons 2001; Firman et al. 2017). More recently, evolutionary biologists have complemented these lines of research with a more holistic approach seeking to understand the fundamental way in which polyandry affects the operation of sexual selection in a population, and the way in which pre- and post-copulatory episodes interact in polyandrous populations (Andersson & Simmons 2006; Evans & Garcia-Gonzalez 2016). This approach is leading to an increased appreciation of more complex ramifications of polyandry. Variation in paternity share generates a more complex architecture of male reproductive success, in which covariances between sources of pre- and post-copulatory reproductive success (e.g. mating success and paternity share) can play an important role in the evolution of sexually-selected traits (Webster et al. 1995). For example, negative covariance between male mating success and paternity share can create the opportunity for disruptive selection promoting alternative strategies (e.g. alternative mating tactics) for males to pursue reproductive success by either investing preferentially in monopolising access to mating opportunities (pre-copulatory) or in achieving a greater share of paternity (post-copulatory; Kvarnemo & Simmons 2013). Moreover, in addition to creating variation in paternity share, polyandry can also impact variation in male mating success and the operation of pre-copulatory sexual selection. For example, to the extent to which it reduces or increases variation in male mating success, polyandry can impact on the opportunity for pre-copulatory sexual selection (Shuster & Wade 2003; Kvarnemo & Simmons 2013; McDonald & Pizzari 2018). It has been suggested that polyandry can modulate the strength of the pre-copulatory male Bateman gradient by reducing reproductive returns of mating (Parker & Birkhead 2013),

and by determining the covariance between male mating success and paternity share (McDonald & Pizzari 2016). This modulation can emerge simply because polyandry determines the degree of saturation of the mating matrix (i.e. the matrix of all possible permutations of mating between individual males and females of a population), which influences the range of random variation in the covariance between male mating success and paternity share (McDonald & Pizzari 2016; 2017). Finally, polyandry will modulate the scale at which intrasexual competition (pre- or post-copulatory) occurs, with higher levels of polyandry globalising the scale of competition. This can have drastic repercussions for sexual selection when populations are phenotypically structured so that the competitive ability of phenotypes is not randomly distributed (McDonald et al. 2013). Unpacking the complex effects of polyandry represents one of the key challenges in the study sexual selection (Andersson & Simmons 2006; Evans & Garcia-Gonzalez 2016).

The goal of this review is to appraise the way studies of fowl populations have contributed to advance our understanding of sexual selection, with particular emphasis on the way polyandry and population structure modulate its operation.

### 1.3 The fowl model system

The red junglefowl, *Gallus gallus*, has long been indicated as the wild species, which gave rise to the modern domestic fowl, *G. domesticus* (Darwin 1868), through domestication and artificial selection, which probably originated multiple independent times starting in the Holocene, as early as ~8 ka calBP (West & Zhou 1988; Peters et al. 2016). This monophyletic view has been corroborated by early molecular phylogenies (Fumihito et al. 1994; 1996). More recent molecular work however, has painted a more nuanced scenario, indicating evidence of genetic contributions from other species of junglefowl to the domestic fowl (Eriksson et al., 2008; Nishibori et al. 2005), confirming earlier suggestions (Wood-Gush 1959; 1971). In light of these findings, red junglefowl and domestic fowl are considered here as separate species, despite a clear lack of reproductive isolation (Henriksen et al. 2018; Nguyen-Phuc & Berres 2018).

202           The socio-sexual behaviour of fowl has been reviewed extensively elsewhere (Kruijt  
203   1964; Wood-Gush 1971; Pizzari et al. 2002; Pizzari 2007; 2016; Birkhead & Pizzari 2009;  
204   Duncan 2009; Nicol 2015; Garnham & Løvlie 2018). Natural groups seldom exceed 30 birds,  
205   although flocks of up to 69 have been reported in semi-natural conditions (Collias et al.  
206   1966). Descriptions of natural populations (wild red junglefowl and feral domestic fowl)  
207   indicate fission-fusion dynamics, in which social groups associated with roosting sites break  
208   up during the day into smaller social units, across spatially differentiated home ranges. Social  
209   units typically comprise of a socially dominant male, several females accompanied by their  
210   young and a number of socially subordinate males orbiting around the dominant male and the  
211   females (Delacour 1951; Collias et al. 1964; 1966; Collias & Collias 1967; 1996; McBride et  
212   al. 1969; Duncan et al. 1978). While socially dominant males monopolise groups of females  
213   (harem polygyny), subordinate males can succeed in mating with these females (McBride et  
214   al. 1969; Cheng & Burns 1988; Thornhill 1988; Collias & Collias 1996; Pizzari et al. 2002).  
215   The mating system is therefore best described as polygamous and more specifically  
216   polygynandrous. However, other social units also occur, including lone individuals,  
217   monogamous pairs or small bands of males (Sullivan 1991; Javed & Rahmani 2000),  
218   indicating substantial flexibility in social structure. These units coalesce into larger social  
219   groups for roosting, and mating activity peaks in the evening and early morning around  
220   roosting sites (McBride et al. 1969; Pizzari & Birkhead 2001). Systematic studies of captive  
221   flocks confirm these observations indicating complex social structure. The seminal work by  
222   Schjelderup-Ebbe (1922) and Banks (1956) revealed tight and persistent sex-specific social  
223   hierarchies, determined by a ‘pecking order’ in domestic flocks, determined by the outcome  
224   of agonistic dyadic interactions between members of the same sex, which are typically  
225   triggered by aggressive behaviour (e.g. pecks) directed by the actor toward a recipient and the  
226   response of the recipient, which can either escalate aggression, leading to a fight, or retreat.  
227   Similar social hierarchies were later confirmed in male red junglefowl (Lill 1966) and in free-  
228   ranging mixed sex groups of both red junglefowl (Collias et al. 1966) and feral domestic fowl  
229   (McBride et al. 1969). Functionally, these social hierarchies were originally interpreted as



emergent properties of competition over, and defence of fitness-related resources (McBride & Foenander 1962). Hence, hierarchies maintain social cohesion by mediating access to resources among socially familiar individuals of varying competitive ability. For example, in domestic flocks, male status is associated with access to food and privileged space utilisation (Masure & Allee 1934), while female status is associated with privileged access to dust-bathing opportunities (Shimmura et al. 2007) and higher fecundity (Sanctuary 1932). Dominant female red junglefowl produce a higher number of offspring over their lifetime compared to subordinate females (Collias et al. 1994).

The study of cognitive mechanisms underpinning social hierarchies in fowl has been extensively investigated (Sanctuary 1932; Zayan 1987a, b). Early work focused on the possibility that the outcome of aggression may reflect a ‘winner-loser effect’, whereby winning (or losing) previous interactions with any bird, may increase (or decrease) the probability that a focal individual will win the next interaction with a new bird (“hysteresis”; Rapoport 1949). Subsequent theoretical and empirical work however, demonstrated that winner-loser effects are insufficient, and the establishment of a social hierarchy requires individual recognition, repeated encounters and memory (McBride & Foenander 1962; Pagel & Dawkins 1997). Evidence for individual recognition in fowl has been mounting since Schjelderup-Ebbe’s (1922) original proposal, through the work by Ryan (1982) and –more recently- Abeyesinghe et al. (2009). These properties allow individual birds to learn their status (i.e. whether they dominant or subordinate toward another bird) through social experience and familiarisation with other flock members. Social experience may also influence the outcome of agonistic interactions through more generalised effects. For example, female red junglefowl with previous experience of being part of small flocks were more aggressive and more likely to be socially dominant when placed in a newly assembled flock compared to females that had been housed with a single male their entire adult life (Kim & Zuk 2000). Consistent individual variation in aggression levels has also been found to be a strong predictor of male social dominance in domestic fowl (Collias 1943; Siegel 1960; Guhl 1962; Favati et al. 2017) and red junglefowl flocks (McDonald et al. 2017). Work in size-

matched paired contests in male domestic fowl has shown that while aggression tends to increase after winning a contest and decrease after losing a contest, consistent with winner-loser effects described above, this did not appear to influence the probability of a male becoming dominant, while his baseline aggression level did (Favati et al. 2017). In general, competitive asymmetries facilitate establishment of status between two birds (Zayan 1987a). As a social hierarchy is developed and birds become familiar with each other, agonistic behaviour tends to be replaced by more nuanced social interactions (e.g. subordinate retreating to the advance of a dominant, “sex crouch” Foreman & Allee 1959), and ritualised behaviours signalling dominance (e.g. crowing, wing-flapping, waltzing in dominant males). Aggression in established hierarchies may persist when two birds are close in competitive ability and repeated challenges of the subordinate may lead to status reversals. In addition to direct interactions, cues of the competitive ability of individual birds can also be gathered indirectly, through social eavesdropping (Potter 1949; Hogue et al. 1996). The role of such indirect cues however, is likely to depend on the linearity of the hierarchy. Early studies documented how flocks may vary in the degree of transitivity of social status. Social transitivity, where if A is dominant over B and B is dominant over C, necessarily means that A is also dominant over C, leads to a linear hierarchy. Intransitivity however, generates more circular social structures (i.e.  $A > B$ ,  $B > C$ ,  $C > A$ , Appleby 1983), where it is presumably more difficult for a bird to gather information about their own social rank based on indirect social cues gathered by eavesdropping the agonistic interactions of other birds.

This work illustrates the central role of social hierarchies in the social structuring of small fowl flocks, which tend to function as single social networks, where dominant males and females in their prime often occupy central positions (Fig. 1). The importance of aggression and social hierarchies is however strongly modulated by group size and density. Studies of domestic lines have been particularly helpful in establishing these effects. Hughes et al. (1997) experimentally studied the relationship between individual rates of aggressive behaviour in 300- and 700-bird flocks, showing limited incidence of aggressive behaviours. Contextualising these results within a considerable body of empirical literature, the authors

showed a positive relationship between aggression rates and group size for groups of up to 12-birds. As group size increases beyond this however, aggression levels drop and are not influenced by further increases in group size (Hughes et al. 1997), and this pattern has been confirmed by subsequent work (Estevez et al. 2003). Hughes et al. (1997) proposed that aggression declines because birds become unable to distinguish familiar from unfamiliar individuals when groups are too large. Similarly, Pagel & Dawkins (1997) predicted that individual recognition may become too costly when groups exceed a certain size. D'Eath & Keeling (2003) provided experimental evidence consistent with this idea for groups of 120 birds. This work is consistent with previous findings that hens are able to individually recognise, and establish a social hierarchy with, 27 other hens (Douglass 1948). Similarly, a study of a flock of 96 birds indicated the presence of an incomplete social hierarchy where the status relationship between several pairs of birds was not clear (Guhl 1953). Together, these results suggest that large (>100-birds) flocks tend to be more socially tolerant (i.e. no discrimination against unfamiliar birds), and while birds might use phenotypic cues to assess the competitiveness of neighbours that do not require familiarity or individual recognition (Pagel & Dawkins 1997), group-wide social hierarchies are unlikely to emerge and influence resource competition. An additional consideration is that increasing group sizes mean that hierarchies are more likely to be intransitive and thus more complex (Wood Gush 1955; Appleby 1983).

The complex social structure of fowl populations (comparable to that of some multi-male mammalian societies) represents a helpful counterpoint to the multitude of studies of sexual selection in socially monogamous species, where social structure and polyandry play a necessarily more limited role. This review has the following aims: i) present a brief synopsis of mechanisms of pre-copulatory sexual selection in fowl, ii) explore patterns and drivers of polyandry, iii) summarise research on post-copulatory sexual selection arising from polyandry, iv) appraise the impact of polyandry on the operation of sexual selection in fowl populations, and vi) outline some caveats and draw some general conclusions on the relevance of fowl research for the study of sexual selection. For this purpose, I focus on

studies of both red junglefowl and domestic fowl populations, with particular emphasis on studies of flocks under close-to-natural conditions. Over the years, our research group has largely worked on two study systems: a feral population of an old Swedish game breed of domestic fowl ('Gammal svensk dvärghöna', Harrison 1987), phenotypically and behaviourally similar to the red junglefowl (Schütz & Jensen 2001), housed at Tovetorp Research Station of Stockholm University, and captive populations of red junglefowl housed at the Swedish University of Agricultural Sciences (Skara) and the John Krebs Field Station of the University of Oxford. While preliminary analyses of this work are contained in several doctoral theses, wherever possible, the review focuses primarily on published results.

## **2. Pre-copulatory sexual selection**

Recent studies of captive red junglefowl groups show that opportunity for pre-copulatory sexual selection largely arises from variance in male mating success (Collet et al. 2012; McDonald et al. 2017), as one would expect in species with polygamous mating systems. Examples of studies investigating pre-copulatory sexual selection in fowl populations are summarised in Table 1a. There is robust evidence that male hierarchies modulate access to mating opportunities. Socially dominant males consistently mate with more females (i.e. have a higher mating success) than socially subordinate males (Guhl et al. 1945; Guhl & Warren 1946; Jones & Mench 1991; Johnsen et al. 2001; Wilson et al. 2008; Collet et al. 2012; McDonald et al. 2017). Both intra-sexual and inter-sexual episodes of sexual selection contribute in favouring socially dominant males.

A direct role of social status in male-male competition is indicated by the fact that social status is strongly predicted by the level of aggression displayed by an individual toward flock members of the same sex (Guhl et al. 1945; Guhl & Warren 1946; Kratzer & Craig 1980; Cheng & Burns 1988; Johnsen et al. 2001; Pizzari 2001; 2003). A recent study of replicate red junglefowl flocks has shown that the mating advantage associated with social dominance is largely explained by the higher propensity of higher-ranking males to attack other males (McDonald et al. 2017). Aggression enables dominant males to more effectively

342 disrupt the mating attempts of subordinate males and discourage subordinates from disrupting  
343 the mating attempts of dominant males (Pizzari 2001). Competitive exclusion of subordinates  
344 is particularly pronounced in the late afternoon (Cheng & Burns 1988), when an insemination  
345 is most likely to result in fertilisation (Pizzari & Birkhead 2001), which results in subordinate  
346 males attempting to mate with females at suboptimal times of the day.

347       Aggression can contribute to maintain and reinforce the mating advantage to the  
348 attacker by encouraging his propensity to attack his rivals again (winner effect) and mate with  
349 females, while discouraging mating propensity in the recipients of his aggression. These  
350 social effects have been suggested to lead to the effective castration of subordinate males in  
351 some domestic populations (Guhl et al. 1945; Duncan 2009), likely through the involvement  
352 of endocrinological pathways. Johnsen & Zuk (1995) have shown that in competitive contests  
353 between two male junglefowl, plasma levels of testosterone prior to a contest do not predict  
354 the outcome of that contest, however following a contest, testosterone plasma levels increase  
355 in the winner and decline in the loser. It is therefore possible that while they have a limited  
356 role in determining social status (see above), winner-loser effects may be more important in  
357 explaining the mating advantage of dominant males over their subordinates.

358       Unpacking the role of social status in intrasexual competition over mating  
359 opportunities in fowl populations has broad relevance for our understanding of mating  
360 systems in social species. For example, in social groups of green woodhoopoes, *Phoeniculus*  
361 *purpureus*, dominant males disrupt the mating attempts of subordinates until females ovulate  
362 (Ligon & Ligon 1990). A similar pattern has been observed in several primates where  
363 subordinate males are often restricted to mating with females who are either not in oestrus or  
364 not yet sexually mature (Hrdy & Witten 1987), and in Soay sheep, *Ovis aries*, groups, where  
365 mate guarding by dominant rams restrict subordinate males to mating opportunities with poor  
366 quality females (Preston et al. 2006). In white-winged trumpeters, *Psophia leucoptera*, a South  
367 American Gruiform bird, dominant males have privileged access to mating opportunities and  
368 aggressively disrupt matings initiated by subordinates (Eason & Sherman 1995). In contrast,  
369 subordinate males manage to mate by approaching females using sneaker tactics, and attempt

to disrupt matings by the dominant male (Eason & Sherman 1995). The formation of coalitions among subordinate males to obtain mating opportunities or curtail those of the dominant male has been reported in a number of social mammals (Smuts 1987), but rarely for birds. Anecdotal information of feral fowl flocks suggests that males of intermediate social status may sometimes interact with each other in a way seemingly consistent with cooperation over mating opportunities (T. Pizzari unpubl.), however the scarcity of such behaviours prevents a systematic study, while indicating that this strategy is rare at best.

Intersexual selection also plays an important role. The fowl represents one of the earliest model systems for the study of mating preferences in both males and females. Early work focused on domestic lines and preferences for mates of the same *versus* different breeds (Lill & Wood-Gush 1965). Female preference has also been implicated in sexual selection for male social status. A number of studies have shown that females prefer to associate with dominant males (Duncan et al. 1978; Graves et al. 1985; Thornhill 1988; Collias & Collias 1996; Johnsen et al. 2001). Preferential mating with dominant males may then arise as simple consequence of proximity. However, females have also been shown to actively bias mating outcome in favour of dominant males (Graves et al. 1985; Pizzari 2001). Female mating behaviour is expressed along a gradient from active solicitation, in the form of a squatting posture in front of a male, which has been shown to trigger male sexual interest (Fisher & Hale 1956), to high levels of active resistance to the advances of a male, and females are more likely to solicit mating with dominant males, and resist less strongly the advances of dominant males (Wood-Gush 1971; Pizzari & Birkhead 2000; Johnsen et al. 2001). In freely-interacting groups the majority of mating attempts tends to be initiated by males and resisted by females to varying degrees (Pizzari & Birkhead 2000; Pizzari 2001; Løvlie & Pizzari 2007). Interactions between intra- and inter-sexual selection episodes have also been documented. Play-back experiments showed that in feral groups of domestic fowl, females use distress calls to attract other males when seized by a socially subordinate male. Attracting male attention increases the chance that a socially dominant male will disrupt the current attempt and replace him in copulating with the female (Pizzari 2001). Subsequent work on the

same population neatly demonstrated that females not only preferentially utter distress calls when approached by subordinate males, but they are especially likely to do so in the presence of other males (Løvlie et al. 2014). Differential distress calling therefore enables female fowl to strategically favour dominant males indirectly, by modulating the level of male-male competition associated with individual copulations. These strategies of ‘indirect’ (*sensu* Wiley & Poston 1996) intersexual selection bear similarities with the behaviour of females in other polygynandrous species where active female resistance and choice may be associated with high costs to females (Cox & LeBoeuf 1977; Semple 1988). For example, Pizzari & Birkhead (2000) showed that in feral flocks females preferentially resist the advances of subordinate males, yet female resistance alone does not appear to have a tangible impact on the mating chances of these males, suggesting that the ability of female fowl to directly prevent mating is limited.

Other studies have indicated a possible role for specific male behaviours in intersexual selection. For example, Leonard & Zann (1998) showed a role of male wing-flapping frequency, a ritualised behaviour that males display to other males and to females, in mating success in mate choice trials of domestic fowl. A similar pattern was observed by Zuk et al. (1995a) in red junglefowl, where females displayed a preference for males that had higher rates of courtship displays (e.g. crowing, wing-flapping, waltzing, “tidbitting”, a ritualized behaviour associated with courtship feeding), following exposure to females. Male display rate however was found to correlate with the expression of several morphological ornaments including comb expression (see below), so it is not clear whether females were responding to male display rates *per se* or displayed a preference for a male phenotype, which also happened to display at a high rate. Wilson et al. (2008) conducted an extensive characterization of male morphological and behavioural phenotypes in flocks of domestic Golden Seabrights, and used multivariate analysis to investigate predictors of both male mating success (measured as frequency of successful mating) and reproductive success (based on paternity assignment). The study showed that variation in both mating success and overall reproductive success was largely explained by a principle component axis capturing male

social status and behaviours associated with status, which included crowing, wing-flapping, and the rate at which males signaled the presence of food items (food calling) or possible aerial and terrestrial predators (alarm calls, Wilson et al. 2008). Wilson & Evans (2008) conducted experiments suggesting that the relationship between male alarm calling rate and mating success may have the opposite causality: rather than females preferring to mate with males that call more frequently, males may increase their calling effort after mating with females. This was originally interpreted as an adaptive strategy, whereby a male invests in the protection of a female with whom he has already mated, as she is likely to produce his offspring (Wilson & Evans 2008). A similar strategy has been proposed in male crickets, *Gryllus campestris*, which expose themselves to a higher risk of predation by giving their mating partners precedence to their own burrows when attacked by predators (Rodríguez-Muñoz et al. 2011). At a proximate level the behaviour observed by Wilson & Evans (2008) may be explained if mating boosts a male's confidence in his attractiveness, promoting investment in courtship and status-related behaviours. Establishing whether functionally this response has been selected as a strategy to invest in the protection of future offspring would require testing if males preferentially invest their vigilance and alarm calling effort in females with whom they have recently mated or whether they indiscriminately increase vigilance and alarm calling effort independently of the identity of the female or their confidence in the paternity of her offspring. Regardless of their adaptive significance, Wilson and Evans (2008) provide an important demonstration of feedback loops between mating and behaviours traditionally associated with courtship or status. A similar causal relation was described in American pintails, *Anas acuta*, where a male's social status is elevated as a consequence of his successfully mating with a female (Sorenson & Derrickson 1994). Collectively, these results confirm a female preference to mate with males that are either of high social status and/or display behaviours (e.g. high rates of alarm calling, courtship-feeding, wing-flapping, waltzing) strongly associated with social dominance.

In addition to social competitive ability, male fowl exhibit a wide range of plumage and morphological traits that are strongly exaggerated compared to females. These include



sickle and hackle feathers, spurs, as well as the comb and wattles, highly vascularised fleshy head ornaments. Extensive work by Marlene Zuk and colleagues on a red junglefowl population originally established at San Diego Zoo (subsequently acquired by the University of New Mexico) focused largely on the role of several such male morphological and plumage traits under experimental conditions. This work often utilised a strongly experimental approach in which individual females were presented with a dyadic choice between two males and where social interactions between males were limited or altogether prevented. This line of research has been instrumental in providing some of the first experimental demonstrations of female choice, revealing a consistent female preference for larger and brighter male comb, while ruling out other traits (Zuk et al. 1990a; 1990b; Zuk et al. 1995a; Ligon & Zwartjes 1995; Parker & Ligon 2003; 2007). The relationship between female preference for male social status and for male comb expression remains unclear. There is some inconsistency over the importance of these two responses reported across different investigations, which has been explained as follows: studies of social groups in which multiple males are able to interact, socially consistently show male status (and related behaviours) as the key predictor of male mating success and female mating preference, with little or no evidence of a role for male comb, while studies where male-male competitive interactions are scarcer or experimentally reduced tend to reveal a role for male comb (Wilson et al. 2008).

It is in principle possible that male comb expression is correlated with male social status and that females may use male comb expression as a proxy for status when male-male competitive interactions are limited (Pizzari 2016). This suggestion is based mainly on: i) some evidence that male comb size and social status are positively correlated in some populations (Graves et al. 1985; Zuk & Johnsen 2000), ii) the notion that both social status and comb size are dependent on steroid plasma levels (Allee et al. 1939; Zeller et al. 1971; Ligon et al. 1990; Fennell & Scanes 1992; Zuk et al. 1995; Verhulst et al. 1999), and iii) the observation that the comb of a male rapidly enlarges or shrinks following a positive or negative outcome of a social challenge by another male in red junglefowl (Parker et al. 2002;

Cornwallis & Birkhead 2008). In addition, males appear to be particularly challenged by rivals sporting both, a dominant behaviour and a large comb, suggesting that these traits may play an integrated role in social signalling (Parker et al. 2002). However, a recent study of replicate red junglefowl groups has shown no relationship between male comb size and social status, and no consistent changes in comb size across males that increased or lost social rank over successive days (R. Carleial, G. C. McDonald, & T. Pizzari unpubl.). Johnsen et al. (2001) have shown that even in small freely-mating red junglefowl groups with only two males and three females, the socially dominant male accounted for most of the matings observed. Interestingly, male comb size was associated with social status only in one of the two study years, confirming the weak relationship between these phenotypes (see above), and female preference for the dominant male was unanimous among all females when status was positively correlated with comb size, whereas when comb size was independent of status, females of the two highest social rank preferred the dominant male, and the bottom-ranking female showed a preference for the subordinate male (Johnsen et al. 2001). A similar interaction between male comb size and social status was described in domestic flocks by Graves et al. (1985). Here, status was positively correlated with comb size. Females that were naïve about male status preferentially associated with relatively large-combed males, while females with information on the male hierarchy demonstrated a preference for dominant males. Collectively, these results indicate that in social units in which individuals can interact freely, male status tends to emerge as the key factor in predicting mating success through both intra- and inter-sexual mechanisms. The extent to which male social status and comb expression are phenotypically integrated and their independent roles in female preference remains unclear. However, it is possible that male comb expression can inform female mating decisions under some conditions.

## 2.1 The significance of female preference

Little is known about the mechanisms promoting the evolution and maintenance of the patterns of female preference described above. It has been speculated that preferential

association with socially dominant males (Graves et al. 1985) may result in better protection against predators and access to nutritious food items (McBride et al. 1969; Pizzari 2003; Wilson et al. 2008). Indeed, describing the behaviour of feral flocks, McBride et al. (1969) suggest that the crow of the dominant male may function as an ‘all-clear’ signal, indicating to females and their chicks that it is safe to forage in the open. Socially dominant males invest more time in vigilance against predators, and their alarm calls tend to be more reliable than those produced by subdominant males (Pizzari 2003). Socially dominant males have also been shown to be more likely than subordinates to courtship-feed females (through tidbitting) when experimentally provided with food items, while subordinates would often attempt to attract females with dishonest food calls (i.e. calls not associated with genuine food items, Pizzari 2003). In this context, dishonest tidbits by subordinate males appears to represent a “sensory trap”. Females may therefore be naturally selected to associate with dominant males in pursuit of commodities such as protection and food, and females may be more likely to mate with these males as the coincidental consequence of physical proximity. Alternatively, females could be actively selected to preferentially mate with dominant males if these males traded vigilance and courtship-feeding for sex. A similar mechanism of retribution seemingly consistent with reciprocity, was suggested for Adelie penguins, *Pygoscelis adeliae*, where females appear to engage in extra-pair copulations with neighbouring males in order to obtain nest material (Hunter & Davis 1998). Pizzari (2003) found no evidence of this hypothesis in feral domestic flocks, showing that variation in the probability of a female mating with a male was predicted exclusively by the male’s social status rather than by his provision of courtship-feeding to the female.

In principle, a preference for male status and for comb phenotype may also be driven by the pursuit of genetic benefits to the offspring. Rather than a phenotypic trait *per se*, dominance is more usefully viewed as a social construct emerging from the outcome of competitive interactions, and thus contingent on the social environment (Moore et al. 2002). However, a number of phenotypic traits, such as pheromones, body size or aggression, predispose individuals to social dominance, by increasing the chances of winning competitive

interactions. There is substantial evidence that phenotypes associated with social competitive ability display some degree of additive genetic variation in a number of species (Kuse and DeFries 1976; Horne and Ylönen 1998; Moore et al 2002), which can contribute to positive parent-offspring relationship in social status (Jonsson 1985; Nol et al. 1996). Early work on domestic fowl indicated that social competitive ability is heritable (Craig et al. 1965), suggesting that females with a preference for socially dominant males may benefit by producing more competitive offspring. This scenario is consistent with a Fisherian-runaway model, in which linkage disequilibrium between genes associated with female preference for socially dominant partners and genes for social competitive ability promote the co-evolution of preference and preferred trait (Andersson 1994). Another non-mutually exclusive possibility is that social status reflects a male's breeding value for condition. First, social contests among males are often injurious and energetically costly. Second, status-related behaviours such as vigilance prevent males from foraging or resting. Physiological and energetic constraints associated with attaining and maintaining high social status can lead to genetic covariance between male competitive ability and condition, creating scope for females that reproduce with dominant males to also benefit through the production of more viable offspring, as predicted by "good genes" models of mate choice. A recent study of red junglefowl groups however, found that over the course of a 10-day experimental trial in which males competed over access to females, more dominant males gained in weight or lost proportionally less weight than socially subordinate males (R. Carleial, G. C. McDonald, & T. Pizzari unpubl.). One possible interpretation of this pattern is that genotypes predisposed to social dominance will find the costs of social competition easier to bear, which would be consistent with the good genes model. An alternative explanation however, is that being socially dominant may not be costlier than being subordinate, which questions the notion of male status as a condition-dependence trait. This may be due in part to the fact that social dominance, while costly, confers better access to fitness-related utilities.

Condition-dependence has been more extensively investigated in comb expression. The comb size of male red junglefowl has been shown to be particularly sensitive to

experimental infection with the nematode *Ascaridia galli* in red junglefowl (Zuk et al. 1990c; but see Chappell et al. 1997). This pattern is consistent with the idea that heightened condition dependence is mediated by parasite infections, which makes sexually-selected ornaments (such as the bright plumage and soft tissue displayed in birds) reliable indicators of a male's breeding value for parasite resistance (Hamilton & Zuk 1982). Two potential pathways underpinning parasite-mediated condition dependence of male comb expression have been proposed. One explanation evokes a role for free-radicals, released by phagocytes activated to combat parasitic infections, which may compromise comb expression by reducing the viscosity of hyaluronic acid, a key factor for the intercellular matrix of this tissue (von Schantz et al. 1999). A second explanation is based on the immune-suppressant effect of steroid hormones (Folstad & Karter 1992). Comb expression is strongly dependent on testosterone plasma levels (Hardesty 1931; Allee et al. 1939). If the testosterone levels required for maintaining large, bright combs depress a male's immune ability to combat pathogens and parasites, comb expression can in principle function as a 'handicap', representing a reliable signal for male breeding values for immune capacity. Patterns consistent with this idea were demonstrated in domestic fowl lines divergently selected for high and low antibody responses to sheep erythrocytes, where males from the high response line had smaller combs and lower testosterone levels than males from the low line (Verhulst et al. 1999; Foo et al. 2017). A similar pattern was described in sexually immature domestic chicks in lines selected for meat production (broilers), where high immunological responses to *Escherichia coli* were associated with smaller combs and lower plasma levels of testosterone (Leitner et al. 1996). Importantly, comb size is heritable (von Schantz et al. 1995; Tufvesson et al. 1999) and a multi-generation pedigree study of red junglefowl detected a strong genetic correlation between comb size and residual body mass, a standard measure of condition (Parker & Garant 2004), creating opportunity for good genes mechanisms of female preference. In principle, linkage disequilibrium between possible female preference genes and genes associated with comb expression would also create scope for the evolution of female preference for large male comb through Fisherian runaway dynamics. A careful study of red

junglefowl has provided some evidence that reproducing with large-combed males may result in both more viable and more attractive sons (Parker 2003). The study used vasectomized control males and artificial insemination experiments to control for differential maternal allocation (i.e. females allocating more resources to eggs fertilized by large-combed males), and showed that sons of large-combed males developed better body condition and larger combs. These paternal effects on sons became more pronounced over ontogenetic development, implicating the causal effect of inherited genetic variation from fathers, rather than mechanisms such as indirect genetic effects (*sensu* Moore et al. 1997) of paternal genotype on offspring development, e.g. from variation in seminal fluid composition (Parker 2003).

The genetic architecture of comb size raises a further possibility for the evolution of a female preference for larger male comb, namely that males sporting large combs may produce more fecund daughters. Quantitative genetic work indicates a positive genetic correlation between male and female comb size (von Schantz et al. 1995; Tufvesson et al. 1999), and more recent Quantitative Trait Loci (QTL) mapping studies of a red junglefowl intercross with a domestic egg-layer line (White leghorn) revealed that QTLs associated with female-specific variation in comb size are linked with QTLs associated with female fecundity (Wright et al. 2008). This indicates that the phenotypic relationship between female comb size and different measures of female reproductive output (e.g. oviposition rate, average egg mass) may have an additive component. Together, these results suggest the novel possibility that genetic benefits obtained through daughters (rather than sons) may contribute to the evolution and maintenance of female preference for male comb expression. This idea however, requires careful consideration; positive intersexual genetic correlations for individual traits do not necessarily translate into positive intersexual genetic fitness correlations (see below).

The fact that other plumage and morphological ornaments do not appear to play a role in female preference raises questions about their maintenance in male fowl. This is a typical pattern in sexual selection studies. The maintenance of seemingly redundant, multiple

exaggerated traits in males has long puzzled evolutionary biologists. It has been suggested that males cannot afford to loose ornament traits even when they are no longer the target of female preference because without such traits females would fail to recognize males as possible reproductive partners (Holland & Rice 1998). Similarly, it is possible that different traits capture complementary aspects of male quality and thus provide females with different information on the quality of the reproductive contribution of a male (Møller & Pomiankowski 1993). There is some evidence that some of the exaggerated traits displayed by male fowl may play a role under specific conditions. For example, experiments using high-definition playbacks and 3D animations have indicated that the display of wattles (fleshy, paired caruncles, which hang from the bird's lower mandible) can help the efficacy with which tidbitting males signal to a female audience (Smith et al. 2009). Studies investigating sexual selection on fowl comb and wattles have been particularly helpful in elucidating the functional significance of fleshy ornaments displayed by many other species of galliformes (Buchholz 1997) and of other avian orders (Lloyd-Jones & Briskie 2015), and more generally on the role of bare parts in social signaling (*sensu* Iverson & Karubian 2017) across different taxa. Fleshy traits differ from more structural traits in their extreme phenotypic plasticity (e.g. in size, shape and colour) and sensitivity to both changes in internal state and in the social environment, which increases potential for more accurately reflecting condition and for social signaling (Iverson & Karubian 2017).

In summary, variation in male mating success is largely explained by intrasexual mechanisms mediated by male social status, especially in larger or more male-biased populations, and intersexual mechanisms driven by female preference, which often reinforces selection for male status, and –when male-male competition is limited- for male comb expression. The adaptive significance of female preference remains unclear. The architecture of both status and comb size offers potential for genetic benefits (good genes and Fisherian runaway). Future research should seek to resolve: 1) the relative female costs of resisting dominant and subordinate males, 2) the genetic correlation between social competitive ability and comb expression, and 3) the intersexual genetic fitness correlation of status and/or comb

size. The need to address the latter point is motivated by recent work indicating that genotypes producing competitive/attractive males often produce less successful females and *vice-versa*. This negative intersexual fitness correlation, which arises as the inevitable consequence of constraints in the evolution of sex limitation and the strong divergence in phenotypic optima between adult males and females, can temper genetic benefits associated with preferred male phenotypes (van Doorn 2009).

## 2.2 Social modulation

A fundamental pattern emerging from studies of fowl populations is that the strength of intra- and inter-sexual selection are highly labile and socially plastic (Craig et al. 1977; Kratzer & Craig 1980; Fig. 2). This plasticity is observed at multiple levels. First, the relative importance of male-male competition versus of female choice changes with group size and sex ratio. Hanne Løvlie studied replicate groups of feral domestic fowl of similar size but different sex ratios, and found that in strongly female-biased groups active female choice was evident through active solicitation of sex from certain males (Cheng & Burns 1988; Løvlie & Pizzari 2007). As groups become less female-biased however, individual females are exposed to increasing levels of sexual advances by males. This results in: more direct competitive interference between males, a drastic reduction in female solicitation and a corresponding increase in female resistance to male advances. Under such conditions the signature of female preference is progressively less perceptible and largely restricted to patterns of differential resistance rather than active solicitation (Pizzari 2001; Løvlie & Pizzari 2007). Therefore, as the number of females that are on average available to males declines in a group, we expect intra-sexual selection to become progressively more important and inter-sexual selection to become progressively less important. Controlling for sex ratio, group size is also likely to play an important role in modulating episodes of sexual selection. For example, observations of feral fowl populations suggest that smaller and more female-biased groups enable dominants to monopolise access to females through direct competitive exclusion of subordinates, and accentuate active female choice in favour of dominant males (Dean et al.



2010). These effects are likely to diminish with unilateral increments in the proportion of males in the population or with population size (Duncan 2009), because the ability of dominant males to monopolise females will diminish with the number of competitors and because large flocks will prevent individual recognition of all flock members, thus preventing the establishment of a stable hierarchy (see above). Sexual selection for male social status is expected to be highest for groups of intermediate size/sex ratios, and diminish when groups are too large and/or male-biased for social males to monopolise access to females. Under such conditions mating opportunities are likely determined by scramble competition, although accurate information on mating dynamics in large groups is currently lacking.

A second level of plasticity is observed at different temporal scales. Over successive days an obvious effect of sexual rest is observed, whereby sexually rested and reproductively active (i.e. ‘in lay’) females demonstrate a strong propensity to mate and little discrimination in the first hours following initial exposure to males. As females accumulate matings, discrimination increases and within 24-48 hrs, females begin to resist the majority of male sexual advances (R. Carleial, G. C. McDonald, & T. Pizzari unpubl.). Løvlie et al. (2005) demonstrated that stimuli associated with the physical act of a male mounting the female trigger inhibition of female propensity to mate again (i.e. lower propensity to solicit and stronger resistance to male advances) over the following 48 hrs in feral domestic fowl. A very similar mechanism has been characterised in *D. melanogaster*, where female mating propensity is reduced through the interaction between an accessory gland product (the Sex Peptide) inseminated by the male and the Sex Peptide Receptor expressed in the female (Sirot et al. 2011).

Therefore, rather than fixed, the relative roles of intra- and inter-sexual episodes of sexual selection are highly dynamic in fowl populations, and research indicates this dynamism is strongly modulated by variation in the social environment.

### **3. Drivers of polyandry**

Unlike socially-monogamous birds, where female polyandry was revealed only relatively

recently through the advent of molecular paternity assignment (Parker & Birkhead 2013), the promiscuity of female fowl had been known long before Darwin. In his *History of Animals* (350 B.C.), Aristotle described hens mating with multiple males and provided the first account of sperm competition, and Pliny the Elder commented on the lustful behaviour of both male and female Galliforms, including domestic fowl, in his *Natural History* (77 A.D.). Subsequent naturalists such as Buffon and Smellie viewed polyandry as a property of domestication (Birkhead & Pizzari 2009). While factors associated with domestication, such as larger, denser populations and artificial selection, may have contributed to promote polyandry (Hulme-Beaman et al. 2018), field studies of red junglefowl demonstrated that some degree of polyandry is typical of ancestral, natural conditions (Collias & Collias 1967; 1996; Collias et al. 1964). Both male- and female-driven mechanisms appear to contribute to polyandry. A male factor is clearly indicated by the observation that the number of mating attempts per female increases with the number of males in the population and that the vast majority of copulation attempts are initiated by males and resisted by females except in small, strongly female-biased groups (Løvlie & Pizzari 2007). This difference in propensity to mate with multiple males is explained by sex-specific selection on mating success (Bateman gradients). Collet et al. (2014) showed that male junglefowl are strongly sexually selected to mate with multiple females (a steep Bateman gradient), while females display a weaker (albeit significantly positive) Bateman gradient. Even this weaker female gradient however is misleading. Experimental tests showed that female Bateman gradients do not indicate that females produce more offspring by mating with more males but rather reflects a male preference to mate with females that are inherently more fecund (Collet et al. 2014). This suggests that polyandry may arise as the consequence of pre-copulatory sexual selection on mating success in males, which is likely to result in multiple males mating with the same female particularly in small fowl populations and in other systems where females cannot be effectively monopolized by a single male. Females may then mate with multiple males simply because they are unable to resist the advances of different males or because strategically it may be beneficial (i.e. less costly) to mate with multiple males rather than resist most

attempts (“convenience polyandry”). The latter possibility may be particularly relevant to female fowl. Relevant potential costs of mating to females include the risk of sexually-transmitted disease, and reproducing with males providing suboptimal genetic paternal contributions. While there is increasing evidence of bacterial exchange among mating partners in several species of birds (Kulkarni & Heeb 2007), few studies have been able to unambiguously attribute this effect to insemination eliminating confounding factors such as shared environment and diet. In addition, very few of the bacterial species that are potentially sexually transmitted have been shown to be pathogenic in avian hosts (Sheldon 2003). It is in principle possible that mating may be costly by disrupting the community of bacteria in the female reproductive tract. Recent work on the microbiota of the male ejaculate and the female reproductive tract of a population of red junglefowl has begun to reveal complex patterns of bacterial diversity dynamically variable in space and time (Eccleston 2017). At present however, we know little about the role that these communities play in the reproductive health and fitness of female hosts and the way inseminations impact these effects.

Another possible cost of mating may arise when a male of ‘low genetic quality’ sires the offspring. To the extent to which heritable traits such as social competitive ability may influence offspring viability and reproductive success (see above), producing offspring sired by males of low competitive ability may compromise a female’s fitness. In addition to the male genotype, the interaction between the genotypes of reproductive partners can also contribute to mating costs, as some combinations of parental genotypes are less compatible than others and suffer impaired offspring viability and/or reproductive success. Well-established cases of such incompatibilities include inbreeding depression (Hedrik & Garcia-Dorado 2016) and loss of heterozygosity at candidate loci such as the Major Histocompatibility Complex (MHC), a critical component of vertebrate immunity (Spurgin & Richardson 2010). Both such factors have been well documented in domestic fowl. Inbreeding depression has been characterized in a number of domestic populations (Craig & Baruth 1965), while the simple structure of the fowl MHC has resulted in clear demonstrations of association between polymorphism at MHC loci and resistance to specific

pathogens or between MHC polymorphism and immune responses (Kaufman 2000; Zelano & Edwards 2002).

The risk of inbreeding is relevant to wild populations of red junglefowl, which are typically philopatric (Nguyen-Phuc & Berres 2018; Collias et al. 1966; Collias & Collias 1996). Such high viscosity can favour inbreeding: around 4% of the copulations observed in the free-ranging red junglefowl population at San Diego Zoo, were among 1<sup>st</sup> order relatives (Collias & Collias 1996). In addition, due to high polymorphism and limited recombination at the MHC, genetic relatedness between individuals is often highly correlated with their similarity at the MHC (Penn & Potts 1999), suggesting that –in addition to inbreeding depression- population viscosity in fowl can also have repercussion for MHC diversity *per se*. In a captive population of red junglefowl we recently demonstrated significant inbreeding depression on a number of fitness-related traits, e.g. male social status (C. K. Tan, G. C. McDonald, L. Spurgin, S. Wigby, D. S. Richardson, & T. Pizzari unpubl.; see Craig & Baruth 1965 for similar results in domestic populations), and a viability cost associated with low MHC heterozygosity during coccidiosis outbreaks (Worley et al. 2010). It is however arguable whether these costs would necessarily increase on average for polyandrous females in fowl populations. In fact, polyandry provides females with the possibility of differentially utilizing sperm from different partners, favouring fertilization by preferred males through cryptic female choice (see below). Compared to pre-copulatory female choice, which requires costly resistance against non-preferred males, cryptic female choice represents an arguably less costly strategy for females to retain some control over the paternity of their offspring (Firman et al. 2017). Compared to potential costs of mating, the costs of resisting mating appear substantial in female fowl. Males are larger than females and resistance involves sustained chases and physical struggles. There is some evidence that these are energetically demanding. Across 10-day trials, freely-mating female red junglefowl, which were exposed to a higher frequency of resisted mating attempts were found to loose more body mass (R. Carleial, G. C. McDonald, & T. Pizzari unpubl.). Interestingly, the overall mating rate of a female did not predict the degree of body mass loss, suggesting that mating *per se* is not

energetically costly (R. Carleial, G. C. McDonald, & T. Pizzari unpubl.). Female resistance can also be associated with risks of injury to females, e.g. leading to the rupture of hard-shelled eggs carried in the oviduct (Møller & Birkhead 1992; Pizzari 2001). These lines of evidence suggest that patterns of polyandry observed in mating flocks, particularly flocks with relatively high proportion of males, are largely driven by sexual selection on male mating success. However, the notion that the levels of polyandry in fowl populations largely reflect selection on males does not rule out the possibility that females too might actively pursue mating with more than one male. Experimental mate choice trials in red junglefowl, where male initiation of mating is prevented, demonstrated that females can actively solicit mating from multiple males (Wood-Gush 1971; Ligon & Zwartjes 1995). One possibility is that female-driven polyandry may arise as a genetic consequence of sexual selection on male mating success. If propensity to mate with multiple partners (promiscuity) is heritable and expressed in both sexes, female propensity for polyandry can evolve even when it delivers no benefit to females provided that there is sufficiently strong sexual selection for male propensity for polygyny and sufficiently large inter-sexual genetic covariance in propensity for promiscuity. Recent experimental work on Zebra finches, *Taeniopygia guttata*, has demonstrated the potential for positive genetic inter-sexual correlations in promiscuity propensity (Forstmeier et al. 2011).

A number of adaptive hypotheses have also been proposed to explain the evolution of female-driven polyandry (Jennions & Petrie 2000; Simmons 2001). The fact that female fowl do not obtain quantitative benefits (i.e. produce more offspring) by mating with multiple males does not exclude the possibility that some degree of polyandry may benefit females. Under some circumstances, polyandry may enable females to reduce the risk of mating with a male that does not deliver sufficient sperm to fertilize the entire set of eggs. This may be particularly relevant in social groups where socially dominant males are old. In a feral population of domestic fowl male reproductive performance (mating propensity, probability of insemination, number of sperm delivered, sperm swimming velocity) begins to decline when males are around 4 years old, partly as a result of population-level processes (i.e. males

with superior reproductive performance suffering shorter lifespan) and partly due to irreversible age-dependent declines in reproductive function within individual males, typical of reproductive senescence (Dean et al. 2010; Cornwallis et al. 2014). This is consistent with our observations of male senescence in captive red junglefowl (McDonald et al. 2017; R. Carleial, G. C. McDonald, & T. Pizzari unpubl.) and with demographic data from the free-ranging junglefowl population at San Diego Zoo (Collias et al. 1966; Collias & Collias 1996). While male aggression also may sometimes decline with male age (McDonald et al. 2017; Rosher et al. 2017), it appears that male social status remains less vulnerable to ageing, at least when male competition is limited, i.e. small groups where the sex ratio is not male-biased (Dean et al. 2010). In these groups, old, dominant males are unable to fertilise all the eggs produced by each female resulting in substantial fertility costs to females (~20% fewer eggs fertilized compared to groups with young dominant male; Dean et al. 2010). Notably, all the eggs in groups with a young dominant male were sired by this male, while ~30% of the eggs fertilized in groups with old, dominant males were sired by subordinate males (Dean et al. 2010). This strongly suggests that polyandry could buffer the costs imposed on females by mating with old, dominant males. It is possible that male strategic sperm allocation (see below) may further exacerbate this problem. For example, a considerable proportion of behavioural successful matings fail to result in sperm transfer (aspermic copulations, Garcia-Gonzalez 2004), and this has been established in several populations of fowl (Parker et al. 1942; Pizzari et al. 2003). In discussing patterns of repeated copulations in birds, Møller & Birkhead (1992) had already argued that, given sperm depletion, copulations are often unlikely to result in sperm transfer and had speculated about the functional significance of aspermic copulations, e.g. through social signalling (Garcia-Gonzalez 2004). In fowl, the propensity of males to perform aspermic copulation after inseminating ejaculates in a female (Pizzari et al. 2003) combined with the observation that mounting alone is sufficient to inhibit female propensity to remate (Løvlie et al. 2005), suggests that aspermic copulations may represent a strategy to reduce the risk of sperm competition faced by their own ejaculates in the near future. When male sperm reserves are limited and multiple mating partners are

available (as is the case for dominant male fowl), a male may benefit by economizing his sperm allocation to a female in order to inseminate other females. Compared to a strategy in which the male invests enough sperm in a female to guarantee fertilization of all her eggs, this behaviour may reduce the proportion of eggs that he fertilizes in each clutch (resulting in a fertility cost to individual females), but can substantially increase his own overall reproductive success as he would be able to fertilise a proportion of the eggs of multiple clutches. Sperm limitation may be relevant to mating systems where high mating skew exposes the most successful males to considerable risk of sperm depletion (Warner et al. 1995; Preston et al. 2001), although the role of mechanism in female-driven polyandry remains to be elucidated.

In principle the pursuit of male courtship-feeding may also contribute to female-driven polyandry in this species. This could represent either a female strategy to mate with different males in exchange for food or might emerge as the consequence of females mating with the nearest male and moving from male to male in order to secure more courtship-feeding. Pizzari (2003) however, found no evidence that the probability of a female mating with a male is predicted by the amount of courtship feeding that she received from the male. Most adaptive hypotheses of female-driven polyandry have focused on possible genetic (or epigenetic) mechanisms of increased offspring performance. These benefits fall into the following categories: i) increased genetic diversity of a brood; ii) genetic benefits determined by linkage with male sperm competitive ability (“sexy sperm” hypothesis, Keller & Reeve 1995; “good sperm” hypothesis, Yasui 1997); and iii) genetic benefits associated with certain paternal genotypes or the compatibility of paternal/maternal genotypes. The first category requires polyandry to result in a close-to-even share of paternity of a brood across multiple, genetically different males. The second category requires polyandry to incite sperm competition and a strong additive components and genetic covariances in male fertilizing efficiency, female-driven polyandry and/or viability. The third category requires the ability of females to bias the outcome of sperm competition in favour of certain male genotypes through mechanisms of cryptic female choice. The relevance of the first two categories

remains unclear for fowl populations as well as more broadly. The third category, based on cryptic female choice, appears more relevant and is discussed in more detail below.

In summary, potential benefits of polyandry to females are tempered by substantial costs of mating and male sexual harassment. The optimal level of polyandry for females is therefore likely lower than what is typically observed, particularly large flocks and those close to unitary or male-biased (Fig. 2).

#### **4. Post-copulatory sexual selection**

Fowl populations have provided unique insights into patterns and mechanisms of post-copulatory sexual selection, due to the following reasons. First, fine-grained studies of mating behaviour in habituated populations has enabled the precise quantification of mating rates and mating success independently of reproductive success. These variables, which are critical to the study of sexual selection, are often inferred indirectly from parentage data in wilder, more vagile and less habituated populations, resulting in potentially severe misestimates and bias (Collet et al. 2014). Second, the availability of techniques developed by poultry scientists to collect semen samples non-invasively, study sperm and seminal fluid *in vitro* and *in vivo* through artificial insemination, has catalysed the experimental study of the mechanisms of sperm competition and cryptic female choice, linking behaviour with ejaculate performance. Third, the sequencing of the fowl genome (International Chicken Genome Consortium 2004) is catalysing our understanding of the genetic architecture of traits potentially important in post-copulatory sexual selection, such as seminal fluid proteins.

Studies of red junglefowl groups have shown that standardised variance in paternity share, arising from polyandry, is consistently the largest source of variation in male reproductive success, accounting for ~42% of the variance in groups of 3 males and 4 females (Collet et al. 2012) and ~57% in groups of 10 males and 12 females (McDonald et al. 2017), consistent with the idea that polyandry may increase with population size and the proportion of males in the population. This variance represents the opportunity of post-copulatory sexual selection on male traits promoting fertilisation success following mating, through sperm



competition and cryptic female choice. Examples of studies investigating post-copulatory sexual selection in fowl populations are summarised in Table 1b. Below, I discuss some of the male traits that have emerged as potentially important targets of post-copulatory sexual selection.

#### 4.1 Male social status

There is increasing evidence that in addition to mating success, male social status and phenotypes conducive to dominance, may also play a key role in predicting paternity share in polyandrous groups. Recent work is helping shed light onto the mechanisms through which social dominance confers an advantage in post-copulatory sexual selection. In small groups dominant males are able to prevent other males from remating with their sexual partners therefore maximising their own share of paternity (Cheng & Burns 1988; Dean et al. 2010, Fig. 2). In larger groups or groups with smaller female bias, dominant males appear to be favoured through other mechanisms. One such mechanism favouring male status after mating is inter-sexual, through cryptic female choice. Randy Thornhill originally provided anecdotal evidence that in a small flock of red junglefowl, females may be more likely to eject semen shortly following mating with socially subordinate males (in Birkhead & Møller 1992; in Eberhard 1996). Consistent with this suggestion, observations in free-ranging flocks of feral fowl indicated that socially dominant males suffered a lower risk of sperm ejection by females, and that these differences in sperm ejection tracked changes in the social status of individual males (Pizzari & Birkhead 2000). Given that this study was based on observations of free-ranging flocks, it failed to control for a number of methodological factors. These included the fact that it could not be performed blind with respect to male status, and could not control for the volume of ejaculates produced by different males. To address these points, Dean et al. (2011) used a more controlled approach in which a female was exposed to a sequence of six different males randomized with respect to social status in experimental trials replicated over two successive days. The female was held in a standardized soliciting position to control for female mating behaviour and males could not interfere with each other's mating

attempts. Sperm ejection was assessed blind through the analysis of video recordings of the female cloaca at each mating, and ejaculate volumes were estimated for each male, by exposing birds to similar trials in which the female was fitted with a harness which, while preventing insemination and sperm ejection, enabled the collection of ejaculates and the quantification of ejaculate volume. The study produced a more nuanced appraisal of sperm ejection behaviour, parsing out factors affecting the probability that an ejaculate would be ejected (risk of ejection) and those affecting the proportion of the volume of semen ejected when ejection did occur (intensity of ejection). The risk of ejection appeared to increase steadily over the mating sequence in the first day of a trial and remained high throughout the second day. Males that on average produced larger ejaculates also suffered a higher risk of ejection. In addition, controlling for the above factors, females ejected a larger proportion of the inseminations by socially subordinate males (Dean et al. 2011). Collectively, these results suggest that a number of mechanisms might contribute to explain the observations of female fowl preferentially ejecting semen of subordinate males in free-ranging groups, which reflect a combination of cryptic female preference for dominant males and behavioural factors which predispose subordinates to higher risk of ejection. First, when multiple males mate with the same female in succession, subordinate males can be more likely to mate after dominant males and produce larger ejaculates. This is partly because the limited access to mating opportunities of subordinate males results in lower sperm depletion, and partly because subordinate males are likely to suffer a higher risk of sperm competition and might therefore need to invest more sperm in individual mating. Second, females appear to neutralize a larger proportion of the ejaculates of subordinate males. An important caveat is that we are assuming that the behaviour observed under experimental conditions captures the same mechanisms driving the patterns observed in free-ranging flocks. Subsequent experimental work on the same population confirmed that sperm ejection increases over successive days of a trial, and revealed that sperm ejection is socially plastic, being much more common in male-biased than female-biased groups (Dean 2009). These patterns can be explained in light of the findings by Dean et al. (2011), in terms of male sperm depletion (high in female-biased

groups), female mating rates (higher in male-biased groups), and the importance of male status and female preference (limited in female-biased groups, higher in male-biased groups). Thus, it would appear that patterns of sperm ejection are highly labile and are likely to promote dominant males only under a limited range of social parameters, namely groups of intermediate size, with close-to-equal sex ratios (Fig. 2). Female sperm ejection and sperm dumping have been described across a range of taxa (Eberhard 1996; Snook and Hosken 2004; Firman et al. 2017). A similar mechanism of differential sperm ejection has been well characterised in the socially monogamous kittiwake, *Rissa tridactyla*, where sperm ejection occurs following within-pair matings and the risk of sperm ejection is maximal at the onset of the mating season and declines progressively as a female approaches egg-laying (Wagner et al. 2004). Experimental manipulations of kittiwake mating behaviour have indicated that differential sperm ejection in favour of more recent inseminations reduces the risk of fertilisation by old sperm, which appears to result in impaired offspring fitness in this (White et al. 2008) and other species (Pizzari et al. 2008a). The adaptive significance of sperm ejection in fowl is less clear. It is however possible that genetic benefits associated with social dominance (see above) may play a role in the evolution and maintenance of this behaviour.

A better-characterised mechanism favours male status after mating through differential remating rates with the same female. Collet et al. (2012) showed that dominant males remate with their partners at a higher rate than subordinate males, and that in groups with more intense postcopulatory sexual selection on male status (i.e. standardised gradient of the regression of paternity share over male status) were also characterised by stronger postcopulatory sexual selection on male remating with the same females (Collet et al. 2012). McDonald et al. (2017) have confirmed these patterns in larger groups and showed that postcopulatory sexual selection favours males remating frequently with their partners, because by doing so males account for a greater proportion of the matings of a female. This study related the “Sperm Competition Intensity” (the harmonic mean of the polyandry of a male’s partners, SCI) and “weighted Sperm Competition Intensity” (weighted by the number of matings between partners,  $SCI_w$ , McDonald & Pizzari 2016; McDonald et al 2017) faced by males, to

their reproductive success and paternity share. There was strong post-copulatory sexual selection against a male's SCI and SCI<sub>w</sub>, i.e. males had lower share of paternity if they mated with more polyandrous females and mated proportionally less with these females relative to rival males. McDonald et al. (2017) also showed that, compared to younger males, older males achieve a lower share of paternity for their remating rates, which indicates that, in addition to impacting a male's status and his ability to mate frequently with a female, advanced age can also impact on a male's fertilising performance by compromising the number of sperm inseminated and their fertilising efficiency (see below). Socially dominant males may therefore be favoured by postcopulatory sexual selection through different pathways, i.e. competitive exclusion of subordinates, cryptic female choice and privileged access to remating opportunities, which enables dominant males to maintain a high number of their sperm within individual females

#### 4.2 Sperm number and differential sperm allocation

Postcopulatory sexual selection for remating rates outlined above suggests that the number of sperm from different males contributing to the 'fertilising pool' is an important predictor of the outcome of sperm competition. Early artificial insemination experiments in commercial lines provided elegant demonstration that under controlled conditions (e.g. frequency and timing of inseminations), paternity share is accurately predicted by the relative number of sperm contributed by competing sperm donors (Martin et al., 1974; Birkhead & Pizzari 2009). Detecting the signature of sperm numbers in variation in paternity share in freely mating populations has been more challenging. However, recent analysis of fine-grained mating behaviour in red junglefowl flocks indicates that –out of the males mating with a female, the male that is more likely to fertilise a specific ovum is the one that mates with the female more frequently in the days preceding ovulation (McDonald 2014; R. Carleial, G. C. McDonald, & T. Pizzari unpubl.). These patterns are consistent with current understanding of sperm competition mechanisms in fowl, where a small proportion of each insemination is stored in the female sperm storage tubules (SSTs) with sperm egressing the SSTs at a constant rate,

moving up the female oviduct to the infundibulum, the site of fertilisation, through oviducal contractions (Birkhead & Pizzari 2009). Competing inseminations are thought to mix randomly within SSTs and –all else being equal- the probability of an ejaculate to fertilise successive eggs can be represented as a temporally discounting function of the number of sperm originally inseminated (Birkhead & Pizzari 2009).

The importance of sperm numbers in sperm competition dynamics is also reflected in the complex patterns of differential sperm allocation described in male fowl. Theory has long predicted that, because the level of sperm allocation promoted by sperm competition is costly, males should evolve strategies of investment in ejaculate expenditure to maximise reproductive returns across different mating opportunities. The development of techniques to obtain ejaculates produced by males during natural matings (Pizzari 2007) has enabled the experimental study of differential sperm allocation in male fowl. This work has revealed considerable dynamic plasticity, whereby males rapidly adjust sperm allocation in response to socio-sexual stimuli, often in status-specific pattern. In particular, three trends have emerged, which are briefly discussed below (Birkhead & Pizzari 2009).

i) Level of sperm competition. Pizzari et al. (2003) found that male feral fowl changed the number of sperm allocated to a female in response to the number of males in the experimental treatment. When housed singly males invested minimally in a female. However, in the presence of competitors dominant and subordinate males responded in different ways. Dominant males maximised their sperm investment in a female when grouped with three other males, while subordinate males maximised their sperm investment when grouped with just one other (dominant) male, and reduced their investment in the presence of three other males (Pizzari et al. 2003). A qualitatively similar pattern was subsequently confirmed in a population of red junglefowl (C. K. Tan, G. C. McDonald, S. Wigby, L. Spurgin, D. S. Richardson, & T. Pizzari unpubl.). The functional significance of this status-specific response is not entirely clear. It has been suggested that the reduced ability of subordinate males to remate with their partners and prevent them from mating with other males (see above) may mean that subordinate males typically face intense sperm competition (*sensu* Parker & Pizzari

2010), i.e. a situation in which sperm competition is always certain (risk of sperm competition is always one), although different mating opportunities will be associated with different levels of sperm competition. Game theoretic models of sperm competition intensity predict males to maximise their investment in the presence of one competitor and gradually reduce investment as intensity increases progressively beyond this point, under certain conditions (i.e. males possess information on the current levels of sperm competition intensity, mating rates are under male control; Parker & Pizzari 2010). By contrast, dominant males are expected to face a risk of sperm competition, i.e. a situation in which the probability that an ejaculate faces sperm competition varies from zero to one, but is not constantly one. Under most conditions, game theoretic models predict a gradual increment in sperm investment under increasing sperm competition risk. While the predictions of the intensity and risk models are broadly consistent with the behaviour of subordinate and dominant male fowl, respectively, the relevance of these models to the fowl system requires further work. Because female fowl ovulate a single egg every day and can store viable sperm in their SSTs for more than two weeks (Etches 1996), it is difficult to assess how the number of males associated with individual mating opportunities can inform on the level of sperm competition that the ejaculate of a male will face throughout the prolonged period of sperm storage by females. Empirical tests of the predictions of sperm competition intensity models, particularly those utilising internally-fertilising species have provided ambiguous support for these predictions (Kelly & Jennions 2011).

ii) Female sexual novelty. Male fowl display a typical Coolidge response, i.e. a decline in a male's sexual interest in a female over successive mating with this female, replaced by a sudden renewal in sexual interest upon exposure to a sexually novel female (Brown 1974). Importantly, this response is recapitulated in patterns of sperm allocation, as males preferentially invest sperm in matings with sexually novel females and reduce their investment with increasing sexual familiarity (i.e. over successive mating) with a female (Pizzari et al. 2003). This means that when males are exposed to a new female, males on average allocate more sperm than one would expect based on the pattern of decline in sperm

numbers observed over successive matings with the previous female. This response was originally shown in both a feral population of domestic fowl and a population of red junglefowl, and has now been replicated in the latter through multiple experimental approaches (Alvarez-Fernandez 2016; Y. Wang, G. C. McDonald, S. Dorus, & T. Pizzari unpubl.). Evidence is beginning to accumulate for similar patterns across a diverse range of polygynandrous species (Joseph et al., 2015; Reinhold et al., 2015)

iii) Female reproductive quality. Female comb size is asymptotically positively correlated with the number and size of eggs that females produce over a period of time across different populations (Pizzari et al. 2003; Wright et al. 2008), indicating that comb size provides males with information on the quality of the reproductive investment of prospective partners. A series of experiments in feral fowl has shown that when simultaneously exposed to a large- and a small-combed female, dominant males bias sperm investment in the large-combed female, while this bias tends to be absent or less marked in subordinate males (Pizzari et al. 2003; Cornwallis & Birkhead 2007; 2008). It is likely that this status difference reflects the differential risk of sperm depletion through access to mating opportunities. Prudent sperm investment is likely more adaptive in dominant males, which have the opportunity to mate more often and with more females than subordinate males. Similar patterns of male choosiness have been reported in other species where attractive males are exposed to considerable risks of sperm depletion and can be discriminant and highly economical in their sperm investment (Wedell et al. 2002).

Although some patterns of differential sperm allocation make adaptive sense (e.g. female novelty and female reproductive quality), we currently lack quantitative evidence of the role that sperm allocation strategies play in variation in paternity share, and thus the strength of postcopulatory sexual selection on these strategies.

#### 4.3 Sperm fertilising efficiency

It has long been known that the outcome of sperm competition is not entirely determined by relative numbers of sperm but also by the differential fertilising efficiency of rival ejaculates,

which may capture various sperm phenotypes such as morphology, metabolic performance and motility (Snook 2005; Pizzari & Parker 2009). Experiments based on the artificial insemination of similar numbers of two competing ejaculates have shown that controlling for relative sperm numbers, the outcome of sperm competition is predicted by relative motile performance, measured through a sperm mobility assay (Birkhead et al. 1999). Subsequent work utilised a similar approach to show that differences in numbers of sperm inseminated are particularly important in determining the outcome of sperm competition over eggs ovulated shortly following insemination, while differences in sperm mobility become the predominant predictor of paternity share for the eggs ovulated several days after insemination (Pizzari et al. 2008). The effect of sperm mobility likely reflects a role in sperm storage within the female SSTs, with high mobility ejaculates being lost from the SSTs at slower rates and thus retaining the ability to fertilise eggs ovulated over a prolonged period of time (Birkhead & Pizzari 2009). These patterns may help explain why it has been difficult to detect a clear signature of sperm fertilising efficiency in freely-mating flocks (Bilcik & Estevez 2005). It is plausible that when remating rates are sufficiently high, differences in sperm numbers are the predominant driver of sperm competition while the importance of sperm fertilising efficiency is expected to emerge in conditions of low remating rates (e.g. small, female-biased populations). It is also possible that measures of sperm fertilising efficiency, such as sperm mobility, are differentially important across male phenotypes. For example, sperm mobility is expected to be particularly influential to the paternity share of males with limited ability to remate with the same females, such as subordinate males. Froman et al. (2002) studied the relationship between sperm mobility and male status in a domestic population with large and consistent inter-male variation in sperm mobility, and found that in experimental male pairs, high-mobility males were more likely to be subordinate, suggesting a possible negative correlation between sperm mobility and social status. Subsequent studies have painted a more nuanced pattern, indicating that negative correlations might some times arise as a consequence of rapid and dynamic changes in sperm fertilising efficiency, associated with changes in male social status (Pizzari et al. 2007) or over a mating sequence



(Cornwallis & Birkhead 2007). It is noticeable that while sperm fertilising efficiency has long been studied as an inherent property of sperm phenotype (Wishart 2009), the dynamic changes in sperm fertilising efficiency described above appear too rapid to be explained by the production of sperm of different quality (Birkhead & Pizzari 2009). This has led to suggestions that part of the variation in sperm fertilising efficiency in male fowl may be determined by the effect of seminal fluid (Birkhead & Pizzari 2009). This is consistent with recent demonstrations in other species that males with different mating strategies produce seminal fluid with strikingly different characteristics. For example, in the Grass goby, *Zosterisessor ophiocephalus*, where males display alternative mating tactics, ‘sneaker’ males, which resort to stealing paternity by outcompeting the ejaculates of ‘territorial’ males, produce sperm that swim faster when exposed to the seminal fluid of territorial males and seminal fluid that preferentially slows down the sperm of territorial males (Locatello et al. 2013). Importantly, Bartlett et al. (2017) recently demonstrated experimentally that in chinook salmon, *Oncorhynchus tshawytscha*, where alternative mating tactics are modulated by social status, males can rapidly increase the swimming velocity of their sperm upon losing a social challenge and becoming subordinate, and that these changes in sperm velocity are determined by seminal fluid.

Therefore, sperm fertilising efficiency, largely in the form of sperm motile performance, can play an important role in sperm competition, generating variation in male paternity share independently of (or possibly even negatively related to) social status. Finally, rapid changes in sperm fertilising efficiency suggest the influence of seminal fluid effects (see below).

#### 4.4 Seminal fluid

Seminal fluid refers to the non-sperm, physiological component of an ejaculate. Seminal fluid molecules, often produced by designated accessory glands have been shown to play critical roles in the coordination of post-mating events, including female sperm storage, ovulation and oviposition (Chapman et al. 2008; Perry et al. 2013). Importantly, several seminal fluid

proteins represent important targets of post-copulatory sexual selection, as they can drastically impact paternity share through their influence on sperm fertilising efficiency, female propensity to remate and oviposition rate (Chapman et al. 2008; Perry et al. 2013). The reproductive tract of male fowl lacks accessory glands, however two distinct exudates contribute to the seminal fluid of natural ejaculates: seminal plasma, produced from the ductus deferens through which developed sperm travel from the testis to the phallus and in which they are stored prior to ejaculation, and a lymph-like transparent fluid, produced by the cloacal folding of the phallus at ejaculation. Early work on the impact of seminal plasma and transparent fluid on sperm fertilising efficiency has produced ambiguous results, with some suggestions that transparent fluid may have a negative impact (Lake 1971; Fujihara 1992; Etches 1996; Wishart 2009).

Indirect evidence for a seminal fluid influence of sperm fertilising efficiency was provided by an *in vitro* experiment in which the seminal fluid and sperm of an ejaculate were separated through centrifuge and remixed across the ejaculates that a male produced with a large- and small-combed female (Cornwallis & O'Connor 2009). This study found that larger ejaculates contained faster-swimming sperm and over a mating sequence, dominant males tended to allocate consistently more and faster-swimming sperm to large-combed females. When sperm and seminal fluid were mixed across ejaculates *in vitro*, seminal fluid appeared to have an impact on sperm swimming velocity. First, the study found a strong effect of ejaculate volume, with sperm swimming velocity increasing upon exposure to seminal fluid from a large ejaculate of the same male. Given that larger ejaculates tend to have faster-swimming sperm, it can be concluded that seminal fluid from larger ejaculates may be of higher quality. However, the study also found that additional effects independent of ejaculate volume. The swimming velocity of sperm allocated to large-combed female did not change upon exposure to seminal fluid from ejaculates allocated to small-combed females, however sperm swimming velocity declined when sperm allocated to small-combed females were exposed to seminal fluid from ejaculates allocated to large-combed females (Cornwallis & O'Connor 2009). This result is somewhat counterintuitive; given that ejaculates allocated to

large-combed females had faster sperm, one would have expected that seminal fluid from these ejaculates would increase (rather than reduce) the swimming velocity of sperm allocated to small-combed females.

Because sperm competition in the fowl is mediated by the potential for protracted interactions between rival ejaculates within the female tract, it is important to investigate how the seminal fluid affects not only the sperm of the same ejaculate (self) but also the sperm of rival males (non-self). Lack of discrimination between self and non-self would create the risk that self-sperm may be damaged by potential deleterious effects of seminal fluid and potential for rival ejaculates to exploit potential beneficial effects of seminal fluid (Hodgson & Hosken 2006; Alonzo & Pizzari 2010). Over the last years, our research group has explored the potential effect of seminal fluid on sperm fertilising efficiency in self *versus* non-self scenarios both *in vitro* and *in vivo* (i.e. through artificial insemination). For example, Alvarez-Fernandez (2016) has shown *in vitro* that exposure to non-self seminal fluid (either related, from a full-sib brother, or from an unrelated male) might increase the motile performance of sperm compared to self seminal fluid. Artificial insemination experiments presented a consistent, albeit non-significant, pattern, with inseminations of sperm and non-self seminal fluid resulting in more sperm reaching the eggs ovulated by inseminated females over successive days. Interactions between the initial swimming velocity of a sperm sample and seminal fluid treatment (i.e. self, non-self related, non-self unrelated) also appeared to influence the number of sperm reaching individual eggs following artificial insemination (Alvarez-Fernandez 2016). The recent characterisation of the seminal fluid proteome of a population of red junglefowl (Borziak et al. 2016) as well as that of some domestic lines (Labas et al. 2015) is beginning to catalise a more systematic approach to investigate the role of specific seminal fluid proteins in sperm competition and sperm fertilising efficiency. The red junglefowl seminal fluid proteome displays considerable functional complexity, comprising of in excess of 1,000 proteins, some of which with known functions in sperm maturation, fertilisation and immune responses (Borziak et al. 2016). The comparisons of proteomes across young and old males with relatively slow- or fast-swimming sperm has

identified seminal fluid proteins associated with sperm swimming velocity and with male age, revealing a highly distinct proteomic profile for the ejaculates of old males with relatively fast sperm, suggesting that some of these proteins may be functionally involved in maintaining high sperm fertilising efficiency in old age (Borziak et al. 2016). Characterising the proteomic profile of the ejaculates produced by individual male junglefowl over a mating sequence has revealed rapid dynamic changes in seminal fluid protein composition, indicating clusters of seminal fluid proteins that are differentially allocated to ejaculates in response to the mating sequence and the sexual novelty of a female (A. Alvarez-Fernandez, K. Borziak, G. C. McDonald, S. Dorus, & T. Pizzari, unpubl.). This work suggests that seminal fluid proteins may be an integral but so far neglected aspect of ejaculate economics. Collectively, these results suggest that the seminal fluid of male fowl is biochemically complex, changes dynamically across ejaculates as a result of depletion and differential allocation, and can potentially have important influences in sperm fertilising efficiency, paternity share and post-copulatory sexual selection. Similar patterns of male adjustment of seminal fluid protein allocation in response to socio-sexual conditions of a mating are beginning to emerge across a range of polyandrous species. *D. melanogaster* males strategically adjust investment in specific seminal fluid protein in relation to the mating status of a female (Sirot et al. 2011). Similarly, seminal fluid composition changes in response to changes in perceived sperm competition risk in male house mice, *Mus musculus domesticus* (Ramm et al. 2015), while Sloan et al. (2018) have shown that in the cricket, *Teleogryllus oceanicus*, males adjust the expression of seminal fluid protein genes in response to experimental changes in the level of sperm competition.

#### 4.5 Genetic compatibility and relatedness

There is increasing evidence that patterns of genetic compatibility, similarity and relatedness may modulate both female patterns of sperm utilisation and male strategies of sperm allocation.

Differential patterns of female sperm utilisation after mating suggest that mechanisms

of cryptic female choice may be at play with regards to genetic incompatibility. First, Birkhead et al. (2004) repeatedly artificially inseminated sets of females with heterospermic inseminations of two different sperm donor males, and showed significant heterogeneity in the paternity share of the offspring produced by these females. This general result was due to the fact that paternity of the offspring produced by some individual females was consistently biased in favour of one sperm donor despite the fact that competing ejaculates were standardised (Birkhead et al. 2004). This pattern is consistent with genetic incompatibilities arising in certain male-female combinations, influencing the outcome of sperm competition. Second, Pizzari et al. (2004) presented experimental evidence that –following natural mating– female red junglefowl bias sperm utilisation against the sperm of related males to avoid inbreeding. There was no evidence that differential sperm ejection through cloacal contractions caused these patterns, raising the possibility that sperm selection may occur further up the oviduct, e.g. through mechanisms involved in the sperm access to the female sperm storage tubules. These patterns were replicated by Løvlie et al. (2013), who utilised MHC data to show that MHC similarity between a female and a male was a better predictor of sperm utilisation than pedigree relatedness. This is not surprising given that MHC similarity between two individuals is often a sensitive measure of their genetic relatedness (Løvlie et al. 2013). Further work has sought to establish the independent roles of MHC similarity and genetic relatedness in sperm selection. A series of experiments using natural matings, and artificial insemination approaches has shown that MHC similarity is associated with differential sperm utilisation only to the degree to which MHC similarity predicts relatedness, while no evidence was found that females discriminated sperm based on the MHC similarity of males when all males were unrelated to the female (M. Gillingham, J. Collet, D. S. Richardson, T. Pizzari unpubl.). Finally, the experiment by Løvlie et al. (2013) also showed that artificial insemination failed to generate patterns of biased sperm utilisation. This could be explained by methodological factors such as the lower number of sperm successfully transferred during artificial insemination, or could reflect biological properties. For example, artificial insemination eliminates phenotypic cues of the male, which might be

required in order to trigger mechanisms of female sperm selection. Similarly, artificial insemination bypasses the first part of the female vagina, and differential responses may be prevented without sperm exposure in this region of the female oviduct. Cryptic female choice may represent an important strategy for females to influence the genetic polymorphism of their offspring given the high viscosity of natural populations (see above) and the ability of males to coerce females into mating (Pizzari 2001). Similar patterns of cryptic female choice against inbreeding have been confirmed in a range of other species, from crickets to guppies (Firman et al. 2017 for discussion).

Males too might respond to their genetic relatedness to a female, however this response is weaker and less consistent. Løvlie (2008) studied feral domestic flocks and found that when a male was with two females that were his own full-sib sisters and two unrelated females, he showed a preference to mate with the two unrelated females, suggesting some degree of kin discrimination. However, when experimentally presented with the opportunity to mate with either a full-sib sister or an unrelated female, red junglefowl males showed no evidence of avoiding mating with their sisters (Pizzari et al. 2004; Løvlie et al. 2013). Pizzari et al. (2004) found that when exposed to a sister, males took slightly longer to initiate mating but then invested on average more sperm in these females compared to unrelated females. Løvlie et al. (2013) however found no evidence of these differential responses, suggesting that males do not discriminate between related and unrelated females under these conditions. It is possible that differences in mate availability can reconcile the discrimination in freely-mating flocks observed by Løvlie (2008) and the lack of discrimination observed when males were experimentally exposed to a single mating opportunity. Theory would predict that males should avoid inbreeding when better reproductive alternatives (i.e. unrelated females) are available (Parker 2006). This possibility was explored by exposing male junglefowl to different regimes of availability of related and unrelated females, however little evidence of male discrimination was found in terms of both mating propensity and ejaculate expenditure (C. K. Tan, G. C. McDonald, L. Spurgin, S. Wigby, D. S. Richardson, & T. Pizzari unpubl.). On the other hand, female availability has been suggested to play a role in male sperm

investment in MHC-similar and MHC-dissimilar females, albeit in a counterintuitive way. One would expect males to become more discriminant when multiple females are simultaneously available, yet Gillingham et al. (2009) found the opposite pattern. No evidence for preferential investment was detected when male red junglefowl were simultaneously presented with the opportunity to mate with an MHC-similar and an MHC-dissimilar female, yet when females were presented sequentially, in random order with respect to MHC similarity, males were found to allocate more sperm to the more MHC-dissimilar of the two females.

Finally, relatedness between males engaged in competition after mating may also play an important role in post-copulatory processes. Male red junglefowl were found to substantially increase their sperm investment in a female when mating after their own full-sib brother compared to when they mated with a female after a male unrelated to the focal male (Tan et al. 2017). This is puzzling because inclusive fitness theory predicts that relatedness should reduce rather than increase investment in selfish competition under certain conditions of population structure (Pizzari et al. 2015). Such reduction in selfish competition, after controlling for social status effects, has been independently confirmed for male pre-copulatory behaviour (i.e. competition over mating opportunities) by two studies of red junglefowl (Rosher et al. 2017; Tan et al. 2017). One possible reason for why male-male relatedness appears to relax male investment in pre-copulatory competition while favouring male investment in post-copulatory competition may have to do with the differential costs associated with pre- and post-copulatory competition. Pre-copulatory competition in this species is associated with fights and injuries with long-term consequences (e.g. males can lose an eye when fighting). While fighting always carries a risk of injury to self (a direct fitness cost), fighting with rivals more related to the focal male than the population average can also result in long-term injuries to the male's close relatives (an additional indirect fitness cost). If males are selected to reduce investment in pre-copulatory competition with related males, they may have the opportunity and need to increase their investment in sperm competition with relatives (because they have saved resources and because reduced pre-

copulatory competition may mean that males are more likely to enter sperm competition with relatives). Other, non-mutually exclusive possibilities evoke female behaviour. For example, if females were more likely to eject the sperm of a male if he mated with the female shortly after his brother, the second to mate of the two brothers may be selected to increase his sperm investment in such mating. Similarly, if clusters of male relatives were distributed discretely in space and a female moved from one cluster of males to the next mating with some males in each cluster, pre-copulatory competition over mating would be largely limited to the local scale (i.e. among relatives), however post-copulatory sperm competition will occur more globally (i.e. among the ejaculates of different clusters of males). It is possible that this scenario may create the conditions for related males to derive inclusive fitness benefits by investing more sperm when mating with the same female, in order to outcompete the ejaculates of unrelated males that the female would receive when moving to other male clusters. Clearly, more empirical and theoretical work is required to better understand this male response.

## **5. The impact of polyandry on sexual selection**

Recent work has investigated the wider repercussions that polyandry has for the operation of sexual selection, in addition to generating opportunity for post-copulatory sexual selection. Studies of fowl populations have contributed to provide empirical demonstrations of these effects.

Collet et al. (2012) studied replicate groups of three male and four female red junglefowl, and quantified variation in average female polyandry across groups and combined this information with fine-grained observation of mating behaviour and molecular parentage assignment of embryos. The study showed that groups with higher levels of average polyandry were characterised by drastically reduced opportunity for sexual selection (i.e. standardised variance in male reproductive success). These findings are consistent with empirical studies of other polyandrous species (Jones et al. 2001; Lesobre et al. 2010), and with theoretical expectations (Shuster & Wade 2003; Kvarnemo & Simmons 2013;



McDonald & Pizzari 2018). This pattern is due to two inter-related mechanisms. First, increasing average polyandry results in all males in a group achieving a similarly high mating success, which erodes the opportunity of pre-copulatory sexual selection on mating success. Second, increasing average polyandry also reduces the reproductive returns associated with mating with a new female, thus reducing the pre-copulatory sexual selection gradient on male mating success (i.e. the Bateman gradient; Parker & Birkhead 2013). Opportunity of post-copulatory sexual selection (i.e. standardised variance in paternity share) on the other hand, was independent of the level of average polyandry (given that all groups were polyandrous to some degree, i.e. average female mating success was always greater than one). This means that as groups become more polyandrous on average males are exposed to progressively weaker sexual selection and a greater component of this selection is likely to be explained by post-copulatory episodes.

In some polyandrous species, a negative covariance between male mating success and paternity share generates antagonistic pre- and post-copulatory selective pressures on males, creating opportunity for alternative mating tactics to co-exist by pursuing reproductive success through preferential investment in either pre- or post-copulatory competition (Danielsson 2001; Demary & Lewis 2007; Katuski et al. 2017). Negative covariance between male mating success and paternity share is therefore expected to maintain genetic variation. Instead, studies of red junglefowl have consistently demonstrated positive covariance between male mating success and paternity share (Collet et al. 2012; McDonald et al. 2017). This indicates that pre- and post-copulatory sexual selection act to a large degree synergistically in this species. Similarly synergistic patterns have been described in some populations of guppies, *Poecilia reticulata* (Devigili et al. 2015) and *D. melanogaster* (Morimoto et al. 2016). In the case of fowl populations, sexual selection favours socially dominant males, which gain higher mating success at pre-copulatory stage and larger share of paternity after copulation. It has been shown that positive covariance between male mating success and paternity share arises as a consequence of negative assortment, a pattern in which males that mate with more females tend to also mate with the least polyandrous females of the

population and remate with females more often (thus suffering low SCI and  $SCI_w$ ), while the males with the lowest mating success tend to mate with the most polyandrous subset of the female population (i.e. high SCI and  $SCI_w$ , McDonald et al. 2017, Fig. 3). In other words, red junglefowl flocks are characterised by a negative relationship between the polygyny of a male and the average polyandry of his partners. Patterns of mating assortment have important consequences for sexual selection: negatively assorted populations should tend to increase pre-copulatory sexual selection on male mating success by reducing the reproductive returns of mating for males with low mating success (who suffer high sperm competition) compared to males with high mating success (who do not have to share the eggs of some of their females with as many males; McDonald & Pizzari 2016; 2017). McDonald & Pizzari (2016) have shown that mating assortment can be captured by the moment correlation between two variables: the male Bateman gradient controlling for variation in SCI and the slope of the regression of male reproductive success over SCI, controlling for mating success (the “Sperm Competition Intensity Correlation”, *SCIC*, McDonald & Pizzari 2016; Fig. 4). Negative *SCIC* values should drive a positive covariance between male mating success and paternity share due to males with high mating success mating with females of low average polyandry. Instead, positive *SCIC* should lead to a negative covariance between mating success and paternity share due to males with high mating success mating with females of high average polyandry (McDonald & Pizzari 2016; 2018). Simulation work indicates that while the average level of polyandry in a population tends to reduce the opportunity of sexual selection on males (see above), for a given level of average polyandry, increasingly positive *SCIC* drastically reduces variation in male reproductive success and the potential strength of sexual selection on male pre-copulatory traits (McDonald & Pizzari 2018). Moreover, in addition to its independent impact on sexual selection, the average polyandry of a population also affects sexual selection indirectly by modulating scope for random variation in *SCIC*. When populations show intermediate levels of polyandry, *SCIC* can vary widely by chance. For example, negative *SCIC* can arise simply because, given a minority of less polyandrous females, males that mate with more females are more likely to mate with these females

simply by chance. As populations become more polyandrous however, the mating matrix (i.e. the matrix of potential mating combinations between individual males and females) becomes progressively saturated, restricting the range of random *SCIC* around zero (McDonald & Pizzari 2018). This theoretical work therefore indicates that polyandry has a dual effect: increases in average polyandry directly erodes opportunity for sexual selection, but polyandrous structures leading to negative *SCIC* tend to buffer this effect indirectly, by increasing the strength of pre-copulatory sexual selection on male mating success. The importance of direct and indirect effects of polyandry likely depends on factors affecting matrix saturation such as population size and sex ratio, and behavioural mechanisms for non-random mating assortment. For example, in small and highly polyandrous groups, *SCIC* will tend towards negative values, meaning null expectations for the covariance between male mating success and male paternity share will on average be positive. As part of a larger empirical review, McDonald & Pizzari (2018) indicated that in red junglefowl groups, negative patterns of *SCIC* may emerge as a consequence of such group level properties. More recent and detailed work on these junglefowl groups suggests that the negative *SCIC* may in part be mediated by female age. Young females are more fecund and more polyandrous, while older females form tight social clusters that avoid male attention and as a result are less polyandrous (G. C. McDonald, L. Spurgin, E. Fairfield, D. S. Richardson, & Pizzari, T unpubl.). When they do mate, older females tend to mate with socially dominant males, which could reflect either an active choice by females or –more parsimoniously– the fact that dominant males by virtue of mating more frequently are simply more likely to mate with older females (G. C. McDonald, L. Spurgin, E. Fairfield, D. S. Richardson, & Pizzari, T unpubl.). Previous work showed that older female red junglefowl tend to be socially dominant (Kim & Zuk 2000), and that in domestic flocks, dominant females mate less frequently (Guhl 1950). The recent work in red junglefowl controls for the effect of female status (G. C. McDonald, L. Spurgin, E. Fairfield, D. S. Richardson, & Pizzari, T unpubl.), indicating that female age may also affect mating and social behaviour through mechanisms independent of status.

A positive covariance between male mating success and paternity share determined by negative *SCIC*, should in principle contribute to erode additive variation, compounding the lek paradox. Consistent with this, a number of studies have shown that in freely-mating groups, socially dominant males tend to sire more offspring overall (Guhl et al. 1945; Jones & Mench 1991; Johnsen et al. 2001; Wilson et al. 2008; Collet et al. 2012; McDonald et al. 2017), as a result of both higher mating success and higher share of paternity. However, several mechanisms are likely to buffer the effect of male status to maintain genetic diversity. First, polyandry means that on average a larger proportion of the male population will contribute to the next generation compared to a strictly polygynous mating system such as a lek. Second, while pre- and post-copulatory sexual selection act synergistically to promote traits associated with male social dominance, other male traits, which are independent of- or negatively related to status will also play a role in determining variation in male reproductive success in these populations, such as male age, comb expression at the pre-copulatory stage and sperm fertilising efficiency at the post-copulatory stage. For example, a study of three small broiler breeder flocks suggested that the males with the highest share of paternity tended to be those with high sperm mobility and producing on average larger ejaculates, rather than those of high social status and high mating frequency (Bilcik & Estevez 2005). While the limited sample of the study precludes robust conclusions, these results are consistent with the idea that polyandry can promote alternative male phenotypes. Third, mechanisms promoting inbreeding avoidance or genetic compatibility typically act to buffer directional sexual selection.

## **6. Concluding remarks**

The study of polyandry in fowl populations has contributed to progress toward a more nuanced and complex understanding of the operation of sexual selection in males, particularly for non-socially monogamous species. The traditional paradigm that males are sexually selected to mate indiscriminately and maximise number of partners is being replaced by a scenario in which males are strongly selected to invest in each one of their sexual partners in

order to defend their paternity, which promotes remating with the same partner, and male choosiness through prudent strategies of ejaculate expenditure. Similarly, the traditional expectation that polyandry exacerbates sexual selection on males is unlikely to apply beyond a restrictive range of social conditions (McDonald & Pizzari 2018), while a more general pattern is emerging, whereby polyandry impacts on the operation of sexual selection in multiple ways: directly, by eroding opportunity for pre-copulatory sexual selection, and indirectly, by modulating patterns of mating assortment in these populations (i.e. *SCIC*).

In addition to mating assortment, studies of fowl populations are contributing to highlight the need to consider non-random population structure (e.g. in terms of genetic relatedness or competing phenotypes) in analyses of sexual selection, as well as consideration of the way in which polyandry influences structure. In this context, polyandry appears to have a dual effect on genetic diversity. On the one hand, genetic variation is likely eroded by intra- and inter-sexual selection episodes, which act in synergy to consistently promote male traits conducive to social status both before and after copulation. On the other, some genetic variance may be preserved by post-copulatory sexual selection for traits, such as sperm fertilising efficiency, which are not positively correlated with status and by non-directional selection driven by inbreeding avoidance and genetic compatibility. Finally, studies of fowl populations have been helpful in providing proof of concept of how females can retain some control of the reproductive outcome, even when overt strategies are curtailed by male sexual coercion, through more covert strategies such as the manipulation of male competition before mating, and post-copulatory mechanisms of cryptic female choice.

Finally, the fowl study system presents a rare opportunity for studies of sexual selection and sexual conflict to have direct applicability, by illuminating mechanisms underpinning better welfare and productivity of commercial populations (Pizzari 2016).

#### 6.1 A cautionary note and future challenges

While recognising the insights into the operation of sexual selection gained by the study of the social organisation of fowl populations, it is important to consider some of the limitations of this study system.

Logistic constraints often limit the scope and scale of studies, and potential for replication. A reassuring number of key patterns have been found reproducible across different studies, however some of these replications are limited to the same study population and research group, while other results remain in need of replication (Table 1). In addition, while a number of genetic tests (e.g. molecular parentage, MHC genotyping, relatedness) and *in vitro* assays can be easily performed blind with respect to experimental treatments and predictions, blinded studies of social traits such as social status or mating success are substantially more challenging. Our research group has resorted to blinding techniques such as the use of video scoring in some cases, however this approach is not always possible or relevant. Importantly, given the extreme plasticity of sexual behaviour and its sensitivity to social and sexual stimuli, replicating studies requires careful standardisation of the socio-sexual environment. For example, it is likely that captivity, through larger populations and higher population densities compared to populations in the wild, may emphasise mechanisms of male-male competition over female choice, and episodes of post- over pre-copulatory sexual selection, by increasing average levels of polyandry. Similarly, captivity will likely emphasise the signature of ageing by prolonging life expectancy beyond that typically experienced in the wild. Therefore, caution should be used when interpreting the results from captive populations in the context of sexual selection in the wild.

Genetic differences across populations and breeds of domestic fowl also deserve consideration as potentially important drivers of variation, as indicated by careful ethological comparisons (Schütz & Jensen 2001; Håkansson et al. 2007). The use of red junglefowl populations, while offering the benefit of a system less affected by domestication and artificial selection, also presents challenges due to possible genetic introgression with domestic fowl (Nguyen-Phuc & Berres 2018) or inbreeding. Future work should seek to investigate patterns of sexual selection across multiple genetically distinct populations from different geographic regions and ecological habitats, to establish the generality of results and –crucially- develop an understanding of the way in which social, ecological and genetic factors account for discrepancies across different studies or populations. In particular, as more

genomic information becomes available regarding the changes associated with the processes of the domestication of red junglefowl and the feralisation of domestic fowl (Johnsson et al. 2016), careful comparisons of well characterised populations present a unique opportunity to elucidate the genetic and genomic architecture of social traits targeted by sexual selection, as well as detect the signatures of domestication and feralisation on the evolution of these traits.

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**Table 1.** Examples of studies investigating the effect of male phenotypic traits in a) pre- and b) post-copulatory sexual selection. Studies testing for an overall effect on total number of offspring produced with unclear parsing across pre- and post-copulatory mechanisms are listed in c). DF: domestic fowl, JF: red junglefowl, AI: artificial insemination, *M*: mating success, *P*: paternity share.

a) pre-copulatory sexual selection			
trait	effect	notes	reference
Social status	Increased overall mating frequency	DF	Cheng & Burns 1988
	Increased female preference	DF. Preference for high wing-flapping rates, correlated with male dominance	Leonard & Zanette 1998
	Increased probability of female solicitation and reduced female resistance	DF	Pizzari & Birkhead 2000
	Positive effect on $M$	DF	Jones & Mench 1991
		JF	Johnsen et al. 2001
		DF	Wilson et al. 2008
		JF	Collet et al. 2012
		JF. Stronger effect of male aggression and age, correlated with status	McDonald et al. 2017
Comb size and brightness	Positive effect on female preference	JF	Zuk et al. 1990a
		JF	Zuk et al. 1990b
		JF	Zuk et al. 1990c
		JF	Ligon et al. 1990
		JF	Zuk et al. 1995
	Weak effect on female preference	JF. Only when associated with status	Johnsen et al. 2001
	No effect on female preference	DF	Leonard & Zanette 1998
		DF	Wilson et al. 2008
b) post-copulatory sexual selection			
trait	effect	notes	reference
Social status	Positive effect on $P$	JF	Collet et al. 2012
		JF. Male aggression and age, correlated with status	McDonald et al. 2017
	Negative effect on female sperm ejection	JF. Anecdotal	R. Thornhill in Birkhead & Møller 1992
		DF. Behavioural observation of free-ranging birds	Pizzari & Birkhead 2000
		DF. Experimental setting	Dean et al. 2011
Re-mating with same female	Positive effect on $P$	JF	Collet et al. 2012
		JF	McDonald et al. 2017
Sperm number	Positive effect on $P$	DF. AI experiment.	Martin et al 1974
		DF. AI experiment, only first days after insemination when ejaculates vary in sperm mobility	Pizzari et al. 2008
Sperm swimming	Positive effect on $P$	DF. AI experiment.	Birkhead et al. 1999

velocity		Sperm mobility assay.	Pizzari et al. 2008
		DF. AI experiment. Sperm mobility assay, only after the first days following insemination when ejaculates vary in sperm numbers	
c) total sexual selection			
Social status	Positive effect on $T$ (conflates $M$ and $P$ )	DF	Guhl et al. 1945
		DF	Guhl & Warren 1946
		DF	Jones & Mench 1991
Overall mating frequency	Positive effect on $T$ in 2 out 6 flocks	DF	Bilcik & Estevez 2005
Sperm swimming velocity	Positive effect on $T$ in 3 out of 6 flocks	DF	Bilcik & Estevez 2005

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## Figure legend

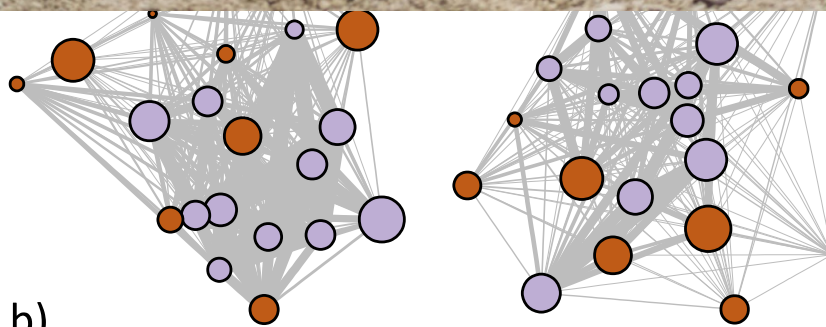
**Figure 1. a)** A small flock of male and female red junglefowl (photo: D. Wilson, T. Pizzari). **b)** Social networks of two larger red junglefowl flocks. Nodes represent individual birds: brown nodes are males, purple nodes are females. The size of the node reflects the social status of a bird. Nodes connected by edges represent social interactions between these individual birds. The thickness of the edge reflects the strength of the connection (G. C. McDonald, unpubl.).

**Figure 2.** Hypothetical qualitative changes in patterns of sexual selection along a social gradient, through both pre-copulatory sexual selection on male mating success (M) and post-copulatory sexual selection on paternity share (P), and their relative contribution to total male reproductive success (T). Red pathways are targeted by intersexual selection, blue pathways represent intrasexual episodes, signs denote negative and positive effects, the weight of the link represents the strength of the effect. **a)** Groups that are small, low density and/or strongly female-biased are characterised by relatively low polyandry, strong pre-copulatory selection on male mating success (M), partly through overt female choice promoting male displays associated with social status and ornament expression (e.g. comb), and little postcopulatory sexual selection on paternity share (P), largely through status-mediated ability to monopolise females, e.g. through prevention or interruption of copulations. **b)** As groups become larger, denser and/or less female-biased, polyandry increases, the opportunity for pre-copulatory selection is reduced, female choice shifts to differential resistance and more covert strategies promoting status, mechanisms of male-male competition, e.g. status-mediated aggression, become more important. Male ability to monopolise females is reduced and post-copulatory sexual selection now favours status-mediated male ability to remate frequently with the same female, sperm numbers and fertilising efficiency through sperm competition, and to some degree, male status through cryptic female choice. Status might impact negatively on ejaculate size and possibly sperm fertilizing efficiency. **c)** When groups are sufficiently large, dense and/or male-biased, male hierarchies are likely to progressively become less important, and access to mates is likely determined by scramble competition promoting traits such as male aggression. Female choice is likely limited, polyandry is high, generating strong sperm competition for traits such as ejaculate size, sperm fertilizing efficiency and remating frequently with the same. Females are likely to display high levels of pre-copulatory resistance and post-copulatory sperm ejection however the latter is unlikely to be biased in favour of social males. Cryptic female choice determined by inbreeding avoidance or genetic compatibility might also occur.

**Figure 3. a)** An example of a negatively assorted mating matrix from a red junglefowl flock. Rows represent individual males, rows individual males, filled cells identify male:female combinations that were observed to mate successfully during a trial. Males are arranged according to mating success in descending order, the least successful males tend to mate with a more polyandrous subset of the females of the group, while the most successful male secures exclusive access to some females (G. C. McDonald & T. Pizzari unpubl.). **b)** Negative assortment modulates pre-copulatory sexual selection on male mating success, as males with high mating success tend to sire more offspring per female as their ejaculates face low intensity of sperm competition, while males with low mating success on average sire fewer offspring per female as they suffer high intensity of sperm competition. The graph shows standardised male reproductive success (i.e. total number of offspring sired) in relation to standardised mating success (i.e. number of females mated), data point represent individual males across trials, colour coded for the average weighted intensity of sperm competition ( $SCI_W$ ) experienced across females (McDonald et al. 2017).

**Figure 4. a)** The reproductive success of individual males is positively affected by the number of females mated (mating success,  $M$ ) and negatively affected by the polyandry of these females (Sperm Competition Intensity, SCI). The structure of the sexual network modulates the relationship between  $M$  and SCI, and characterising this modulation is critical to the study of sexual selection in structured populations.  $\beta_{M \cdot SCI}$  represents the slope of the partial regression of male reproductive success over  $M$ , controlling for SCI, while  $\beta_{SCI \cdot M}$  represents the slope of the partial regression of male reproductive success over SCI controlling for  $M$ . SCIC is the correlation between  $\beta_{M \cdot SCI}$  and  $\beta_{SCI \cdot M}$  (McDonald & Pizzari 2016). **b)** SCIC has critical repercussions on sexual selection as it ranges from negative to positive. The Bateman gradient on male  $M$  is stronger under negative SCIC values, which reflect negative mating assortment where males with high  $M$  on average suffer lower sperm competition resulting in a positive covariance between male pre- and post-copulatory performance (i.e.  $M$  and  $P$ , paternity share). Positive SCIC values on the other hand, reflect positive mating assortment, which exposes males with high  $M$  to higher sperm competition (i.e.  $Cov(M, P) < 0$ ), thus weakening the Bateman gradient (McDonald & Pizzari 2018). Red junglefowl flocks are often characterised by negative mating assortment (Fig. 3b).

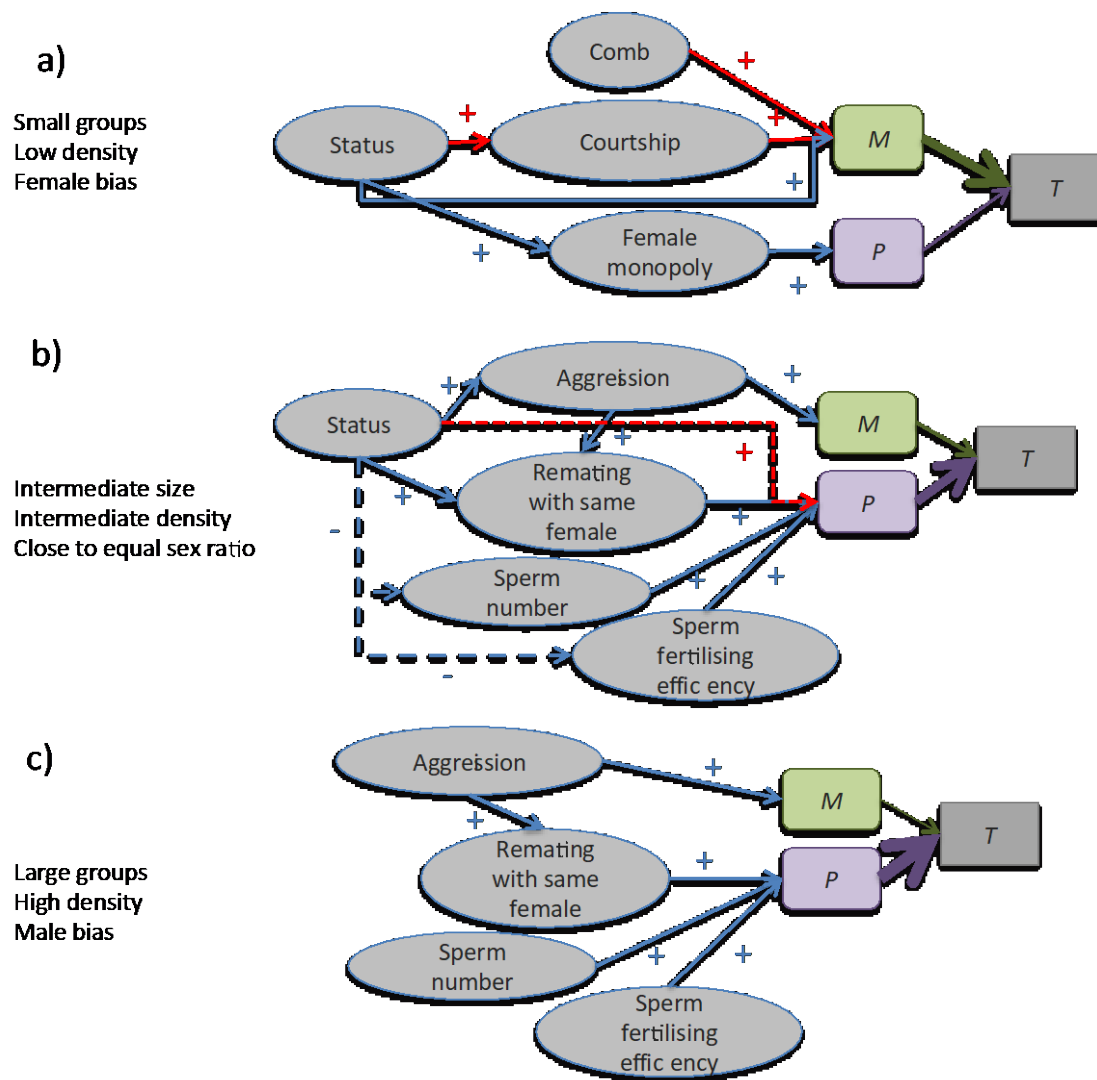




b)  
Figure 1

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Figure 2

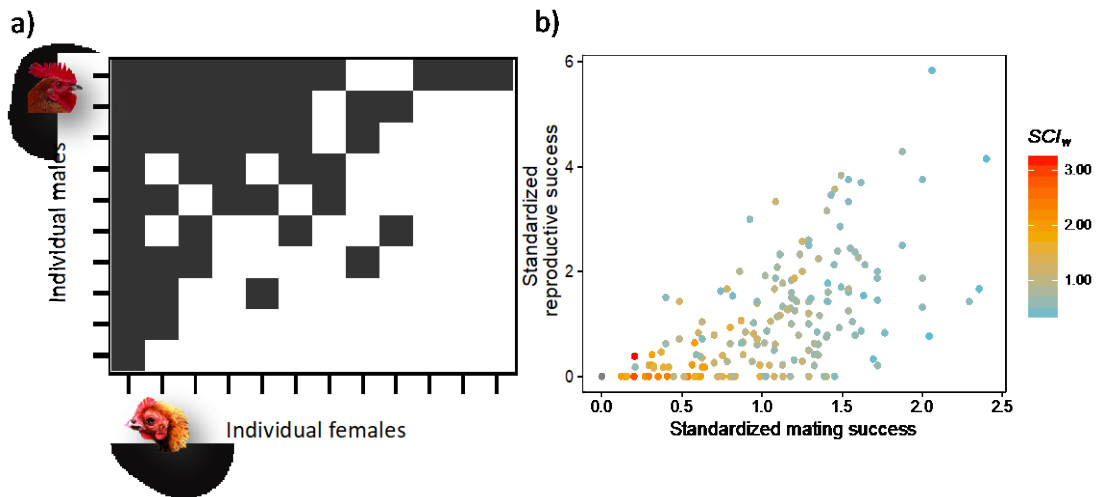
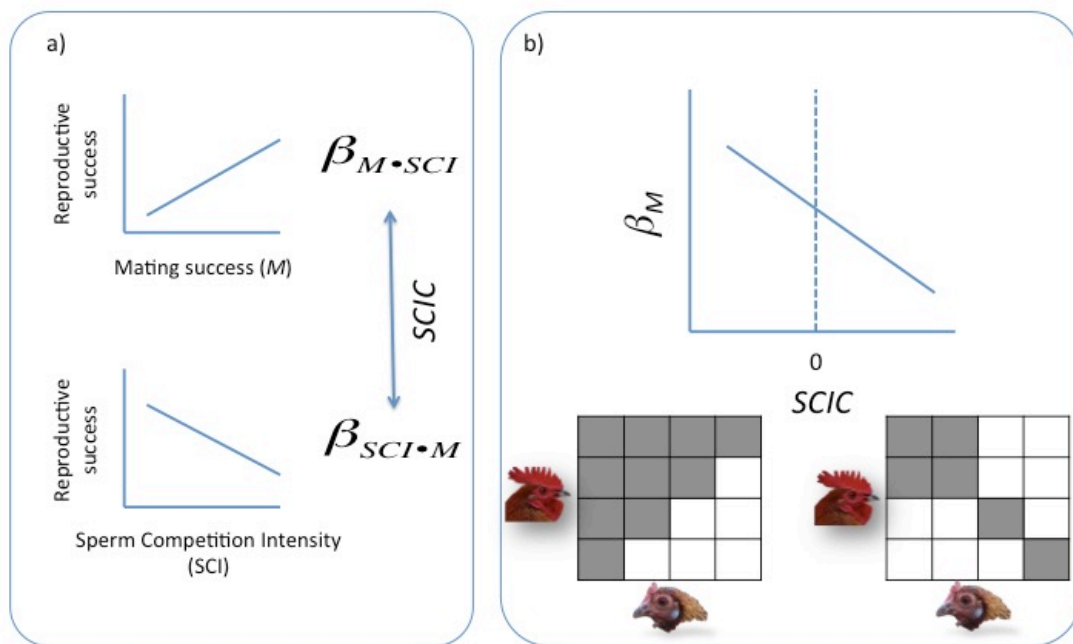


Figure 3

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