# Social evolution
# and
# sex allocation theory

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## Declaration

This work has been composed by me, is the result of my own research and contains no work done in collaboration except where stated otherwise. In all chapters, I have carried out the greater part of the work, except chapter 2, where I led the theoretical work, and the empirical work was led by Ramiro. The text does not exceed 50,000 words. No part of this thesis has been submitted to any other university in application for a higher degree. My supervisors certify that all these statements are correct.

Details of collaborative work:

Chapter 2 is a published paper: Ramiro, R. S., J. Alpedrinha, L. Carter, A. Gardner, and S. E. Reece. 2011. Sex and Death: The Effects of Innate Immune Factors on the Sexual Reproduction of Malaria Parasites. Plos Pathog 7. I participated in the original idea for the paper, led the development of the theoretical section and contributed for the writing up of all parts of the paper.

Chapter 3 is in review at *Evolution* as a research paper. The original idea is shared with my co-authors, I led the developmental of the theory and performed the analysis. AG and SW contributed to the write up.

Chapter 4 is being prepared for publication. The original idea is shared with my co-authors, I led the developmental of the theory and performed the analysis. The work presented has entirely composed by me.

Chapter 5 is being prepared for publication. The work presented has entirely composed by me.

The Appendix contains two other publications. Section 1 is a published paper: Gardner A, Arce A & Alpedrinha J (2009) Budding dispersal and the sex ratio. Journal of Evolutionary Biology 22, 1036-1045. AG had the original idea, led the development of the theory and analysis, and wrote the paper. I contributed to the development of the theory and analysis, wrote some sub-sections and provided extensive comments. Section 2 is a published paper: Gardner A, Alpedrinha J & West SA (2012) Haplodiploidy and the evolution of eusociality: split sex ratios. American Naturalist 179, 240-256. AG led the development of the theory and analysis, and wrote the paper. Together with AG and SW, I contributed to the development of the idea, and helped to develop some of the theory. I also provided extensive comments.

This thesis is composed of 49992 words, excluding Acknowledgements, Literature Cited and Appendix.

## Acknowledgments

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I dedicate this work to my Mother.

## Abstract

The study of sex allocation is one of the most successful areas in evolutionary biology: its theoretical predictions have been supported by experimental, observational and comparative approaches. Here, I develop sex allocation theory as follows: (1) I use fertility insurance theory to predict the sex ratio strategy of the malaria parasite, in response to human medical interventions that increase mortality and decrease fertility of the parasite's various sexual stages; (2) Haplodiploidy has been suggested as a driver of the evolution of eusociality, as under this genetic system a female may be more related to her sister than to her own offspring. I examine a model considering queen versus worker control over the sex ratio of the colony and show that haplodiploidy alone does not explain the evolution of helping; (3) I follow up this study of the haplodiploidy hypothesis by examining the idea that split-sex ratios may favour the evolution of eusociality in haplodiploid species. I study the two mechanisms of split sex ratios, that are found in natural populations and may have been important in the transition to eusociality: queen virginity and queen replacement. I focus on the impact of worker reproduction by considering the effect of woker producing a fraction of the colony offspring and by considering variation in the workers' offspring sex ratio. My analysis shows that worker reproduction does not promote the evolution of helping in haplodiploid species; (4) I examine the evolution and function of a sterile soldier caste in parasitoid wasps from the genus Encyrtidae. Two main functions have been hypothesized for the emergence of soldiers: spiteful mediation of a sex ratio conflict in mixed-sex broods, and altruistic protection and

facilitation of the development of relatives. I develop a model considering variation in the oviposition behaviour of females, that may produce single-sex or mixed-sex broods. I show that, in accordance with previous theory, females are expected to produce more soldiers than males, under the sex ratio conflict hypothesis. I also show that one of the consequences of this costly conflict is that females are favoured to produce single-sex broods over mixed-sex broods.

# 1. Introduction

**Summary**

The object of this thesis is the study of the action of natural selection, in particular in the context of social interactions, applied to problems of sex allocation. Sex allocation is the study of the allocation of resources between male versus female reproduction (Charnov 1979, 1982; West 2009). This allocation depends on the fitness consequences of factors such as the breeding system within a species, the mechanisms of sex-determination and ecological or demographic factors, such as the rate of dispersal or condition of individuals (West 2009). The field of social evolution studies the fitness consequences of the interaction between individuals and the outcome of this for their fitness. The overlap of these two areas of study is considered one of the most productive areas of study in evolutionary biology, where theoretical predictions have been supported by experimental, observational and comparative approaches (Charnov 1982; Leigh et al. 1985; Godfray 1994; Hamilton 1996; Frank 2002; West 2009).

The major part of my thesis is divided into four chapters. In these, I investigate how natural selection has shaped sex allocation in malaria parasites (Chapter 2), eusocial insects (Chapter 3 and 4) and polyembryonic parasitoid wasps (Chapter 5). In the remainder of this chapter, I provide a brief summary of these four chapters, and explain how social evolution theory is applied to sex allocation. A more detailed introduction to the relevant background literature is provided in each chapter.

In Chapter 2, I investigate how malaria parasites change sex allocation strategy in response to changes in male and female mortality and fertility during gametogenesis. To be transmitted, these parasites produce male and female sexual stages that differentiate into gametes and mate when taken up in a mosquito blood meal. In order to develop effective transmission-blocking therapies it is necessary to understand how parasites adjust their sex allocation in response to treatments that affect the development of these sexual stages. I developed a model showing how the parasite sex allocation strategy changes with differential mortality or fertility in gametocyte, gamete and offspring stages of the life cycle.

In Chapters 3 and 4 I study the importance of haplodiploidy in the evolution of eusociality. Hamilton suggested that haplodiploid genetics, where unfertilized (haploid) eggs develop into males and fertilized (diploid) eggs develop into females, may raise the relatedness between siblings and hence make it easier for kin selection to favour altruistic workers, who help rear their mothers offspring, rather than reproduce themselves. In Chapter 3, I follow the scenario developed by Trivers and Hare (1976), where sex allocation becomes biased due to worker control of sex allocation and/or worker reproduction, and I assess the overall effect of haplodiploidy on the evolution of helping. In Chapter 4 I focus on the effect that split sex ratios have on the evolution of eusociality. In a haplodiploid population with the sex ratio in every colony, a female biased sex ratio means that the worker is more related to her siblings, but this effect is exactly cancelled by the concomitant increase in the reproductive value of her male relatives. However, the haplodiploidy hypothesis may work if some broods are relatively male biased and others relatively female biased – termed split sex ratios – as there is higher relatedness to siblings in female-biased

colonies without the concomitant reproductive value effect. I investigate the consequences for the evolution of helping of two empirically relevant mechanisms that can drive the evolution of split sex ratios, queen virginity and queen reproduction, focusing on the role of population with worker reproduction.

In Chapter 5, I study the evolution and function of a soldier caste in polyembryonic parasitoid wasps. Wasps from the genus *Encyrtidae* may lay one or two eggs inside caterpillar eggs. These each develop clonally to form up to a thousand individuals. Within each egg, a fraction of individuals may develop precociously to form a sterile cast of soldiers (Grbic et al. 1992; Ode and Strand 1995; Gardner et al. 2007). I investigate the existence of a sex ratio conflict and soldier function in a population composed of both mixed and single-sex broods, and consider the coevolution of solider activity, sex ratio, and the maternal allocation between single-sex and mixed-sex broods.

**Social evolution**

Social evolution is the study of interactions between individuals (Frank 1998; West et al. 2007). Social interactions can be classified in four different categories, according to the effect on the actor and on the recipient of the behaviour or trait (Hamilton 1964a, b). A behaviour or a trait can be seen as mutually beneficial (+/+), when both the actor and the recipient benefit from the action/trait (Maynard Smith 1982); altruistic (-/+) when the action/trait is costly to the actor and beneficial to the recipient; selfish (+/-), when the actor benefits from the action/trait but it is costly to the recipient (Hamilton 1964a, b); and spiteful (-/-) when both actor and recipient

suffer costs from the action/trait (Hamilton 1970). While mutually beneficial and selfish behaviours or traits are easy to explain, as they provide a direct benefit to the actor, costly traits are less easy to explain as they decrease the fitness of the actor (Hamilton 1964a, b).

Hamilton offered a solution to this problem by showing the importance of a genetic association between the actor and the recipient (Hamilton 1964a, b, 1972). Taking a gene's-eye view (Dawkins 1976), Hamilton showed that fitness can be measured through the gene's point of view, and a gene's fitness is not measured solely by its bearers' reproductive success but instead is measured by his impact on the reproductive success of others (Hamilton 1964a, b; Grafen 2004). If averaging over all carriers of the gene, the bearers have greater personal fitness than the non-carriers, then it doesn't matter that some of the individuals are actually losing fitness through their actions. This led to the development of the concept of neighbour-modulated fitness (Hamilton 1964a, b), where an individual's fitness is measured by the sum of his phenotype effect on his reproductive success (direct fitness) plus the effect of others on his reproductive success (indirect fitness). Taking an organismal perspective, Hamilton defined inclusive fitness as the effect of the behavior of an individual on the fitness of all relatives, including self. In this way, inclusive fitness is measured by all the offspring (from the actor and neighbours) that can be attributed to the actor's own behaviour, each valued according to genetic relatedness. Relatedness serves as a type of exchange rate that allows non-descendant offspring to be translated into effective numbers of offspring (Frank 1998; Gardner and Foster 2008).

Using this new conceptualization for an individual's inclusive fitness, Hamilton

defined an inequality quantitatively describing the rate of success of a given social behaviour. This relation provides a strikingly simple expression that is known as Hamilton's rule (Charnov 1978). This states that a given trait will be selected if the sum of its direct fitness (-*c*) plus its indirect fitness weighted by the relatedness (*rb*) between the actor and the recipient(s) is bigger than zero: $-c + br > 0$ (Hamilton 1964a, b). In the case of altruism, a given altruistic trait may spread in a population if its cost to the actor is smaller that the benefits to the recipients weighted each one by their relatedness to the actor. This can be extended to any number of recipients by summing multiple rb terms. Although devised to understand social adaptations, inclusive fitness theory provides a mean of understanding the evolution of any behavioural, morphological or physiological trait, which impact upon the fitness of individuals other than the bearer.

**Sex allocation**

*Fisher's model*

In an outbred, diploid population, with equal costs of producing males or females, the expected sex ratio of the population is 1:1. This follows from the fact that, if a population is male biased, a female will in average have more chances to mate than a male and therefore an individual producing a female biased offspring will have a fitness advantage over the average of the population. The opposite applies to a female biased population and this effect holds until the population sex ratio is equal. This frequency dependent argument shows that on this case, producing a 1:1 sex ratio is the unbeatable sex ratio strategy. Fisher (1930) also showed that the theory holds when the costs of producing males and females are unequal if each sex is weighted by

its cost. Thus, if producing a female is twice as costly producing a male, then the population will have twice as much males as females. This means that even if the sex ratio is biased, there is still an equal investment in producing males and females.

### *Local mate competition*

Fisher (1930) assumed a very simple framework that allows a remarkable simplicity and provides a strong conceptual value. However, real populations often don't follow some of the assumptions of his model, and biased sex ratios can be favoured when relatives compete or cooperate for a resource (Hamilton 1967). Local resource competition occurs when the production of one sex leads to an increase of competition between relatives over a limiting resource (Taylor 1981). In this case, natural selection will favour the production a biased sex ratio towards the sex that leads to less competition (Taylor 1981; Wild and Taylor 2004). Local resource enhancement is the opposite situation, and occurs when there are cooperative interactions between relatives (Trivers and Hare 1976). In this case, natural selection will favour a sex ratio biased towards the sex that leads to higher cooperation (Taylor 1981; Charnov 1982; Pen and Weissing 2000).

Local mate competition is a particular case of local resource competition, first described by Hamilton (1967). In a population composed by an infinite number of breeding patches, where each patch is colonized by a single fertilized female, if mating occurs within the patch, relatedness between each of the members of the patch will be higher among members of the same patch with outsiders. In such a population, if there is no pre mating dispersal all mating events will involve siblings, and the best strategy for a female is to produce the least amount of males necessary to mate with

all the females in the patch. With no male dispersal, the optimal sex ratio was shown by Hamilton to be very close to zero, which is translated in having the minimal number of males to mate with the females from the patch. As dispersal rates increase there are more opportunities for sons to outbreed and as a result the sex ratio becomes less female biased.

In Chapter 5, I study the existence of a sex ratio conflict between brothers and sisters in polyembryonic wasps from the genus *Encyrtidae*. In these species, females may lay either one or two opposite sex eggs in the host (Ode and Strand 1995). Each egg develops clonally and produces a large number of male and female offspring. From these, a fraction undergoes a precocious development into a sterile soldier caste (Ode and Strand 1995). As the host dies, emerging adults may disperse or mate in the same patch. This translates in a range that goes from local mate competition, if the individuals in the same patch are all siblings, to a fully panmitic population if they all disperse. The function of the soldier caste has been associated with a sex ratio conflict, where brothers favour a less female biased sex ratio than sisters (Grbic et al. 1992; Gardner et al. 2007; Giron et al. 2007b). I develop a model to investigate this conflict, in a population with both single-sex (one egg) and mixed-sex (two opposite sex eggs) broods, and investigate the possible role of soldiers in the resolution of that sex ratio conflict.

### *Fertility insurance*

The local mate competition models described above assume that there are always enough males to mate with the females. That may not be the case, either due to mortality affecting males during their development or to a low number of eggs formed

by the female that might lead her to accidentally not producing a male (Green et al. 1982). In this case, a less female sex ratio may be favoured by selection ensure that all females are fertilized (West et al. 2002). Fertility insurance theory predicts that the evolutionary stable sex ratio strategy decreases increasing clutch size and increases with higher developmental mortality (Green et al. 1982; West et al. 1997). This is in accordance with empirical observations in parasitoid wasps (Griffiths and Godfray 1988; Hardy and Cook 1995), fig wasps (Herre et al. 1997) and mites, where the sex ratio becomes increasingly female biased with an increase of the brood size (Nagelkerke 1996).

Fertility insurance has also been studied in the context of malaria infections (Read et al. 1992; West et al. 2001). In malaria parasites, the gametocytes are taken up from the host in the blood meal of the vector. Within the vector, female and male gametocytes develop into gametes and fuse, forming zygotes. Fertility insurance may occur due to either a small number of gametes formed per gametocyte, gamete or gametocyte mortality or the risk of absence of male gametocytes due to small blood meals. In Chapter 2, I study a fertility insurance model to predict the effect of nitric oxide (NO) and of tumor necrosis factor-α (TNF-α), two known immunity factors produced by the host that can reduce and even block successful gametogenesis (Luckhart et al. 1998a; Long et al. 2008). I develop theory to predict how the evolutionary trajectories of parasite sex ratio strategies are shaped by sex differences in gamete production, fertility and offspring development.

# 2. Sex and Death: the Effects of Innate Immune Factors on the Sexual Reproduction of Malaria Parasites

This chapter appears as the following publication: Ramiro, R. S., J. Alpedrinha, L. Carter, A. Gardner, and S. E. Reece. 2011. Sex and Death: The Effects of Innate Immune Factors on the Sexual Reproduction of Malaria Parasites. Plos Pathog 7.

## Abstract

Malaria parasites must undergo a round of sexual reproduction in the blood meal of a mosquito vector to be transmitted between hosts. Developing a transmission-blocking intervention to prevent parasites from mating is a major goal of biomedicine, but its effectiveness could be compromised if parasites can compensate by simply adjusting their sex allocation strategies. Recently, the application of evolutionary theory for sex allocation has been supported by experiments demonstrating that malaria parasites adjust their sex ratios in response to infection genetic diversity, precisely as predicted. Theory also predicts that parasites should adjust sex allocation in response to host immunity. Whilst data are supportive, the assumptions underlying this prediction – that host immune responses have differential effects on the mating ability of males and females – have not yet been tested. Here, we combine experimental work with theoretical models in order to investigate whether the development and fertility of male and female parasites is affected by innate immune factors and develop new theory to predict how parasites' sex allocation strategies should evolve in response to the observed effects. Specifically, we demonstrate that reactive nitrogen species

impair gametogenesis of males only, but reduce the fertility of both male and female gametes. In contrast, tumour necrosis factor-α does not influence gametogenesis in either sex but impairs zygote development. Therefore, our experiments demonstrate that immune factors have complex effects on each sex, ranging from reducing the ability of gametocytes to develop into gametes, to affecting the viability of offspring. We incorporate these results into theory to predict how the evolutionary trajectories of parasite sex ratio strategies are shaped by sex differences in gamete production, fertility and offspring development. We show that medical interventions targeting offspring development are more likely to be 'evolution-proof' than interventions directed at killing males or females. Given the drive to develop medical interventions that interfere with parasite mating, our data and theoretical models have important implications.

## Author summary

Malaria and related parasites cause some of the most serious infectious diseases of humans, domestic animals and wildlife. To be transmitted, these parasites produce male and female sexual stages that differentiate into gametes and mate when taken up in a mosquito blood meal. Despite the need to develop a transmission-blocking intervention, remarkably little is understood about the evolution of parasite mating strategies. However, recent research demonstrates that producing the right ratio of male to female stages is central to mating success. Evolutionary theory predicts that sex ratios are adjusted in line with a variety of factors that affect mating success, including host immunity. We test this theory by investigating whether ubiquitous

immune factors differentially affect the production and fertility of males and females. Our experiments demonstrate that immune factors have complex, sex-specific effects, from reducing gamete production to affecting offspring viability. We use these results to generate theory predicting how such effects shape the evolutionary trajectories of parasite sex ratio strategies. Given the drive to develop medical interventions that prevent transmission by blocking parasite mating, our results have important implications. Specifically, we suggest that medical interventions targeting offspring development are more likely to be 'evolution-proof' than interventions with sex-specific effects.

## Introduction

Malaria parasites are obliged to undertake a single round of sexual reproduction in the mosquito vector before they can transmit to new hosts, making this stage of their life-cycle a potential target for medical interventions (Paul et al. 2003b; Saul 2008). The success of interventions aiming to disrupt mating success will depend upon a variety of epidemiological parameters (e.g. transmission intensity/seasonality), but will also be strongly determined by the parasites' behavioural and evolutionary responses (Paul et al. 2003b; Smith et al. 2007; Saul 2008). Current candidates for transmission-blocking vaccines (TBV) involve targeting proteins, expressed on the surface of sexual stages, that are essential for the fertility of males (e.g. P48/45 and P230) (Carter 2001; van Dijk et al. 2001; Outchkourov et al. 2008; Chowdhury et al. 2009; van Dijk et al. 2010). However, theory predicts that the efficacy of a vaccine that reduces the fertility of one sex may be eroded if parasites respond by adjusting their sex ratios in favour of the targeted sex. The study of sex allocation has been one of

the most successful areas of evolutionary biology, with empirical data matching clear theoretical predictions across a variety of taxa (West 2009). Before describing evolutionary theory for sex allocation strategies we outline the relevant aspects of *Plasmodium* mating biology.

Every asexual replication cycle, a small proportion of parasites differentiate into male and female sexual stages – termed gametocytes – which are developmentally arrested gamete precursors (Taylor and Read 1997; Talman et al. 2004). Gametogenesis of both sexes begins as soon as gametocytes are taken up in a mosquito blood meal, fertilization occurs within 30 minutes, and zygotes develop into the stages infective to vectors (ookinetes) after 18-20 hours (Alano and Carter 1990; Vaughan 2007). To differentiate into gametes, gametocytes must leave the relative safety of their red blood cells (RBCs), becoming exposed to host- and mosquito-derived factors that can block mating (Alano and Carter 1990). Males are expected to be more vulnerable than females to transmission-blocking factors due to their more complex gametogenesis and mating activities (West et al. 2001; Gardner et al. 2003). Whereas female gametocytes only have to leave their RBCs to become gametes, male gametogenesis also includes three rounds of mitosis and flagellum construction to produce a (rarely achieved) maximum of eight 'sperm-like' gametes (Sinden 1983b; Janse et al. 1986; Sinden 1998; Schall 2000; Reece et al. 2008). Mature male and female gametocytes are easily distinguished by their phenotypes as their reproductive roles result in different cellular contents (Mons 1986; Khan et al. 2005). Mature males are terminally differentiated, only having pre-synthesized proteins and machinery for gamete production (e.g. α-tubulin II, cell cycle proteins, dynein) (Sinden 1983a; Talman et al. 2004; Khan et al. 2005). In contrast, mature female gametocytes are

prepared for continued development after fertilization, having high levels of ribosomal proteins, mitochondria (which are absent in mature males) and pools of translationally repressed messenger RNAs (mRNAs; similar to P bodies in metazoan oocytes) (Talman et al. 2004; Khan et al. 2005; Mair et al. 2010). Therefore, male and female gametocytes are primed for gametogenesis and zygote development, respectively (Janse and Waters 2004).

Sex allocation is an important fitness-related trait in *Plasmodium* and could play an important role in the response of malaria parasites to medical interventions that aim to reduce mating success (Paul et al. 2000; Reece et al. 2008; Mitri et al. 2009; Reece et al. 2009). Parasites could respond to transmission-blocking interventions by adjusting their sex allocation strategies via two evolutionary processes. First, if conditions within hosts are unpredictable, invariant, or if variation in within-host conditions is not a good proxy for variation in the mating conditions experienced within vectors, parasites evolve fixed (i.e. canalised) sex allocation strategies that reflect the average environment. Second, if in-host conditions reliably predict in-vector conditions, parasites will evolve to facultatively adjust their sex ratios (proportion of male gametocytes) through phenotypic plasticity. In this scenario, if asexual stage parasites detect an increase in a factor (or correlate of) that reduces mating ability in a sex-specific way, parasites will benefit from adjusting the production of male and female gametocytes in response. Given that once parasites are taken up by a vector, no further gametocyte production can occur and gametogenesis and fertilization are completed within 30 minutes, the mating environment within the blood meal is 'imported' from the host. Therefore, the within-host conditions will be good

predictors for mating conditions and so facultative sex ratio adjustment is both predicted and observed (West et al. 2001).

Currently, two complementary evolutionary theories predict how and why parasites should adjust their investment into male and female gametocytes to maximise fertilization success. These theories – Fertility Insurance and Local Mate Competition – predict that parasites adjust sex ratios in response to environmental (e.g. transmission-blocking immunity) and social factors (inbreeding rate), respectively (Hamilton 1967; Read et al. 1992; Read et al. 1995; West et al. 2000; West et al. 2001; Nee et al. 2002; West et al. 2002; Gardner et al. 2003). The ability of parasites to facultatively adjust their sex ratios in response to variation in the inbreeding rate has recently been verified (Reece et al. 2008; Reece et al. 2009). Additionally, data also suggest that sex ratios are altered in response to the development of immunity (Reece et al. 2008). Host-derived immune factors make mating challenging for parasites because they can reduce and even block fertilization (Carter et al. 1979; Naotunne et al. 1991). This phenomenon, called 'transmission-blocking immunity' (TBI), has been extensively observed and documented across a variety of malaria parasite species (Carter et al. 1979; Mendis et al. 1987; Targett 1988; Naotunne et al. 1991; Naotunne et al. 1993; Drakeley et al. 1998). The mechanisms of TBI are varied and include damaging gametocytes, preventing successful gametogenesis (Naotunne et al. 1991; Naotunne et al. 1993; Cao et al. 1998; Gardner et al. 2007; Long et al. 2008), decreasing the ability of gametes to interact (Carter et al. 1979; Mendis and Targett 1981) and preventing post-fertilization development (Targett 1988; Luckhart et al. 1998b). Fertility Insurance predicts that when hosts mount an immune response, the fertility of male gametocytes and/or gametes is most affected, therefore parasites

should produce more males to compensate (West et al. 2001; Gardner et al. 2003). Two lines of empirical data support this prediction. First, Paul *et al.* (2000) showed that *P. gallinaceum* and *P. vinckei* increase their sex ratio in response to erythropoiesis, which is thought to act as a cue for the appearance of TBI factors. Second, Reece *et al.* (2008) provided indirect support by suggesting that sex ratio variation observed during infections of different *P. chabaudi* genotypes is a mechanism to ensure fertility in face of within-host competition, host anaemia and TBI factors. Fertility Insurance currently provides the best explanation for the observed within-infection variation in the sex ratios of malaria parasites. However, the theory is based upon the untested assumption that TBI factors reduce the fertility of males more than females. Here we provide the first direct test of this key assumption by investigating whether reactive nitrogen species and pro-inflammatory cytokines, influence gametogenesis, gamete fertility and ookinete production.

Levels of reactive nitrogen species (RNS) and pro-inflammatory cytokines vary during malaria infections. These immune factors, which are ubiquitous components of the innate immune system, have been specifically implied in the sudden loss of infectivity to vectors that occurs during paroxysms and infection crisis (Naotunne et al. 1993; Long et al. 2008). Specifically, tumour necrosis factor-α (TNF-α) is a potent pro-inflammatory cytokine and several studies have revealed a role for this cytokine in mediating the killing of *Plasmodium* gametocytes, across a variety of host-parasite systems (Naotunne et al. 1991; Karunaweera et al. 1992; Long et al. 2008). This could occur through the stimulation of phagocytosis and nitric oxide (NO) production by white blood cells (Naotunne et al. 1993; Tracey and Cerami 1994; Muniz-Junqueira et al. 2001), as these are capable of phagocytosing opsonized gametes in the mosquito

midgut (Lensen et al. 1997) and the inhibition of NO synthesis by white blood cells reduces in 60% the inactivation of *P. falciparum* and *P. vivax* gametocytes (Motard et al. 1993; Naotunne et al. 1993). NO is produced by the enzyme inducible nitric oxide synthase in response to infection, in both hosts and vectors, and is extremely toxic at high doses. NO is a highly reactive molecule, thus a significant extent of the damage it causes is indirect, through the production of RNS (such as peroxynitrite, nitrates, nitrites or S-nitrosothiols) that frequently function as the ultimate effectors (Bogdan 2001). Hereafter, unless otherwise stated, we use the term 'RNS' to refer to NO and its reaction products. During *Plasmodium* infections, RNS appears to impair asexual replication, gametogenesis and zygote development (Naotunne et al. 1993; Cao et al. 1998; Luckhart et al. 1998b; Wang et al. 2009). Levels of RNS increase during *P. yoelii* infections and reduce ookinete production when either gametocytes or gametes are exposed (Cao et al. 1998). Furthermore, RNS have been shown to induce the programmed cell death of *P. berghei* ookinetes (Ali et al. 2010) and to extensively reduce *P. berghei* oocyst burdens in *Anopheles* mosquitoes (Luckhart et al. 1998b). This is, at least in part, the result of a pro-inflammatory response, in which host cytokines induce the mosquito to increase NO (and therefore RNS) production (Luckhart et al. 2003).

Here, we use the rodent malaria parasite *Plasmodium berghei* to conduct a series of experiments to investigate how RNS and TNF-α influence mating success and ookinete production and develop theoretical models that predict the evolution of sex allocation strategies, given the effects observed in our experiments. Therefore, we use these immune manipulations as 'proof-of-principle' for other factors with similar effects on the sexual reproduction and transmission of malaria parasites. Specifically, we test whether: (1) RNS and TNF-α have dose dependent effects on male

gametogenesis (exflagellation) and ookinete production; (2) exposure of male and female gametocytes to both RNS and TNF-α influences their sexual development; (3) the greater effect of RNS we observe on male gametogenesis results in sex-specific fertility effects; and (4) the observed effects of RNS depend on the developmental stage at which parasites are exposed. Our results reveal that RNS reduces male but not female gametogenesis and impairs the fertility of both sexes, whereas TNF-α only affects zygote development. The relative importance of reduced gametogenesis, impaired mating ability and reduced post-mating development have not been explicitly considered by Fertility Insurance theory. Therefore we develop a new mathematical model to derive predictions for how the effects of immune factors generated naturally or by a medical intervention are likely to impact upon parasite sex ratio evolution (a schematic of the biological effects included in the model is presented in Figure 2.1).

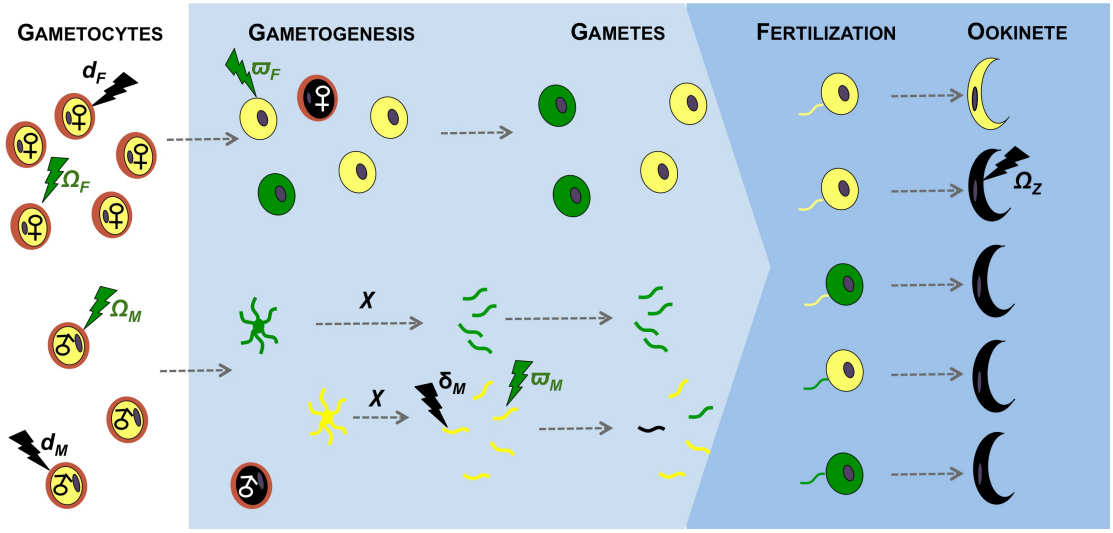



Figure 2.1. Effects of immunity on gametogenesis and fertility of malaria parasites. The effects of transmission-blocking immune factors on the sexual development of malaria parasites investigated in our model. Female and male gametocytes circulating in the host

(white background) undergo gametogenesis when taken up by a mosquito vector (blue background). Each male gametocyte differentiates into $\chi$ gametes ($\chi \leq 8$) and each female gametocyte produces one gamete. Male gametes locate and fertilise female gametes, and the resulting zygotes develop into ookinetes. Immune factors circulating in the host can act on males and females throughout their sexual development, from gametocytes to zygotes. The developmental stages of females are shown above the stages of males and each individual gametocyte/gamete is shown in the same relative position throughout development. The effects of immune factors (lighting) on sexual stages can either be cryptic (i.e. render gametocytes/gametes dysfunctional; green), or fatal (i.e. gametocytes/gametes die; black). Healthy, unaffected, parasites are represented in yellow, dysfunctional parasites in green, and dead parasites in black. Immune factors kill female gametocytes with probability $d_F$ and male gametocytes or gametes with probabilities $d_M$ or $\delta_M$, respectively. Dead sexual stages do not participate further in the mating pool. Immune factors render female gametocytes and gametes dysfunctional with probabilities $\Omega_F$ and $\varpi_F$ respectively, and male gametocytes and gametes with probabilities $\Omega_M$ and $\varpi_M$, respectively. Dysfunctional gametocytes/gametes participate in the mating pool and can be fertilized as for healthy gametes, however zygotes are unviable and die before reaching the ookinete stage. Immune factors can also directly lead to zygote death with probability $\Omega_Z$. All possible fertilization scenarios are represented: mating between two healthy gametes, mating between one healthy and one dysfunctional gamete and mating between two dysfunctional gametes.

## Results

All the experiments we describe below were performed *in vitro*, using gametocytes harvested form *Plasmodium berghei* infected mice. Parasites were either cultured in conditions that 'mimicked the vector' (in which they immediately became activated

and underwent gametogenesis and mating; media at pH 8 and 21 ºC), or conditions that 'mimicked the host' (in which gametocytes remained developmentally arrested; pH 7.25, 37 ºC) (Reece et al. 2008). Parasites cultured in host mimicking conditions became activated and underwent gametogenesis if subsequently exposed to vector mimicking conditions. We manipulated exposure to TNF-α with recombinant mouse TNF-α and RNS exposure with L-ana (L-Arginine p-nitroanilide dihydrochloride) and SIN-1 (3-morpholinosydnonimine hydrochloride). L-ana is an inhibitor of NO synthesis and SIN-1 donates RNS in solution (see methods for details) (Singh et al. 1999). We exposed parasites to RNS and TNF-α treatments in 1 ml cultures with 15 or 20 μl parasitized blood.

*Experiment 1: dose-dependent effects of RNS and TNF-α*

We first tested whether RNS and TNF-α influence sexual reproduction by exposing parasites to different concentrations of these factors and assaying exflagellation and ookinete production. We incubated parasites in vector mimicking media across seven concentrations of SIN-1 (ranging from 0 to 1 mg/ml) (Dea-Ayuela et al. 2009) and five concentrations of recombinant mouse TNF-α (from 0 to 1 μg/ml; see Methods). Increasing concentrations of SIN-1 caused a significant linear decrease in the densities of exflagellating males ($F_{(1,35)} = 16.28$, $P < 0.0001$; transformed y = 0.16-0.10x) and ookinetes ($F_{(1,35)} = 25.86$, $P < 0.0001$; transformed y = 0.17-0.18x). Similarly, TNF-α also caused a significant linear decrease in the densities of exflagellating males ($F_{(1,15)} = 6.83$, $P = 0.012$; y = 0.23-0.09x) and ookinetes ($F_{(1,15)} = 17.53$, $P < 0.0001$ ; transformed y = 0.54-0.37x).

*Experiment 2: effects of RNS and TNF-α on gametogenesis and ookinete production*

Having found significant negative effects of RNS and TNF-α on exflagellation and ookinete production we investigated whether these factors interacted with each other to further reduce parasite mating success and if these effects depended on the developmental stage at which parasites were exposed (i.e. in host or vector conditions). For this set of experiments we used a fully cross-factored design, consisting of two RNS and two TNF-α levels (see Methods).

First, we investigated the effects of RNS and TNF-α on gametocytes by incubating parasites for 60 minutes in host mimicking media. We then replaced treatment media with vector mimicking media (without RNS or TNF-α manipulations) to stimulate gametogenesis and quantified the development of male and female gametocytes into gametes using the following classifications: (a) mature gametocytes still inside their RBC, (b) gametocytes that had emerged from the RBC and (c) exflagellating male gametes (see Methods for criteria). We present the proportion of a given developmental stage relative to the total number of observed gametocytes/gametes of the same sex (Figure 2.2). The proportion of emerged female gametocytes was not significantly influenced by either RNS ($\chi^2_1 = 2.72$, P = 0.099) or TNF-α ($\chi^2_1 = 0.12$, P = 0.731; or their interaction $\chi^2_1 = 3.38$, P = 0.066). In contrast, the proportion of male gametocytes that emerged from RBCs was significantly reduced by RNS ($F_{(1, 59)}$ = 81.29; P < 0.0001; mean 'RNS -' 0.55 ± 0.02; 'RNS +' 0.32 ± 0.02) but not by TNF-α ($\chi^2_1 = 0.16$, P = 0.689; or their interaction $\chi^2_1 < 0.01$, P = 0.982). Similarly, the ability of males to exflagellate was significantly reduced by RNS ($F_{(1, 59)} = 33.40$;

P < 0.0001; mean 'RNS-' 0.15 ± 0.01; 'RNS +' 0.09 ± 0.01) but not by TNF-α ($\chi^2_1$ = 0.85, P = 0.36; or their interaction $\chi^2_1$ = 0.02, P = 0.885).

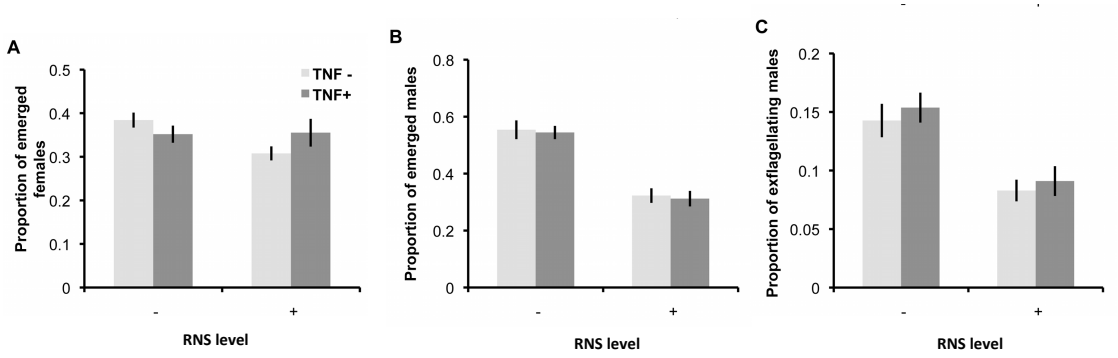
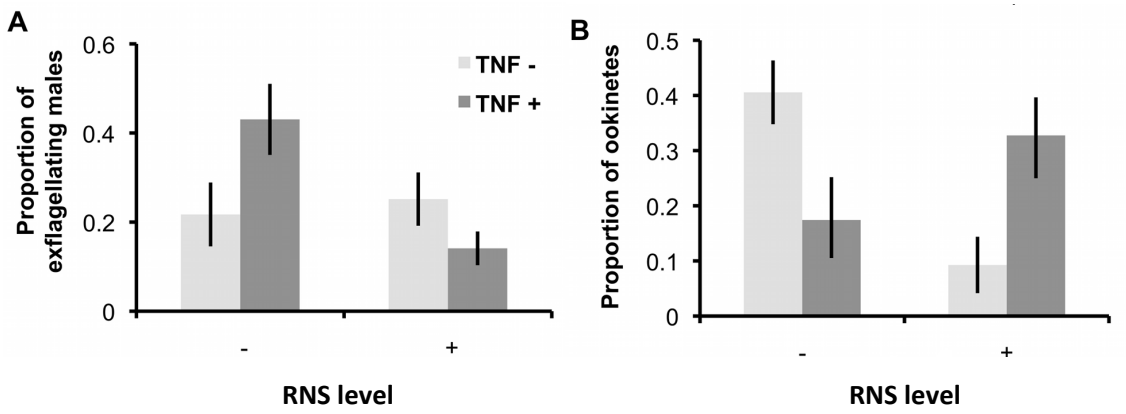



Figure 2.2. Ability of gametocytes to undergo gametogenesis after exposure to RNS and TNF-α. Mean (± S.E.) proportion (n = 20) of emerged female gametes (A), emerged male gametocytes (B), and exflagellating male gametes (C), relative to the total number of male or female gametocytes/gametes observed, when gametocytes are exposed to immune factors during incubation in 'host conditions' and then activated in un-manipulated 'vector conditions' media.

Second, we investigated the effects of RNS and TNF-α on exflagellation and ookinete production by incubating parasites in culture media mimicking the vector environment (Figure 2.3). In line with the results from our previous experiments, the proportion of exflagellating males was significantly reduced by RNS ($F_{(1, 45)}$ = 11.24, P = 0.002; mean 'RNS -' 0.32 ± 0.06; 'RNS +' 0.12 ± 0.03). This effect was enhanced by TNF-α (interaction: $F_{(1, 45)}$ = 6.67, P = 0.014) but in the absence of RNS, TNF-α had no significant effect ($F_{(1, 45)}$ = 1.90, P = 0.175). Conversely, the effect of RNS and TNF-α on ookinete production depended on each others presence (interaction $F_{(1, 24)}$ = 14.91, P = 0.001). Specifically, ookinete production was reduced by TNF-α but only in the absence of RNS (mean 'TNF-α-' 0.41 ± 0.06; 'TNF-α+' 0.17 ± 0.07), whereas

RNS reduced ookinete production but only when TNF-α was absent (mean 'RNS -' 0.41 ± 0.06; 'RNS +' 0.09 ± 0.05).




2. 2.3. Exflagellation rates and ookinete production after exposure to RNS and TNF-α during gametogenesis. Mean (± S.E.) proportion of exflagellating male gametes (A; n=16) or ookinetes (B; n=9) produced when parasites are exposed to RNS and TNF-α during gametogenesis (in-vector conditions media). Proportions are relative to the total number of exflagellating male gametes or ookinetes produced from each infection, across treatments.

*Experiment 3: sex-specific effects of RNS on fertility*

Experiment 2 revealed that only RNS had a significant effect on gametogenesis, in which male but not female development was impaired. Therefore, we tested whether these effects translated into sex-specific differences in fertility (i.e. whether matings with RNS exposed gametocytes/gametes resulted in fewer ookinetes), when parasites were exposed as gametocytes (in host-mimicking media) or during gametogenesis (in vector-mimicking media). We separately exposed each sex to RNS using two genetically transformed (knock-out; KO) *P. berghei* lines: Pbs48/45ko and Pbs47ko (van Dijk et al. 2001; Khan et al. 2005; van Dijk et al. 2010), which produce unviable

male and female gametes, respectively. This allowed us to assay the fertility consequences of exposing one sex to RNS by providing exposed parasites with a surplus of unexposed mates from the opposite sex and assaying ookinete production (Figure 2.4).

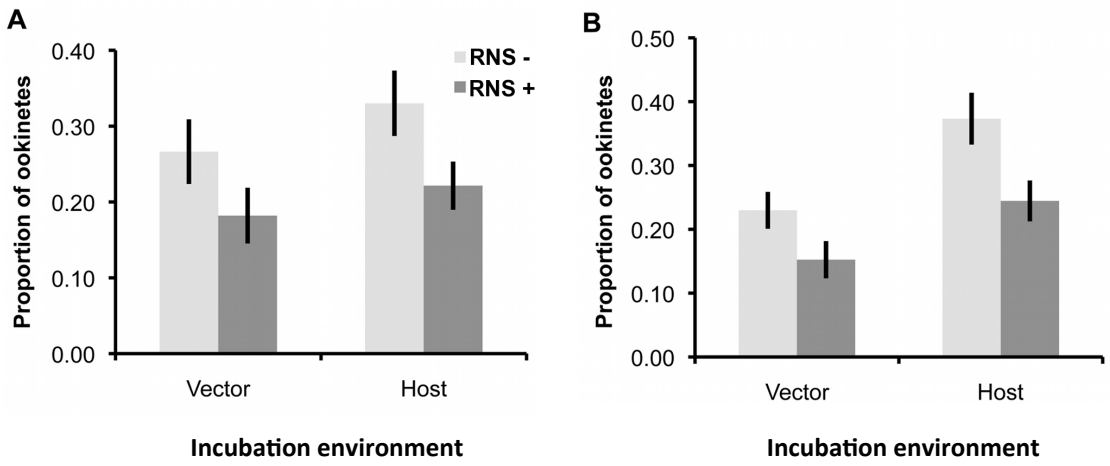



Figure 2.4. Ookinete production after exposure of males or females to RNS, before or during gametogenesis. Mean (± S.E.) proportion (n=19) of ookinetes produced, when females (A) or males (B) are exposed to RNS as gametocytes (in-host conditions media) or during gametogenesis (in-vector conditions media). Proportions are relative to the total number of ookinetes produced by the focal sex from each pair of infections.

We observed that RNS exposure significantly reduced fertility of both males and females regardless of whether parasites were exposed as gametocytes or during gametogenesis ($F_{(1,131)} = 15.87$, P = 0.0001; mean 'RNS -' 0.30 ± 0.02; 'RNS +' 0.20 ± 0.02). In contrast to our predictions, RNS did not have sex-specific effects (treatment:sex interaction: $\chi^2_1 = 0.023$, P = 0.88), nor was this effect influenced by exposing parasites to RNS in host- or vector-mimicking environments (treatment:environment interaction: $\chi^2_1 = 0.366$, P = 0.55). However, across all

treatments, parasites exposed in host conditions produced significantly more ookinetes than those exposed in vector conditions ($F_{(1,131)} = 10.19$, $P = 0.0018$; mean 'Host' 0.29 ± 0.02; 'Vector' 0.21 ± 0.02).

*Theoretical model*

We incorporate our experimental results into Fertility Insurance theory by developing a mathematical model to explore the impact of transmission-blocking factors on the evolution of parasite sex allocation strategies. Specifically, we examine whether sex ratio adjustment could compensate for transmission-blocking factors with the following effects on males or females: preventing male or female gametocytes from undergoing gametogenesis (as each female gametocyte only produces one gamete, killing of these stages is mathematically equivalent); blocking the mating ability of male gametes; and causing damage to gametocytes or gametes such that mating can occur but zygotes are not viable. We term the latter phenomenon, of cryptic damage to gametocytes or gametes that results in a dead zygote, as dysfunction. Note that, although we do not observe all of the effects on all stages and all sexes, we incorporate them all in the model (illustrated in Figure 2.1), as they are theoretical possibilities. Also, our model makes no assumptions about whether parasites evolve fixed (i.e. canalised) or facultative (i.e. plastic) sex allocation strategies.

First, we show that all zygote mortality effects (i.e. treatments leading to $0 < p < 1$) have no impact on the evolutionarily stable (ES) sex ratio (Maynard Smith and Price 1973; Maynard Smith 1982). We write $W = \zeta(z)\, p$, i.e. fitness is the product of zygote production and zygote viability, where zygote production depends upon sex ratio but

zygote viability does not. The direction of selection is given by the derivative of fitness with respect to sex ratio (Taylor 1996), and this 'marginal fitness' is $dW/dz = (d\zeta/dz)p$. The ES sex ratio $z^*$ satisfies $dW/dz|_{z=z^*} = 0$, i.e. selection does not favour an increase or decrease in sex ratio when the population is at the ES sex ratio, and this is equivalent to the condition $d\zeta/dz|_{z=z^*} = 0$ for all $p>0$. Since $\zeta$ is not a function of $p$, it follows that $z^*$ is not a function of $p$ (and hence is not a function of $\Omega_Z$, $\Omega_M$, $\Omega_F$, $\varpi_M$ or $\varpi_F$; see Methods and Figure 2.1 for symbol definitions). Therefore, treatments that simply impact upon the viability of zygotes (e.g. cause gametocyte/gamete dysfunction) are not expected to have an evolutionary impact upon parasite sex ratios.

Second, to investigate the impact of model parameters arising from gametocyte or gamete killing on the ES sex ratio, we write an explicit expression for expected fitness:

$$W = \sum_{\alpha=0}^{q}\sum_{\Gamma=0}^{\alpha}\sum_{\gamma=0}^{\chi\Gamma}\sum_{\phi=0}^{q-\alpha}\binom{q}{\alpha} z^{\alpha}(1-z)^{q-\alpha}\binom{\alpha}{\Gamma} d_M^{\alpha-\Gamma}(1-d_M)^{\Gamma}\binom{\chi\Gamma}{\gamma}\delta_M^{\chi\Gamma-\gamma}(1-\delta_M)^{\gamma}\binom{q-\alpha}{\phi} d_F^{q-\alpha-\phi}(1-d_F)^{\phi}\min\{\gamma,\phi\}p \qquad (2.1)$$

The condition $dW/dz|_{z=z^*} = 0$ can be solved numerically for $z^*$ for any numerical parameter set ($q$, $d_M$, $d_F$, $\delta_M$). An exploration of the ES sex ratio $z^*$ across this parameter space is presented in Figures 2.5 and 2.S1-3. Specifically, we recover the prediction that the gametocyte ES sex ratio will be biased towards the more limiting sex when factors prevent male or female gametocytes from undergoing gametogenesis or block the mating ability of male gametes.

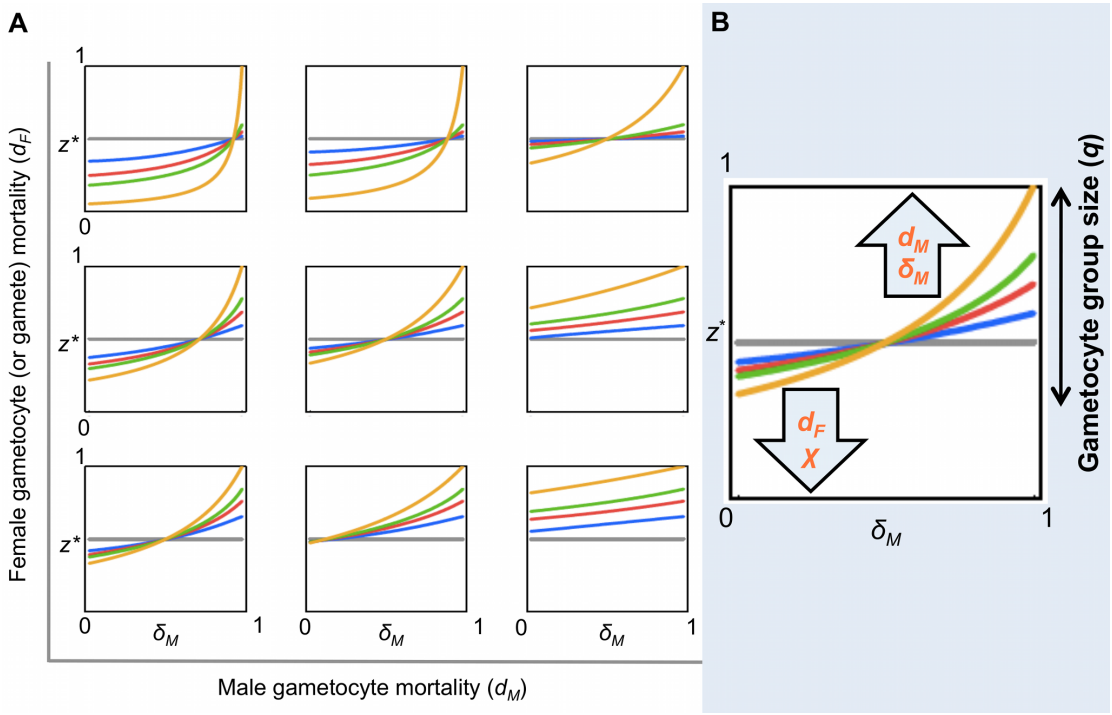



Figure 2.5. Evolutionarily stable sex allocation strategies when sex- and stage-specific mortality rates vary. Effect of male and female gametocyte mortality and male gamete mortality on the ES gametocyte sex ratio ($z^*$), for a clonal population, when the number of gametes produced per male gametocyte ($\chi$) is 2 (this fecundity has been estimated for this system by other studies; see ref. (Reece et al. 2008)). Figures 2.S1-3 show similar patterns to Figure 2.5A for $\chi$ = 1; 4; 8, respectively. (A) For each plot within the panel, $z^*$ varies with male gamete mortality rate ($\delta_M$). The coloured lines represent different gametocyte group sizes ($q$): 2 (grey), 5 (blue), 10 (red), 20 (green) and ∞ (yellow). Each plot depicts different parameter combinations of male gametocyte ($d_M$ = 0.1; 0.5; 0.9) and female mortality rate ($d_F$ = 0.1; 0.5; 0.9), with $d_M$ increasing left to right and $d_F$ increasing bottom to top. (B) Cartoon summarizing the effects observed in Figures 2.5A and 2.S1-3. The set of possible values for $z^*$ is strongly influenced by $q$. The number of gametes of each sex reaching the mating pool (which depends on the mortality parameters and on $\chi$) influences $z^*$ within the constraints determined by $q$. Within each plot, the effects of $\delta_M$ and $q$ on $z^*$ can be clearly observed: the magnitude of sex ratio change increases with $q$ and $z^*$ increases to compensate for higher $\delta_M$. The effects of $d_M$ and $d_F$ can be observed by comparing the points where the lines cross the y

axes (i.e. $\delta_M$=0) across the plots: $z^*$ increases along rows with increasing $d_M$ and decreases up the columns with increasing $d_F$. The effect of $\chi$ on $z^*$ can be observed by comparing plots that are in the same position in different figures: sex ratio becomes more female biased as $\chi$ increases.

## Discussion

Evolutionary theory developed to explain the sex allocation strategies of metazoan taxa has enjoyed huge success. Recently, there has been growing interest in whether this theory could be applied to protozoans, particularly malaria parasites (West et al. 2001). The sex ratios of malaria parasites are normally female biased, but extensive variation occurs during the course of infections (Reece et al. 2009). Evolutionary theory offers an explanation for this variation and predicts that in-host conditions will influence parasite sex allocation strategies if host-derived immune factors disproportionately reduce the fertility of males relative to females (Hamilton 1967; Read et al. 1992; Read et al. 1995; West et al. 2000; West et al. 2001; Nee et al. 2002; West et al. 2002; Gardner et al. 2003). Here, we tested this assumption by quantifying the effects of two well-known innate TBI factors (RNS and TNF-α) on sexual development and fertility of malaria parasites (West et al. 2002; Gardner et al. 2003). We show that: (1) RNS and TNF-α reduce the densities of exflagellating males and ookinetes in a dose-dependent manner; (2) TNF-α can reduce ookinete densities, but only in the absence of RNS (Figure 2.3); (3) RNS impairs male but not female gametogenesis (Figure 2.2 and 2.3), and reduces the fertility of both males and females independently of whether parasites are exposed as gametocytes or during

gametogenesis (Figure 2.4). We then explored the consequences of our results for parasite sex ratio evolution, by incorporating them into Fertility Insurance theory (Figures 2.1 and 2.5) (West et al. 2002; Gardner et al. 2003). Specifically, our model demonstrates that the ES sex ratio will be biased towards the sex that has a lower number of surviving gametes reaching the mating pool and that the extent of this bias increases as the number of gametocytes in the mating group ($q$) increases. We also show that factors causing gametes to become dysfunctional (resulting in inviable zygotes) do not affect the ES sex ratio. Below, we discuss the results of our experiments, explain the evolutionary predictions of our model and its implications for the development of transmission-blocking interventions.

*RNS, TNF-α and the sexual development of malaria parasites*

In our experiments, RNS reduced male but not female gametogenesis while impairing the fertility of both sexes. How can these results be explained? In parasitic infections, high levels of RNS may cause: oxidative damage of DNA (leading to mutations and strand brakes); inhibition of DNA repair and synthesis; inhibition of protein synthesis; inhibition of mitochondrial activity; down- or up-regulation of cytokine (e.g. TNF-α) levels (Clark and Rockett 1996; Bogdan 2001). As described in the introduction, male and female gametocytes are prepared for gametogenesis and zygote development respectively (Janse and Waters 2004). If RNS can impair DNA synthesis and/or microtubule assembly, males would not be able to produce gametes. In contrast, female gametogenesis does not involve these activities and females 'simply' need to leave their RBCs, for which they use the contents of pre-synthesized secretory organelles called osmiophilic bodies (de Koning-Ward et al. 2008). Therefore, whilst female gametogenesis and mating *per se* is unlikely to be influenced by RNS, the

development of fertilized females into zygotes and ookinetes is likely to be affected. For example, damage to stored mRNA and inhibition of protein synthesis or mitochondrial activity (e.g. cytochrome oxidases) would impair meiosis (at ~3h after fertilization) and zygote development, but not impair fertilization (Sinden 1983b; Clark and Rockett 1996; Bogdan 2001). These effects could explain the observed results, because instead of reducing the ability of females to differentiate into gametes, the effects of RNS would be expressed after fertilization (which we term dysfunction) and lead to female-dependent zygote death, resulting in fewer ookinetes. Here we did not identify the causal RNS and their relative contributions. However, this will be important if transmission-blocking interventions cause or mimic the activities of RNS.

Our experiments show that TNF-α consistently reduces ookinete production and whilst we observed a reduction in exflagellation in some experiments, this effect was inconsistent. Why does TNF-α reduce ookinete production? As TNF-α functions are mainly modulatory and need time to develop, it is possible that gametogenesis and mating occur before the effects of TNF-α manifest. Ookinete development takes about 18-20 hours from fertilization and during this time TNF-α could exert its effects, which could also involve the activation of apoptotic-like death (Janse et al. 1985; Hurd and Carter 2004). Recent experiments provide support for our interpretations, as the deletion of genes coding for proteins essential for the storage and stabilization of translationally repressed mRNAs, in female gametocytes/gametes, do not reduce fertilization success, but substantially reduce the differentiation of zygotes into ookinetes (Mair et al. 2006; Mair et al. 2010). Interestingly, deletion of different genes can affect zygotes throughout development, suggesting that damage to

stored mRNA could abort zygote development at multiple stages (e.g. before or after meiosis) (Mair et al. 2010).

*Evolution of parasite sex allocation strategies: theoretical predictions*

The results of our experiments show that TBI factors can affect the sexual development and fertility of male and female parasites and that the stage at which this occurs is sex-specific. As illustrated in Figure 2.1, we incorporated the observed and potential effects of transmission-blocking factors on males and females, at all stages of development, into Fertility Insurance theory and generated new predictions for the evolution of parasite sex allocation strategies. Our model predicts that the ES gametocyte sex ratio will be insensitive to variation in gametocyte or gamete dysfunction and zygote mortality. This means that treatments that impact upon the viability of zygotes are not expected to have an evolutionary impact upon parasite sex ratios. In contrast, we predict that the best (ES) sex ratio strategy will vary depending on an interaction between gametocyte group size ($q$), number of gametes formed per male gametocyte ($0 \leq \chi \leq 8$) and gamete and/or gametocyte mortality. Although, our model makes no assumptions about whether parasites achieve an ES sex ratio through the evolution of facultative or fixed sex allocation strategies, facultative sex allocation is predicted for reasons already outlined in the introduction.

In the context of clonal infections, the ES sex ratio maximises the expected number of viable zygotes, i.e. maximises the expected number of gametes of the minority sex present in the mating pool (this excludes dead gametocytes/gametes, but includes dysfunctional gametocytes/gametes). For an infinite gametocyte group size (i.e.

$q \to \infty$), that behaves deterministically, the ES sex ratio is one that leads to the same number of male and female gametes being present in the mating pool. This is the sex ratio $z^*$ that satisfies $cz^* = 1-z^*$, i.e. $z^* = 1/(c+1)$, where $c$ is the number of male gametes able to mate, produced per male gametocyte, equivalent to $\chi$ in the model presented here (Read et al. 1992; Gardner et al. 2003). Thus, the ES sex ratio is female biased if $c>1$, and male biased if $c<1$ (Figures 2.5 and 2.S1-3). However, for finite mating groups ($q<\infty$) – that behave stochastically – the expectation of mating success must be calculated over the whole distribution of possible outcomes. This will tend to reduce the extent to which the sex ratio is biased towards the sex favoured in the deterministic case (West et al. 2002; Gardner et al. 2003). For example, in the extreme of a gametocyte group size of two ($q = 2$; the lowest mating group size for which mating success is possible), the ES sex ratio is always $z^* = 0.5$ (regardless of other parameter values), to maximise the probability of both sexes being present (Figures 2.5 and 2.S1-3). Additionally, we reveal that, in a small portion of parameter space – corresponding to very small gametocyte group sizes, low female mortality, and high male gametocyte mortality and fecundity ($\chi$) – fertility insurance can even lead to a sex ratio bias in the opposite direction (i.e. producing a female biased sex ratio, despite the risk of the absence of males in the mating pool; Figures 2.S2 and 2.S3). This non-intuitive result is due to the way stochastic variation in the number of gametocytes of each sex alters the variance as well as the expected number of gametes of each sex that reach the mating pool. Although the conditions under which this occurs are restrictive, they may be met in natural infections, as many individuals carry gametocytes at extremely low densities (Drakeley et al. 2006). In the context of our experiments and assuming parasites can facultatively adjust sex ratios, our model

predicts that if $q$ is high enough to allow for sex ratio adjustment, then RNS should induce parasites to increase the production of male gametocytes.

Our data suggest that RNS reduced female fertility by rendering gametocyte/gametes dysfunctional, so that their fertilisation results in the production of unviable zygotes. The reduction in ookinete production by TNF-α could also be due to male or female dysfunction or, more likely, through increasing zygote mortality. Therefore, we examined the influence of gametocyte and gamete dysfunction and zygote mortality on the evolution of parasite sex allocation strategies. We found that the ES gametocyte sex ratio is independent of these factors (i.e. the occurrence of gametocyte/gamete dysfunction and zygote mortality does not change the relative fitness of different sex ratio strategies). Put simply, this suggests that zygote mortality or gametocyte/gamete dysfunction will not impose selection on parasite sex allocation strategies as parasites cannot compensate for the loss of reproductive success through sex ratio adjustment. More broadly, other immune factors, such as antibodies or complement, could also impair the sexual reproduction of malaria parasites and the effects of such factors should be easily interpreted in light of our theoretical models.

To bring our mathematical modelling in line with our experiments we have focused on the importance of mortality and dysfunction throughout the sexual development of malaria parasites. However two additional factors have an important impact in sex allocation strategies of malaria parasites: (1) the inbreeding rate and (2) the rate at which asexually replicating parasites commit to gametocyte production (conversion rate). The effect of inbreeding on the ES sex ratio is well understood, with theory

(Local Mate Competition) enjoying strong empirical support (Read et al. 1992; Read et al. 1995; West et al. 2000; West et al. 2001; Nee et al. 2002; Reece et al. 2008). For clonal mating groups, the ES sex ratio strategy is the one that maximises the overall mating success of the infection as the parasites behave as a single, unified decision maker (West et al. 2001; Reece et al. 2009). In contrast, in mixed infections, conflicts between clones occur, such that the ES sex ratio is the one that maximises each individual clone's inclusive fitness and not the overall mating success of the infection (West et al. 2001; Reece et al. 2009). But for the work we present here, extending our model to allow for a finite number of independent clones per host would not change the qualitative results we present. Fertility Insurance theory predicts that if a low conversion rate results in a small number of gametocytes being taken up by the vector (i.e. small $q$), parasites should produce a less female biased sex ratio than expected by the inbreeding rate alone. This is due to the stochastic risk of too few males being present in the blood meal to fertilize the females when sex ratios are female biased (Gardner et al. 2003). One intuitive solution for this would be to produce more gametocytes. However, given that gametocyte production comes at a cost to asexual replication, parasites face a trade-off between investment in in-host survival and reproduction (i.e. transmission). Increasing gametocyte conversion is a solution that will not always be available and might be impossible when parasites are 'stressed' (e.g. by in-host competition and low doses of anti-malarial drugs) (Mideo and Day 2008; Reece et al. 2010). Therefore, if transmission-blocking interventions also affect asexual stages and reduce in-host survival, parasites are likely to reduce conversion rates and produce fewer gametocytes.

*Implications for transmission blocking interventions*

Our model reveals that an intervention with a sex-specific effect on mating ability will elicit an evolutionary response. However, sex ratio adjustment cannot fully rescue zygote production, given that an increase in the number of male gametocytes comes at the cost of decreasing the number of female gametocytes. Nevertheless, in a scenario of widespread transmission-blocking vaccination or treatment with gametocidal drugs with a sex-specific effect, natural selection will "compare" the fitness of parasites that do, and do not, adjust their sex allocation strategies, leading to an increase in the frequencies of the former. Therefore, quantifying the impact of sex ratio adjustment on rescuing fertility and thus, fitness is now required. In contrast, our model also reveals that a transmission-blocking factor resulting in zygote mortality or gametocyte/gamete dysfunction will be 'evolution proof' with respect to parasite sex allocation strategies. Therefore, we suggest that current efforts to prevent fertilization by targeting proteins with sex-specific phenotypes, such as P230, P48/45 (involved in gamete attachment) or Pfg377 (female emergence from the RBC), will be less effective than vaccines targeting zygote development (e.g. P28) (Carter 2001; Saxena et al. 2007; de Koning-Ward et al. 2008). An alternative transmission-blocking approach could cause dysfunctional female gametes by targeting the expression of female-specific translationally repressed mRNAs (Mair et al. 2010). Furthermore, a transmission-blocking intervention combining targets for gamete dysfunction and zygote death would minimize possible redundancy effects, which have been observed in several knock-outs of malaria parasites (e.g. P48/45) (van Dijk et al. 2001).

## Conclusions

Given the drive to develop transmission-blocking interventions that disrupt sexual reproduction in malaria parasites, there is an urgent need to evaluate how their short- and long-term success will be influenced by parasite mating strategies. Here, we combined experiments with mathematical modelling to predict how transmission-blocking factors influence parasite sex allocation strategies. Our model predicts that transmission-blocking interventions causing gametocyte/gamete dysfunction and/or zygote mortality will be 'evolution-proof' from the perspective of imposing selection on parasite sex ratio strategies, i.e. parasites may still evolve other strategies or traits to cope with a transmission-blocking intervention, but these will have to be independent of sex allocation. Put simply, understanding the behavioural strategies that parasites have evolved to cope with naturally occurring transmission-blocking immune factors, will inform predictions for how they will respond to a transmission-blocking factor. More broadly, understanding how, when and why parasites respond to changes in their in-host environment will facilitate the development of interventions that induce parasites to make decisions that are suboptimal for their transmission success, but that are clinically or epidemiologically beneficial. For efficient progress, synergy between research directed at evolutionary and mechanistic explanations for parasite traits and strategies is required.

## Methods

*Hosts and parasites*

We maintained MF1 mice, aged 8-10 weeks (Harlan-Olac, UK; or in house supplier, University of Edinburgh), on *ad libitum* food (RM3(P), DBM Scotland Ltd, UK) and water (supplemented with 0.05% PABA to enhance parasite growth), with a 12 hour light cycle, at 21 ºC. We initiated infections by intra-peritoneal inoculation of $10^7$ parasitized RBCs in 100 µl carrier consisting of 50% Ringers (27 mM KCl, 27 mM $CaCl_2$, 0.15 M NaCl), 47.5% heat-inactivated foetal bovine serum and 2.5% heparin (5 units $ml^{-1}$). For experiments 1 and 2, we inoculated female mice, previously (day -3 or -4) treated with 60 mg/kg of phenylhydrazine (PHZ), with *P. berghei* line 820 (Ponzi et al. 2009). For experiment 3 we inoculated male mice (PHZ treatment: 125 mg/Kg, day -2) with one of two *P. berghei* KO lines: Pbs48/45ko or Pbs47ko (van Dijk et al. 2001; Khan et al. 2005; van Dijk et al. 2010). We treated mice with PHZ because the resulting release of young RBCs increases gametocyte production in *P. berghei*, which maximises the number of gametocytes that can be harvested for *in vitro* mating experiments (Gautret et al. 1997). For each experiment, parasites were collected from mice on day 3 or 4 post-infection, and each infection contributed parasites to all treatments to control for any potentially confounding influences of differences between infections.

*Animal Ethics Statement*

All the protocols involving mice passed an ethical review process and were approved by the U.K. Home Office (Project License 60/3481). Work was carried according to the Animals (Scientific Procedures) Act, 1986.

*Culture conditions*

In order to manipulate the levels of RNS and TNF-α we used the following chemicals: recombinant mouse TNF-α (Sigma, UK), L-ana (Sigma, UK) and SIN-1 (Sigma, UK). We dissolved all chemicals in phospate buffered saline and exposed parasites to treatments in 1 ml cultures with 15 or 20 μl parasitized blood. L-ana is a specific inhibitor of the activity of the enzyme inducible nitric oxide synthase which becomes active in response to infection. SIN-1 donates NO and/or superoxide, in solution, at different rates depending on the specific conditions in which SIN-1 is incubated (Feelisch et al. 1989; Noack and Feelisch 1989; Singh et al. 1999). However, given that superoxide and NO react with each other at an extremely fast rate to produce peroxynitrite ($ONOO^-$), SIN-1 is likely to act as a donor of either NO or peroxynitrite, depending on the rates at which SIN-1 generates NO and superoxide (Singh et al. 1999). The oxygen concentration of the solution is one of the major determinants of whether SIN-1 behaves as a NO or peroxynitrite donor, donating mostly NO in anaerobic conditions and peroxynitrite in aerobic conditions (Singh et al. 1999). In our cultures, oxygen concentrations were in-between fully anaerobic and aerobic conditions, as parasites were incubated in closed 1.5ml tubes. Biological agents, such as human plasma or heme proteins, which are similar to components of our cultures (e.g. mouse plasma, haemoglobin) increase the capacity of SIN-1 to

donate NO (Singh et al. 1999). Furthermore, as peroxynitrite can react to produce several RNS (e.g. nitrite, nitrate, S nitrosothiols or nitrosyl-metal complexes) and as we did not measure the specific contributions of each of these factors, we use the term RNS to refer to the factors present in cultures exposed to SIN-1 (Bogdan 2001; Hurd and Carter 2004; Novo and Parola 2008). We did not measure RNS and TNF-α levels in our cultures for three reasons. First, our focus is on testing the effects of RNS and TNF-α on the sexual development of parasites. As our experiments were designed so that each host contributed blood and parasites to all treatment groups in a given experiment, this controls for any variation between infections and ensures that our results are due to the RNS and TNF-α manipulations each culture was subjected to. Second, TNF-α levels were directly manipulated with recombinant mouse TNF-α. Third, we are not aware of any method that would allow us to measure total levels of the different RNS in small volume cultures.

*Experiment 1*

We set up cultures with vector mimicking media for the following SIN-1 concentrations: 0, 0.00001, 0.0001, 0.001, 0.01, 0.1 and 1 mg/ml (Dea-Ayuela et al. 2009), with 6 mice contributing parasites to each treatment. We tested the following concentrations of recombinant mouse TNF-α: 0, 0.005, 0.01, 0.5 and 1 μg/ml with 4 mice contributing parasites to each treatment. We recorded the densities of exflagellating males after 15-20 minutes and ookinetes after 18-20 hours using a haemocytometer.

*Experiment 2*

We used the following RNS and TNF-α levels: 1 mg/ml SIN-1 (RNS +), 1 mg/ml of L-ana (RNS -), and presence (TNF-α +) or absence (TNF-α -) of 1 μg/ml recombinant mouse TNF-α. Parasites from each of 20 mice were exposed to all four combinations of treatments. We used the following criteria to classify developmental stages of gametogenesis after 15 minutes incubation in vector mimicking media: (1) Mature gametocytes: still inside their RBC; females have blue-purple cytoplasm, small, well defined purple nucleus surrounded by a small nucleolus; males have pink-yellow cytoplasm and large disperse pale-pink nucleus. (2) Emerged females: female gamete condensed into a more circular shape, without a vacuole, cytoplasm staining a more intense blue and a less obvious nucleolus than in a female gametocyte. (3) Emerged male: male gamete with a large circular nucleus in the centre of the cell surrounded by a ring of cytoplasm. (4) Exflagellating male: emerged male gamete progressed to forming up to 8 flagella that protrude from the cell and stain red-purple (Kawamoto et al. 1991; Kawamoto et al. 1992; Reece et al. 2003). We also recorded the densities of exflagellating males and ookinetes as described for experiment 1.

*Experiment 3*

We infected 38 mice with Pbs47ko (n=19) or Pbs48/45ko (n=19). We set up mating cultures following Reece et al. (Reece et al. 2008), by pairing infections according to proximity of their sex ratios, calculated from the densities of Pbs48/45ko female gametocytes in giemsa stained smears (using criteria described for Experiment 2) and Pbs47ko exflagellating males (as for Experiment 1). To avoid pseudo-replication, each infection was only used in 1 pair. For each pair of mice, we made 8 sets of 1 ml

cultures, either with (RNS +) or without (RNS -) $1x10^{-5}$ mg/ml SIN-1, mimicking host (60 min. incubation) or vector conditions (15 min. incubation), to which we added 15 μl of parasites from one of the infections in each pair. These single-sex cultures provided 'exposed' parasites for fertility testing, and corresponded to the following factorial design: 2 conditions (host/vector) x 2 SIN-1 exposures (RNS +/-) x 2 sexes (male/female). After incubation we replaced media in all cultures with 1 ml vector mimicking media (without any SIN-1 manipulation). While 'exposed' parasites were incubating, we collected 60 μl of blood from each infection's pair and added these 'unexposed' parasites to 4 ml cultures in vector mimicking media (without SIN-1). Each 1 ml culture of the 'exposed' parasites was then added to a 4 ml culture containing its 'unexposed' pair and incubated to produce ookinetes (as for Experiment 1). This allowed us to ensure that the mating success of the 'exposed' sex would not be limited by the availability of 'unexposed' gametocytes from the opposite sex. All the cultures were timed so that 'exposed' parasites were added to the cultures containing their 'unexposed' mates at the same developmental stage. For example, a final 5 ml culture could contain 15 μl of blood from a RNS exposed Pbs48/45ko infection (in which females are the 'exposed' sex) and 60 μl of blood from a Pbs47ko infection (in which ~4 times more males are provided as 'unexposed' mates). We also set up cultures in vector mimicking media to verify that 'unexposed' parasites from each line are unable to produce ookinetes on their own. We recorded the densities of ookinetes as described for experiment 1.

*Statistical analysis*

We used linear mixed effects models (R version 2.7.0; The R Foundation for Statistical Computing; www.R-project.org) because, by treating each infection (or pair of infections in Experiment 3) as a 'random' effect, we can account for problems associated with pseudoreplication arising from repeated measurements of each infection. In order to meet the assumptions made by parametric tests we arcsine square root transformed response variables where necessary. We minimised models following stepwise deletion of the least significant term and using log-likelihood ratio ($\chi^2$) tests to evaluate the change in model deviance until only significant terms remained, and we present F-ratios for fixed effects remaining in minimal models. We then re-ran minimal models using restricted maximum likelihood to estimate the effect sizes reported in the text. Unless otherwise indicated, data and estimated effect sizes are presented as proportions of the focal parasite stage produced in a given treatment, relative to that produced across all treatments for each infection.

*Theoretical model*

We assume an infinite host population, divided into infected and uninfected individuals, with infected hosts containing a single infection producing haploid gametocytes that circulate in the blood. We assume that $q$ gametocytes are transferred from host to vector during blood feeding, and that these gametocytes form a single mating group. The expected proportion of males in the mating group is $z$, i.e. the sex allocation strategy of the parasite strain that contributed the gametocytes. Hence, the actual number of males is a random variable $\alpha \sim Bi(q,z)$ (i.e. binomially distributed with $q$ trials and probability of success $z$). Consequently, the number of female

gametocytes is $q$-$\alpha$. Male and female gametocytes are killed with probability $d_M$ and $d_F$ respectively, leaving $\Gamma \sim Bi(\alpha,1\text{-}d_M)$ surviving males and $\phi \sim Bi(q\text{-}\alpha,1\text{-}d_F)$ surviving females. We assume every surviving male produces $\chi$ gametes, and every surviving female produces a single gamete. We consider that male gametes are killed with probability $\delta_M$, and hence $\gamma \sim Bi(\chi\Gamma,1\text{-}\delta_M)$ male gametes enter the mating pool. We assume that all $\phi$ female gametes enter the mating pool (death of female gametes is formally equivalent to that of female gametocytes, and hence is implicitly included in the parameter $d_F$). Therefore, the number of zygotes is equal to the number of gametes of the limiting sex, i.e. $\zeta = min(\gamma,\phi)$. Finally, we assume that only a proportion $p$ of zygotes are viable, due to either: (a) factors that kill each zygote with probability $\Omega_Z$; (b) factors acting on gametocytes resulting in the production of dysfunctional gametes at rate $\Omega_M$ for males and $\Omega_F$ for females; or (c) factors acting on gametes and causing them to become dysfunctional at rate $\varpi_M$ for males and $\varpi_F$ for females, i.e. $p = (1\text{-}\Omega_Z)(1\text{-}\Omega_M)(1\text{-}\Omega_F)(1\text{-}\varpi_M)(1\text{-}\varpi_F)$. In this context, we use the term 'dysfunctional' to refer to a gamete that achieves fertilisation but carries sufficient damage to render the resulting zygote inviable (i.e. unable to develop as an ookinete). Inviable zygotes will result when one or both of the parental gametes are dysfunctional. Hence, the number of viable zygotes produced by the mating group is $W = \zeta p$, and this is our measure of fitness (Read et al. 1992; West et al. 2002; Gardner et al. 2003).

**Supporting material**

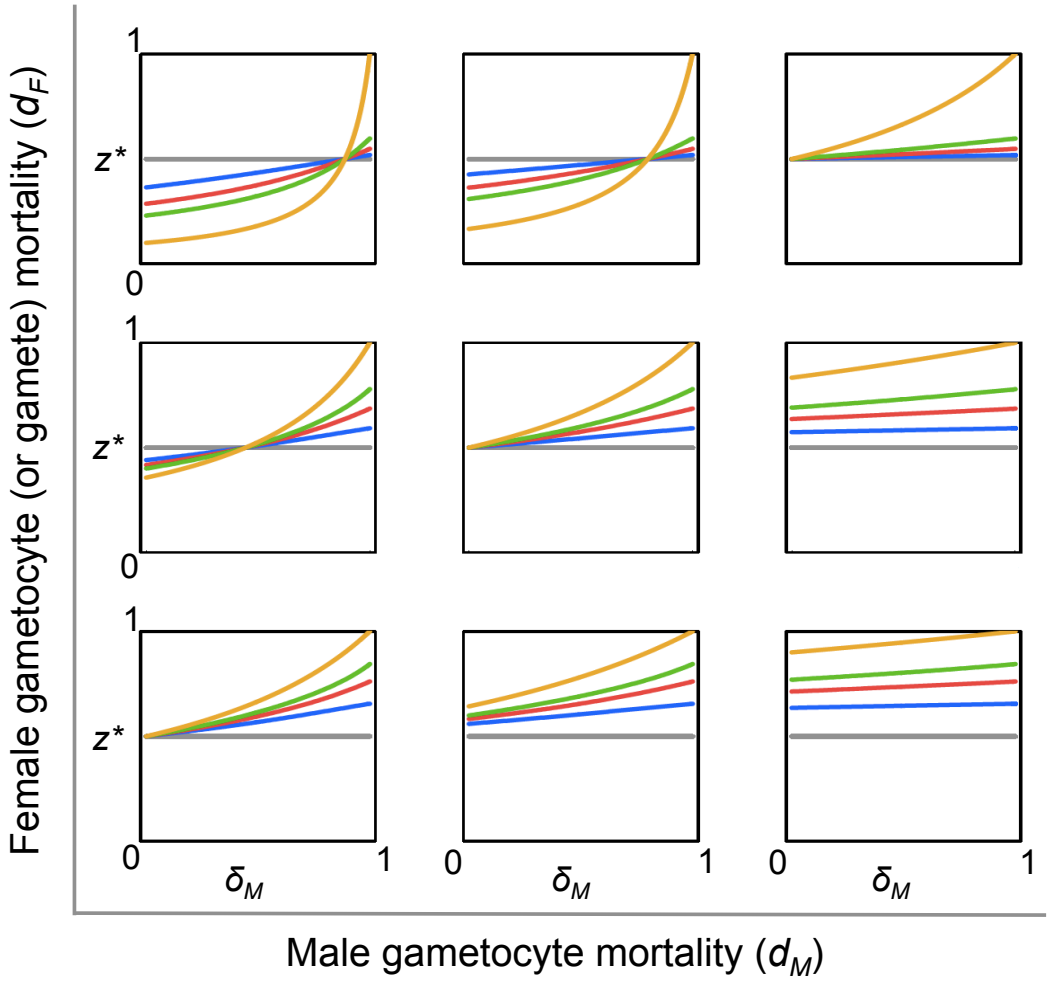



Figure 2.S1. Evolutionarily stable sex allocation strategies when sex- and stage-specific mortality rates vary ($\chi = 1$). Effect of male and female gametocyte mortality and male gamete mortality on the ES gametocyte sex ratio ($z^*$), for a clonal population, when the number of gametes per male gametocyte ($\chi$) is 1. On each plot, $z^*$ varies with male gamete mortality rate ($\delta_M$). The coloured lines represent different gametocyte group sizes (q): 2 (grey), 5 (blue), 10 (red), 20 (green) and ∞ (yellow). Every plot depicts different parameter combinations of male gametocyte ($d_M = 0.1; 0.5; 0.9$) and female mortality rate ($d_F = 0.1; 0.5; 0.9$), with $d_M$ increasing left to right and $d_F$ increasing bottom to top.

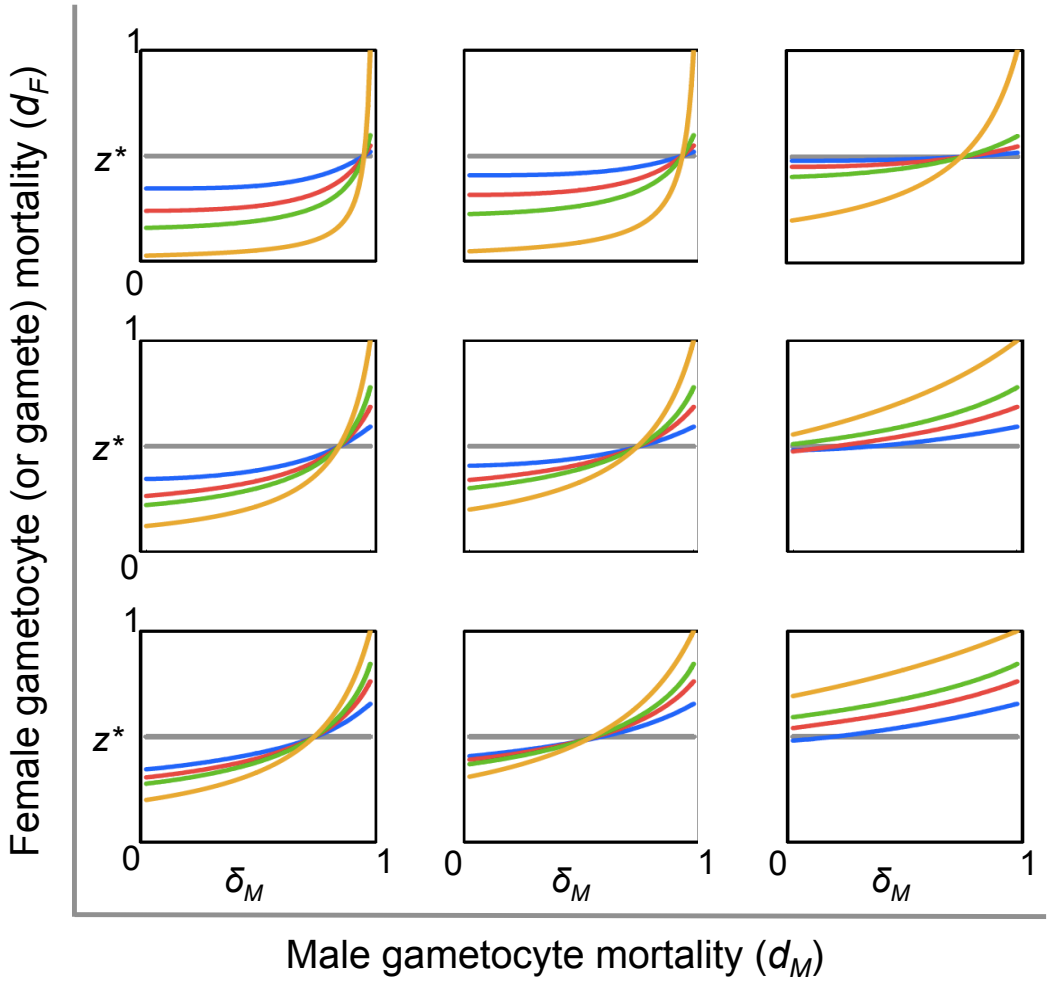



Figure 2.S2. Evolutionarily stable sex allocation strategies when sex- and stage-specific mortality rates vary ($\chi$=4). Effect of male and female gametocyte mortality and male gamete mortality on the ES gametocyte sex ratio ($z^*$), for a clonal population, when the number of gametes per male gametocyte ($\chi$) is 4. On each plot, $z^*$ varies with male gamete mortality rate ($\delta M$). The coloured lines represent different gametocyte group sizes ($q$): 2 (grey), 5 (blue), 10 (red), 20 (green) and ∞ (yellow). Every plot depicts different parameter combinations of male gametocyte ($d_M$ = 0.1; 0.5; 0.9) and female mortality rate ($d_F$ = 0.1; 0.5; 0.9), with $d_M$ increasing left to right and $d_F$ increasing bottom to top.

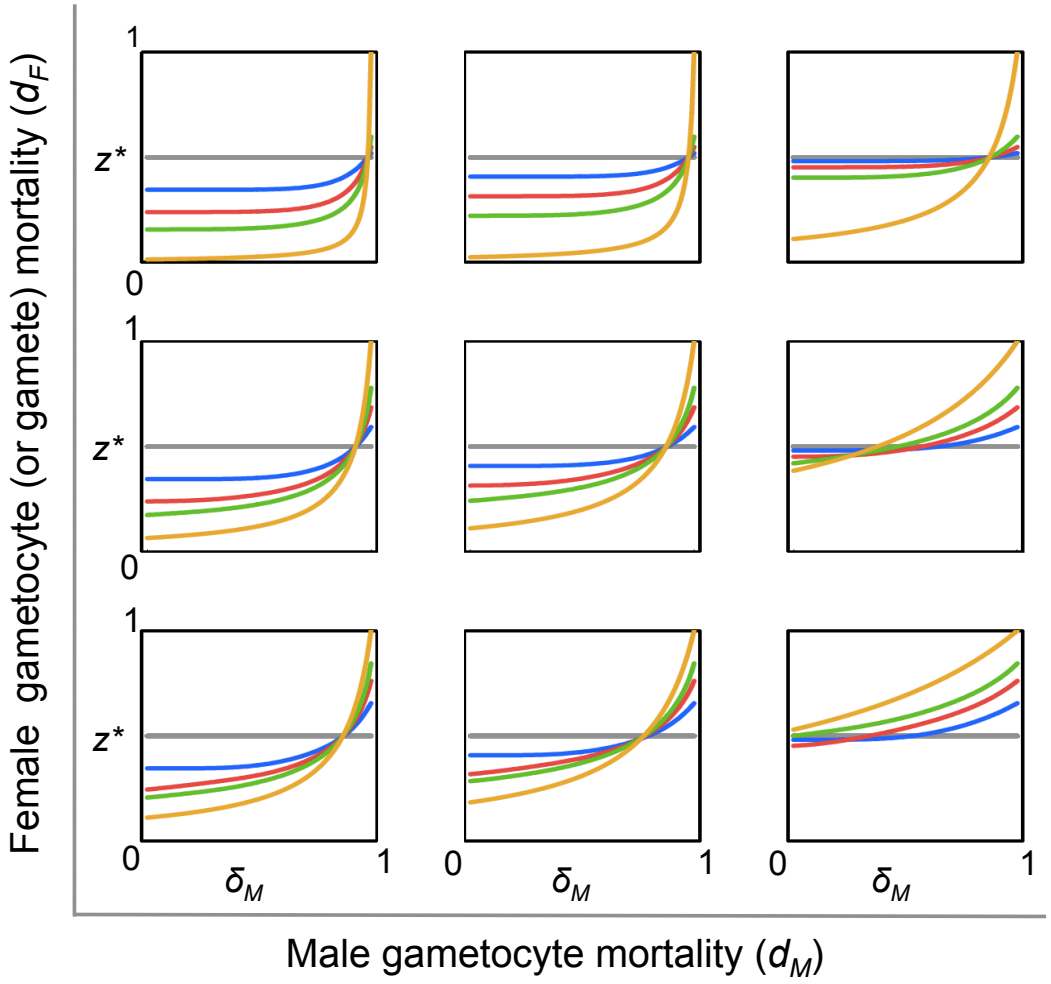



Figure 2.S3. Evolutionarily stable sex allocation strategies when sex- and stage-specific mortality rates vary ($\chi$=8). Effect of male and female gametocyte mortality and male gamete mortality on the ES gametocyte sex ratio ($z^*$), for a clonal population, when the number of gametes per male gametocyte ($\chi$) is 8. On each plot, $z^*$ varies with male gamete mortality rate ($\delta M$). The coloured lines represent different gametocyte group sizes ($q$): 2 (grey), 5 (blue), 10 (red), 20 (green) and ∞ (yellow). Every plot depicts different parameter combinations of male gametocyte ($d_M$ = 0.1; 0.5; 0.9) and female mortality rate ($d_F$ = 0.1; 0.5; 0.9), with $d_M$ increasing left to right and $d_F$ increasing bottom to top.

# 3. Haplodiploidy and the evolution of eusociality: worker revolution

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## Abstract

Hamilton suggested that haplodiploidy predisposes species to the evolution of eusociality. This is because, under female biased sex allocation, haplodiploidy increases the genetic similarity of workers to the brood that they could help to raise. Trivers and Hare built upon this idea by proposing two biological scenarios whereby haplodiploidy could promote eusociality: (a) workers biasing the sex allocation of the queen's brood towards females; and (b) workers replacing the queen's sons with their own sons. However, biased sex allocation and worker reproduction can have multiple consequences for both the genetic structure of colonies and the reproductive values of males and females. Here we determine the net effect of all these consequences, for the two scenarios whereby the workers seize control of reproduction. We find that, in both cases, haplodiploidy tends to inhibit rather than promote helping. This is because female biased sex allocation increases the relative reproductive value of males, such that the value of producing sons is generally greater than the value of helping to rear a female biased mixture of sisters, brothers and/or nephews.

## Introduction

The eusocial taxa are dominated by the social Hymenoptera: the ants, bees and wasps (Crozier 2008). Hamilton (Hamilton 1964a, b, 1972) suggested that this owes to their haplodiploid genetics, whereby unfertilized (haploid) eggs develop into males and fertilized (diploid) eggs develop into females. All workers are female, and haplodiploidy leads to a worker being more related to her full sisters (life-for-life relatedness $R = ¾$, assuming an even sex ratio) than to her own daughters ($R = ½$). This could raise the relatedness to siblings and hence make it easier for kin selection to favour altruistic workers, who help with the rearing of their mothers offspring, rather than reproducing for themselves. However, things are not so simple, because haplodiploidy also makes a worker less related to her brothers ($R = ¼$) than to her sons ($R = ½$) so that, with an unbiased sex allocation, the average relatedness to siblings would be exactly equal to that of offspring ($R = ½$; Hamilton 1972; Trivers and Hare 1976).

Subsequent versions of Hamilton's (Hamilton 1964a, b, 1972) "haplodiploidy hypothesis" have relied upon biased sex allocation. Trivers and Hare (1976) showed that, when the population sex ratio is unbiased, the higher relatedness between sisters ($R = ¾$) than between sisters and brothers ($R = ¼$) means that workers will be favoured to bring about a female biased sex allocation. As worker control of sex allocation spreads through a population, it increases the average genetic similarity between workers and the siblings that they help to raise. Another factor suggested by Trivers and Hare (1976) was that workers could replace the queens sons with their own sons. This could further raise the relatedness between workers and the offspring

that they help to raise, because they are more related to the sons of their sisters (nephews, $R = 3/8$) than to the sons of their mother (brothers, $R = ¼$). Since Trivers and Hare's (1976) landmark paper, other researchers have suggested a range of scenarios that could lead to sex ratio variation between broods – termed "split sex ratios" – that could make the haplodiploidy hypothesis work (reviewed by(Grafen 1986; Boomsma and Grafen 1991; Gardner et al. 2012). Overall, this has led to widespread acceptance that – from a theoretical perspective – haplodiploidy favours the evolution of eusociality (Seger 1991; Krebs and Davies 1993; Bourke and Franks 1995; Queller and Strassmann 1998; Alcock 2005).

However, we suggest that it is necessary to re-assess the theoretical basis of the haplodiploidy hypothesis, for two reasons. First, biased sex allocation can have multiple consequences for genetic similarity of nestmates and the reproductive value of different classes of individuals. For example, if the population sex allocation is biased towards females, this increases the genetic similarity of workers to their siblings, but also increases the reproductive value of males. This in turn would favour those workers who breed independently to bias their sex allocation towards sons, which would increase the inclusive fitness payoff of personal reproduction and act to disfavour helping. Consequently, heuristic arguments can be misleading (Taylor 1992; Frank 1998), making it necessary to formally work through all the inclusive fitness consequences of biased sex allocation and worker reproduction. Second, the last 30 years have seen a leap forward in our empirical knowledge of both sex allocation in the hymenoptera and the route by which eusociality evolved (reviewed by (Boomsma 2007, 2009; West 2009). This means that we now have a much better

understanding of which scenarios need to be considered and which assumptions can realistically be made.

Our aim here is to determine, from a theoretical perspective, the overall influence of haplodiploidy on the evolution of eusociality. We focus upon the scenarios envisioned by Trivers and Hare (1976), in which sex allocation is biased owing to worker control and/or worker reproduction. We first examine a revolution in control over sex allocation, whereby worker control spreads through a population, leading to a female biased sex allocation. We assess the robustness of this mechanism to changes in assumptions regarding the underlying genetics, strength of selection, extent of worker reproduction and rate of sex ratio evolution. We then examine the consequences of workers replacing some of the queen's offspring with their own sons. We consider that workers might replace brothers only, or that they might replace both brothers and sisters. In contrast to most theory on this topic, our aim is not to see if the haplodiploidy hypothesis can be made to work: it can (Grafen 1986). Instead, our aim is to assess the likely impact of haplodiploidy in biologically relevant contexts.

## General methods

We determine the inclusive fitness consequences for a potential worker who chooses to help rear *b* additional juveniles in her mother's nest at a personal cost of *c* of her own offspring. Hamilton (1963, 1964a, b, 1970, 1972) showed that the value of a social partner to a focal actor is measured by how well the former transmits copies of the latter's genes to future generations. This is the product of two quantities: the expected asymptotic genetic contribution made by the recipient to future generations,

termed reproductive value ($v$; Fisher 1930); and the concentration of the actor's genes in the recipient, termed consanguinity ($p$; Bulmer 1994). We assume that the sex ratio (proportion male) among the reproductive juveniles in the mother's nest is $z_C$, and that the workers produce a sex ratio of $z_O$ if they breed independently. The worker has an average consanguinity: $p_M$ to the juvenile males in her mother's nest; $p_F$ to the juvenile females in her mother's nest; $p_S$ to her sons; and $p_D$ to her daughters. We calculate these coefficients of consanguinity in Appendix 3A. The reproductive value of reproductive juvenile males and females are $v_m$ and $v_f$, respectively, which we calculate in Appendix B. Table 3.1 presents a summary of the model notation used in this work.

Helping improves the worker's inclusive fitness when $b(z_C p_M v_m+(1 - z_C)p_F v_f) > c(z_O p_S v_m+(1-z_O)p_D v_f)$. We may rewrite this condition as $c/b < \alpha$, where $\alpha$ defines the potential for helping, and is given by:

$$\alpha = \frac{z_C p_M v_m + (1 - z_C) p_F v_f}{z_O p_S v_m + (1 - z_O) p_D v_f}. \qquad (3.1)$$

The potential for helping ($\alpha$) represents the threshold $c/b$ at which the potential worker is indifferent between raising juveniles in her mother's nest versus her own offspring (Charnov 1978; Grafen 1986; Gardner et al. 2012). Under diploidy, strict monogamy leads an individual to be equally related to her siblings and her own offspring, such that helping is favoured when $c/b < 1$. Hence, haplodiploidy can be said to promote the evolution of helping, relative to diploidy, when $\alpha > 1$.

| Symbol | Definition |
|---|---|
| $\bar{z}$ | Population sex ratio |
| $z_C$ | Colony offspring sex ratio |
| $z_O$ | Sex ratio strategy of a focal worker breeding independently |
| $z_Q$ | Queen-controlled colony sex ratio strategy for queen-derived offspring |
| $z_W$ | Worker-controlled colony sex ratio strategy for queen-derived offspring |
| $z_A$ | Sex ratio strategy of workers for worker-derived offspring |
| $\mu$ | Proportion of worker reproduction in the population |
| $\upsilon$ | Proportion of colonies where queen-derived offspring is worker-controlled |
| $\phi$ | Paternal kinship index. The probability that any two sperm cells present in the queen spermatheca derive from the same male |
| $p_x$ | Consanguinity between a focal female and a reproductive individual x, present in the same colony. |
| $R_{x,y}$ | Life-for-life relatedness coefficient of individual x to individual y |
| $v_m$ | Value of a male, given by the class reproductive value $c_m$ over the number of males in the population $\bar{z}$ |
| $v_f$ | Value of a female, given by the class reproductive value $c_f$ over the number of females in the population 1- $\bar{z}$ |
| $c_m$ | Male class reproductive value |
| $c_f$ | Female class reproductive value |
| $K$ | Number of colonies in the population |
| $N$ | Population size |

Table 3.1: Summary of model notation.

We measure genetic similarity using Hamilton's (1970) regression coefficient of relatedness, which is simply the consanguinity of the actor and recipient divided by the consanguinity of the actor to herself ($r = p/p_{self}$; Bulmer 1994). Thus, full sisters

are related to each other by $r = ¾$, and the relatedness that a female places upon her brother is $r = ½$. This regression form provides the general definition of relatedness (Grafen 1985), that is at the heart of modern theoretical methodology (Taylor and Frank 1996; Frank 1998; Grafen 2006), and is the basis for how relatedness is measured using molecular markers such as a microsatellites (Queller and Goodnight 1989).

Before proceeding, we clarify how this measure of relatedness relates to other versions of relatedness that are often encountered in the social insect literature. Regression relatedness is sometimes combined with the ratio of the recipient and actor reproductive values ($R = r\,(v'/v)$), where $v'$ is the reproductive value of the recipient and $v$ is the reproductive value of the actor). This gives a "life-for-life" relatedness coefficient that expresses the value of the recipient from the perspective of the actor relative to the value the actor places upon herself (Hamilton 1972; Bulmer 1994). For example, when sex ratio is unbiased and workers do not reproduce, the reproductive value of a female is twice that of a male in a haplodiploid population, so the life-for-life relatedness of the actor to a female recipient is twice that of the actor to a male recipient, assuming that the regression relatedness to each recipient is the same (Crozier and Pamilo 1993). So, provided that the sex ratio is unbiased and workers do not reproduce, the life-for-life relatedness of full sisters is $R = r\,(v'/v) = (3/4)(1/1) = ¾$ and the life-for-life relatedness that a female places upon her brother is $R = r\,(v'/v) = (1/2)(1/2) = ¼$. If the sex ratio is biased, or if workers reproduce, then this may change the individual reproductive values of females and males, and hence cause the life-for-life relatedness coefficients to vary from ¾ and ¼ respectively.

Relatedness has also been defined as the probability that a gene chosen at random from the actor is also present in the recipient, as can be calculated with pedigree drawings (e.g. Trivers and Hare 1976; Charlesworth 1980; Grafen 1986, 1991; Krebs and Davies 1993). Although this probability definition is different from that given in the previous paragraph, it is also sometimes used interchangeably with or referred to as life-for-life relatedness. In haplodiploids, the ploidy of a female is twice that of a male so, assuming no inbreeding, the life-for-life relatedness of the actor to a female recipient is twice that of the actor to a male recipient, if the regression relatedness to each recipient is the same (the female contains more genes, and hence is more likely to carry the actor's gene). Thus, both definitions for life-for-life relatedness often arrive at the same answer ($R$ = ¾ for full sisters, and $R$ = ¼ for sister and brother), owing to a mathematical coincidence between the ratios of female versus male reproductive value and female versus male ploidy.

However, this agreement breaks down when there are biased sex ratios or there is worker reproduction (Grafen 1986; Bulmer 1994; Gardner et al. 2012). Whilst it is relatively easy to correct the probability approach for a biased sex ratio, using a scaling factor, it is more difficult to correct for worker reproduction. This is because biased sex ratios do not impact upon the reproductive value of each class, but only upon how reproductive value is shared between individuals within each class, whereas worker reproduction changes class reproductive values directly. Thus, whilst we could add a 'correction factor', this would change from case to case, depending upon the extent of worker reproduction and the sex ratio of their offspring, and would have no conceptual depth. Nonetheless, to facilitate comparison with Trivers and Hare

(Trivers and Hare 1976), we illustrate how our results can also be explained with their probabilistic form of life-for-life relatedness, when assuming no worker reproduction.

**Worker control of sex ratio**

*Female biased sex allocation*

We start by considering the simplest case, where all colonies produce the same sex ratio ($z_C = \bar{z}$) and worker reproduction is negligible. We assume a population composed of an infinite number of colonies, each founded by a mated queen. Queens could be singly or multiply mated, and we define $\phi$ as the probability that two maternal sisters are also paternal sisters. Unless stated otherwise, we assume full monogamy ($\phi = 1$), as eusociality has evolved only in monogamous species (Boomsma 2007; Hughes et al. 2008; Boomsma 2009). Upon founding the colony, the queen produces a cohort of females, defined as workers, who mate at random with non-related males. These workers may stay and help the queen, or leave the colony to breed independently. If workers breed independently, their offspring sex ratio is $z_O$, which can depart from the colony and population sex ratios ($z_C$ and $\bar{z}$).

Our aim here is to examine how selection for helping is influenced by colony sex ratio ($z_C$). We focus on the range from $z_C = ¼$ to $z_C = ½$ (75% to 50% female), because this represents the continuum between the optima from the points of view of the queen and her workers (Trivers and Hare 1976). In terms of the life-for-life measure of relatedness used by Trivers and Hare, the queen is equally related to sons and daughters ($R = ½$), and so she prefers equal investment in sons and daughters ($z_C^* = ½$), but the workers are three times more related to sisters ($R = ¾$) than brothers ($R = ¼$), and so would prefer to raise three times as many sisters as brothers ($z_C^* = ¼$).

Consequently, we are not making assumptions about the mechanisms of sex ratio conflict, but rather examining the consequences of whether it is won by queens, by workers, or resolves somewhere between these two extremes. There is considerable empirical evidence that workers can gain control of sex allocation, and adjust it to their optimum, away from that of the queen (Meunier et al. 2008).

In this scenario, a female biased sex ratio has three consequences for the evolution of helping behaviour. First, it increases the genetic similarity of workers to potential siblings (Fig. 3.1a). Second, it increases the average number of mates for each male, and so increases the reproductive value of males relative to females (Fig. 3.1b). Third, this change in reproductive value will favour workers who breed independently to bias their offspring sex ratio towards males ($z_O > ½$). If we replace the reproductive values and consanguinity coefficients by model parameters, the potential for helping is $\alpha = 2z_C(1-z_C)/(z_O+z_C-2z_Oz_C)$.

In Figure 3.1c we show how the potential for helping ($\alpha$) is influenced by the colony and population sex ratio ($z_C = \bar{z}$). Assuming that worker reproduction is rare, workers who breed independently will be favoured to produce offspring of the rarer sex, and so whenever the population sex ratio is biased towards females, the workers are favoured to produce only sons ($z_O^* = 1$). However, for explanatory purposes, we plot lines for when the offspring produced by independently reproducing workers are either all males ($z_O = 1$), 50% male ($z_O = ½$) or the same sex ratio as that in colonies with queens ($z_O = z_C$). Overall, we obtain:

**Result 1.** As the population sex ratio becomes more female biased ( $\bar{z} < ½$), haplodiploidy increasingly inhibits helping ($\alpha < 1$; Fig. 3.1c). This is because the female biased sex ratio raises the reproductive value of males ($v_m$; Fig. 3.1b) and decreases the reproductive value of females ($v_f$), which makes the value of producing sons greater than the value of helping to rear a female biased mixture of sisters and brothers.

We will explain this result in detail, as it is central to all subsequent sections of the paper. As we move from a population sex ratio of $z_C = ½$ to $z_C = ¼$ (50% female to 75% female), the average relatedness to siblings increases from $r = 5/8$ to $r = 11/16$ (Fig. 3.1a), and the reproductive value of a male increases from 0.5× to 1.5× that of a female (Fig. 3.1b). The overall influence of these two factors upon selection for helping will depend upon the sex ratio of the offspring that the potential worker would produce if she were to reproduce independently (Fig. 3.1c). If she produces the same sex ratio as the colonies with queens (i.e. equally female biased), then these two factors exactly cancel out, and siblings are worth the same as offspring ($\alpha = 1$), which was the result found by Craig (1979). For example, when the population sex ratio is $\bar{z} = 1/4$ (75% female), then the relative value of siblings (daughters + sons = $3/4 \times 3/8 \times 8/9 + 1/4 \times 1/4 \times 4/3 = 1/3$) is the same as the relative value of offspring ($3/4 \times 1/4 \times 8/9 + 1/4 \times 1/2 \times 4/3 = 1/3$). However, because the population sex ratio is female biased, the reproductive value of males is greater, and so the potential workers who reproduce independently are favoured to produce only sons. Consequently, as the population becomes more female biased, offspring become increasingly more valuable than siblings (Fig. 3.1c). In the extreme, if the population sex ratio is $\bar{z} = 1/4$ (75% female), then the relative value of sons ($1 \times ½ \times 4/3 = 2/3$) is twice that of

siblings (3/4 × 3/8 × 8/9 + 1/4 × 1/4 × 4/3 = 1/3). Consequently, as worker control of sex allocation spreads through a population, and it moves from the queen's optimum of 50% females to the workers' optimum of 75% females, the effect of the overall population level bias would be to disfavour helping behaviour.

Exactly the same conclusions are reached if we use the life-for-life approach of Trivers and Hare (1976). Consider the scenario where the population sex ratio is $\bar{z}$ = ¼ (75% female), and that a focal breeding worker produces the same the same sex ratio as her queen. In this case, reproductive success of a male is three times that of a female, and so we find that the relative value of siblings (3/4 × 3/4 × 1 + 1/4 × 1/4 × 3 = 3/4) is the same as the relative value of offspring (1/2 × 3/4 × 1 + 1/2 × 1/4 × 3 = 3/4). In contrast, if we assume that the focal worker produces only sons, then we find, as above, that sons (1 × 1/2 × 3 = 3/2) are worth twice as much as female biased mixture of sisters and brothers (3/4 × 3/4 × 1 + 1/4 × 1/4 × 3 = 3/4).

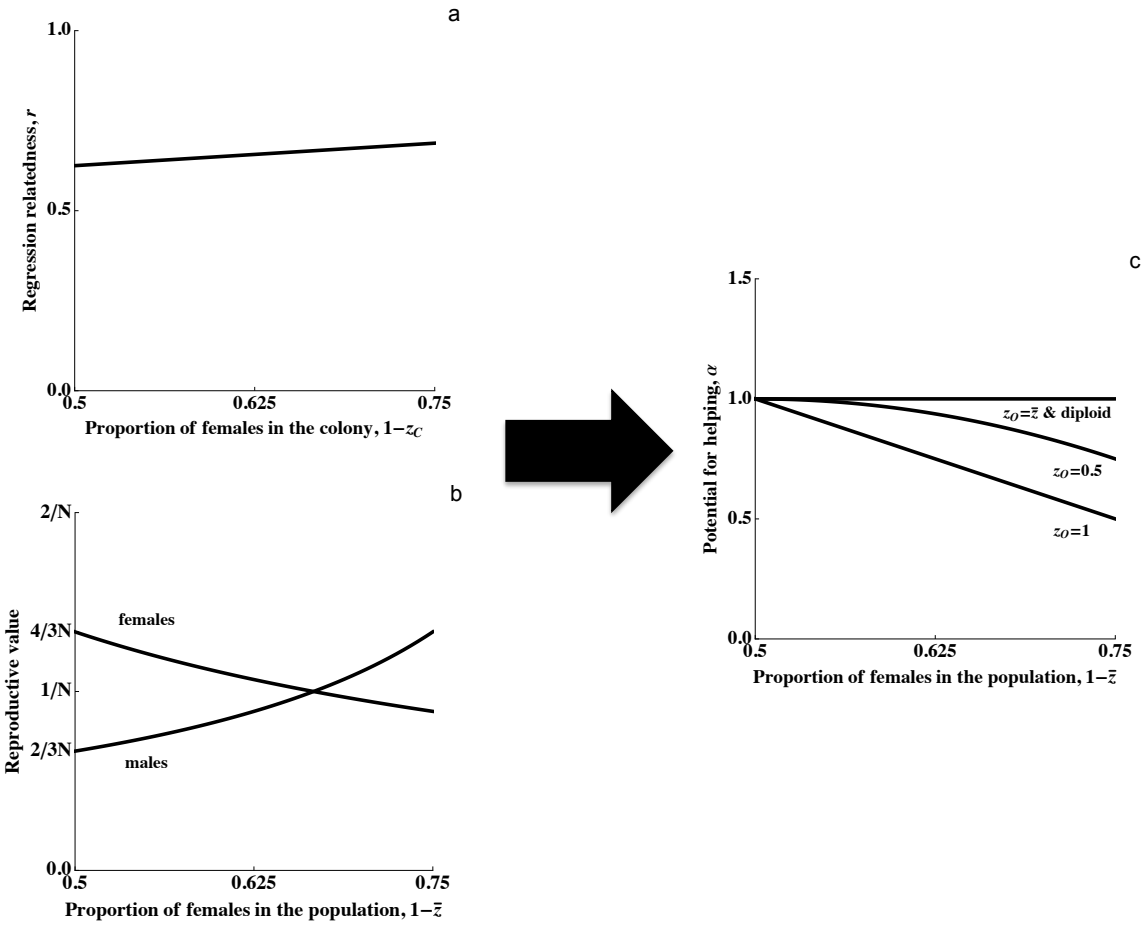


Figure 3.1. Biased sex ratio at the population level. As the sex ratio produced in colonies with queens, and therefore the population sex ratio, becomes increasingly female biased, this leads to: (a) an increase in the relatedness ($r$) between workers and their siblings; (b) an increase in the reproductive value of males ($v_m$) and a decrease in the reproductive value of females ($v_f$) – when $z = ¼$ $v_m = 4/3N$ and $v_f = 8/9N$; when $z = ½$ $v_m = 2/3N$ and $v_f = 4/3N$ ; (c) reduced selection for helping, as shown by a lower potential for helping ($\alpha$). Diploidy leads to the potential for helping being $\alpha = 1$, which is equivalent to when $z_C = \bar{z}$. In this and subsequent figures, unless stated otherwise, we assume full monogamy ($\phi = 1$).

### *Queen versus worker control over the sex ratio*

We now consider the early evolution of the eusociality scenario suggested by Trivers and Hare (1976, pp. 251-252) where some level of helping already occurs in the

population. Trivers and Hare (1976) argued that as worker control of sex allocation spreads through a haplodiploid population, this worker revolution would temporarily cause sex ratio variation between the colonies that allows haplodiploidy to promote the evolution of eusociality. We describe Trivers and Hare's scenario in the following steps:

1. Some daughters stay and help their mothers, rather than breeding independently.
2. The queen allocates equal resources into male and female offspring.
3. A mutation (or a small number of mutations) occurs that allows workers to bias the sex ratio of the brood that they are helping to raise towards females (sisters).
4. While the mutation is spreading, colonies that contain workers with the mutation will be raising broods of reproductive offspring that are biased towards females, relative to the population as a whole.
5. Consequently, these workers gain the relatedness benefit of rearing a greater proportion of sisters, without this being exactly cancelled by an increased reproductive success of males, and so greater levels of helping are favoured.
6. As worker control spreads, workers evolve specialized adaptations to cooperative breeding, which increases the efficiency benefit of cooperation (higher $b/c$), which helps drive the evolution of eusociality.

In order to formally model this scenario, it is necessary to make a number of assumptions about factors such as the underlying genetics, strength of selection, etc. We will examine one of the simplest possible cases in this section, and then examine

the consequences of relaxing these assumptions in later sections. We extend our above model by assuming that, with regards to sex allocation, a proportion $\upsilon$ of the colonies are controlled by workers and produce a sex ratio of $z_W$, whereas the other proportion 1-$\upsilon$ of colonies are queen-controlled and produce a sex ratio $z_Q$.

A key implicit assumption here is that worker control arises from one or a small number of mutations, spreading quickly through the population. If this were not the case, then we would not see such a clear distinction between worker-controlled versus queen-controlled colonies. In the extreme, if worker control owed to many mutations of small effect, then we would expect to see all colonies producing a similar sex ratio, which would become more female biased as worker control spreads. In that case, the spread of worker control would be captured by the model analyzed in the previous section (Fig. 3.1).

We also need to make assumptions about the sex ratios produced in queen and worker-controlled colonies. We assume that worker control spreads rapidly relative to the timescale of sex ratio evolution, such that queen-controlled colonies maintain the production of an unbiased sex ratio ($z_Q$ = ½) but worker-controlled colonies produce a female bias ($0 < z_W < ½$). We first assume that independent reproduction by workers within the colony is rare, and so the population sex ratio is given by $\bar{z} = \upsilon z_w+(1-\upsilon) z_Q$. Consequently, if there are any worker-controlled colonies ($\upsilon > 0$), then the population sex ratio will be female biased ($\bar{z} < ½$). When we make these substitutions into equation 3.1, we find that the average potential for helping, across all workers in all colonies, is $\alpha = [1-\upsilon^2(1-2z_W)^2]/[1-\upsilon(1-2z_O)(1-2z_W)]$.

The results of this scenario are similar to our previous model. As worker control spreads through the population, the population sex ratio becomes increasingly female biased (Fig. 3.2a). Workers would therefore be raising an increased proportion of sisters, and so are more related to the brood that they would help to raise (Fig. 3.2b) However, at the same time, the female bias in the population sex ratio (Fig. 3.2a) leads to a relative increase in the reproductive value of males ($v_m$) and a decrease in the reproductive value of females ($v_f$; 3. 2c). This favours independently reproducing workers to produce only sons ($z_O^* = 1$). Figure 3.2d shows how the potential for helping ($\alpha$) is influenced by the proportion of worker-controlled colonies, when the independently reproducing workers produce either only sons ($z_O = 1$) or an equal mixture of sons and daughters ($z_O = 0.5$). Overall, this gives:

**Result 2.** The spread of worker-controlled sex allocation through the population inhibits helping ($\alpha < 1$; Fig. 3.2d). The reason for this is that it causes the population sex ratio to become female biased (Fig. 3.2a), which increases the relative reproductive value of males (Fig. 3.2c) such that sons become more valuable than a female biased mixture of sisters and brothers. If independently reproducing workers produce an unbiased sex ratio ($z_O = 0.5$), then helping is still inhibited, although to a lesser degree (Fig. 3.2d).

**Result 3.** The greater the degree of female bias in worker-controlled colonies, the more helping is disfavoured (Fig. 3.2d). This occurs because the larger the female bias at the population level is, the larger the relative reproductive value of males (see Fig. 3.1b), which favours the production of sons over a female biased mixture of brothers and sisters.

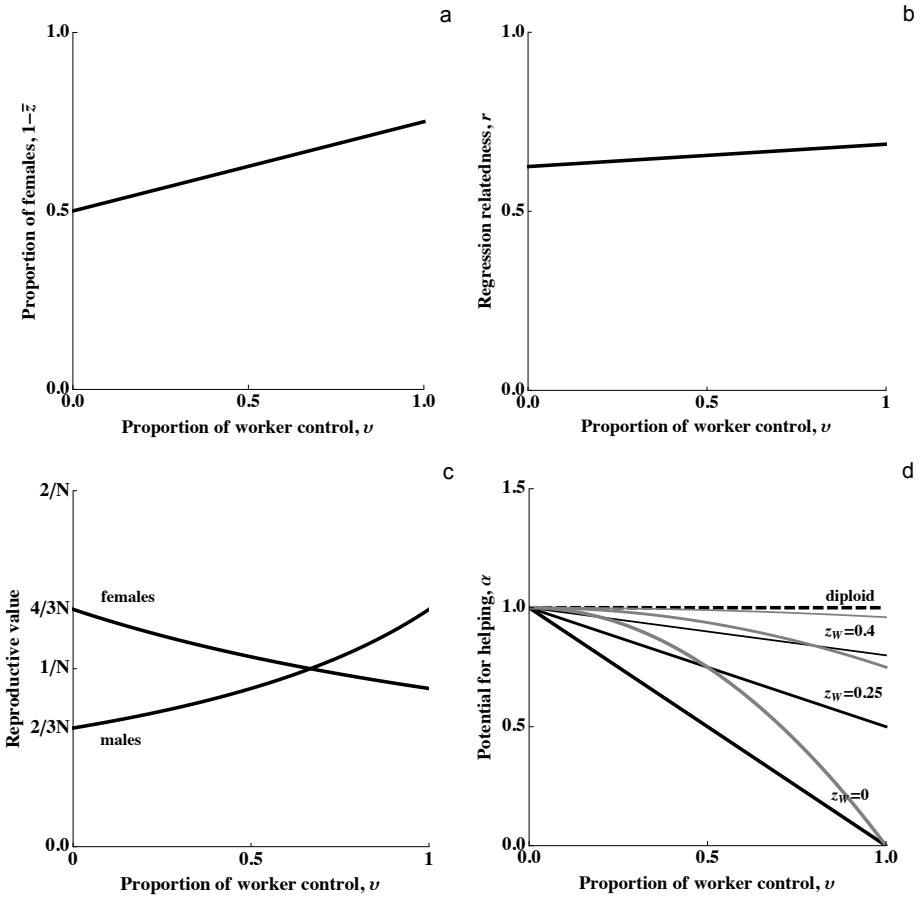


Figure 3.2. Worker revolution with fixed sex allocation strategies. Queen-controlled colonies produce an unbiased sex ratio ($z_Q = ½$) and worker-controlled colonies produce a female biased sex ratio ($z_W < ½$). As worker control υ increases in the population, this: (a) leads to a female bias in the population sex ratio; (b) increases the genetic relatedness between workers and the siblings that they could help raise; (c) increases the reproductive value of males, whilst decreasing the reproductive value of females – when $z = ¼$ $v_m = 4/3N$ and $v_f = 8/9N$; when $z = ½$ $v_m = 2/3N$ and $v_f = 4/3N$ –; (d) disfavors helping (independently reproducing workers produce either only sons (black lines; $z_O = 1.0$), or an unbiased mixture of sons and daughters (grey lines; $z_O = 0.5$). This effect against helping increases as the sex ratio of worker-controlled colonies becomes more female biased. In panels a-c, we assume that $z_W = ¼$.

In Appendix C and D we show that similar results are obtained with an alternative scenario, where the spread of worker control is relatively slow, such that the sex ratio at queen and worker-controlled colonies evolves in response to the proportion of worker control in the population. So, for example, as worker control spreads through the population, queens that are still in control of their colony sex ratio are favoured to produce a male biased sex ratio ($z_Q > ½$). Although such rapid sex ratio evolution maybe relatively unrealistic, it allows us to investigate the scenario where there is the greatest possible differences in sex ratio between queen and worker-controlled colonies, and hence places an upper bound on the possible consequences of haplodiploidy. Under this model, worker control leads to a female bias in the population sex ratio (Fig. 3.S2a) and, due to the increase in male reproductive value (Fig. 3.S2b), a worker is favoured to produce sons over helping to rear the colony offspring. In this case, the potential for helping is never higher than the unity and it decreases as worker control spreads in the population ($\alpha < 1$; Fig. 3.S2d).

*Worker reproduction*

Our above results assume that worker reproduction within the colony is negligible. Here, we relax this assumption, allowing for a proportion $\mu$ of offspring to be produced by workers, and the remaining proportion 1-$\mu$ by queens. We assume that workers are produced earlier than the colony reproductive offspring and thus can only mate with males from the previous generation. Also, we consider that sex ratios in queen and worker-controlled colonies are fixed parameters ($z_Q = ½$ and $0 < z_W < ½$), and that workers reproducing within the colony are constrained to produce the same

sex ratio as the queen ($z_A = z_Q$ or $z_W$). We assume a large number of workers such that the probability of a worker helping her own offspring is negligible.

Worker reproduction has two consequences. First, it leads to generation overlap such that both offspring and grand-offspring of the queen coexist as reproductives. In a haplodiploid system with no worker reproduction, the total reproductive value of females is twice that of males. This is due to the particular genetics where males don't transmit any genes to their sons. When there is worker reproduction, the proportion of genes in the next generation of male reproductives that trace back to males in the previous generation of reproductives increases above zero, increasing the reproductive value of males (3.3b). Second, as a result of rearing nephews instead of siblings, worker reproduction leads to a decrease in the relatedness of a focal worker to the larvae she could help to rear (Fig. 3.3a).

These two effects combine with the change in population sex ratio due to worker control. The female bias at the population level increases the reproductive value of males, (Fig. 3.3b), and so any workers who breed independently will be favoured to produce only sons ($z_O = 1$). The overall consequence of these different effects is that the potential for helping, $\alpha = [(1-\upsilon(1-2z_W))(4-(2-\mu)\mu)]/[2(2+\mu)]$, is never higher than the unity, and gives:

**Result 4.** Worker reproduction inhibits helping ($\alpha < 1$; Fig. 3.3c). This is because worker reproduction decreases the relatedness to the brood they would help to rear (Fig. 3.3a), and makes males relatively more valuable (Fig. 3.3b). Both of these

effects make sons relatively more valuable than a mixture of siblings, nieces and nephews.

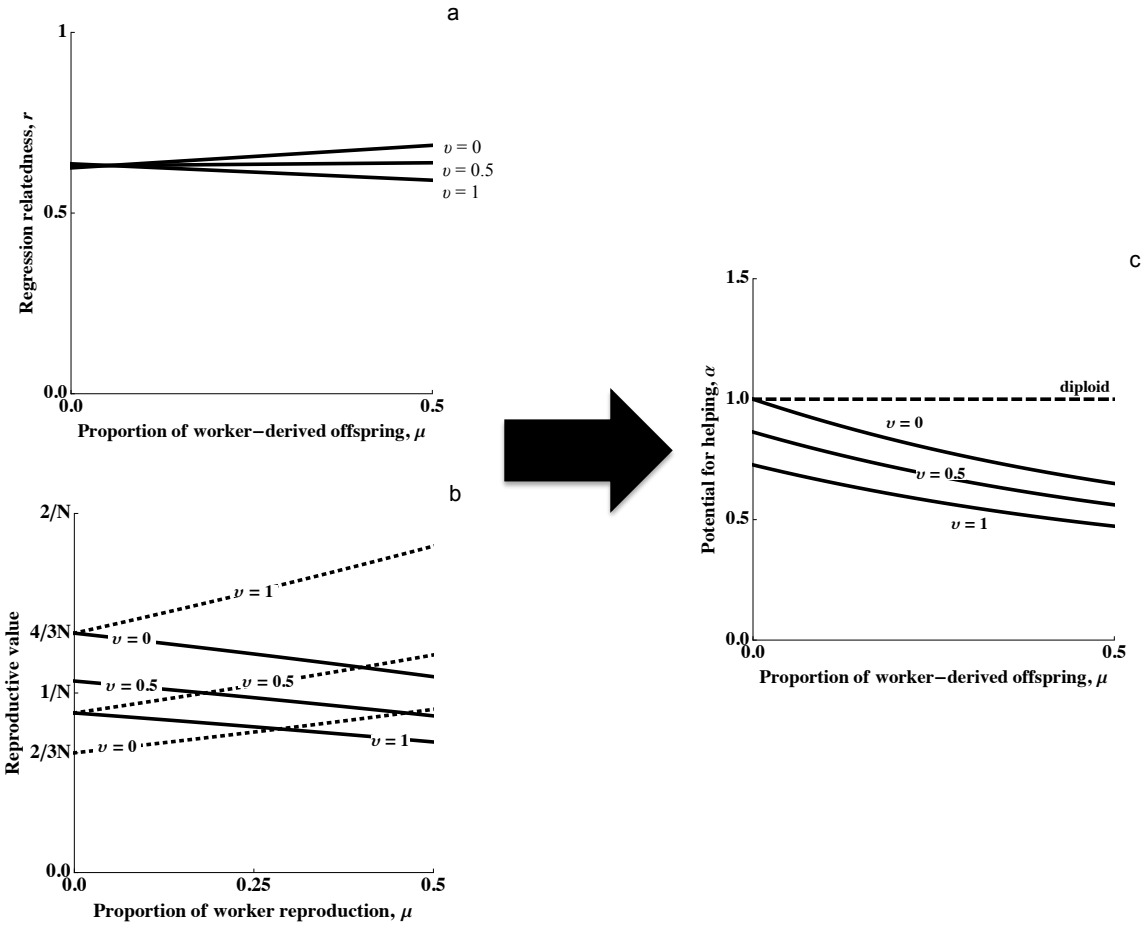


Figure 3.3. Worker revolution with worker reproduction. Worker reproduction: (a) decreases the regression relatedness of workers to the offspring that they could help rear, because sisters and brothers are replaced with nieces and nephews; (b) increases the reproductive value of males and decreases the reproductive value of females; (c) disfavors helping ($\alpha < 1$). We assume $z_Q = ½$ and $z_W = 5/14$ ($z_W = 5/14$ is the optimal sex ratio strategy for a worker-controlled population when $\upsilon = 1$ and $\mu = ½$).

In Appendices C and D, we show that this same qualitative result holds if we assume that the spread of worker control is slow, such that the sex ratios at queen and worker-controlled colonies evolve in response to the proportion of worker-controlled colonies.

*Association between helping and sex allocation*

We now study the scenario where worker control of sex allocation and tendency to help always co-occur. This association between the two traits follows from steps 4 & 5 of the Trivers and Hare (1976) scenario, outlined above, and could arise for two reasons. First, the two traits might be genetically linked. Second, the helping behavior might be facultative, and only employed when in relatively female biased broods. We assume that the sex ratios produced in queen and worker-controlled colonies are fixed, and given by $z_Q$ and $z_W$ respectively, and that reproduction by workers is negligible ($\mu = 0$). In this case, the potential for helping is $\alpha = [z_W+(3-4z_W)((1-\upsilon)z_Q+\upsilon z_W)]/[2(z_Q-\upsilon z_Q+\upsilon z_W+z_I(1-2(1-\upsilon)z_Q-2\upsilon z_W))]$. If we assume that queen-controlled colonies produce an even sex ratio ($z_Q = ½$) and worker-controlled colonies produce a female biased sex ratio of $z_W = ¼$, then the population sex ratio becomes increasingly female biased as the proportion of worker-controlled colonies increases. Consequently, any workers who breeds independently will be selected to produce only sons ($z_O^* = 1$), in which case, assuming monogamy, the potential for helping is $\alpha = (5-2\upsilon)/(4+2\upsilon)$. This leads to:

**Result 5.** When worker control is rare, helping is initially promoted by haplodiploidy ($\alpha > 1$), but then as worker control spreads, helping is inhibited ($\alpha < 1$; Fig. 3.4b). Helping is promoted when worker control is rare, because workers in worker-

controlled colonies gain a relatedness advantage of rearing a higher proportion of sisters (Fig. 3.4a), without their reproducing value being decreased by a female-biased sex ratio at the population level, as originally argued by Trivers and Hare (1976). However, this is negated and eventually reversed as worker control becomes more common, because the relative value of females decreases (Fig. 3.2b), making sons more valuable than a female biased mixture of sister and brothers. Overall, averaging across different amounts of worker control ($\upsilon$), the average potential for helping is $\alpha \approx 33/40$, which, all else being equal, would inhibit helping.

To facilitate comparison, we now show how the same conclusions can be reached with Trivers and Hare's (1976) version of life-for-life relatedness. When worker control is rare ($\upsilon \approx 0$), the sex ratio in worker-controlled colonies ($z_W$) is biased towards females, but the overall population sex ratio is not biased ($\bar{z} = ½$). If we assume that worker-controlled colonies produce 75% female reproductive offspring ($z_W = ¼$), then the relative value of offspring ($¾ \times ¾ \times 1 + ¼ \times ¼ \times 1 = 10/16$) is 1.25 times the reproductive value of a mixture of sons and daughters ($½ \times ½ + ½ \times ½ = ½$). Consequently, in this case, haplodiploidy promotes the evolution of helping. However, as worker control becomes more common, the population sex ratio becomes female biased, which raises the relative reproductive value of males selects for independently reproducing workers to produce only sons ($z_O^* = 1$). In the extreme, with complete worker control ($\upsilon = 1$), when all colonies reproductive that are 75% female ($\bar{z} = ¼$), we obtain the same result, already discussed for Figure 3.1, that sons are worth twice as much as a female biased mixture of sisters and brothers.

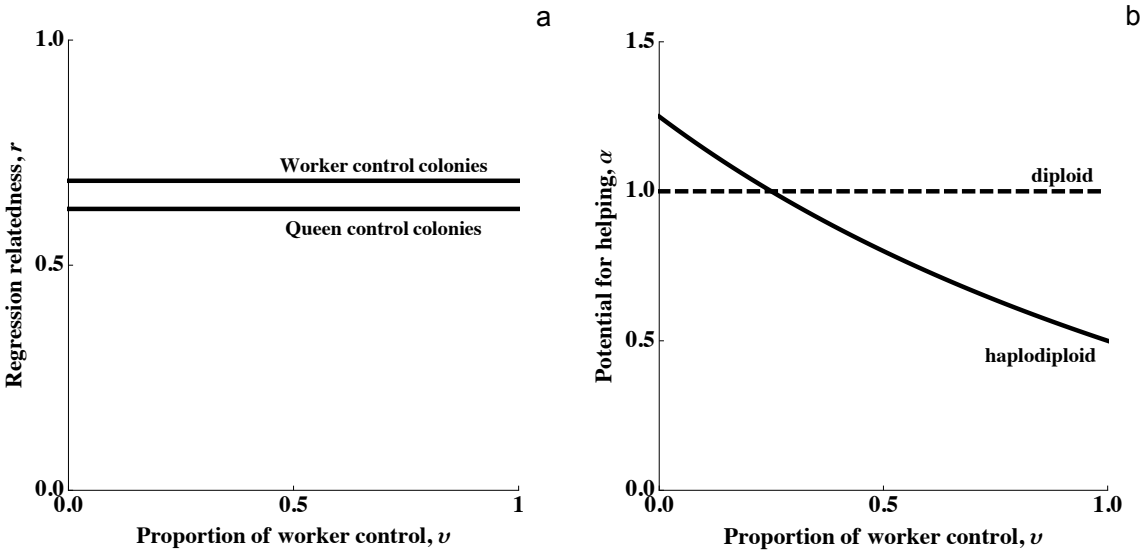


Figure 3.4. Worker revolution with association between helping and sex allocation. (a) The relatedness of a potential worker to the siblings that they could help rear is higher in worker-controlled colonies. This is because workers are more closely related to sisters, and a higher proportion of sisters are produced in worker controlled colonies. (b) Helping is favoured when worker control is relatively rare ($\alpha > 1$ when $\upsilon < ¼$), but disfavoured as worker control becomes more common ($\alpha < 1$ when $\upsilon > ¼$).

In Appendices C and D, we show that the same qualitative result is obtained if we assume that the spread of worker control is relatively slow, such that the sex ratios at queen and worker-controlled colonies evolve to their sex ratio strategies, in response to the proportion of worker-controlled colonies.

## Worker replacement

The other scenario that Trivers and Hare (1976; pp. 250-251) suggested could lead to haplodiploidy favouring the evolution of eusociality is if workers "deny to the queen the production of males". The idea here is that workers replace the sons of the queen

with their own sons, and that this would favour helping because workers are more related to the sons of their sisters (nephews, $r = ¾$, under monogamy) than to the sons of their mother (brothers, $r = ½$). Consequently, workers could benefit by raising sisters and nephews rather than sisters and brothers.

*Brothers replaced by nephews*

We assume that, in each colony, a fraction $\mu$ of the queen's sons are replaced by the workers' sons. We assume a large number of workers such that a focal worker's own offspring is vanishingly rare and the colony offspring is mainly composed of siblings and nephews. We are interested in the effect of worker reproduction *per se*, and so examine the two extreme scenarios regarding how sex allocation is controlled: complete queen control and complete worker control.

Queen control of sex allocation

Replacing brothers with nephews doesn't change the queen's convergence stable sex ratio strategy for the colony offspring (3.5a), because the decrease in relatedness for rearing grandsons (sons: $r = ½$; grandsons: $r = ¼$) is exactly cancelled by the fact that worker reproduction also increases the reproductive value of males (Fig. 3.5c), as described previously. Consequently, $z_Q^* = 1/2$. From the point of view of the worker, worker reproduction has two consequences. First, it leads to the replacement of brothers with nephews and so increases their relatedness to the offspring that they could help raise (Fig. 3.5b). Second, it increases male reproductive value (Fig. 3.5c), and so any independently reproducing workers would be selected to produce only

sons ($z_O^* = 1$). Given this, we find that the potential for helping is $\alpha = (4-\mu)/4$, which decreases with worker reproduction (Fig. 3.5d). This leads to:

**Result 6.** If queens control sex allocation, then the replacement of the queen's sons with the sons of workers inhibits helping (Fig. 3.5d). Although workers become more related to the offspring that they could help rear (Fig. 3.5b), the increased reproductive value of males means that sons become increasingly more valuable than a mixture of sisters, brothers and nephews.

Worker control of sex allocation

We now consider the scenario where workers are in control of sex allocation. In the absence of worker reproduction, the convergence stable colony sex ratio strategy for the workers is $z_W^* = 1/4$ (75% females; Trivers and Hare 1976). Worker reproduction increase the relatedness of the workers to the offspring that they could help raise (Fig. 3.5b), and increases the reproductive value of males. These effects select for workers to produce a less female biased colony sex ratio, as worker reproduction becomes more common (Fig. 3.5a). As the sex ratio becomes less female biased this decreases the relative reproductive value of males (Fig. 3.5c). However, the reproductive value of a male is always higher than that of a female, and so any independently reproducing workers would be selected to produce only sons ($z_O^* = 1$). Hence, the potential for helping is $\alpha = (2+\mu)/4$. Overall, these different effects lead to the potential for helping increasing from $\alpha = ½$, when there is no worker reproduction (analogous to Fig. 3.2d), to $\alpha = ¾$, when all the male reproductive offspring is worker-derived (Fig. 3.5d). This leads to:

**Result 7.** In a population with worker-control over sex allocation and worker replacement of brothers by nephews, helping is inhibited. Worker replacement increases the value of $\alpha$ but the potential for helping is always below the unity ($\alpha <$ 1), with a maximum value of $\alpha = ¾$ (Fig. 3.5d). This is a result of the increased reproductive value of males leading to sons being always more valuable than a mixture of sisters, brothers and nephews.

*Brothers and sisters replaced by nephews*

We have assumed above that workers are able to replace brothers with their own sons. However, another biologically plausible scenario is that they replace siblings of both sexes with their own sons (or, equivalently, just add their sons to the brood of the queen). We analyze this scenario in Appendices C & D, considering both queen and worker control of sex allocation, as well as when sex allocation is either fixed or evolving. In all of these scenarios, we found that the potential for helping is never higher than the value of one found for diploid populations (i.e. $\alpha < 1$ for haplodiploids). Consequently, these scenarios do not lead to haplodiploidy promoting helping, relative to the scenario in diploids.

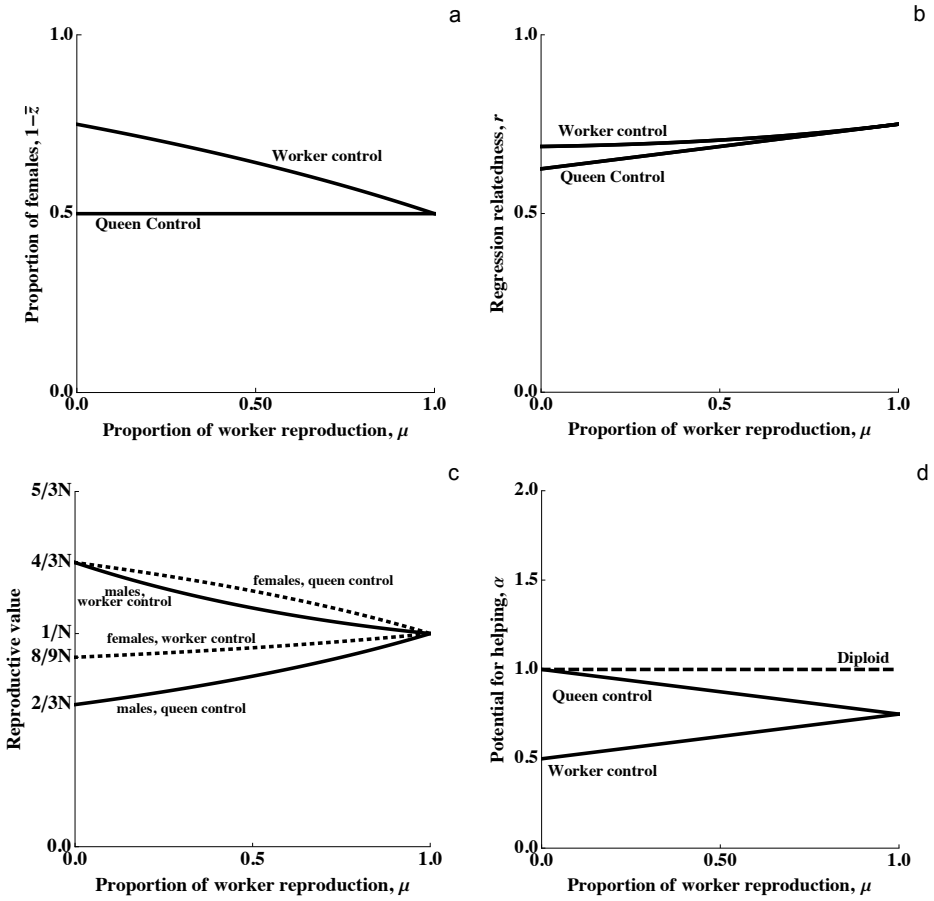


Figure 3.5. Worker replacement. If workers replace the sons of the queen with their own sons, this: (a) does not alter the ESS sex ratio if the queen is in control of her offspring sex ratio, but leads to a less female biased sex ratio if workers are in control; (b) increases the relatedness between a potential worker and the brood that they could help rear; (c) raises the reproductive value of males ($v_m$), while decreasing that of females ($v_f$) under queen control and the opposite under worker control; (d) inhibits helping in queen-controlled populations but promotes in worker-controlled populations. Overall, the potential for helping is never greater than 1.0 ($\alpha = 1$ if $\mu = 0$ and $\alpha < 1$ if $\mu > 1$, in queen-controlled populations; $\alpha < 1$ in worker-controlled populations).

## Discussion

We have found that, when there is worker control of sex allocation or worker reproduction, haplodiploidy can inhibit or promote the evolution of helping. Specifically: (1) female biased sex allocation inhibits the evolution of helping (Figs. 3.1-3.2) (2) worker reproduction inhibits the evolution of helping by other workers (Fig. 3.3); (3) an association at the colony level between worker control of sex allocation and helping initially promotes helping when worker control is rare, but then inhibits helping as helping becomes more common (Fig. 3.4); (4) worker replacement of sons or siblings with their own sons never leads to a scenario where haplodiploidy promotes helping relative to diploidy (Fig. 3.5). Overall, our results suggest that, via these mechansims, haplodiploidy is more likely to inhibit rather than promote the evolution of helping, and hence eusociality.

We found that when workers bias the sex allocation of the queen's brood towards females, haplodiploidy inhibits the evolution of helping (Figs. 3.1 & 3.2). Female biased sex allocation has two consequences: (a) it increases the genetic similarity of workers to the brood that they could help raise (Figs.3.1a & 3.2b); (b) it increases the reproductive value of males (the rarer sex), whilst decreasing that of females (Figs. 3.1b & 3.2c). Craig (1979) suggested that these two effects exactly cancel, such that haplodiploidy has no net effect on the evolution of helping. However, his result was based upon the assumption that if they bred independently, workers would produce offspring with the same (female biased) sex ratio as the one produced in their colonies. (i.e. the line with $z_O = \bar{z}$ in fig. 3.1c). If workers who breed independently produce a less female biased, an unbiased sex ratio, or a male biased sex ratio, then

this increases the relative value of breeding independently, and so haplodiploidy inhibits helping (Fig. 3.1c). Independently reproducing workers are selected to produce a male biased sex ratio, because the female biased sex ratio at the population level means that sons are worth more than daughters (Fig. 3.1b). However, even before there had been time to respond to this selection, we would expect females to be producing unbiased sex ratios, in which case helping is still disfavoured (Fig. 3.1c). However, our sex ratio predictions could change depending upon life history details, and so it would be worth analyzing any specific cases of biological relevance. For example, independently producing workers could be favoured to produce females if there was a chance that they would help their mother rather than breed independently, and these workers could, in turn, bias the sex ratio of their siblings.

The only scenario where we found haplodiploidy was able to promote the expression of the helping trait, for some portion of parameter space, was when worker control of sex allocation was linked to helping (Fig. 3.4). In this case, when worker-controlled colonies are rare, then workers in those colonies gain a relatedness advantage of rearing a higher proportion of sisters (Fig. 3.4a), without the reproductive value of females being appreciably decreased by a female biased sex ratio at the population level. This is the argument of Trivers and Hare (1976, pp. 251-252). However, this is negated and eventually reversed as worker control becomes more common, because this leads to a female bias in the population sex ratio, (Fig. 3.2a) which decreases the value of females and increases the value of males (Fig. 3.2c), as described above. Eventually, when worker control is sufficiently common (>25% of colonies; fig. 3.4b), then even in a worker-controlled colony, sons are more valuable to a potential worker than a female biased mixture of sister and brothers. This suggests that, under

this scenario, there is a short period of time during which haplodiploidy can promote helping, as worker control spreads, but that haplodiploidy subsequently inhibits helping as worker control becomes common. The relative importance of these promotion and inhibition periods will depend upon life history details. The promotion period could give time for the evolution of more efficient helping, which alter the $b/c$ ratio, such that the net effect is positive. Alternatively, the time that is spent when worker control is common ($\mu > 0.25$; helping disfavoured) would include all the time afterwards, when worker control is fixed ($\mu = 1$), which would be much greater than the time when worker control is rare ($\mu < 0.25$; helping favoured), and hence lead to a negative net effect.

We found that worker replacement of the queen's sons with their own sons doesn't lead to the situation where selection for helping is greater in haplodiploids than in diploids (Fig. 3.5d). Worker replacement leads to an increase in the relatedness of potential workers to any offspring that they could help rear (Fig. 3.5b). However, worker replacement also changes the reproductive value of males, such that it increases with worker reproduction when the queen controls the sex ratio, and is always higher than the reproductive value of females when workers control the sex ratio. Consequently, in all the scenarios we examined, sons are more valuable to workers than a mixture of sisters, brothers and nephews (Fig. 3.5d). We reached the same conclusion when considering the scenario where workers replace siblings of both sexes with their own sons.

In order to assess the overall impact of haplodiploidy upon the evolution of eusociality, we need to consider the likelihood of the different scenarios that we have

modelled. Overall, we suggest that haplodiploidy will inhibit helping and hence impede the transition to eusocial socities. First, in all cases, except when sex allocation and helping are linked, the unambiguous effect of haplodiploidy is inhibitory. Second, as described above, even in the linked-traits scenario, the overall impact of haplodiploidy (averaged over evolutionary time) is likely to inhibit helping. Third, the linked-traits scenario is likely to be unrealistic because it implicitly relies upon sex allocation being controlled by a very small number of mutations of large effects. If this was not the case, then we would not see appreciable sex ratio variation between colonies as worker control spreads, and hence would obtain a situation closer that considered in Fig. 3.1. Almost all quantitative traits that have been studied, including the sex ratio behavior of a wasp, have been to shown to be underlain by many loci each contributing a small effect (Lynch and Walsh 1998; Pannebakker et al. 2008). Fourth, the linked-traits scenario requires either genetic linkage between sex allocation and helping loci or that helping is only expressed within colonies in which workers control sex allocation. There is no reason to expect genetic linkage to be common across these traits, let alone that this should be the norm (our aim is to understand whether haplodiploidy has tended to promote eusociality, not whether it can promote eusociality). Facultative helping seems more plausible, but this could only the aid of facultative helping behaviours, and not obligate traits such as sterility, which are the hallmark of eusociality.

To conclude, we have found that both worker control of sex allocation and worker reproduction lead to haplodiploidy hindering the evolution of eusociality. Similar conclusions have been reached when considering the other mechanisms which could lead to sex ratio variation between colonies, termed split sex ratios (Gardner et al.

2012). Consequently, whilst Trivers and Hare's (1976) predictions about sex ratio conflict have led to one of the most successful and productive areas of evolutionary ecology (West 2009), we suggest that the resultant biased investment is more likely to have inhibited than promoted the evolution of helping and eusociality. Haplodiploidy could influence the evolution of eusociality for other reasons (Wade 2001; Lehmann et al. 2008; Fromhage and Kokko 2011; Johnstone et al. 2012), but none of these appears to be generally applicable (Gardner et al. 2012). This suggests that the preponderance of eusociality within the Hymenoptera is due other factors of their biology, such as life-time monogamy and extended parental care (Stubblefield and Charnov 1986; Queller 1994; Boomsma 2007).

## Appendix A – Genetic associations

In this section we define and determine the different measures of genetic similarity used in this paper: consanguinity and regression relatedness.

Consanguinity

Here we derive the consanguinity coefficients used through this study. The consanguinity coefficient between individual i and individual j, denoted $p_{ij}$, is the probability that two genes picked at random from the same locus from individuals i and j respectively are identical by descent (Bulmer 1994). A summary of all consanguinity coefficients is presented in Table A2.

*Diploidy* – Assuming outbreeding, the consanguinity of a diploid individual to themselves is $p_{FF} = p_{MM} = ½$. The consanguinity of two maternal sisters is given by the probability ¼ of drawing a maternal gene from each sister times the consanguinity ½ of the mother to herself, plus the probability ¼ of drawing a paternal gene from each sister, times the probability $\phi$ that they share the same father, times the consanguinity ½ of the father to himself, i.e. $p_F = (1+\phi)/8$. Owing to the sexually symmetrical inheritance, the consanguinity of two maternal brothers is also $p_M = (1+\phi)/8$. Similarly, the consanguinity $p_D$ between mother and daughter is the same as the consanguinity $p_S$ between mother and son, and is given by the probability ½ of picking the maternal gene from the offspring times the consanguinity ½ of the mother to herself, i.e. $p_D = p_S = ¼$.

*Haplodiploidy* – Due to the haplodiploid genetics, the consanguinity of a male to himself is different from the consanguinity of the female to herself. Assuming outbreeding, the consanguinity of a (diploid) female to herself is $p_{FF} = ½$.The consanguinity of a (haploid) male to himself is $p_{MM} = 1$. The consanguinity of two maternal sisters is given by the probability ¼ of drawing their two maternal genes times the consanguinity ½ of their mother to herself, plus the probability ¼ of drawing their two paternal genes, times the probability $\phi$ that they share the same father, times the consanguinity 1 of their father to himself, i.e. $p_F = (1+2\phi)/8$. The consanguinity between opposite sex siblings is the probability ½ of drawing their two maternal genes times the consanguinity ½ of the mother to herself, i.e. $p_M = ¼$. The consanguinity between mother and daughter is given by the probability ½ of drawing the maternal gene from the daughter, times the consanguinity ½ of the mother to herself, i.e. $p_D = ¼$. The consanguinity between mother and son is given by the

probability 1 of drawing the maternal gene from the son times the consanguinity ½ of the mother to herself, i.e. $p_S = ½$. The consanguinity between aunt and niece is given by the consanguinity $(1+2\phi)/8$ between two maternal sisters times the probability ½ of drawing the maternal gene from the niece, i.e. $p_{Ni} = (2\phi+1)/16$. Equally, the consanguinity between aunt and nephew is given by the consanguinity $(1+2\phi)/8$ between two maternal sisters times the probability 1 of drawing the maternal gene from the nephew, i.e. $p_{Ne} = (1+2\phi)/8$. The consanguinity between grandmother and grandson, $p_{Gs}$ is given by the consanguinity ¼ between a female and her daughter times the probability 1 of drawing the maternal gene from the grandson, i.e. $p_{Gs} = ¼$.

Regression relatedness

The coefficient of relatedness, $r_{ji}$, "the relatedness of the recipient j to the actor i", is defined as the regression coefficient of the recipient's phenotype on the actor's genotype (Hamilton, 1970; Bulmer 1994). In our analysis we use the regression definition of relatedness (Bulmer 1994), where the relatedness of j to i is $r_{ji} = (q_j – q)/(q_i – q)$, where $q_i$ is the probability of drawing the same gene at random from i twice, $q_j$ is the probability of drawing the same gene in i and j and $q$ is the probability of drawing the same gene from i and from a random individual in the population. Notice that, for now, we are focusing on alleles identical in state. The identity in state of two alleles can be due to them sharing an identity by descent (when they share a common ancestor defined in a pedigree map) or they can be identical due to the frequency of that allele in the population. This way, we can write $q_j = p_{ij} + (1 – p_{ij})\, q$ and $q_i = p_{ii} + (1 – p_{ii})\, q$, where $p_{ij}$ is the consanguinity shared between i and j, and $p_{jj}$

is the consanguinity of j to herself. If we expand this into our definition of the relatedness of j to i, we find that $r_{ji} = p_{ij} / p_{ii}$.

| | | Consanguinity | | Regression relatedness |
|---|---|---|---|---|
| Relationship | | In terms of model parameters | | |
| Son to mother | $p_S$ | ½ | $r_S$ | ½ |
| Daughter to mother | $p_D$ | ¼ | $r_D$ | ½ |
| Female to sister | $p_F$ | $(1+2\phi)/8$ | $r_F$ | $(1+2\phi)/4$ |
| Brother to sister | $p_M$ | ¼ | $r_M$ | ½ |
| Nephew to aunt | $p_{Ne}$ | $(1+2\phi)/8$ | $r_{Ne}$ | $(1+2\phi)/4$ |
| Niece to aunt | $p_{Ni}$ | $(1+2\phi)/16$ | $r_{Ni}$ | $(1+2\phi)/8$ |
| Grandson to grandmother | $p_{Gs}$ | ¼ | $r_{Gs}$ | ½ |
| Female and herself | $p_f$ | ½ | $r_f$ | 1 |
| Male and himself | $p_m$ | 1 | $r_m$ | 1 |

**Table A1:** Genetic associations in haplodiploid populations

## Appendix B – Reproductive value

Reproductive value is defined as the asymptotic genetic contribution of an individual or class to the future generations (Fisher 1930). We calculate first the reproductive value for a class, and then this is shared equally over all individuals in that class. In this paper we present models defining two classes: males and females. Hence, the individual reproductive value of males is $v_m = c_m / N_m$, where $c_m$ is the class reproductive value and $N_m$ is the number of males in the population. Equally, the reproductive value of females is $v_f = c_f / N_f$, where $c_f$ is the female class reproductive

value and $N_f$ is the number of females in the population. To make quantities more manageable, we can multiply all individual reproductive values by the total number of individuals in the population, $N$, and obtain $v_m = c_m / \bar{z}$ and $v_f = c_f /(1- \bar{z})$, where $\bar{z}$ is the proportion of reproductive males in the population.

We consider a population of $K$ colonies each founded by a single female (the queen) that can be multiply mated. The female produces a first cohort of non-dispersing females (workers) followed by reproductive males and females (reproductive offspring). We allow for worker reproduction by assuming that the queen produces a proportion $1-\mu$ of the colony's reproductive offspring and the workers produce a proportion $\mu$ of the colony's reproductive offspring. The sex ratio in a proportion $\upsilon$ of colonies is worker-controlled and given by $z_W$, whilst the sex ratio in a proportion $1-\upsilon$ of colonies is queen-controlled and given by $z_Q$. The reproductive offspring matures and disperse, mating at random; $K$ mated females found a new set of nests and form the next generation of queens.

Worker reproduction increases the male class reproductive value. In a population with overlapping generations, the class reproductive values for haplodiploids may change from the traditional $c_m = 1/3$ and $c_f = 2/3$. Female class reproductive value is twice the male class reproductive value because a female contributes to half the genes of her daughters and to all the genes present in her sons, while a male doesn't transmit genes to male offspring. Worker reproduction allows reproductive males to contribute in average with ½ of the genes on worker-derived grandsons. Worker-derived femle reproductives may be produced if workers mate. We assum that workers mate with males from their mother's generation, in which case a female reproductive from the

next generation has on average, ¼ of her genes from grand paternal origin and ½ from paternal origin. Hence, through worker reproduction, ¾ of the genes in a worker-derived female have originated from males of the previous generation of reproductives.

In order to calculate the class reproductive values, we census the population at the moment of production of offspring and we account for the contribution of both queens and workers in queen-control and worker control colonies. The contribution of a individual of class *i* to class *j* of the next generation $\wp_{j\leftarrow i}$ depends on the different sex ratio strategies followed by queen-control and worker-control colonies and worker-derived offspring, as well as the frequency of these. Specifically, the probability of picking a queen-derived male juvenile is $\zeta_{Q,m} = ((1-\mu)((1-\upsilon)z_Q+\upsilon z_W))/((1-\mu)((1-\upsilon)z_Q+\upsilon z_W)+\mu z_A)$, the probability of picking a queen-derived female juvenile is $\zeta_{Q,f} = ((1-\mu)((1-\upsilon)(1-z_Q)+ \upsilon(1-z_W)))/((1-\mu)((1-\upsilon)(1-z_Q)+ \upsilon(1-z_W))+\mu(1-z_A))$, the probability of picking a worker-derived male juvenile is: $\zeta_{W,m} = \mu z_A/((1-\mu)((1-\upsilon)z_Q+\upsilon z_W)+\mu z_A) = 1-\zeta_{Q,m}$, and the probability of picking a worker-derived female juvenile is $\zeta_{W,f} = \mu(1-z_A)/ ((1-\mu)((1-\upsilon)(1-z_Q)+ \upsilon(1-z_W))+\mu(1-z_A)) = \zeta_{Q,f}$. Thus, the proportion of genes in female larvae at the time of the census that derive from females of the last census is $\wp_{f\rightarrow f}= ½\zeta_{Q,f}+ ¼\zeta_{W,f}$ and hence the proportion of genes in female larvae that derive from males is of the last census is $1-\wp_{f\rightarrow f} = \wp_{m\rightarrow f}= ½\zeta_{Q,m}+ ¾\zeta_{W,m}$ . The proportion of genes in male larvae that derive from females in the last census is $\wp_{f\rightarrow m} = \zeta_{Q,m}+ ½ \zeta_{W,m}$, and hence the proportion of genes in male larvae that derive from males in the last census is $1-\wp_{f\rightarrow m} = ½\zeta_{W,m}$ . These quantities can be summarized in a gene-flow matrix **G**:

$$\mathbf{G} = \begin{bmatrix} \wp_{f\to f} & \wp_{m\to f} \\ \wp_{f\to m} & \wp_{m\to m} \end{bmatrix} .$$

(3.A1)

The class reproductive values are given by the dominant left eigenvector of the gene-flow matrix, i.e. the solution to $(c_f \ c_m) = (c_f \ c_m) \bullet \mathbf{G}$. Using model parameters to describe the class reproductive values, we have:

$$c_f = \frac{1}{\Psi}(2((1-\mu)z_A + 2\mu(1-\upsilon)z_Q + 2\mu\upsilon z_W)((1-\mu)z_A + \mu(1-\upsilon)z_Q + \mu\upsilon z_W - 1)),$$

(3.A2)

and

$$c_m = \frac{1}{\Psi}(((1-\mu)z_A + \mu(1-\upsilon)z_Q + \mu\upsilon z_W)(3z_A + \mu(1-3z_A + 2(1-\upsilon)z_Q + 2\upsilon z_W) - 3)),$$

(3.A3)

where $\Psi = 5(1-\mu)^2 z_A{}^2 - (1-\mu)z_A(5-\mu(1+11(1-\upsilon)z_Q+11\upsilon z_W)) - \mu((1-\upsilon)z_Q+\upsilon z_W)(7-\mu(1+6(1-\upsilon)z_Q+6\upsilon z_W))$.

## Appendix C – Convergence stable sex ratios

Worker control of sex ratio

*Slow spread of worker control* – Here we analyze the evolution of split sex ratios in haplodiploid populations. We consider that in $\upsilon$ colonies the sex ratio of queen-derived offspring is worker-controlled (producing $z_W$ males) and in $1-\upsilon$ colonies is queen-controlled (producing $z_Q$ males). We start by considering worker reproduction to be negligible, such that the population sex ratio is $\bar{z} = \upsilon z_W + (1-\upsilon) z_Q$ and the class reproductive values are $c_f = 2/3$ and $c_m = 1/3$. We use a neighbour-modulated fitness (personal fitness) approach to kin selection analysis (Taylor and Frank 1996; Frank 1997a, 1998), to determine the convergence stable sex ratio strategies for worker-control and queen-control colonies. To determine the sex allocation decision we consider that an equal number of male and female reproductive larvae are created but only half of these survive, depending on the sex allocation strategy followed in the colony. Hence, the expected fitness of a random, focal female is $w_f = \upsilon (1 - z_W) + (1-\upsilon)(1 - z_Q)$. The average fitness among all females is $\bar{w}_f = \upsilon (1 - \bar{z}_W) + (1-\upsilon)(1 - \bar{z}_Q)$, and hence the relative fitness of the focal female is $W_f = w_f / \bar{w}_f = [\upsilon (1 - z_W) + (1-\upsilon)(1 - z_Q)]/[\upsilon (1 - \bar{z}_W) + (1-\upsilon)(1 - \bar{z}_Q)]$. We use the same method to determine the relative fitness of a random, focal male, $W_m = [\upsilon z_W + (1-\upsilon) z_Q]/[\upsilon \bar{z}_W + (1-\upsilon) \bar{z}_Q]$. Natural selection maximizes the quantity $W = c_f W_f + c_m W_m$, (Taylor 1996; Taylor and Frank 1996; Frank 1997a, 1998; Rousset 2004; Taylor et al. 2007).

We consider genetic variation at a locus *G,* with genic value *g*, controlling for the sex ratio strategy of workers from worker-controlled colonies. Assuming vanishingly

small genetic variation around a population average of $\bar{g}$, natural selection will act to increase the population average genic value if $dW/dg\big|_{g=\bar{g}} > 0$. Hence, the direction of selection acting on the sex ratio of worker-controlled colonies is given by:

$$\frac{\mathrm{d}W}{\mathrm{d}h} = c_{\mathrm{f}}\frac{\mathrm{d}W_{\mathrm{f}}}{\mathrm{d}g} + c_{\mathrm{m}}\frac{\mathrm{d}W_{\mathrm{m}}}{\mathrm{d}g} =$$

$$= c_{\mathrm{f}}\frac{\partial W_{\mathrm{f}}}{\partial z_{\mathrm{W}}}\frac{\mathrm{d}z_{\mathrm{W}}}{\mathrm{d}\hat{g}}\frac{\mathrm{d}\hat{g}}{\mathrm{d}g_{\mathrm{f}}} + c_{\mathrm{m}}\frac{\partial W_{\mathrm{m}}}{\partial z_{\mathrm{W}}}\frac{\mathrm{d}z_{\mathrm{W}}}{\mathrm{d}\hat{g}}\frac{\mathrm{d}\hat{g}}{\mathrm{d}g_{\mathrm{m}}}, \qquad (3.A4)$$

where: $\partial W_{\mathrm{f}}/\partial z_{\mathrm{W}}$ and $\partial W_{\mathrm{m}}/\partial z_{\mathrm{W}}$ are respectively the partial derivatives of female and male relative fitness with the worker-controlled sex ratio strategy, $\mathrm{d}z_{\mathrm{W}}/\mathrm{d}\hat{g} = 1$ is the genotype-phenotype map; and $\mathrm{d}\hat{g}/\mathrm{d}g_{\mathrm{f}} = p_{\mathrm{F}}$ and $\mathrm{d}\hat{g}/\mathrm{d}g_{\mathrm{m}} = p_{\mathrm{M}}$ are the coefficients of consanguinity between sisters and between sister and brother, respectively (Taylor 1990, 1996; Taylor and Frank 1996; Frank 1997a, 1998; Rousset 2004; Taylor et al. 2007). We use an analogous approach to determine how selection acts upon the sex allocation of queen-control colonies. We consider a locus *H*, with a genic value *h*, that controls the sex ratio expressed in queen-control colonies. In this case, d*W*/d*h* can be written as:

$$\frac{\mathrm{d}W}{\mathrm{d}h} = c_{\mathrm{f}}\frac{\mathrm{d}W_{\mathrm{f}}}{\mathrm{d}h} + c_{\mathrm{m}}\frac{\mathrm{d}W_{\mathrm{m}}}{\mathrm{d}h}$$

$$= c_{\mathrm{f}}\frac{\partial W_{\mathrm{f}}}{\partial z_{\mathrm{Q}}}\frac{\mathrm{d}z_{\mathrm{Q}}}{\mathrm{d}\hat{h}}\frac{\mathrm{d}\hat{h}}{\mathrm{d}h_{\mathrm{f}}} + c_{\mathrm{m}}\frac{\partial W_{\mathrm{m}}}{\partial z_{\mathrm{Q}}}\frac{\mathrm{d}z_{\mathrm{Q}}}{\mathrm{d}\hat{h}}\frac{\mathrm{d}\hat{h}}{\mathrm{d}h_{\mathrm{m}}}, \qquad (3.A5)$$

where $\partial W_{\mathrm{f}}/\partial z_{\mathrm{Q}}$ and $\partial W_{\mathrm{m}}/\partial z_{\mathrm{Q}}$ are respectively the partial derivatives of female and male relative fitness with the queen-controlled sex ratio strategy. The coefficients of

consanguinity between mother and daughter and between mother and son are, respectively, $\mathrm{d}\hat{h}/\mathrm{d}h_{\mathrm{f}} = p_{\mathrm{D}}$ and $\mathrm{d}\hat{h}/\mathrm{d}h_{\mathrm{m}} = p_{\mathrm{S}}$. Using equations 3.A4 and 3.A5, we find that the joint convergence stable sex ratio strategy is:

$$\left(z_Q^*, z_W^*\right) = \begin{cases} \left(\dfrac{1}{2(1-\upsilon)}, 0\right) & \upsilon \le \dfrac{1}{2} \\ (1,0) & \text{if} \quad \dfrac{1}{2} < \upsilon \le \dfrac{1+2\phi}{2(1+\phi)} \\ \left(1, \dfrac{1-2(1-\upsilon)(1+\phi)}{2\upsilon(1+\phi)}\right) & \upsilon > \dfrac{1+2\phi}{2(1+\phi)} \end{cases} . \qquad (3.A6)$$

These solutions can be used to compute the population sex ratio as a function of the paternity index $\phi$ and of the proportion of worker-control colonies, $\upsilon$ (Fig. 3.2a).

*Slow spread of worker control and worker reproduction* – We allow for workers to reproduce by mating with unrelated males from the queen's generation, and consider that a proportion $\mu$ of the colony offspring are worker derived and the remainder 1-$\mu$ are queen-derived. We assume that the worker-derived offspring and queen-derived offspring have the same sex ratio, which depends upon whether it is queen or worker controlled. Consequently, the population sex ratio is $z = \upsilon z_{\mathrm{W}} + (1-\upsilon) z_{\mathrm{Q}}$. The main difference between this and the above model lies in the change in male and female class reproductive value due to worker reproduction. We determine the partial fitness functions for male and female juveniles in a similar fashion as the above model (Equations 3.A4 and 3.A5) and we find that the joint convergence stable sex ratio strategies are:

$$\left(z_Q^*, z_W^*\right) = \begin{cases} \left(\dfrac{2+\mu}{4(1-\upsilon)}, 0\right) & \upsilon \le \dfrac{2-\mu}{4} \\ (1,0) & \text{if} \quad \dfrac{2-\mu}{4} < \upsilon \le \dfrac{2-\mu+2\phi+2\phi(1-\mu)}{2(2+\phi+\phi(1-\mu))} \\ \left(1, \dfrac{1}{2\upsilon}\left[\dfrac{2+\mu}{2+\phi+\phi(1-\mu)} - 2(1-\upsilon)\right]\right) & \upsilon > \dfrac{2-\mu+2\phi+2\phi(1-\mu)}{2(2+\phi+\phi(1-\mu))} \end{cases}$$

.(3.A7)

Worker replacement

*Brothers replaced by nephews* – We now consider that workers replace brothers with sons and we determine the convergence stable sex ratio strategy. We study two cases: (i) queen has full control over the sex ratio of her offspring and (ii) workers have full control over the sex ratio of their siblings. In both cases we assume that the information on replacement is available to the actor controlling the sex allocation strategy. The relative fitness of a juvenile male and of a juvenile female are then given by $W_m = z/\ \bar{z}$, and $W_f = (1-z)/(1-\ \bar{z})$. As before, natural selection maximizes $W$ which given by the sum of the relative within-class fitness of females $W_f$ and males $W_m$ weighted respectively by their class reproductive values, $c_f$ and $c_m$, i.e., $W = c_f W_f + c_m W_m$. We determine the partial fitness functions for male and female juveniles in a similar fashion as before.

In a population with queen-control over sex allocation, the direction of selection acting upon the sex allocation trait is given by:

$$\frac{\mathrm{d}W}{\mathrm{d}g} = c_{\mathrm{f}} \frac{\mathrm{d}W_{\mathrm{f}}}{\mathrm{d}g_{\mathrm{f}}} + c_{\mathrm{m}} \frac{\mathrm{d}W_{\mathrm{m}}}{\mathrm{d}g_{\mathrm{m}}} =$$

$$= c_{\mathrm{f}} \frac{\partial W_{\mathrm{f}}}{\partial z} \frac{\mathrm{d}z}{\mathrm{d}\hat{g}} \frac{\mathrm{d}\hat{g}}{\mathrm{d}g_{\mathrm{f}}} + c_{\mathrm{m}} \frac{\partial W_{\mathrm{m}}}{\partial z} \frac{\mathrm{d}z}{\mathrm{d}\hat{g}} \frac{\mathrm{d}\hat{g}}{\mathrm{d}g_{\mathrm{m}}} = c_{\mathrm{f}} \frac{\partial W_{\mathrm{f}}}{\partial z} p_{\mathrm{D}} + \left((1-\mu)p_{\mathrm{S}} + \mu p_{\mathrm{GS}}\right), \qquad (3.A8)$$

where $\mathrm{d}g / \mathrm{d}g_{\mathrm{f}} = p_{\mathrm{D}}$ is the coefficient of consanguinity between the queen and her daughter, and $\mathrm{d}g / \mathrm{d}g_{\mathrm{m}} = ((1-\mu)p_{\mathrm{S}} + \mu p_{\mathrm{Gs}})$ is the average consanguinity shared between the queen and a male reproductive larvae picked at random (an can be either the queen's son or grandson). Setting this expression to zero and solving for $z$ we find that the convergence stable sex ratio strategy is $z_{\mathrm{Q}}^{*} = ½$.

Similarly, if we consider a population with worker control over sex allocation, we find that the convergence stable sex ratio strategy is given by:

$$\frac{\mathrm{d}W}{\mathrm{d}g} = c_{\mathrm{f}} \frac{\partial W_{\mathrm{f}}}{\partial z} p_{\mathrm{F}} + c_{\mathrm{m}} \frac{\partial W_{\mathrm{m}}}{\partial z} \left((1-\mu)p_{\mathrm{M}} + \mu p_{\mathrm{Ne}}\right), \qquad (3.A9)$$

where $p_{\mathrm{F}}$ is the consanguinity between a worker and her sister and $p_{\mathrm{M}}$ is the consanguinity between a worker and her brother and $p_{\mathrm{Ne}}$ is the consanguinity between a worker and her nephew. In this case, we set A9 to zero, solve it in order to $z$ and find that the convergence stable sex ratio strategy for worker control is: $z_{\mathrm{W}} = (2-\mu +2\phi-2\phi(1-\mu))/(2(2-\mu+2\phi))$ (Fig. 3.5).

*Brothers and sisters replaced by nephews* – Now we consider that a proportion $\mu$ of queen-derived offspring is replaced by sons of the workers. Again, we consider both:

(i) queen controlled offspring sex ratio ($z_Q$), and (ii) workers control the sex ratio of the queen's offspring ($z_W$). In both cases worker reproduction changes the sex ratio in the colony and hence it impacts on the partial fitness functions defined for male and female juveniles. Hence, the queen offspring sex ratio evolves as a function of worker reproduction $\mu$. In this way, we can define the fitness of a male juvenile as $W_m = ((1-\mu)z+\mu)/\ \bar{z}$ and the fitness of a female juvenile as $W_f = ((1-\mu)(1-z))/\ (1-\ \bar{z})$, where $z = z_Q$ in a queen-controlled population and $z = z_W$ in a worker-controlled population. As worker derived offspring are produced following a fixed strategy, we can define the sex ratio strategy for queen-derived offspring following equation 3.A4, in the scenario with queen control over the sex ratio and equation 3.A5 with worker control over the sex ratio. In the former scenario, $z_Q^* = (2+3\mu)/(4(1-\mu))$ if $\mu < 2/3$ and $z_Q^* = 0$ otherwise. In the latter, $z_W^* = (2-3\mu-2\mu\phi)$ if $\mu < 2/(3+2\phi)$ and $z_W^* = 0$ otherwise.

## Appendix D – Potential for helping in haplodiploid populations

*Worker control of sex ratio*

Queen versus worker control over the sex ratio

*Female-biased sex allocation* – In the main text we present the potential for helping, considering a monogamous population ($\phi = 1$). Here, we present a more general result and allow for $0 \leq \phi \leq 1$. In this case, $\alpha = [(1+\upsilon(1-2z_W))(1-\upsilon(1-2z_W))(1+\phi)]/[2(1-\upsilon(1-2z_O)(1-2z_W))]$.

*Fast spread of worker control* – Here we generalize the result presented in the main text, by relaxing the assumption of monogamy in the population. As a consequence, the probability of two maternal sisters sharing the same paternal allele is: $0 \leq \phi \leq 1$. In this case, the potential for helping is $\alpha = [(1-\upsilon(1-2z_W))(2-\mu+(2-(1-\mu)\mu)\phi]/2(2+\mu)$.

*Slow spread of worker control* – In the previous section we found the convergence stable sex ratio strategy for worker-controlled and queen-controlled colonies. As the spread of worker-control leads to a female bias in the population sex ratio, a worker reproducing within her colony is favoured to produce only sons ($z_O^* = 1$). In this case, the potential for helping is:

$$\alpha = \begin{cases} \dfrac{1+\phi}{2} & \upsilon < \dfrac{1}{2} \\ (1-\upsilon)(1+\phi) & \text{if} \quad \dfrac{1}{2} < \upsilon < \dfrac{1+2\phi}{2(1+\phi)}, \\ \dfrac{1}{2} & \upsilon > \dfrac{1+2\phi}{2(1+\phi)} \end{cases} \tag{3.A10}$$

which is never higher than the unity ($\alpha \leq 1$; Fig. 3.S1).

*Slow spread of worker control and worker reproduction* – We assume that workers are limited to produce offspring with the same sex ratio as their colony and that the colony is composed by a large number of workers, such that the probability of a focal worker allocating help to her own offspring is negligible. In Appendix C we find the convergence stable sex ratio strategies (equation 3.A7), shown in Fig. 3.S2. If we assume that an independently reproducing worker produces only sons ($z_O^* = 1$), the potential for helping is:

$$\alpha = \frac{1}{\frac{c_m}{\bar{z}} p_S}\Bigg( (\upsilon\big(z_W \frac{c_m}{\bar{z}}\big(p_M(1-\mu) + p_{Ne}\mu\big) + \big(1-z_W\big)\frac{c_f}{1-\bar{z}}\big(p_F(1-\mu) + p_{Ni}\mu\big)\big) +$$

$$\Big((1-\upsilon)\big(z_Q \frac{c_m}{\bar{z}}\big(p_S(1-\mu) + p_{Ne}\mu\big) + \big(1-z_Q\big)\frac{c_f}{1-\bar{z}}\big(p_D(1-\mu) + p_{Ni}\mu\big)\big)\Big)\Bigg),$$

(3.A11)

where $\upsilon$ is the proportion of worker control and $\mu$ the proportion of worker reproduction. Substituting for the consanguinity coefficients, sex ratios and class reproductive values, the potential for helping is:

$$\alpha = \begin{cases} \dfrac{2-\mu+\phi(2-\mu(1-\mu))}{4} & \upsilon \le \dfrac{2-\mu}{4} \\ \dfrac{(1-\upsilon)(2-\mu+\phi(2-\mu(1-\mu)))}{2+\mu} & \text{if} \quad \dfrac{2-\mu}{4} < \upsilon \le \dfrac{2-\mu+2\phi+2\phi(1-\mu)}{2\phi(2+\phi+\phi(1-\mu))} \\ \dfrac{2-\mu+\phi(2-\mu+\mu^2)}{4+2\phi(2-\mu)} & \upsilon > \dfrac{2-\mu+2\phi+2\phi(1-\mu)}{2\phi(2+\phi+\phi(1-\mu))} \end{cases},$$

(3.A12)

and this is never higher than the unity ($\alpha \le 1$; Fig. 3.S2).

*Association between helping and sex allocation, with coevolving sex ratios* – We first consider a population where worker control spreads very rapidly relative to changes in sex allocation, such that queen-control and worker-control sex ratio strategies are fixed in the population. We extend the result presented in the main text by dropping the assumption of monogamy in the population. In this case, the potential for helping is:

$$\alpha = \frac{z_W + (3-4z_W)((1-\upsilon)z_Q + \upsilon z_W)}{2(z_Q - \upsilon z_Q + \upsilon z_I(1-2(1-\upsilon)z_Q - 2\upsilon q z_W))}. \quad (3.A13)$$

In a population where the spread of worker control is relatively slow, such that the sex ratio evolves in response to the proportion of worker control in the population (Appendix C; Fig. 3.S3), we study the effect of an association between helping and worker control. We assume that worker reproduction is negligible. In this case, the potential for helping is:

$$\alpha = \begin{cases} \frac{2\phi+1}{2} & \upsilon \le \frac{1}{2} \\ \frac{(1-\upsilon)(1+2\phi)}{2\upsilon} & \text{if} \quad \frac{1}{2} < \upsilon \le \frac{1+2\phi}{2(1-\phi)} \\ \frac{1}{2} & \upsilon > \frac{1+2\phi}{2(1-\phi)} \end{cases}. \quad (3.A14)$$

In this case, the potential for helping may be higher than the unity if the proportion of worker control in the population is lower than a threshold value ($\alpha$ >1 if $\upsilon$ < (1+2$\phi$)/(3+2$\phi$); Fig. 3.S3).

*Worker replacement*

*Brothers and sisters replaced by nephews* – We make the same assumptions as the worker replacement model in the main text, except that we now assume workers replace $\mu$ of the queen's offspring (rather than just sons) with their own sons. We first focus on a population with queen control over the sex ratio. If the queen's sex allocation strategy is fixed, replacing siblings by nephews results in a male-biased

population sex ratio and independently reproducing workers are selected to produce only daughters. In this case, the potential for helping is $\alpha = (1-\mu)(1+\phi+\mu\phi)/2$ (Fig. 3.S4). This is always lower than one if there is worker replacement ($\mu > 0$), meaning that worker reproduction disfavors the evolution of helping. If the queen's sex allocation strategy evolves in response to the proportion of worker replacement, the potential for helping is $(2-\mu+\phi(2+\mu))/4$ if $\mu \leq 2/3$ and $(1-\mu)(1+2\phi)$ if $\mu > 2/3$, and hence helping is never promoted ($\alpha \leq 1$, Fig. 3.S5).

We now focus on a population where workers control the sex ratio of queen-derived offspring and where the sex allocation strategy changes with the proportion of worker replacement. In this case, the population sex ratio is female biased if worker replacement is not high, and it becomes male biased as worker replacement increases. If we assume that independently reproducing workers produces only sons when $\mu <$ 2/3, and only daughters when $\mu > 2/3$, then the potential for helping is:

$$\alpha = \begin{cases} \dfrac{2-\mu(1-2\phi)}{4} & \mu < \dfrac{2}{3+2\phi} \\ \dfrac{\mu(1+2\phi)}{2} & \dfrac{2}{3+2\phi} < \mu < \dfrac{2}{3} \\ (1-\mu)(1+2\phi) & \mu > \dfrac{2}{3} \end{cases}, \qquad \text{(3.A15)}$$

which is never higher than the unity ($\alpha \leq 1$; Fig. 3.S6).

## Appendix E – Convergence stable strategies in diploid populations

In diploid populations there is no relatedness asymmetry. This means that a focal female shares the same consanguinity with opposite sex siblings ((1+α)/8) and with opposite sex offspring (¼). Also, if we consider a full monogamous population, these values will be exactly the same. This means that changes in the value of male and female offspring are due to changes in consanguinity instead of being due to relatedness asymmetry as well, as in haplodiploid populations. As an outcome of this, we can state that in a population with queen and worker-controlled colonies, the convergence stable sex ratio strategy is the same for both types of colony. For instance, consider a population where a female mates with *N* males. In this case, the consanguinity to offspring is twice the consanguity siblings, but it the same between opposite sexes. This means that both types of colony produce the same convergence stable sex ratio (½). Introducing worker reproduction changes things a little as it increases male reproductive value. In this case, the whole population will produce a sex ratio of (1-*a*)/4, where *a* is the proportion of queen-derived offspring. However, this effect is due to changes in class reproductive value and not to relatedness asymmetry and thus the convergence stable sex ratio will be the same for queen-controlled, worker-controlled and worker derived offspring. Multiple mating reduces the value of siblings; a focal female worker is indifferent between raising own offspring or siblings if full monogamy ($\alpha = 1$), but it will be favoured to produce own offspring if there is multiple mating due to the decrease in relatedness with siblings ($\alpha = (1+\phi)/2$). Hence, we define a standard diploid model, which consists of the simplest scenario for eusociality to be favoured in diploidy and where the potential for helping

is given by $\alpha = 1$, in full monogamy and where the worker values offspring and siblings equally.

## Appendix F – Supporting material

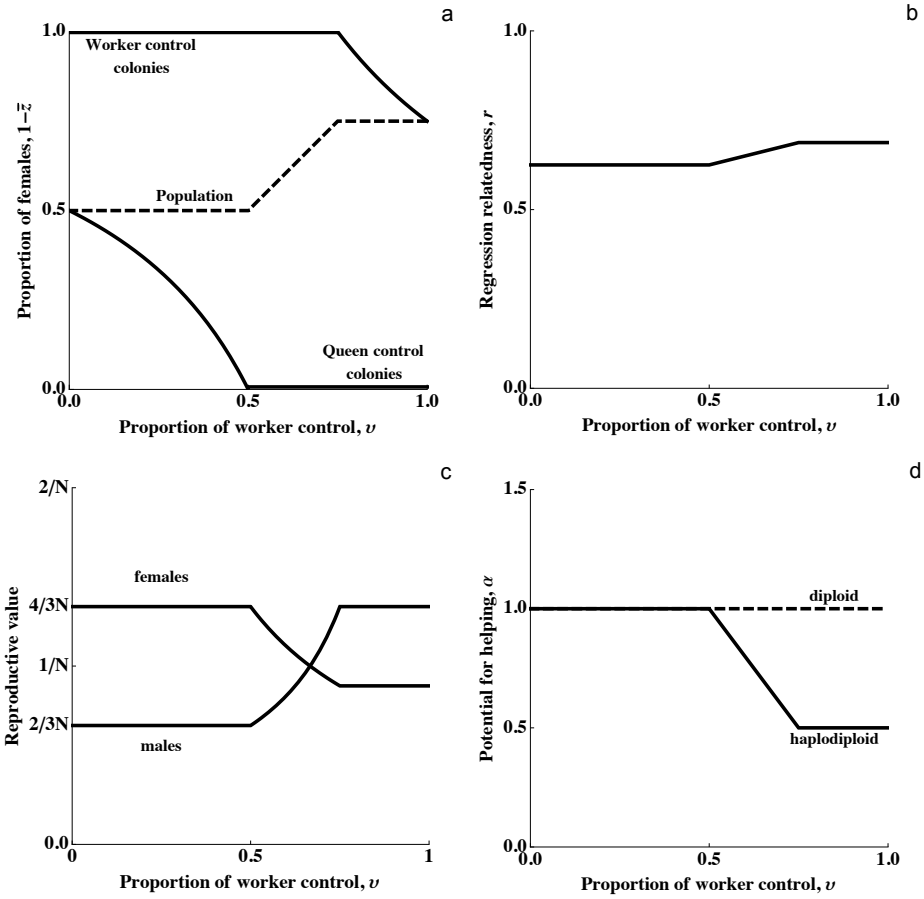


Figure 3.S1. Worker revolution with evolving sex ratios. Worker-controlled colonies are selected to produce a female biased sex ratio and queen-controlled colonies to produce a male biased sex ratio. As worker control spreads through the population, this: (a) leads to a female bias in the population sex ratio; (b) increases the relatedness between workers and the reproductive siblings that they could help raise; (c) increases the reproductive value of males and decreases the reproductive value of females; (d) disfavors helping, with the potential for

helping $\alpha$ being smaller than in the standard diploid model if worker-controlled colonies are more common than queen-controlled colonies ($\alpha < 1$ if $\upsilon > ½$).

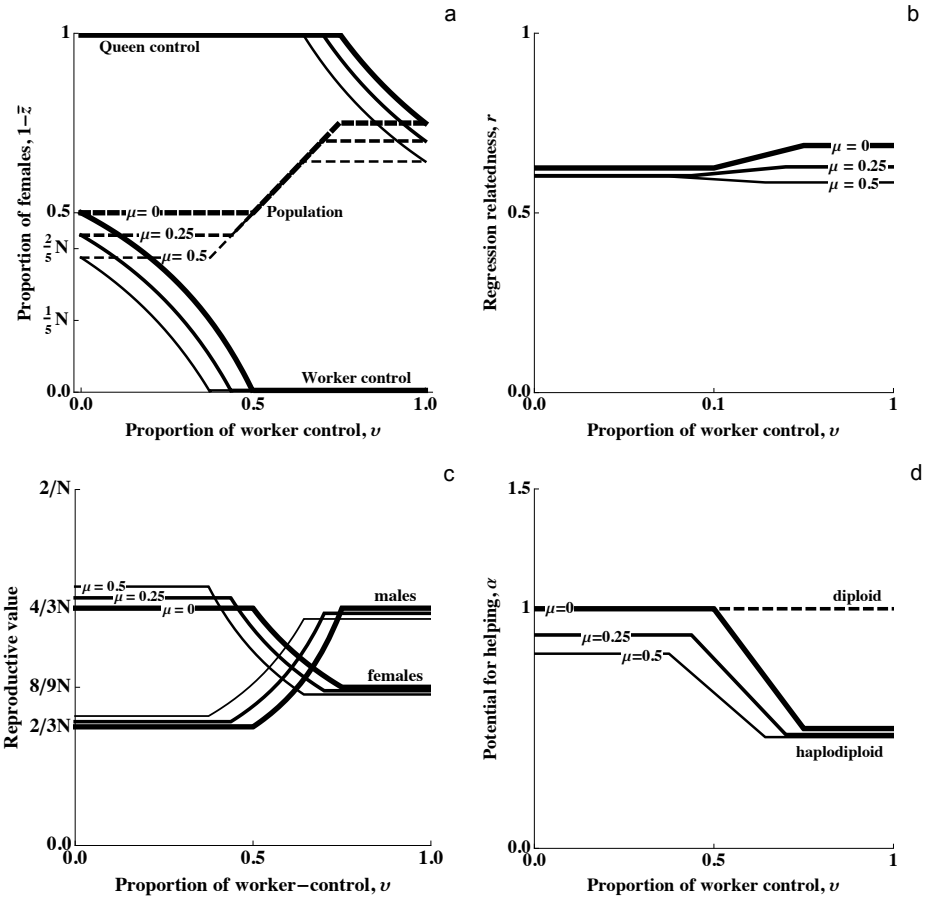


Figure 3.S2. Worker revolution with evolving sex ratios and worker reproduction. Worker reproduction leads to: (a) an increase of male bias in both queen and worker-controlled colonies and thus in the population's sex ratio. As a result, the population sex ratio is male biased if queen-controlled colonies are dominant and female biased if worker-controlled colonies are more common; (b) leads to an decrease of the relatedness ($r$) between potential worker and the colony brood he could help to raise; (c) leads to an increase of male reproductive value ($v_m$) if worker control is rare but to a decrease when worker control is common, and to the opposite for female reproductive value ($v_f$); (d) disfavors helping by decreasing the potential for helping $\alpha$ bellow the unity (the value for the diploid model). The different width of the lines represents different proportions of worker reproduction, where the

thicker line is the scenario with no worker reproduction, the medium line 25% of worker reproduction and the thinner line 50% of worker reproduction.

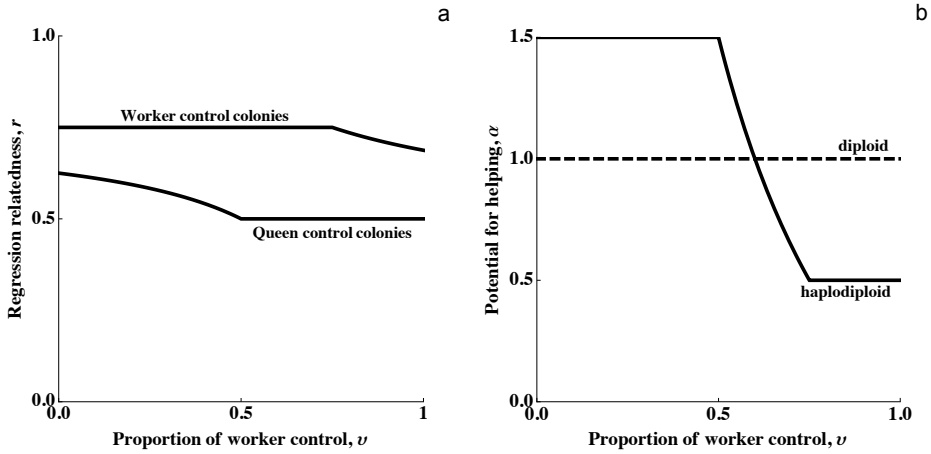


Figure 3.S3. Worker revolution with association between helping and sex allocation, and evolving sex ratios. As worker control spreads through the population, this: (a) leads to a decrease of the relatedness between a worker and her reproductive siblings; (b) initially increases ($\alpha > 1$), but then decreases ($\alpha < 1$) selection for helping ($\alpha = 1$ when $\upsilon = 2/3$).

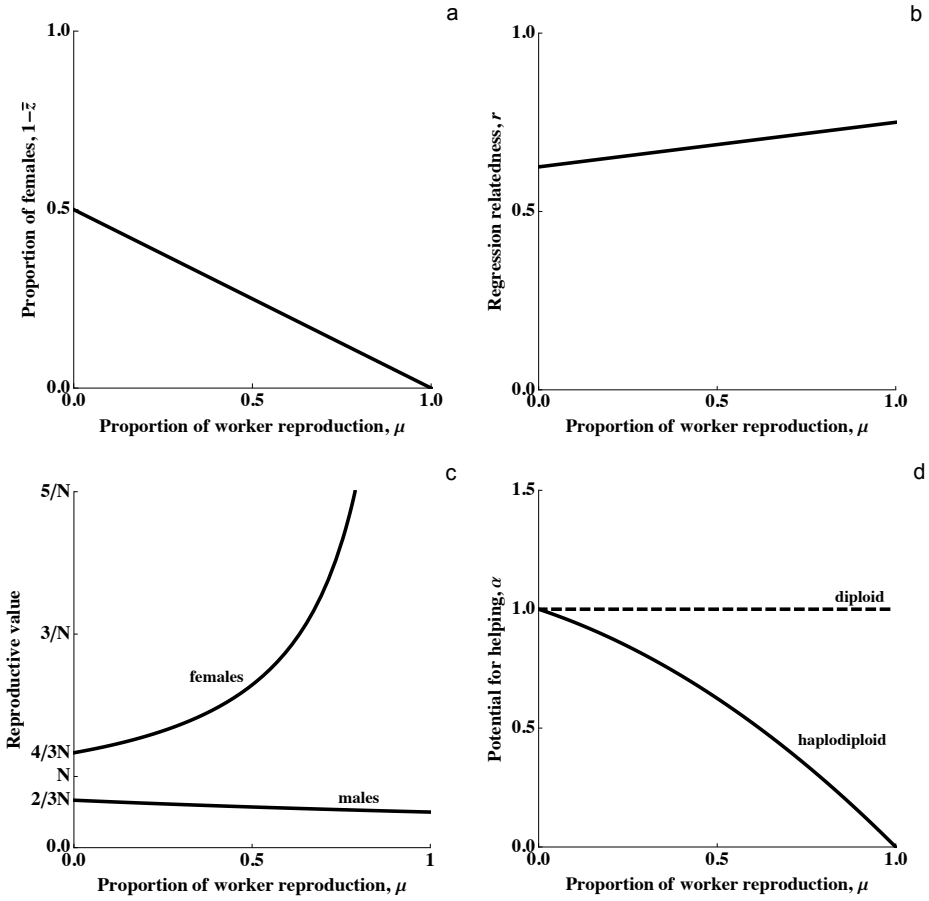


Figure 3.S4. Worker replacement. If workers replace siblings (both sexes) with their own sons, then this leads to: (a) a male bias in the population sex ratio ( $\bar{z} < ½$); (b) an increase the genetic relatedness between workers and the colony offspring (siblings and nephews) they could help raise; (c) an increase in the reproductive value of females ($v_f$) and a decrease in the reproductive value of males ($v_m$); (d) helping being disfavoured ($\alpha < 1$).

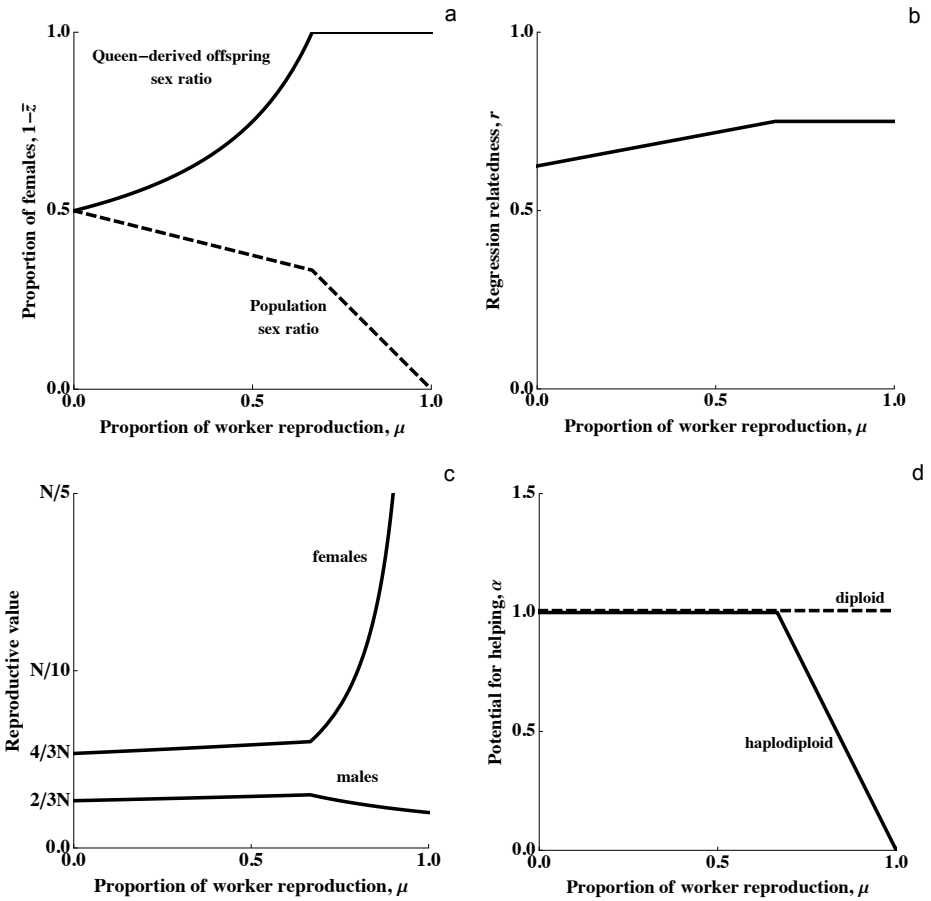


Figure 3.S5. Worker replacement with coevolving sex ratios and queen control. If workers replace siblings (both sexes) with their own sons, then this leads to: (a) queen's producing a female biased offspring sex ratio, but an increasingly male biased population sex ratio; (b) an increase in the relatedness between a potential worker, and the brood that they could help rear; (c) the reproductive value of females increasing, while that of males decreases; (d) disfavors helping if worker replacement is higher than 75% ($\alpha < 1$ if $\mu > ¾$).

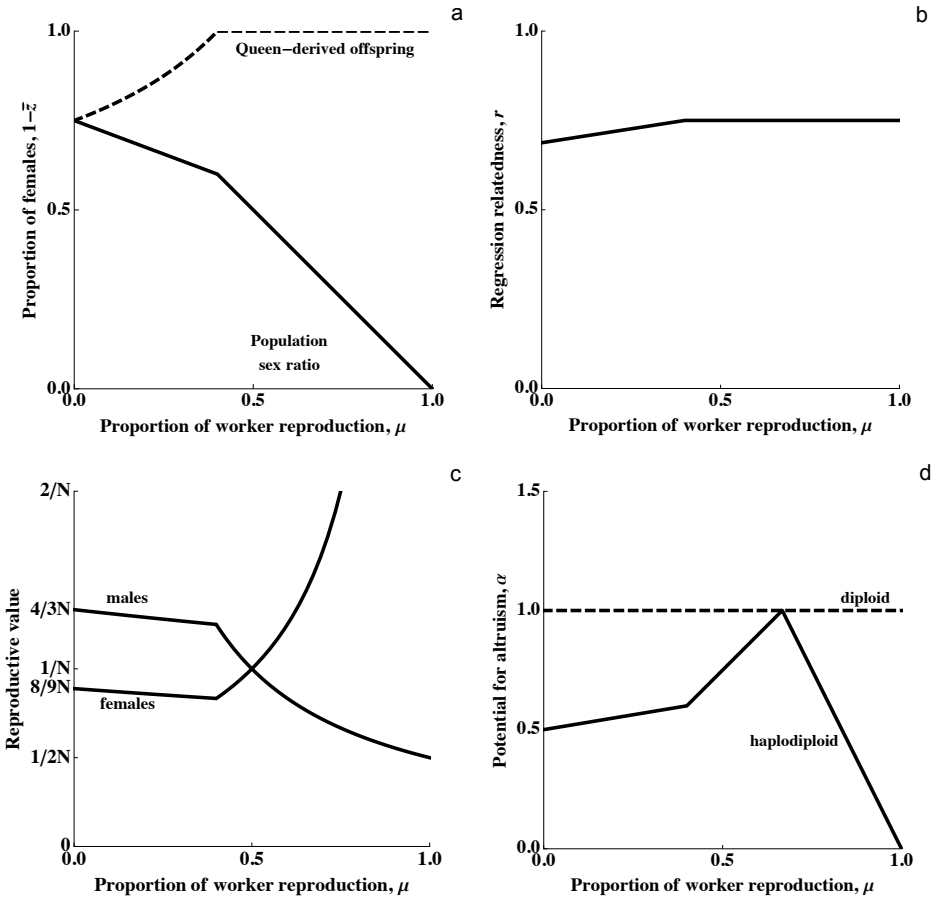


Figure 3.S6. Worker replacement with coevolving sex ratios and worker control. If workers replace siblings (both sexes) with their own sons, then this: (a) leads to an increasingly female biased sex ratio for queen derived offspring, and an increasingly male biased population sex ratio; (b) increases the relatedness between a potential worker, and the brood that they could help rear; (c) increases the reproductive value of females; (d) disfavors helping ($\alpha < 1$ through all the parameter space except when $\mu = 2/3$, $\alpha = 1$).

# 4. Haplodiploidy, and the evolution of eusociality: worker reproduction


## Abstract

The haplodiploidy hypothesis states that the differences in relatedness found between haplodiploid and diploid populations favour the evolution of altruistic helping and eusociality. The haplodiploidy hypothesis requires that some broods are relatively male biased and others relatively female biased, termed split sex ratios. This allows the workers at female biased broods to gain the relatedness advantage of helping a greater proportion of sisters, without being exactly cancelled by a female bias at the population level that reduces the reproduced reproductive value of females. There is empirical evidence for two scenarios, which would lead to split sex ratios: queen virginity and queen replacement. However, previous theoretical analyses have ignored two factors that could influence the evolution of eusociality in these cases. In a previous paper (Gardner et al. 2012), we have shown that the effect of these mechanisms on the facilitation of helping vary with the underlining assumptions. Here we extend those models focusing on the role of worker reproduction. First, worker reproduction within colonies can influence both the relatedness structure within colonies and the relative reproductive value of males and females. Second, independently reproducing workers can be selected or constrained to produce offspring sex ratios that differ from those produced in social colonies. We analyze the consequences of both of these factors and find that in general these factors hinder the evolution of helping. Overall, our results suggest that haplodiploidy may promote or

inhibit the evolution of altruistic helping, depending on other biology-relevant factors, such as multiple mating, association between helping and colony type, or the level of promiscuity in the population.

## Introduction

Advanced or obligate eusociality is defined by the occurrence of: (i) cooperative care of young by individuals other than the mother; (ii) the existence of one or more sterile castes; and (iii) overlap of generations so that a mother, her adult offspring and her young offspring are all alive at the same time (Wilson 1971, 1975 but see Crespi and Yanega 1995). Obligate eusociality is found in the social Hymenoptera (Wilson 1971), termites (Thorne 1997), aphids (Aoki 1977), thrips (Crespi 1992), shrimps (Duffy 1996), and beetles (Kent and Simpson 1992). These groups have achieved a remarkable ecological success, with ants and termites together making up for more than half of insect biomass (Wilson and Hölldobler 2005). The evolution of such an extreme forms of cooperation has puzzled biologists since Darwin drew his attention to the problem (Darwin 1859).

The problem of eusociality is to explain why individuals altruistically give up their own ability to reproduce in order to help others achieve reproductive success (Hamilton 1964a, b, 1972). Hamilton's (1963, 1964a, b, 1970) theory of inclusive fitness reveals that, by helping a relative to reproduce, an individual is able to transmit copies of its own genes to future generations, albeit indirectly. Hamilton (1972) also noted that, under haplodiploidy, fertilized eggs develop into (diploid) females, and

unfertilized eggs develop into (haploid) males. Thus, haplodiploidy leads to a female being more related to her sisters (life-for-life relatedness $R = ¾$) than to her daughters ($R = ½$), assuming an outbred panmictic population where females mate once and an even population sex ratio. This could make it easier for Hamilton's rule to be satisfied and explain the evolution of altruistic traits in haplodiploid populations. Trivers and Hare (1976) pointed out that this "haplodiploidy hypothesis" doesn't necessarily hold, because haplodiploidy also leads to a female being less related to her brothers ($R = ¼$) than to her sons ($R = ½$), which under the assumption of an even sex ratio exactly cancels the benefit of increased relatedness to sisters. Trivers and Hare (1976) suggested that the haplodiploidy hypothesis could still hold if workers preferentially help sisters. However, Craig (1979) showed that this would lead to a female-bias sex ratio in the population resulting in a decrease of the reproductive value of females, and this exactly cancels any relatedness advantage of raising siblings instead of offspring.

One possibility for rescuing the haplodiploidy hypothesis is the existence of split sex ratios in the population, where there is a female bias among an individual's siblings that is not reflected in the population sex ratio (Trivers and Hare 1976; Seger 1983; Grafen 1986). Split sex ratios may be favoured for a number of reasons and have been found in natural populations (Boomsma 1991; Mueller 1991; Meunier et al. 2008; West 2009; Gardner et al. 2012). The argument for the haplodiploidy hypothesis is that variation between colonies in the relatedness of workers to queen-derived offspring may favour workers to preferencially help to rear sisters over own offspring, in those colonies where the sex ratio produced is female biased, while the population sex ratio remains fairly unbiased (Boomsma and Grafen 1990; Boomsma 1991;

Boomsma and Grafen 1991). However, theoretical work has tended to rely on verbal arguments or heuristic arguments, which only address the qualitative question of when could haplodiploidy help, rather than quantitative question of how does haplodiploidy influences the emergence of helping. In addition, because sex ratio variation alters the reproductive value of males relative to females, verbal arguments can even be misleading (Bulmer 1986; Frank 1986).

Gardner et al. (2012) present a framework for the study of the effect of split sex ratios in the evolution of altruistic traits in haplodiploid populations. Of the possible mechanisms that could lead to split sex ratios, several lack empirical support or could arise only when eusociality has already evolved. There are only two mechanisms for split sex ratios where there is evidence that they could have played a role on the route to eusociality: queen virginity and queen replacement (Gardner et al. 2012). Analysing these two cases, Gardner et al. (2012) showed that haplodiploidy may promote or hinder the evolution of altruistic and that the effect of haplodiplody in relation to diploidy depends on ecological factors as the proportion of unmated queens, queen replacement or an association between helping and presence in a female-biased sex ratio colony. However, their analysis made two restrictive asumptions: negligible worker reproduction and that an independently reproducing focal worker would produce the same offspring sex ratio as her colony.

Here, we consider the maintenance and further elaboration of helping, i.e., the conditions necessary for juvenile females to remain in their colony helping to raise the colony offspring. We extend those results presented in Gardner et al. (2012) by focusing on the impact of worker reproduction within the colony. We study the two

biological scenarios described above and divide this article in two parts, the first one dedicated to queen virginity and the second one to queen replacement. In both parts we first relax the assumption of equal sex ratio and allow the focal worker to evaluate her own potential offspring considering two other biologically relevant scenarios: worker producing sons only, as in a population where workers have no possibility of mating and workers producing their convergence stable (optimal) offspring sex ratio. We consider the cases where helping can be either an obligate or a facultative trait.

## General Methods

We consider a newly eclosed female who is choosing to either help her mother to rear the colony's juveniles, or to rear her own offspring, within the same colony. All the notation used in this paper is shown in Table 1. We focus on the maintenance of helping and consider a population where mated queens produce a first cohort consisting of a large number of female workers, followed by a cohort of reproductive juveniles. To account for multiple mating, we define a paternity index $\phi$ as the probability that two maternal sisters share the same father as well. We assume that workers have control over the queen's offspring sex ratio. We allow for worker reproduction and denote $\mu$ as the proportion of worker-derived offspring in the colony (thus a proportion 1 - $\mu$ of the colony offspring is queen-derived). We take an inclusive fitness approach to evaluate from the worker's perspective both the colony offspring and her own offspring. In the first case, the average value to a non-laying worker of an average colony juvenile is $\mu(z_A v_m p_{Ne} + (1 - z_A)v_f p_{Ni}) + (1-\mu)(z_Q v_m p_M + (1 - z_Q)v_m p_F)$, where $z_A$ and $z_Q$ are the sex ratios produced for worker-derived and queen-derived offspring, $v_m$ and $v_f$ are the male and female individual reproductive

values (Appendix A for the derivations), $p_{Ne}$ and $p_{Ni}$ are the consanguinity coefficients of a worker and her nephews and to her nieces, and $p_M$ and $p_F$ are the consanguinity coefficients of a worker to her brothers and to her sisters (Table 2, Appendix B for derivations). The average value of one of the focal female's offspring is $z_O v_m p_S + (1 - z_O) v_f p_D$, where $z_O$ is the sex ratio produced by the worker to her own offspring and $p_S$ and $p_D$ are the consanguinity coefficients of a worker and her son and her daughter. Helping is favoured if the inclusive fitness of the worker increases when the benefit of helping $b$ of the colony offspring is smaller than the cost of not producing $c$ own offspring, i.e. if $b[(\mu(z_A v_m p_{Ne} + (1 - z_A) v_f p_{Ni}) + (1-\mu)(z_Q v_m p_M + (1 - z_Q) v_m p_F)] > c[z_O v_m p_S + (1 - z_O) v_f p_D]$. We may rewrite this condition as $c/b < \alpha$, where $\alpha = [(\mu(z_A v_m p_{Ne} + (1 - z_A) v_f p_{Ni}) + (1-\mu)(z_Q v_m p_M + (1 - z_Q) v_m p_F)]/[ z_O v_m p_S + (1 - z_O) v_f p_D]$ is the potential for helping and represents the threshold at which the worker is indifferent between helping rearing the colony juveniles or producing own offspring. Through this paper, we present general results that consider multiple mating ($\phi \leq 1$). However, in order to distinguish the effects of other biological factors as worker reproduction and queen replacement, we analyze these results under the assumption of monogamy ($\phi = 1$).

Table 1: Summary of model notation used in this article.

| Symbol | Definition |
|---|---|
| $\bar{z}$ | Population sex ratio |
| $z_Q$ | Queen derived offspring sex ratio |
| $z_O$ | Sex ratio strategy of a focal worker breeding independently |
| $z_M$ | Sex ratio strategy for mated-queen colonies |
| $z_R$ | Queenright colony sex ratio strategy for queen-derived offspring |
| $z_L$ | Queenless colony sex ratio strategy for queen-derived offspring |
| $z_A$ | Sex ratio strategy of workers for worker-derived offspring |
| $\mu$ | Proportion of worker reproduction in the population |
| $q$ | Proportion of queen replacement in the population |
| $\phi$ | Paternal kinship index. The probability that any two sperm cells present in a laying female spermatheca (queen or worker) derive from the same male. |
| $p_x$ | Consanguinity between a focal female and a reproductive individual x, present in the same colony. |
| $R_{x,y}$ | Life-for-life relatedness coefficient of individual x to individual y |
| $v_m$ | Value of a male, given by the class reproductive value $c_m$ over the number of males in the population $\bar{z}$ |
| $v_f$ | Value of a female, given by the class reproductive value $c_f$ over the number of females in the population 1- $\bar{z}$ |
| $c_m$ | Male class reproductive value |
| $c_f$ | Female class reproductive value |
| $N$ | Population size |

Table 2: Summary of consanguinity coefficients used in the kin selection analysis.

| Relationship | | In terms of model parameters |
|---|---|---|
| Mother and son | $p_S$ | ½ |
| Mother and daughter | $p_D$ | ¼ |
| Two sisters | $p_F$ | $(1+2\phi)/8$ |
| Brother and sister | $p_M$ | ¼ |
| Nephew and aunt | $p_{Ne}$ | $(1+2\phi)/8$ |
| Niece and aunt | $p_{Ni}$ | $(1+2\phi)/16$ |
| Female and herself | $p_m$ | ½ |
| Male and himself | $p_f$ | 1 |

## Queen Virginity

We consider a population composed of a large number of colonies, each founded by a single female (queen). We assume that a fraction $u$ of the queens do not mate and hence produce only male offspring. The other 1-$u$ females are able to mate multiply and produce workers, who control the sex allocation strategy of the queen's offspring. In this first section we assume worker reproduction to be negligible. As unmated-queen colonies don't have workers, we define $a$ as the productivity of these colonies, as a proportion of the productivity of mated-queen colonies ($0 \leq a \leq 1$). Gardner et al. (2012) found that, if there is no worker reproduction, the convergence stable sex ratio strategy for queen-derived offspring is always female-biased, with the extent of bias increasing with the increase of frequency of unmated queens ($z_M^* = ¾$ if $u = 0$ and $z_M^*$

< ¼ if $u > 0$). We extend this results and found that: $z_M^* = (1-(u(1+a+2a\phi))/(2(1-\mu)(1+\phi)))$ if $u \leq 1/(1+a+2a\phi)$ and $z_M^* = 0$, otherwise.

In order to evaluate the focal worker's offspring, Gardner et al. (2012) assumed that she is constrained to produce the same sex ratio as her colony. Here, we extend that by considering two other scenarios: the worker produces her convergence stable sex ratio strategy and worker producing sons. Due to worker control, the population sex ratio is female biased if unmatedness is rare. However, as the probability of unmatedness increases the production of male offspring by unmated queens leads to a male bias in the population sex ratio (Fig. 1a). Thus, if the focal worker produces her convergence stable sex ratio strategy, she is favoured to produce sons if $u < 1/(1+a)$ and daughters otherwise. In this case, the potential for helping is $\alpha = [\bar{z}_M v_m p_M + (1-\bar{z}_M) v_f p_F]/[z_O^* v_m p_S + (1- z_O^*) v_f p_D]$, where $z_O^*$, where $z_O^*$ is the worker convergence stable sex ratio strategy. In terms of the model parameters, this is:

$$\alpha = \begin{cases} \frac{1}{2} & u \leq \frac{1}{1+a(1+2\phi)} \\ \frac{au(1+2\phi)}{2(1-u)} & \text{if} \quad \frac{1}{1+a(1+2\phi)} < u < \frac{1}{a+1} \\ \frac{1+2\phi}{2} & u \geq \frac{1}{a+1} \end{cases}, \qquad (4.1)$$

**Result 1:** If the focal worker produces the same sex ratio as her colony then haplodiploidy promotes helping if $u > ((1-u)(1+\phi))/(1-\phi(1-2a))$. This means that under monogamy ($\phi = 1$) and equal productivity ($a = 1$) helping is always promoted ($\alpha > 1$; Fig. 1b). However, if the worker produces her convergence stable sex ratio strategy or

if she is limited to produce sons, haplodiploidy promotes helping only if the proportion of unmated females is higher than a threshold ($u > 2/(2+a+2a\phi)$; Fig. 1b). If we consider the empirical range of unmatedness ($0 \leq u \leq 0.06$), the potential for helping is marginally promoted, with a maximum of $\alpha = 1.068$ (assuming full monogamy and equal productivity between colonies). However, if the focal worker produces either sons or her convergence stable sex ratio, helping is never promoted at the empirical range of unmatedness ($\alpha = ½$). In both cases, the potential for helping increases its value as the paternity index increases, showing that monogamy promotes helping rearing the colony offspring.

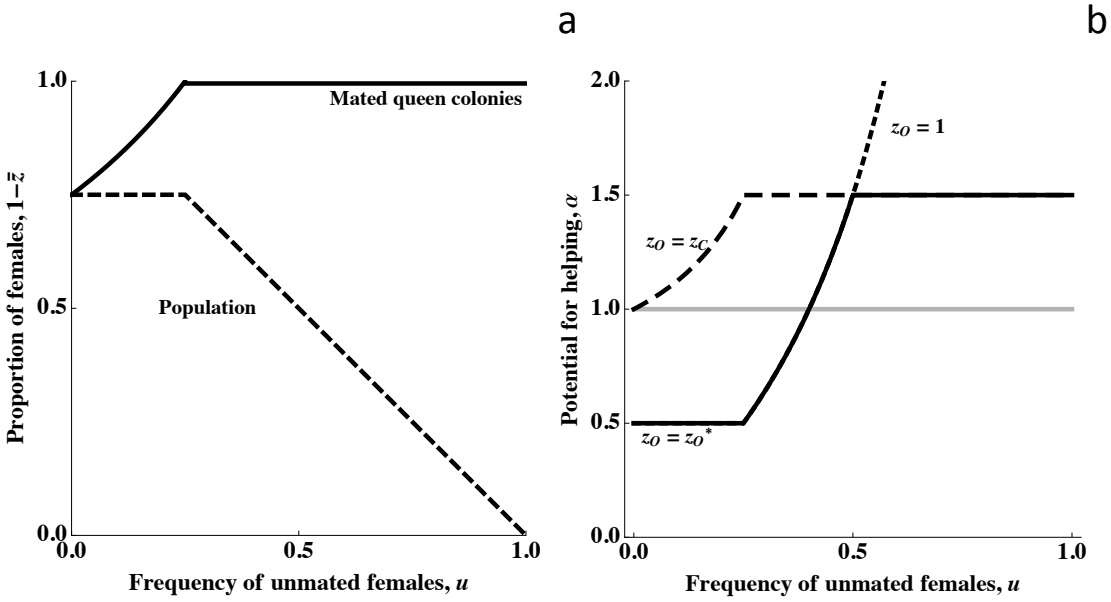


Figure 4.1. Queen virginity. (a) As the frequency of unmated queens increases, the convergence stable sex ratio for mated queen colonies turns increasingly female-biased and the population sex ratio becomes male biased. (b) Helping is always favoured if the worker produces the same sex ratio as her colony. However, if she produces the convergence stable sex ratio for her own offspring or if she is limited to the production of sons, helping is only favoured if $u > 2/(2+a+2a\phi)$. Filled line: focal worker produces convergence stable sex ratio;

dashed line: focal worker produces the same sex ratio as her colony; dotted line: focal worker produces sons.

*Workers produce sons only*

We now introduce worker reproduction by assuming that workers are able to lay a proportion $\mu$ of the colony offspring. Queens from mated-queen colonies may produce female workers while unmated queen colonies don't. If workers are limited to the production of sons, we found that the convergence stable sex ratio strategy for queen-derived offspring is:

$$\bar{z}_M^* = \begin{cases} \dfrac{2-\mu(3+2\phi)-u(2-3\mu-2\mu\phi+2a(1+2\phi))}{4(1-u)(1-\mu)(1+\phi)} & \text{if} \quad u \le \dfrac{2-3\mu-2\mu\phi}{2+2a-3\mu+4a\phi-2\mu\phi} \\ 0 & \text{if} \quad u > \dfrac{2-3\mu-2\mu\phi}{2+2a-3\mu+4a\phi-2\mu\phi} \end{cases} \quad .(4.2)$$

Worker reproduction influences the outcome of the potential for helping. As the production of male offspring by the workers increases, the population sex ratio goes from a female bias, due to worker control, to a male bias. In this sense, the effect of worker reproduction is similar to the effect of unmatedness (Fig. 4.S2a). However, worker production of sons leads as well to an increase of the relatedness between the worker and her colony offspring due to the higher relatedness shared with nephews than with brothers (Fig. 4.S3b). Both worker reproduction and unmatedness also lead to an decrease of male reproductive value as these become common and to an increase of female reproductive value, as these become rarer in the population (Fig. 4.S3c).

As with the other workers, we assume that the focal worker may either produce sons or help rearing the colony offspring. Thus, the inclusive fitness valuation made by a focal worker of the colony offspring is $(1-\mu)[\ \bar{z}_M v_m p_M + (1-\ \bar{z}_M) v_f p_F] + \mu v_m p_{Ne}$, and the valuation made of her own offspring is $v_m p_S$. In this case, the potential for helping is given by:

$$\alpha = \begin{cases} \dfrac{8(1+\phi) - 2\mu(7+6\phi) + \mu^2(5-4\phi^2) - u\mathrm{K}}{16(1-u)(1-\mu)(1+\phi)} & \text{if} \quad u \le \dfrac{2-3\mu-2\mu\phi}{2+2a-3\mu+4a\phi-2\mu\phi} \\ \dfrac{(2au+\mu-u\mu)(1+2\phi)}{4(1-u)} & \text{if} \quad u > \dfrac{2-3\mu-2\mu\phi}{2+2a-3\mu+4a\phi-2\mu\phi} \end{cases} \quad ,(4.3)$$

where $K = 8(1+\phi)-\mu(14-5\mu+12\phi+4\mu\phi^2-2a(1+2\phi)^2)$.

**Result 2:** Worker reproduction may promote the evolution of helping. If the focal worker is limited to the production of sons, helping is promoted if the proportion of unmated females is higher than a threshold ($\alpha > 1$ if $u > (4-\mu-2\mu\phi)/(4-\mu-2\mu\phi+2a(1+2\phi))$; Fig. 4.2a). Considering monogamy and equal productivity, helping is favoured if $u > (4-3\mu)/(10-3\mu)$. This means that, as worker reproduction increases, so does the range at which the potential for helping is higher than the unity and helping is promoted. However, if we consider the empirical range of unmatedness ($0 \le u \le 0.06$), helping is never promoted ($\alpha < 1$).

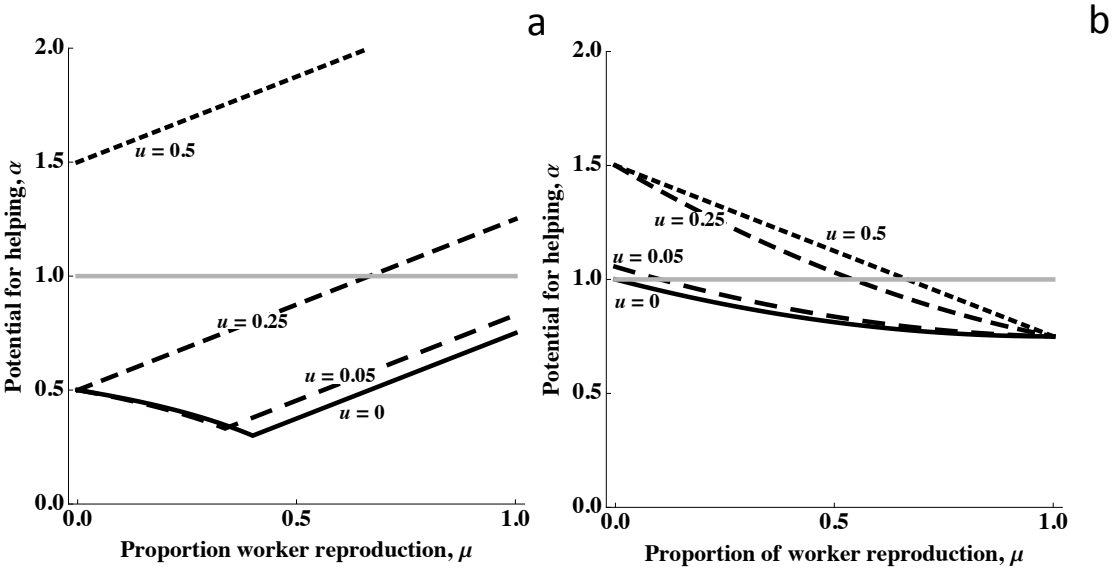


Figure 4.2. Queen virginity and worker reproduction. If workers from mated-queen colonies produce a proportion $\mu$ of the colony offspring then: (a) if workers produce sons, worker reproduction increases the potential for obligate helping. However, for the empirical range found for unmatedness ($0 \leq u \leq 0.1$), obligate helping is never promoted ($\alpha \leq 0$) independently of the proportion of worker-derived offspring. (b) If workers produce the same sex ratio as their colony, helping may be favoured for if unmatedness is relatively high ($\alpha > 1$ if $u > (4-\mu-2\mu\phi)/[4-2\mu(1+2\phi)+2a(1+2\phi)]$), but the potential for helping decreases with an increase of the proportion of worker-derived offspring.

In appendix E we show that if the focal worker produces her convergence stable sex ratio strategy helping is favoured under the same condition as the above scenario. However, in this case as worker reproduction increases, the maximum value of the potential for helping decreases as worker reproduction increases, and helping is only favoured if $\mu < [2(-u(2+a(1+2\phi)))]/[(1-\mu)(1+2\phi)]$. As before, considering monogamy and equal productivity among colonies, helping is never favoured ($\alpha < 1$). Also, monogamy increases the maximum value of the potential for helping.

*Workers produce the same sex ratio as their colony*

We relax the assumption of unmatedness by the workers and assume that workers produce the same sex ratio as the colony. In this case, the effect of worker reproduction is to increase the class reproductive value of males, leading the colony and the population sex ratio to become less female-biased, when unmatedness is rare. As before, an increase of the proportion of unmated queens leads to an increase of males in the population, resulting in a male-biased population sex ratio (Fig. 4.S5a). This also leads to an increase of the reproductive value of females (Fig. 4.S5b) and to mated queen colonies to produce only female reproductive (Fig. 4.S5a). Thus, as the colony convergence stable sex ratio is to produce an increasingly higher proportion of female reproductive, worker reproduction leads to a decrease of the relatedness shared between the worker and her colony offspring (Fig. 4.S5c). The convergence stable sex ratio strategy for mated queen colonies is:

$$\bar{z}_M^* = \begin{cases} \dfrac{2+\mu-u(2+\mu+a(2+4\phi))}{4(1-u)(2-\mu+(2-(1-\mu)\mu)\phi)} & \text{if} \quad u \le \dfrac{2+\mu}{2+2a+\mu+4a\phi} \\ 0 & \text{if} \quad u > \dfrac{2+\mu}{2+2a+\mu+4a\phi} \end{cases} . \qquad (4.4)$$

In this situation, a focal worker limited to produce the same sex ratio as her colony the inclusive fitness evaluation she does of the colony offspring is $\bar{z}_M v_m((1-\mu)p_M + \mu p_{Ne}) + (1-\bar{z}_M)v_f((1-\mu)p_F + \mu p_{Ni})$ and the inclusive fitness evaluation of her own offspring is $z_M v_m p_S + (1-\bar{z}_M)v_f p_D$. In this case, the potential for helping is:

$$\alpha = \begin{cases} \dfrac{2-\mu+(2-\mu(1-\mu))\phi-u(2(1+\phi)-\mu(1+\phi(1-\mu-a(1+2\phi))))}{4(1-u(1+a\phi))} & \text{if} \quad u \le \dfrac{2+\mu}{2+\mu+2a(1+2\phi)} \\ \dfrac{(2+\mu)(1+2\phi)}{4} & \text{if} \quad u > \dfrac{2+\mu}{2+\mu+2a(1+2\phi)} \end{cases}$$

(4.5)

**Result 3:** Worker reproduction decreases the value of the potential for helping and thus inhibits helping. Result 1 showed that, under full monogamy and equal colony productivity, helping is always favored when there is no worker reproduction ($\alpha > 1$ when $\mu = 0$). As worker reproduction increases, the potential for helping decreases and is no longer always higher than the unity. In this case, helping is favoured if the frequency of unmatedness is higher than a threshold ($\alpha > 1$ if $u > (2\text{-}\mu)\mu/[4\text{-}\mu(1\text{-}\mu)]$; Fig. 4.2b). Considering as well the empirical range of unmatedness ($0 \le u \le 0.06$), we have seen in Result 1 that the maximum value for helping is $\alpha = 1.068$. Helping is favoured if the proportion of worker-derived offspring is lower than 12% ($\alpha > 1$ if $\mu <$ 0.123).

In Appendix C, we show that if the focal worker produces her convergence stable sex ratio strategy, the value of the potential for helping decreases with worker reproduction as well. In this case, helping is promoted only for high values of unmatedness and low values of worker reproduction ($\alpha > 1$ if $u >$ $[2(2+\mu)]/[2(2+\mu)+a(2\text{-}\mu)(1+2\phi)]$; Fig. 4.S2b).

## Queen Replacement

We follow Gardner et al. (2012) by considering a population composed of an infinite number of colonies, each one founded by a single multiply mated female (queen). The queen produces a first cohort of non-dispersing females (workers) who can help raise the queen's offspring or raise their own offspring within the colony. We assume that the workers have full control over the operational sex ratio produced for the queen's offspring. While in a proportion of $q$ colonies the queen survives to produce a proportion of $z_R$ males and of 1-$z_R$ females (queenright colonies), in a proportion 1-$q$ of the colonies the queen is replaced by one of her first cohort daughters and these queenless colonies produce a proportion of $z_L$ males and of 1-$z_L$ females (queenless colonies). Females can mate with multiple males and we define the paternity index $\phi$ as the probability that two sisters share the same paternal allele for a given gene. We start following Gardner et al. (2012) by assuming that worker reproduction is negligible ($\mu = 0$). After we relax this assumption ($\mu > 0$) and consider that workers produce a proportion $\mu$ of the colony offspring, with a sex ratio $z_A$. Also, we consider that the paternity index $\phi$ is the same for original and replacement queens. In that case, the population sex ratio is: $\bar{z} = \mu z_A+(1-\mu)(qz_R+(1-q)z_L)$. We focus on two situations concerning worker sex allocation: workers producing sons ($z_A = 1$) and workers producing the same sex ratio as their colony ($z_A = z_C$). After reaching maturity, male and female reproductive offspring disperse and mate at random. Mated females found new colonies and give origin to the next generation of individuals.

Gardner et al. (2012) showed that, if worker reproduction is negligible, queen replacement leads to split sex ratios between queenright and queenless colonies, with

a female biased sex ratio being favoured by workers in queenright colonies, and a male biased sex ratio in queenless colonies. To find the potential for helping, they assumed that the focal worker produces the same sex ratio as her colony and found that: if helping is obligate it is never promoted ($\alpha \leq 1$); if helping is facultative then it is always promoted ($\alpha > 1$ if $q < 1$). We extend these results by looking at two other scenarios: the focal worker is limited to produce sons and the worker produces her convergence stable sex ratio strategy. As the frequency of queen replacement increases, the population sex ratio goes from a female bias (as queenright colonies produce a female-biased sex ratio) to a male bias (queenless colonies produce a male-biased sex ratio; Fig. 4.3a). Through the range of queen replacement, a focal worker values more or equally the production of sons than the production of daughters and thus the conditions for the potential for helping are equal for these two scenarios, i.e., the valuation that a worker does to her own offspring is $v_m p_S$. Hence, if helping is an obligate trait, the potential for helping is:

$$\alpha_{\mathrm{OBL}} = \begin{cases} \dfrac{(1+q)(1+2\phi)}{4} & q < \dfrac{1}{3+\mu} \\ \dfrac{(1-q)(1+2\phi)}{2} & \text{if} \quad \dfrac{1}{3} \leq q \leq \dfrac{1+2\phi}{3+2\phi} \\ \dfrac{1+q+2\phi-2q\phi}{4} & q > \dfrac{1+2\phi}{3+2\phi} \end{cases} . \qquad (6)$$

This result is a generalization of Gardner et al. (2012) relaxing the assumption of monogamy (in this case $0 \leq \phi \leq 1$).

If helping is a facultative trait conditional to the type of colony to which the worker belongs to, we is expected it to be expressed in queenright colonies, as these produce

a female biased sex ratio – increasing the relatedness of the focal worker to the colony offspring. In this case, we found that the potential for helping is:

$$\alpha_{\mathrm{FAC}} = \begin{cases} \dfrac{1+2\phi}{2} & q < \dfrac{1}{3+\mu} \\ \dfrac{(1-q)(1+2\phi)}{4q} & \text{if} \quad \dfrac{1}{3} \le q \le \dfrac{1+2\phi}{3+2\phi} \\ \dfrac{1}{2} & q > \dfrac{1+2\phi}{3+2\phi} \end{cases} . \qquad (7)$$

This result follows Gardner et al. (2012), but relaxing the assumption of monogamy (in this case $0 \le \phi \le 1$).

**Result 3:** If the focal worker produces her convergence stable sex ratio strategy or if she is limited to the production of sons, then this reduces the potential for helping. Considering obligate helping, queen replacement always inhibits the evolution of helping ($\alpha \le 1$; Fig. 4.3b). Facultative helping is favoured in queenright colonies, if the focal produce the same sex ratio as their colony. If the focal worker produces sons or her convergence stable sex ratio, helping is favoured if $q \le (1+2\phi)/(5-2\phi)$. Considering monogamy $q < 3/7$, meaning that within the empirical range for the probability of queen survival ($0.6 \le q \le 0.8$), facultative helping is promoted only if the focal worker produces the same sex ratio as her colony (Fig. 4.3c). An increase of the paternity index $\phi$ results in an increase of the potential for helping, and thus monogamy ($\phi = 1$) promotes helping.

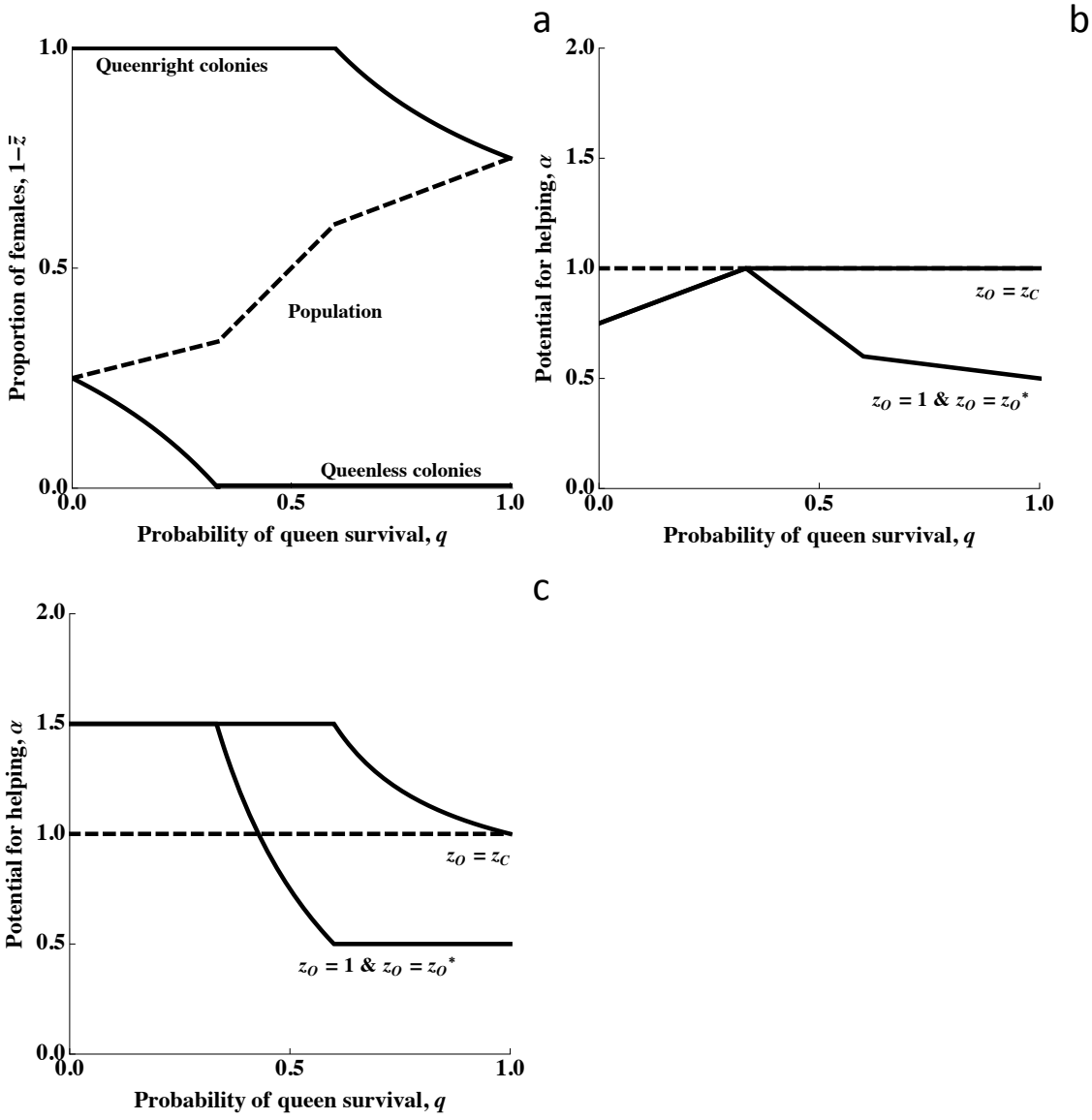


Figure 4.3. Queen replacement. (a) Worker reproduction leads to split sex ratios in the population, with queenright colonies producing a female-biased sex ratio and queenless colonies producing a male-biased sex ratio. The population sex ratio depends on the proportion of queenright and queenless colonies in the population. (b) Obligate helping is never promoted, independently of the focal worker producing either sons, both sex siblings with the same sex ratio as her colony or both sex siblings at her convergence stable sex ratios strategy. (c) Facultative helping is always promoted if the focal worker produces the same sex ratio as her colony and may be promoted in the other two scenarios, if the frequency of queen replacement is lower than a threshold ($q \leq (1+2\phi)/(5+2\phi)$).

*Workers produce sons only*

Consider a population where workers can produce sons only. In this case, the workers from a given colony have not only the control over the sex ratio of the queen's offspring but also produce a proportion $\mu$ of the colony offspring. As a result, male class reproductive value increases. This is because there is an increase of the proportion of paternal genetic contribution found in workers in the census of the next generation's reproductive offspring. However, worker production of sons also leads to a male bias in the population sex ratio and in this way to a decrease in the male individual reproductive value.

We found that the unbeatable sex ratio strategies for queenright and queenless queen-derived offspring are given by:

$$(\bar{z}_L,\bar{z}_R)=\begin{cases}(0,0) & \text{if} \quad \mu>\dfrac{3-q}{4-q} \\ \left(\dfrac{3-q(1-\mu)-4\mu}{4(1-q)(1-\mu)},0\right) & \text{if} \quad \mu\le\dfrac{3-q}{4-q} \quad \text{and} \quad q\le\dfrac{1}{3(1-\mu)} \\ (1,0) & \text{if} \quad \mu\le\dfrac{3-q}{4-q} \quad \text{and} \quad \dfrac{1}{3(1-\mu)}<q\le\dfrac{1+2\phi}{(1-\mu)(3+2\phi)} \\ \left(1,-\dfrac{1+2\phi-q(1-\mu)(3+2\phi)}{4q(1-\mu)(1+\phi)}\right) & \text{if} \quad \mu\le\dfrac{3-q}{4-q} \quad \text{and} \quad q>\dfrac{1+2\phi}{(1-\mu)(3+2\phi)}\end{cases}$$

.(4.8)

Worker production of sons increases the number of males in the population and thus drives both queenright and queenless to have a more female biased colony sex ratio. If there is no worker reproduction, the sex ratio of queenright colonies varies between 75% and 100% females and the sex ratio of queenless colonies varies between 0%

and 25% females, depending on the probability of queen survival. If workers produce 25% of of each colony offspring, the sex ratio of queenright colonies varies between 87.5% and 100% females, while the sex ratio in queenless colonies varies between 0% and 33.3% of females produced (Fig. 4.S6a). In terms of colony relatedness, worker reproduction means that, from the point of view of a worker, the colony offspring is now constituted not only of siblings but of nephews as well. Thus, if queen replacement is rare in the population, as the proportion of queen replacement increases, there will be a higher proportion of reproductive sisters and nephews being raised and as a result there is an average increase in relatedness between a worker and her colony reproductive juveniles (Fig 4.S6c). However, as queen replacement increases, the average relatedness to the colony offspring decreases. In this case worker reproduction decreases the number of siblings produced in queenright colonies leading to a slight decrease on relatedness. Due to its male biasing effect in the population sex ratio, worker reproduction also leads to a decrease in the reproductive value of males, when queen replacement is rare (Fig. 4.S6b).

If helping is an obligate trait, not influenced by the type of colony the worker belongs to, then the average inclusive fitness valuation of the colony offspring value, made by the worker is given by $(1-\mu)(q(z_R v_m p_M + (1-z_R)v_f p_F)) + (1-q)(z_R v_m p_{Ne} + (1-z_R)v_f p_{Ni})) + \mu v_m p_{Ne}$. If workers produce sons, then the average inclusive fitness valuation that a worker does to her own offspring is $v_m p_S$. In this case, the potential for helping is:

$$\alpha_{\mathrm{OBL}} = \begin{cases} \dfrac{\mu + 2\mu\phi}{3-q} & \text{if} \quad \mu > \dfrac{3-q}{4-q} \\ \dfrac{1+q(1-\mu)(1+2\phi)}{4} & \text{if} \quad \mu \le \dfrac{3-q}{4-q} \quad \text{and} \quad q \le \dfrac{1}{3(1-\mu)} \\ \dfrac{1+q(1-\mu)(1+2\phi)}{2} & \text{if} \quad \mu \le \dfrac{3-q}{4-q} \quad \text{and} \quad \dfrac{1}{3(1-\mu)} < q \le \dfrac{1+2\phi}{(1-\mu)(3+2\phi)} \\ \dfrac{1+2\phi+q(1-\mu)(1+2\phi)}{4} & \text{if} \quad \mu \le \dfrac{3-q}{4-q} \quad \text{and} \quad q > \dfrac{1+2\phi}{(1-\mu)(3+2\phi)} \end{cases} . \tag{4.9}$$

**Result 4:** In a population with queen replacing and workers producing sons, obligate helping may be promoted if the frequency of worker reproduction is higher than a threshold ($\mu > (3\text{-}q)/(4\text{-}q)$; Fig. 4.4a). However, considering monogamy ($\phi = 1$) and the empirical range of queen replacement ($0.6 \le q \le 0.8$), helping is only favoured if the proportion of worker-derived offspring in the colony is higher than about 69% ($\alpha > 1$ if $\mu > 0.6875$). As in previous models, monogamy ($\phi = 1$) increases the value for the potential for altruism.

In appendix D we show that the potential for helping is similar to the previous scenario, with the exception of when $\mu \ge (3\text{-}q)/(4\text{-}q)$. In this case, the focal worker is favoured to produce daughters and the potential for helping is never higher than the unity (Fig. 4.S5a). Thus, in this case worker reproduction never promotes helping.

Now we consider helping as a facultative trait. As queenright colonies produce a more female-biased sex ratio than queenless colonies, helping could potentially be promoted if there is an association between the helping trait and presence in queenright colonies. In this case, the valuation of the colony offspring made by a

queenright colony worker is $(1-\mu)(z_R v_m p_M + (1-z_R)v_f p_F) + \mu p_{Ne}$. In the case the focal worker is limited to produce her colony sex ratio the potential for helping is:

$$\alpha_{FAC} = \begin{cases} \dfrac{(5-q)\mu(1+2\phi)}{4(3-q)} & \text{if} \quad \mu > \dfrac{3-q}{4-q} \\ \dfrac{(2-\mu)(1+2\phi)}{4} & \text{if} \quad \mu \le \dfrac{3-q}{4-q} \quad \text{and} \quad q \le \dfrac{1}{3(1-\mu)} \\ \dfrac{(1-q(1-2\mu))(1+2\phi)}{4q} & \text{if} \quad \mu \le \dfrac{3-q}{4-q} \quad \text{and} \quad \dfrac{1}{3(1-\mu)} < q \le \dfrac{1+2\phi}{(1-\mu)(3+2\phi)} \\ \dfrac{2-\mu(1-2\phi)}{4} & \text{if} \quad \mu \le \dfrac{3-q}{4-q} \quad \text{and} \quad q > \dfrac{1+2\phi}{(1-\mu)(3+2\phi)} \end{cases} . \tag{4.10}$$

**Result 5:** In a population where workers produce sons, helping as a facultative trait may be promoted by haplodiploidy. In particular, helping is favoured by high proportion of worker reproduction and low frequency of queen survival ($\alpha > 1$ if $\mu > (3-q)/(4-q)$) or if $q < (1+2\phi)/(5-2\mu+2\phi-4\mu\phi)$)(Fig. 4.4c).

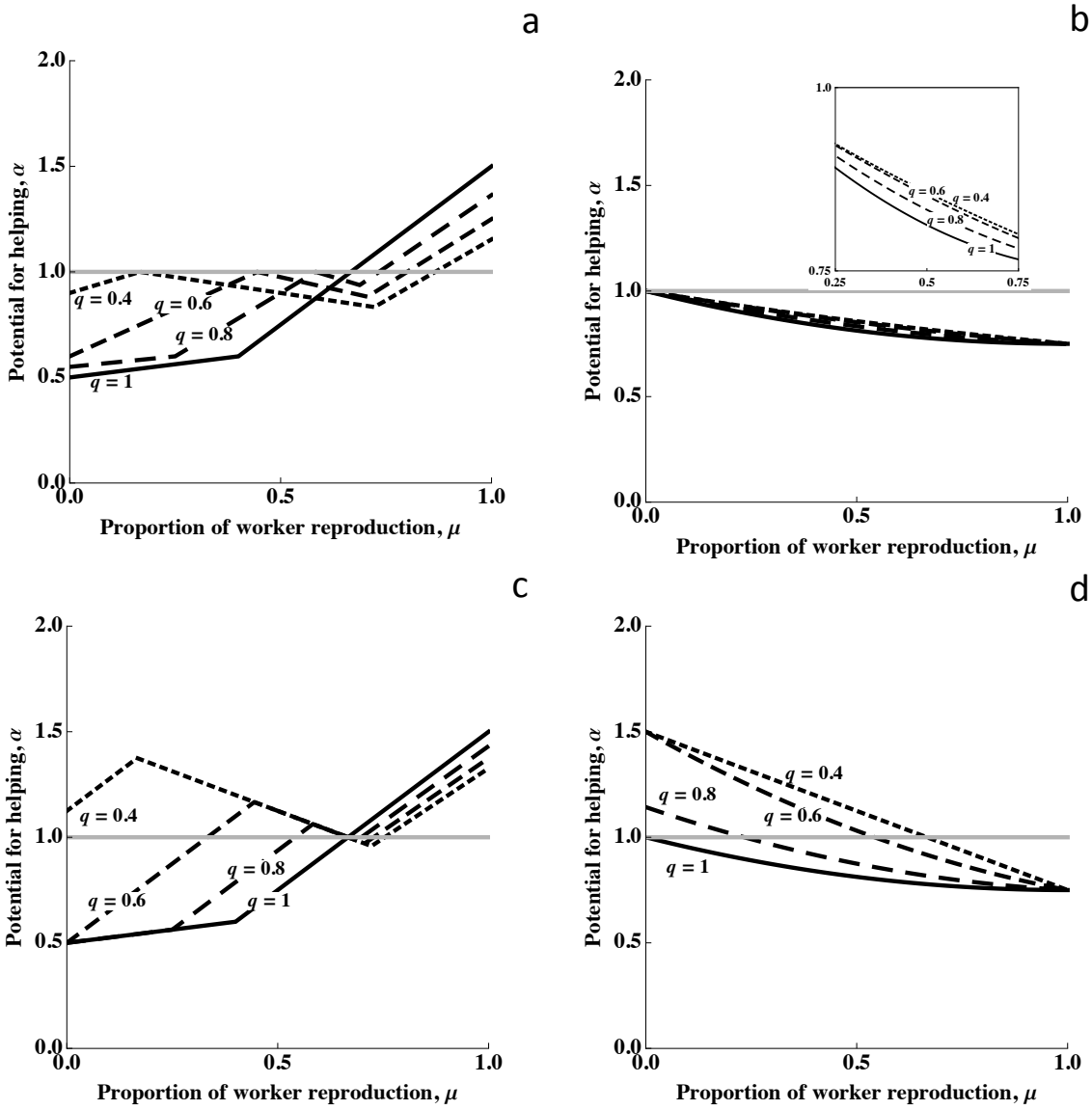


Figure 4.4. Queen replacement and worker reproduction. (a) If workers produce sons, obligate helping may be promoted by worker reproduction if the proportion of worker reproduction is high ($\mu > (3-q)/(4-q)$). In this case, queen-derived offspring are composed only of females and the focal worker is favoured to help raising her sisters. (b) If workers produce the same sex ratio as their colonies, obligate helping is never favoured and the potential for helping decreases with worker reproduction. (c) If workers produce sons facultative helping may be promoted by queen replacement, in particular for lower values of unmatedness ($\alpha > 1$ if $q < (1+2\phi)/(5-2\mu+2\phi(1-2\mu))$) in the population or if the proportion of worker-derived offspring in the population is high ($\alpha > 1$ if $\mu > (3-q)/(4-q)$). (d) If workers produce the same sex ratio as

their colonies, faculative helping may be promoted, in particular, considering the empirical range of queen survival ($0.6 \leq q \leq 0.8$), if the frequency of queen survival is low.

*Workers produce sons and daughters*

Here we relax the assumption of workers producing offspring only from unfertilized eggs and assume that they can mate, but where their sex allocation strategy is the same as the one found in their colony for queen-derived offspring. Worker reproduction increases male class reproductive value to increase, as the proportion of genes censored in the next generation of paternal origin increases with worker reproduction. This leads worker reproduction changing the convergence stable colony sex ratio strategies for both queenright and queenless colonies. We found that the joint convergence stable sex ratio strategies are:

$$\left(\bar{z}_L,\bar{z}_R\right)=\begin{cases}\left(\dfrac{3-q+q\mu}{4(1-q)},0\right) & q<\dfrac{1}{3+\mu}\\ (1,0) & \text{if}\quad \dfrac{1}{3+\mu}\leq q\leq\dfrac{1+2\phi}{3+\mu+2\phi}\\ \left(1,-\dfrac{1+2\phi-q(3+\mu+2\phi)}{2q(2+(2-\mu)\phi)}\right) & q>\dfrac{1+2\phi}{3+\mu+2\phi}\end{cases}. \quad (4.11)$$

Worker reproduction decreases the female bias in queenright colonies, if the frequency of queen replacement is low, and it decreases the male bias in queenless colonies if queen replacement is common (Fig. 4.S7a). As in the previous model, queen replacement leads to an initial increase of the average relatedness between a random worker and the juveniles in her colony due to the high relatedness between a worker and her nephew (Fig. 4.S7c). However, in this case, worker reproduction

decreases the average colony relatedness, due to the lower relatedness shared between a worker and her nieces (Fig. 4.S7b). As queen replacement increases in the population, female individual reproductive value increases and in a population with complete queen replacement, females are worth twice as males ($q = 1$, $v_m = ½\ v_f$; Fig. 4.S7c).

We now focus on the worker's decision of whether or not to help rear the reproductive juvelines of her colony. First we look at helping as an obligate trait. In this case, the focal worker's valuation of her colony offspring is $q(z_R v_m((1-\mu)\, p_M + \mu p_{Ne}) + (1-z_R)v_f\,((1-\mu)\, p_F+\mu p_{Ni})) + (1-q)(z_L v_m p_{Ne} + (1-z_L)v_f p_{Ni})$. If the focal worker produces the same sex ratio as her colony, then her valuation of the colony offspring is $q(z_R v_m p_S + (1-z_R)v_f p_D) + (1-q)(z_L v_m p_S + (1-z_L)v_f p_D)$. In this case, the potential for helping is:

$$\alpha_{OBL} = \begin{cases} \dfrac{(1+q(1-\mu))(1+2\phi)}{4} & q < \dfrac{1}{3+\mu} \\ \dfrac{1+2\phi}{3+\mu} & \text{if} \quad \dfrac{1}{3+\mu} \le q \le \dfrac{1+2\phi}{3+\mu+2\phi} \\ \dfrac{(1+2\phi)(1+(1-\mu)\phi)+q(1-\mu)(1-\phi(1+\mu+2\phi))}{4+2(1-q)(1-\mu)\phi} & q > \dfrac{1+2\phi}{3+\mu+2\phi} \end{cases}. \tag{4.12}$$

**Result 6:** In a population where workers produce offspring with the same sex ratio as their queen, obligate helping is never favoured ($\alpha \le 1$; Fig. 4.4b). Worker reproduction decreases the potential for helping.

In the Appendix we consider the scenario of the focal working producing her convergence sex ratio strategy, and obtain a similar result ($\alpha \leq 1$; Fig. 4.S4b).

If we consider facultative helping, that is only given in queenright colonies, we find that the potential for helping is:

$$\alpha_{\mathrm{FAC}} = \begin{cases} \dfrac{(2-\mu)(1+2\phi)}{4} & q \leq \dfrac{1+2\phi}{3+\mu+2\phi} \\ \dfrac{\mu\phi(1+2\phi) - q(4(1+\phi) - \mu(2+\phi(1-2\mu-2\phi)))}{4\phi - 4q(2+\phi)} & q > \dfrac{1+2\phi}{3+\mu+2\phi} \end{cases} . \tag{4.13}$$

**Result 7:** Facultative helping may be promoted in a population where workers produce the same sex ratio as their colony. However, as worker reproduction increases, the potential for helping decreases. If there is no worker reproduction, helping is always favoured, independently of the frequency of queen survival ($\alpha > 1$ if $\mu = 0$). As worker reproduction increases, the parameter range where helping is promoted decreases as well ($\alpha > 1$ if $q < [\phi(4-\mu-2\mu\phi)]/([4+\mu(2+\phi(1-2\mu-2\phi))]$; Fig. 4.4d).

In appendix we show that, if the worker produces her convergence stable sex ratio strategy, helping is favoured if the proportion of queenright colonies is below a threshold ($\alpha > 1$ if $q < [2(1-\mu)(1+2\phi)]/[10-\mu^2+2(2-\mu(4+\mu))]$; Fig 4.7d). However, the potential for helping decreases as the proportion of worker reproduction increases in the population (Fig. 4.A7d).

## Discussion

Here, we have considered how worker reproduction influences the evolution of helping in haplodiploid populations. We have shown that: (1) queen virginity always promotes helping if the focal worker produces the same sex ratio as her colony but it may inhibit helping if she produces either sons or her convergence stable sex ratio; (2) obligate queen replacement inhibits helping but facultative queen replacement may promote helping; (3) worker reproduction generally inhibits the evolution of altruistic helping; (4) as the paternal index increases, so does the potential for helping and thus monogamy promotes helping.

Queen virginity leads to split sex ratios between mated-queen colonies, producing a female bias and unmated-queen colonies that produce male-only colonies. This bias may promote helping, as workers may value more the offspring sex ratio, composed mainly of high-related sisters instead of their own offspring. We found this to be true if the focal worker produces the same sex ratio as her colony. However, we found that the convergence stable sex ratio strategy for the worker offspring is to produce sons, if unmatedness is not common in the population. In this case, considering the empirical range of unmatedness found in natural populations ($0 \leq u \leq 0.05$), helping is inhibited with the potential for helping being half the value found for the general diploid case ($\alpha = ½$ for haplodiploidy whereas $\alpha = 1$ in the general diploid case). Thus, a simple scenario of workers not mating drives helping to be inhibited instead of promoted (Fig. 4.1). We also found that, in general, an increase in the frequency of worker reproduction decreases the potential for helping. The only situation where worker reproduction increases the potential for helping is in a scenario where workers

and the focal worker produce sons (Fig. 4.3a). However, when considering the empirical range of unmatedness, the potential for helping is well bellow the unity.

Split sex ratios may also arise due to queen replacement, as workers in queenright colonies are favoured to produce a female-biased sex ratio due to their higher relatedness to sisters than to brothers, while workers in queenless colonies are favoured to produce a male-biased sex ratio due to their higher relatedness to nephews than to nieces (Yanega 1989; Boomsma 1991; Mueller 1991). Gardner et al. (2012) studied this mechanism and derived the potential for helping. Assuming that the focal worker produces the same sex ratio as her colony, they have shown that this mechanism promotes the evolution of facultative helping. Here we extended their approach by considering another: worker reproduction by having workers producing sons or by having workers producing their convergence stable sex ratio. In both cases, we have shown that queen replacement can promote but also hinder helping and indeed, if we consider the empirical range for queen survival, helping is inhibited.

We address the effect of worker reproduction in both scenarios and found that generally worker reproduction leads to a decrease in the potential for helping. Wenseleers & Ratnieks (2006) present data on 48 species of bees, wasps and ant species, and found that worker egg-laying is widespread, varying between 0.07% and 36.4%. Trivers & Hare (1976) have argued that worker production of males could favour the evolution of altruistic helping in haplodiploid species. This because a focal worker is more related to her sisters than her daughters and at the same time to her nephews than to her brothers. However, we have shown that the effect of worker reproduction is more complicated. Worker reproduction leads workers to be rearing

nephews as well as siblings, and this impacts on the reproductive value of individuals. Both relatedness and reproductive value will change depending on the sex ratio produced by workers and the proportion of worker-derived offspring, influencing the sex ratio strategy of queen-derived offspring. Thus, the effect of worker reproduction is complex and particular to specific biological scenarios. However, we show that the only situation where worker replacement showed some degree of promoting altruistic helping was in facultative helping in a population where workers produce sons. In this case, worker reproduction increases the range of queen survival where altruistic helping was favoured (where the potential for helping, $\alpha > 1$) but decreases the strength of that advantage (it decreases the value of $\alpha$).

In general, our results also support the importance of monogamy as a consistent factor that maximizes the relatedness between the worker and her colony offspring. The importance of monogamy in the evolution of eusociality as been pointed conceptually and empirically (Strassmann 2001; Boomsma 2007; Hughes et al. 2008; Boomsma 2009; Cornwallis et al. 2010). Unlike the traditional view where haplodiploidy is seen as a critical factor alone responsible for the origin of eusocial species (Krebs and Davies 1993), our results add on to the contemporary view that in haplodiploid societies, monogamy together with particular ecological conditions, have shaped the way to the evolution of helping and help-related traits (Boomsma 2007, 2009; Cornwallis et al. 2010; Gardner et al. 2012).

## Appendix A – Reproductive value

In a class-structured population, individuals may have different reproductive values, independently of the genes they carry (Fisher 1930; Taylor 1996). Reproductive value is first calculated for a class and then shared equally by all the individuals of that class, i.e, $v_j = c_j / N_j$. We can scale this by an arbitrary constant, as the total number of individuals in the population, and obtain $v_j = c_j / u_j$, where $u_j$, depends on the sex allocation strategy played by workers in queenright and queenless colonies and also on the proportion and sex allocation strategy for worker-derived offspring. Hence, we define the individual reproductive value of a male and of a female as $v_m = c_m / \bar{z}$, and $v_f = c_f/(1- \bar{z})$, respectively, where $\bar{z}$ is the population sex ratio, and $c_f$ and $c_m$ are the class reproductive values of females and males, defined as the expected asymptotic contribution to the gene pool of future generations (Fisher 1930; Grafen 2006). The class reproductive values also depend on the proportion of queen versus worker reproduction, and on the sex allocation strategies of the different parties. In order to calculate reproductive values we census the population at the moment of production of reproductive offspring (Gardner et al. 2012).

We now derive the class reproductive values for males and females, in a haplodiploid population with queen replacement and worker reproduction. We census the population at the moment of producing offspring. The proportion of genes in female larvae that derive from the females of the last census is: $\wp_{f\to f} = \frac{1}{2} \varsigma_{Q,f} + \frac{1}{4} \varsigma_{W,f}$ (where $\varsigma_{J,i}$ is the probability of picking a class i derived individual from the J colony type). Thus, the proportion of genes in a female that derive from the males from the last census is $\wp_{m\to f} = 1-\wp_{f\to f} = \frac{1}{2} \varsigma_{Q,f} + \frac{3}{4} \varsigma_{W,f}$. The proportion of genes in a male that

derive from the females from the last census is: $\wp_{f\to m} = \varsigma_{Q,m} + \frac{1}{2}\varsigma_{W,m}$. Therefore, proportion of genes in a male that derive from the males from the last census is $\wp_{m\to m} = 1 - \wp_{f\leftarrow m,} = \frac{1}{2}\varsigma_{W,m}$.

The probability of picking a queen-derived male, among all the male juveniles in the population is:

$$\zeta_{Q,m} = \frac{(1-\mu)qz_R}{(1-\mu)qz_R + (1-\mu)(1-q)z_L + \mu z_A}. \quad (4.A1)$$

The probability of picking a queen-derived female among the female juveniles in the population is:

$$\zeta_{Q,f} = \frac{(1-\mu)q(1-z_R)}{(1-\mu)q(1-z_R) + (1-\mu)(1-q)(1-z_L) + \mu(1-z_A)}. \quad (4.A2)$$

The probability of a worker-derived male among the male juveniles in the population is:

$$\zeta_{W,m} = \frac{(1-\mu)(1-q)z_L + \mu z_A}{(1-\mu)qz_R + (1-\mu)(1-q)z_L + \mu z_A}. \quad (4.A3)$$

The probability of picking a worker-derived female among the female juveniles in the population is:

$$\zeta_{W,m} = \frac{(1-\mu)(1-q)(1-z_L) + \mu(1-z_A)}{(1-\mu)q(1-z_R) + (1-\mu)(1-q)(1-z_L) + \mu(1-z_A)}, \quad (4.A4)$$

where $\mu$ represents the average proportion of worker-derived offspring found in the population, $q$ the probability of queen survival, $z_R$ is the sex ratio strategy in queenright colonies, $z_L$ the sex ratio strategy in queenless colonies and $z_A$ the sex ratio strategy for worker-derived offspring.

These quantities can be summarized in a gene flow matrix:

$$\mathbf{G} = \begin{bmatrix} \wp_{f\to f} & \wp_{m\to f} \\ \wp_{f\to m} & \wp_{m\to m} \end{bmatrix} \quad (4.A5)$$

The class reproductive values are given by the dominant left eigenvector of the gene-flow matrix, i.e. the solution to $(c_f \ c_m) = (c_f \ c_m) \bullet \mathbf{G}$ (Taylor 1996). Using model parameters to describe the class reproductive values, we write $c_f$ as:

$$c_f = \frac{1}{\Omega} 2(((1-q)z_L + 2qz_R)(1-\mu) + z_A\mu)((1-q)z_L + qz_R)(1-\mu) + z_A\mu - 1), \quad (4.A6)$$

and the male class reproductive value, $c_m$ is:

$$c_m = \frac{1}{\Omega}((3z_L + q(1-3z_L+2z_R)(1-\mu) + 3(z_A - z_L)\mu - 3)(qz_R(1-\mu) + z_A\mu + z_L(1-q-\mu+q\mu))) \quad ,(4.A7)$$

where $\Omega = 5(1-q)^2 z_L^2 (1-\mu)^2 + q^2 z_R(1-6z_R)(1-\mu)^2 - (1-q)z_L(1-\mu)(5-q(1+11z_R)(1-\mu)-10z_A\mu) - 5z_A\mu(1-z_A\mu) - q(1-\mu)(7z_R - z_A\mu - 11z_A z_R\mu)$.

## Appendix B – Genetic associations

In this section we make clear how to determine the relatedness coefficients used in the models presented in this paper and the relatedness coefficients used in the figures to illustrate the change in relatedness with worker reproduction and worker control. Bulmer (1994) defines the coefficient of consanguinity $p_{ij}$ between two individuals, i and j, as "the probability that two homologous genes drawn randomly, one from i and one from j, are identical." As an example, in a diploid population, the coefficient of consanguinity between two sisters (defined as $p_F$) is the probability of picking the maternal allele in both sisters and the probability that they received the same allele from their mother, times the probability of picking the paternal allele in both sisters times the probability of sharing the same father (given by $\phi$) times the probability of picking the same paternal allele from the father. This is $½ \times ½ \times ½ + ½ \times ½ \times \phi \times ½ = (1+\phi)/8$. In haplodiploids, has males only have one copy of the genome, this probability becomes $(1+\phi)/8$.

Through this paper, we use the regression definition of relatedness, where the relatedness of j to i is $r_{ij} = (q_{ij} - q)/(q_{ii} - q)$, where $q_{ii}$ is the probability of drawing the same gene at random from i twice, $q_{ij}$ is the probability of drawing the same gene in i and j and $q$ is the probability of drawing the same gene from i and from a random individual in the population. Notice that, for now, we are focusing on alleles identical

in state. The identity in state of two alleles can be due to them sharing an identity by descent (when they share a common ancestor defined in a pedigree map) or they can be identical due to the frequency of that allele in the population. This way, we can write $q_j = p_j + (1-p_j)q$ and $q_i = p_i + (1-p_i)q$, where $p_i$ is the consanguinity shared between i and j, and $p_j$ is the consanguinity of j to herself. If we expand this into our definition of the relatedness of j to i, we find that $r_{ij} = p_j / p_i$.

## Appendix C – Queen virginity

Here we derive the convergence sex ratio strategies (Taylor 1996) for the queen virginity models. We use a neighbour-modulated fitness approach (Taylor 1996; Taylor and Frank 1996; Frank 1997a, 1998; Rousset 2004; Taylor et al. 2007) to determine the direction of selection for the sex allocation trait. We model this by assuming that the queen produces a large number $N$ of male and female reproductive juveniles, but can only rear half of these. We also assume that workers control this sex allocation decision. As workers also reproduce, the number of juvenile reproductive males present in the colony is $(1-\mu)z_M + \mu z_A$ and the proportion of juvenile female reproductives is $(1-\mu)(1-z_M) + \mu(1-z_A)$, where $\mu$ is the proportion of worker reproduction in the colony, $z_M$ is the sex ratio strategy followed for queen-derived offspring and $z_A$ is the sex ratio strategy followed for worker-derived offspring. We start by describing the approach used when considering that worker-derived offspring follows a fixed sex ratio strategy. In this case, the fitness of a queen-derived female is the probability that she will be reared to maturity, which is $(1-\mu)(1-z_M)$. The average fitness of her class is $(1-\mu)(1-\bar{z}_M) + \mu(1-\bar{z}_W)$ and thus, her relative fitness is $W_f = [(1-$

$\mu)(1-z_M) + \mu(1- \bar{z}_W)]/[(1-\mu)(1- \bar{z}_M) + \mu(1- \bar{z}_W)]$. The probability of survival of a male is found in a similar way and the relative fitness of a male egg is $W_m = [(1-u)((1-\mu)z_M + \mu \bar{z}_W) + uz_A]/ [(1-u)((1-\mu)z_M + \mu \bar{z}_W) + uz_A]$. We define a class-structure population where fitness is derived by averaging over the fitness of individuals in each class, weighting by the class reproductive value, that is, $W = c_f W_f + c_m W_m$ (Taylor 1996; Taylor and Frank 1996; Frank 1997a, 1998; Rousset 2004; Taylor et al. 2007). Natural selection favours the increase of a trait in the population if individuals carrying that trait are in average more fit than the population average. If we consider that a trait $G$ with genic value $g$ affects the sex allocation strategy of the individuals carrying it, $G$ is favoured if $dW/dg > 0$. Hence, the marginal fitness of $G$ is given by:

$$\frac{dW}{dg} = c_f \frac{dW_f}{dg_f} + c_m \frac{dW_m}{dg_m},$$

$$= c_f \frac{\partial W_f}{\partial z_M} \frac{dz_M}{d\hat{g}} \frac{d\hat{g}}{dg_{fM}} + c_m \frac{\partial W_m}{\partial z_M} \frac{dz_M}{d\hat{g}} \frac{d\hat{g}}{dg_{mM}}, \qquad (4.C1)$$

where $dz_M/d\hat{g} = 1$ is the genotype-phenotype map; and $d\hat{g}/dg_{fM} = p_S$ and $d\hat{g}/dg_{mM}$ are the coefficients of consanguinity between sisters and between brother and sister. Replacing these expressions by the model parameters allow us to find the convergence stable sex ratio strategy for mated queen colonies.

If worker-derived sex allocation strategy is not fixed in the population (due to workers producing only sons or being limited to produce the same sex ratio strategy as their colony), worker sex allocation strategy for own offspring is found in a similar way as the sex allocation strategy for queen-derived offspring. We consider a trait $H$ acting

on worker-derived offspring sex allocation strategy, with a genic value $h$. As in the previous case, $H$ is favoured if $\mathrm{d}W/\mathrm{d}h > 0$ and the marginal fitness of $H$ is given by:

$$\frac{\mathrm{d}W}{\mathrm{d}h} = c_{\mathrm{f}} \frac{\mathrm{d}W_{\mathrm{f}}}{\mathrm{d}h_{\mathrm{f}}} + c_{\mathrm{m}} \frac{\mathrm{d}W_{\mathrm{m}}}{\mathrm{d}h_{\mathrm{m}}},$$

$$= c_{\mathrm{f}} \frac{\partial W_{\mathrm{f}}}{\partial z_{\mathrm{A}}} \frac{dz_{\mathrm{A}}}{\mathrm{d}\hat{h}} \frac{\mathrm{d}\hat{h}}{\mathrm{d}h_{\mathrm{fA}}} + c_{\mathrm{m}} \frac{\partial W_{\mathrm{m}}}{\partial z_{\mathrm{A}}} \frac{dz_{\mathrm{A}}}{\mathrm{d}\hat{h}} \frac{\mathrm{d}\hat{h}}{\mathrm{d}h_{\mathrm{mA}}}, \qquad (4.C2)$$

where $\mathrm{d}z_{\mathrm{A}}/\mathrm{d}\hat{g} = 1$ is the genotype-phenotype map; and $\mathrm{d}\hat{h}/\mathrm{d}h_{\mathrm{fA}} = p_{\mathrm{S}}$ and $\mathrm{d}\hat{h}/\mathrm{d}h_{\mathrm{mA}} = p_{\mathrm{D}}$ are the coefficients of consanguinity between sisters and between brother and sister, respectively. Together with equation C1, replacing these expressions by the model parameters allow us to find the join convergence stable sex ratio strategy for queen-derived and worker-derived offspring in mated queen colonies.

## Appendix D – Queen replacement

As in the previous section use a neighbour-modulated (direct fitness) approach (Taylor 1996; Taylor and Frank 1996; Frank 1997a, 1998; Rousset 2004; Taylor et al. 2007) to determine the unbeatable sex ratio strategies for queenright and queenless colonies. We define a class-structure model where the fitness of a juvenile queenright colony male is defined as the probability of a male to survive, i.e. $(1-\mu)(qz_{\mathrm{R}} + (1-q)z_{\mathrm{L}}) + \mu\ \bar{z}_{\mathrm{A}}$. The average fitness of a male is $(1-\mu)(q\ \bar{z}_{\mathrm{R}} + (1-q)\ \bar{z}_{\mathrm{L}}) + \mu\ \bar{z}_{\mathrm{A}}$, thus the relative fitness of a male is $W_{\mathrm{m}} = [(1-\mu)(qz_{\mathrm{R}} + (1-q)z_{\mathrm{L}}) + \mu\ \bar{z}_{\mathrm{A}}]/\ [(1-\mu)(q\ \bar{z}_{\mathrm{R}} + (1-q)\ \bar{z}_{\mathrm{L}}) + \mu\ \bar{z}_{\mathrm{A}}]$. Equally, the fitness of a juvenile female is $(1-\mu)q(1-z_{\mathrm{R}}) + (1-q)(1-z_{\mathrm{L}})) + \mu(1-\ \bar{z}_{\mathrm{A}})$, the average fitness of a female is $(1-\mu)q(1-z_{\mathrm{R}}) + (1-q)(1-z_{\mathrm{L}})) + \mu(1-\ \bar{z}_{\mathrm{A}})$ and

thus, her relative fitness is $W_f = [(1-\mu)q(1-z_R) + (1-q)(1-z_L)) + \mu(1- \bar{z}_A)]/[ (1-\mu)q(1-z_R) + (1-q)(1-z_L)) + \mu(1- \bar{z}_A)]$ . Following the same reasoning as in the previous section, the average fitness of an individual is $W = c_f W_f + c_m W_m$, where $c_m$ and $c_f$ are the male and the female class reproductive values, derived in Appendix A (Taylor 1996; Taylor and Frank 1996; Frank 1997a, 1998; Rousset 2004; Taylor et al. 2007). Consider a gene $G$ with genic value $g$, controlling sex allocation in queenright colonies. Natural selection favours an increase of frequency of this trait in the population if $dW/dg > 0$. Hence, the marginal fitness of $G$ is given by:

$$\frac{dW}{dg} = c_f \frac{dW_f}{dg_f} + c_m \frac{dW_m}{dg_m},$$

$$= c_f \frac{\partial W_f}{\partial z_R} \frac{dz_R}{d\hat{g}} \frac{d\hat{g}}{dg_{fR}} + c_m \frac{\partial W_m}{\partial z_R} \frac{dz_R}{d\hat{g}} \frac{d\hat{g}}{dg_{mR}}, \qquad (4.D1)$$

where $dz_R/d\hat{g} = 1$ is the genotype-phenotype map; and $d\hat{g}/dg_{fR} = p_F$ is the consanguinity between a worker and her sister, and $d\hat{g}/dg_{mR} = p_F$ is the consanguinity between a worker and her brother. We use the same rationale to model the action of natural selection on the sex allocation strategy in queenless colonies. In this case, we consider a gene $H$ with genic value $g$, controlling sex allocation in queenless colonies. Through natural selection, this trait increases its value in the population if $dW/dh > 0$. Hence, the marginal fitness of $H$ is given by:

$$\frac{dW}{dh} = c_f \frac{dW_f}{dh_f} + c_m \frac{dW_m}{dh_m},$$

$$= c_f \frac{\partial W_f}{\partial z_L} \frac{dz_L}{d\hat{h}} \frac{d\hat{h}}{dh_{fL}} + c_m \frac{\partial W_m}{\partial z_R} \frac{dz_L}{d\hat{h}} \frac{d\hat{h}}{dh_{mL}}, \qquad (4.D2)$$

where $\mathrm{d}z_L/\mathrm{d}\hat{h}=1$ is the genotype-phenotype map; and $\mathrm{d}\hat{h}/\mathrm{d}h_{\mathrm{fL}}=p_{\mathrm{Ni}}$ and $\mathrm{d}\hat{h}/\mathrm{d}h_{\mathrm{mL}}$ are the coefficients of consanguinity between a worker and her niece and her nephew, respectively. By replacing in equations D1 and D2 expressions by the model parameters, we find the joint convergence stable sex ratio strategy for queen-derived and worker-derived offspring in mated queen colonies.

## Appendix E – Potential for helping when focal worker produces her convergence stable sex ratio strategy

*Queen virginity – workers produce sons only*

If the focal worker is given the possibility of producing offspring of both sexes at her convergence stable sex ratio strategy, the valuation made by the focal worker for her own offspring is $z_{\mathrm{O}}^{*}v_{\mathrm{m}}p_{\mathrm{S}}+(1-z_{\mathrm{O}}^{*})v_{\mathrm{f}}p_{\mathrm{D}}$. A focal worker will be favoured to produce sons if $u\leq(2-3\mu)/(2+2a-3)$, and daughters otherwise (Fig. A1a). In this case, the potential for helping is:

$$\alpha=\begin{cases}\dfrac{8(1+\phi)-2\mu(7+6\phi)+\mu^2(5-4\phi^2)-u\mathrm{K}}{16(1-u)(1-\mu)(1+\phi)} & \text{if } u\leq\dfrac{2-3\mu-2\mu\phi}{2+2a-3\mu+4a\phi-2\mu\phi}\\ \dfrac{(2au+\mu-u\mu)(1+2\phi)}{4(1-u)} & \text{if } \dfrac{2-3\mu-2\mu\phi}{2+2a-3\mu+4a\phi-2\mu\phi}<u\leq\dfrac{2-3\mu}{2+2a-3\mu}\\ \dfrac{(1-\mu)(1+2\phi)}{2} & \text{if } u>\dfrac{2-3\mu}{2+2a-3\mu}\end{cases} \quad .(4.\mathrm{E1})$$

*Queen virginity – workers produce the same sex ratio as their colonies*

If the focal worker is allowed to produce her convergence stable sex ratio strategy, she is favoured to produce daughters if $u > (2+\mu)/(2+2a+\mu)$, otherwise she is favoured to produce sons. Her evaluation for own offspring is $z_O^* v_m p_S + (1-z_O^*) v_f p_D$, and the potential for helping is:

$$\alpha = \begin{cases} \dfrac{2-\mu+(2-(1-\mu)\mu)\phi - u(2-\mu+(2-\mu(1-a-\mu))\phi - 2a\mu\phi^2)}{2(1-u)(2+(2-\mu)\phi)} & u \le \dfrac{2+\mu}{2+2a+\mu+4a\phi} \\ \dfrac{au(2-\mu)(1+2\phi)}{2(1-u)(2+\mu)} & \text{if } \dfrac{2+\mu}{2+2a+\mu+4a\phi} \le u \le \dfrac{2+\mu}{2+2a+\mu} \\ \dfrac{(2-\mu)(1+2\phi)}{4} & u > \dfrac{2+\mu}{2+2a+\mu} \end{cases} . \quad (4.E2)$$

If the focal worker produces the convergence stable sex ratio strategy for her colony, helping is promoted ($\alpha > 1$) if $u > [2(2+\mu)]/[2(2+\mu)+a(2-\mu)(1+2\phi)]$ (Fig. A1b).

*Queen virginity – Workers produce the convergence sex ratio strategy for their offspring*

Workers producing their convergence sex ratio strategy is a case of split sex ratios within each mated colony, where queen-derived and worker-derived offspring are both controlled by workers but produced with different sex ratios. We found that the joint convergence stable sex ratio for queen-derived ($z_M^*$) and worker-derived offspring ($z_A^*$) is:

$$\left(z_M^*, z_A^*\right) = \begin{cases} (0,0) & \text{if} \quad u \geq \dfrac{2+\mu}{2+2a+\mu} \\ \left(\dfrac{2-\mu(3+2\phi)-u(2-3\mu-2\mu\phi+2a(1+2\phi))}{4(1-u)(1-\mu)(1+\phi)}, 1\right) & \text{if} \quad u < \dfrac{2+\mu}{2+2a+\mu} \quad \text{and} \quad \mu \leq \dfrac{2(1-u-au-au\phi)}{(1-u)(3+2\phi)} \\ (0,1) & \text{if} \quad u < \dfrac{2+\mu}{2+2a+\mu} \quad \text{and} \quad \dfrac{2(1-u-au-au\phi)}{(1-u)(3+2\phi)} < \mu \leq \dfrac{2(1-u-au)}{3(1-u)} \\ \left(0, \dfrac{2+\mu-u(2+2a+\mu)}{4\mu(1-u)}\right) & \text{if} \quad u < \dfrac{2+\mu}{2+2a+\mu} \quad \text{and} \quad \mu > \dfrac{2(1-u-au)}{3(1-u)} \end{cases}$$

.(4.E3)

In this case, if worker reproduction is rare, workers are favoured to produce the rarer sex, if the population sex ratio is female-biased, and only sons if the population sex ratio is male-biased. This depends on the proportion of unmated queens in the population. Worker reproduction leads the convergence stable sex ratio for queen-derived colony to be less female-biased, if the proportion of unmated queens is rare, becoming increasingly female biased as the population sex ratio becomes increasingly male biased due to an increase in the proportion of unmated queens. Due to this male bias, the convergence stable sex ratio for worker-derived offspring allows for the production of some daughters, even if still significantly male-biased (Fig. A4a). Due to the production of females by the workers, worker reproduction leads to a decrease of the relatedness between a worker and her colony offspring (Fig. A4b). In general, we found that the reproductive value of males decreases both as worker-reproduction and the proportion of unmated queens increases. If the proportion of unmated queens is low, the workers' convergence stable sex ratio strategy is to produce a male biased sex ratio and thus the population sex ratio becomes male biased leading to an increase of the female reproductive value. Also, an increase of the proportion of unmated queens in the population leads to an increase of the male bias in the population sex ratio and thus to an increase of the female reproductive value (Fig. A4c).

In this model, the worker inclusive fitness evaluation of the colony offspring is $(1-\mu)[\ \bar{z}_M v_m p_M + (1-\ \bar{z}_M) v_f p_F] + \mu[\ \bar{z}_W v_m p_M + (1-\ \bar{z}_W) v_f p_F]$ and the evaluation of her own offspring is $\bar{z}_W v_m p_M + (1-\ \bar{z}_W) v_f p_F$. In this case, the potential for helping is:

$$\alpha = \begin{cases} \dfrac{(2-\mu)(1+2\phi)}{4} & \text{if} \quad u \geq \dfrac{2+\mu}{2+2a+\mu} \\ \dfrac{2-\mu(1-2\phi)}{4} & \mu \leq \dfrac{2(1-u-au-au\phi)}{(1-u)(3+2\phi)} \\ \dfrac{(au+\mu-u\mu)(1+2\phi)}{2(1-u)} & \text{if} \quad u < \dfrac{2+\mu}{2+2a+\mu} \quad \text{and} \quad \dfrac{2(1-u-au-au\phi)}{(1-u)(3+2\phi)} < \mu \leq \dfrac{2(1-u-au)}{3(1-u)} \\ \dfrac{(2-\mu)(1+2\phi)}{4} & \mu > \dfrac{2(1-u-au)}{3(1-u)} \end{cases}$$

(4.E4).

If workers produce their convergence stable sex ratio strategy for their own offspring, worker reproduction may be favoured if the proportion of unmated queens is higher than a threshold (Fig. A1c). If we consider monogamy ($\phi = 1$) and equal production by mated and unmated colonies ($a = 1$), helping is favoured if $u > (2-3\mu)/(5-3\mu)$. Worker reproduction increases this threshold and thus inhibits the potential for helping. As before, an increase of the proportion of unmated queens promotes altruistic helping (Fig. A1c).

*Queen replacement – workers produce sons only*

*Obligate helping:* If the worker is favoured to produce her convergence stable sex ratio strategy, then the valuation she does of her own offspring is $z_O^* v_m p_S + (1-z_O^*) v_f p_D$. Depending on the population sex ratio, a worker is favoured to raise either daughters or sons. If $\mu > (3-q)/(4-q)$ then the population is composed essentially by males and the value of females is very high due to a rare sex effect. In this case, a

worker is favoured to produce daughters. Otherwise a worker is either indifferent or prefers to raise sons – where the value of sons is $v_m p_S$. Thus, the only difference from the above scenario (Equation 13) is that, if $\mu \geq (3\text{-}q)/(4\text{-}q)$, $\alpha_{OBL} = (1\text{-}\mu)(1+2\phi)$ (Fig. A5a).

*Facultative helping:* If the worker produces her convergence stable sex ratio strategy, the potential for helping is given by equation 14, except if $\mu > (3\text{-}q)/(4\text{-}q)$, where $\alpha_{FAC} = (5\text{-}q)(1\text{-}\mu)(1+2\phi)/4$ (Fig. A5c).

*Queen replacement – workers produce the same sex ratio as their colonies*

*Obligate helping:* If we consider that the worker has full control over her own offspring sex ratio such that she produces her convergence stable sex ratio strategy we find that the value of a daughter is never higher than the value of a son, and thus we compare the value of the colony offspring with the value of rearing sons. If helping is an obligate trait, not influenced by the type of colony the worker belongs to, the potential for helping is:

$$\alpha_{OBL} = \begin{cases} \dfrac{(1+q(1-\mu))(1+2\phi)}{4} & q < \dfrac{1}{3+\mu} \\ \dfrac{(1-q)(1+2\phi)}{2+\mu} & \text{if} \quad \dfrac{1}{3+\mu} \leq q \leq \dfrac{1+2\phi}{3+\mu+2\phi} \\ \dfrac{(1+2\phi)(1+(1-\mu)\phi)+q(1-\mu)(1-\phi(1+\mu+2\phi))}{4+2(2-\mu)\phi} & q > \dfrac{1+2\phi}{3+\mu+2\phi} \end{cases} .$$

(E5)

The potential for helping is illustrated in Fig. A5b.

*Facultative helping:* If the worker produces her convergence stable sex ratio strategy, we find that the potential for helping is:

$$\alpha_{\mathrm{FAC}} = \begin{cases} \dfrac{(2-\mu)(1+2\phi)}{4} & q < \dfrac{1}{3+\mu} \\ \dfrac{(2-2\mu-q(2-\mu(4+\mu)))(1+2\phi)}{4q(2+\mu)} & \text{if} \quad \dfrac{1}{3+\mu} \le q \le \dfrac{1+2\phi}{3+\mu+2\phi}. \\ \dfrac{2-\mu(1-2\phi)}{4} & q > \dfrac{1+2\phi}{3+\mu+2\phi} \end{cases} \qquad \text{(E6)}$$

This is illustrated by Fig. A5d.

## Supporting material

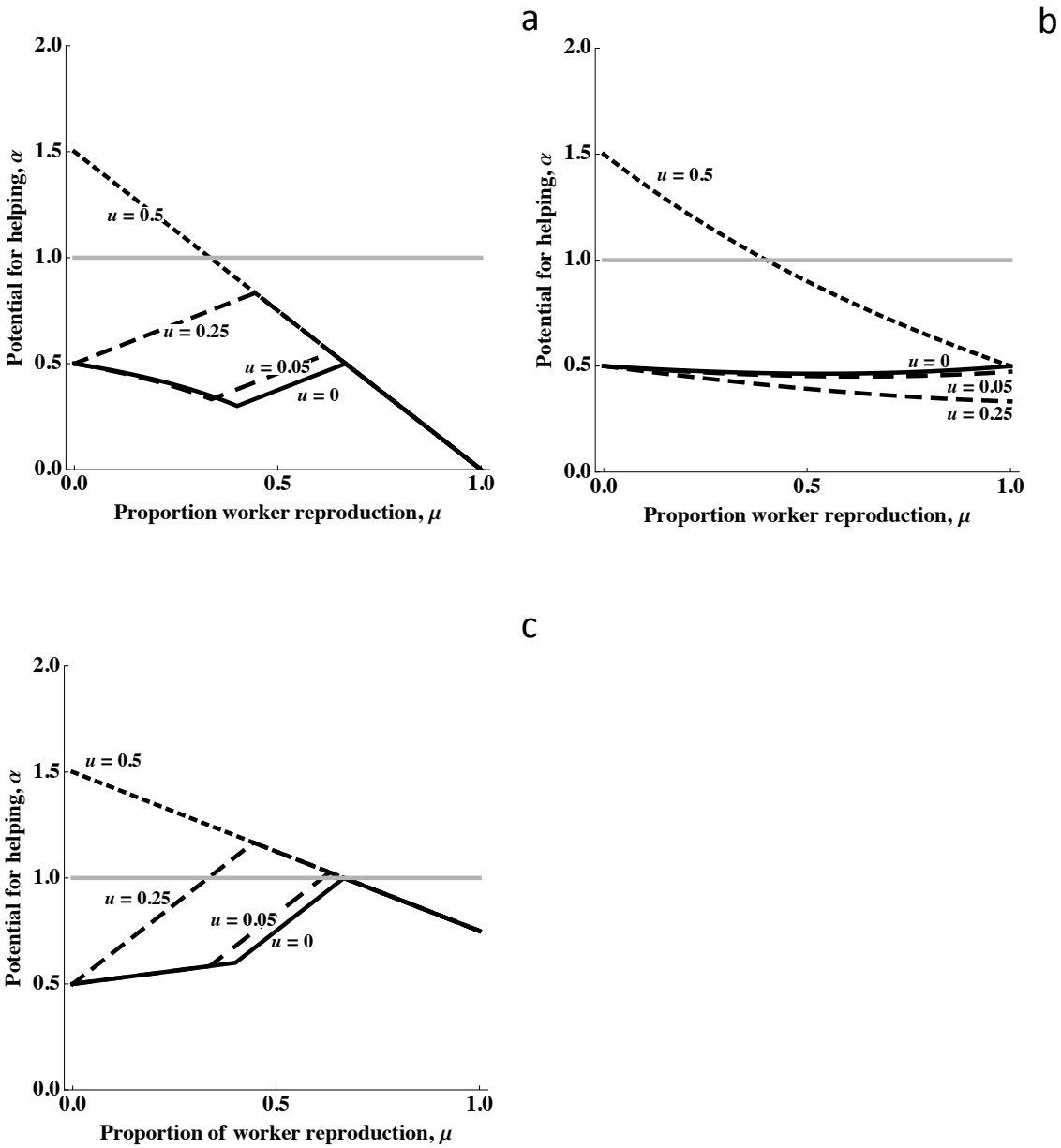


Figure 4.S1. Queen virginity and worker reproduction – Focal worker produces her convergence stable sex ratio strategy. (a) If workers produces sons, then helping is only favoured for high levels of worker reproduction. (b) If workers produce the same sex ratio as their colony then worker reproduction decreases the potential for helping. However, helping may be promoted at the empirical range for unmatedness found in natural populations, if the

proportion of worker reproduction is small. (c) If all workers produce their convergence sex ratio strategy for their own offspring, then helping may be favoured if there are unmated queens in the population.

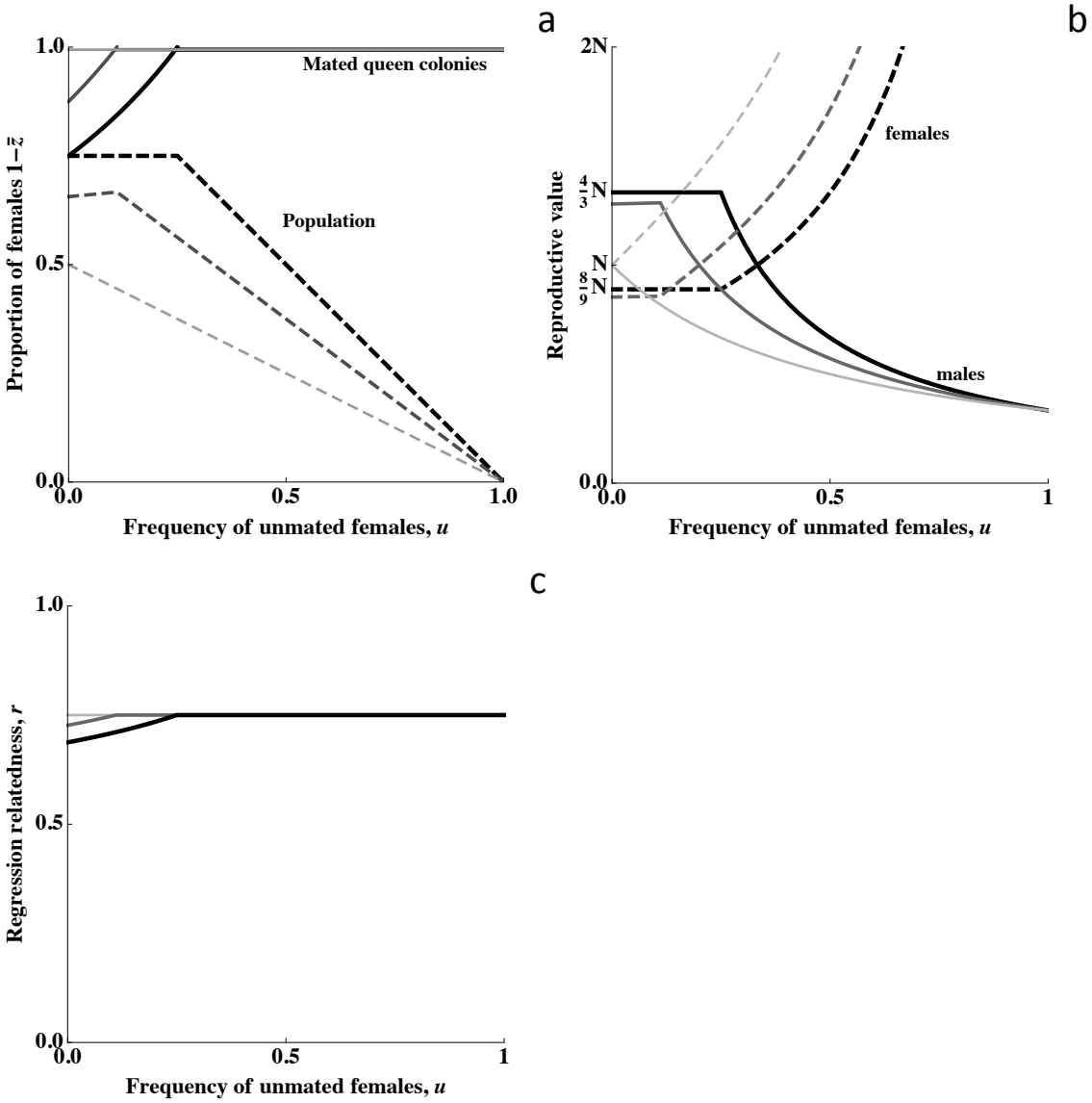


Figure 4.S2. Queen virginity and worker reproduction – workers produce sons. (a) Both unmatedness and worker reproduction lead to a male bias in the population and to mated-queen colonies convergence stable sex ratio to be more female biased. (b) As worker reproduction and unmatedness increase the male bias in the population, the reproductive

value of males decrease and the reproductive value of females increase. (c) Worker reproduction leads to an increase of the relatedness between a worker and her colony offspring, for low values of unmatedness in the population. Worker reproduction increases the proportion of males in the population leading to a female-bias in the mated-queen colonies convergence stable sex ratio and thus to an increase in relatedness.

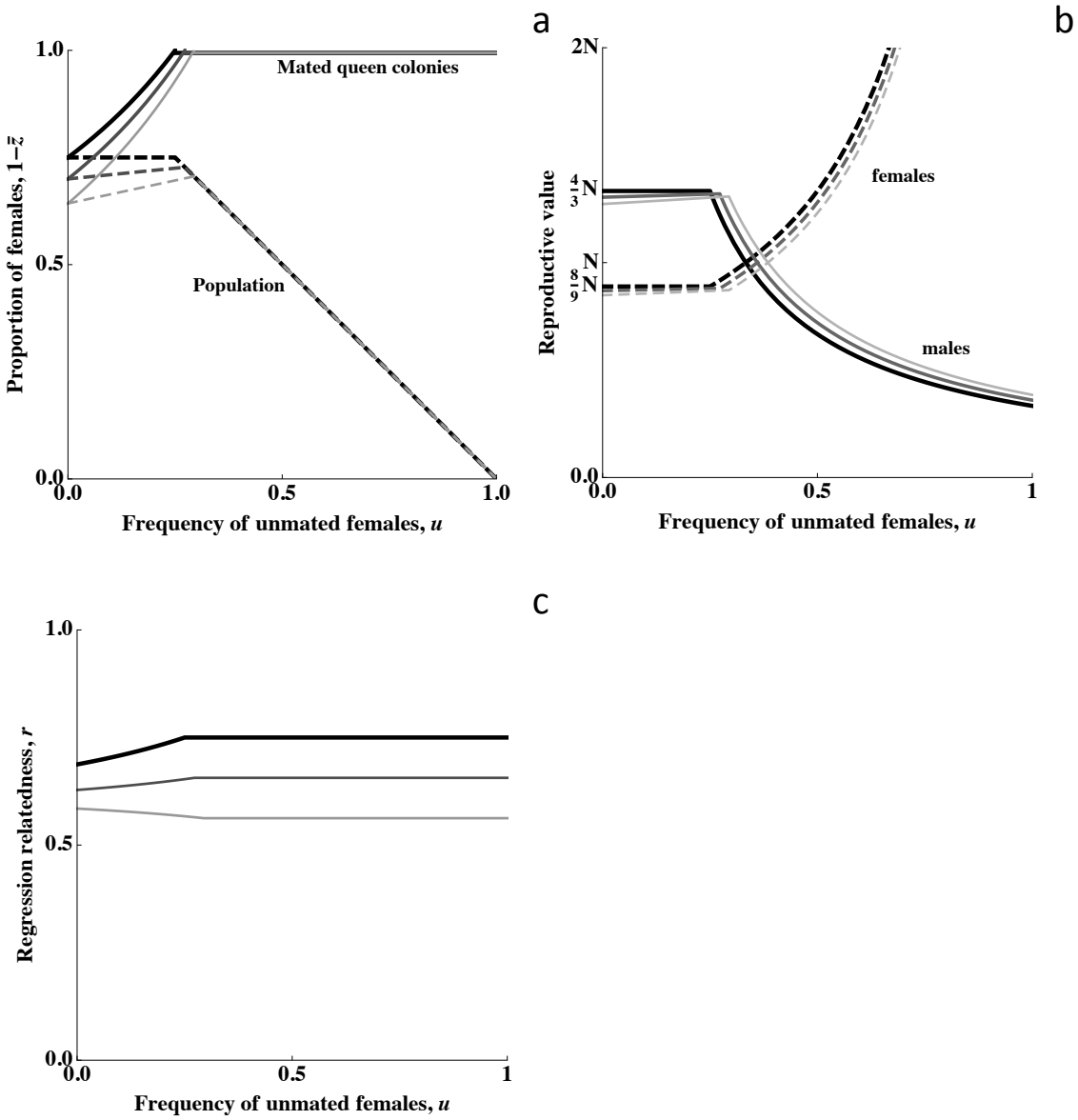


Figure 4.S3. Queen virginity and worker reproduction – workers produce the same sex ratio as their colony. (a) Unmatedness leads to male bias in the population sex ratio and consequently to a higher female bias in the convergence stable sex ratio strategy for the

mated-queen offspring. Worker reproduction lead to a less female bias in the sex ratio of both the colony and mated-queen offspring. (b) The reproductive value of females increases and the reproductive value of males decreases, as the proportion of unmated queens increases in the population. (c) Worker reproduction decreases the relatedness between a worker and her colony offspring.

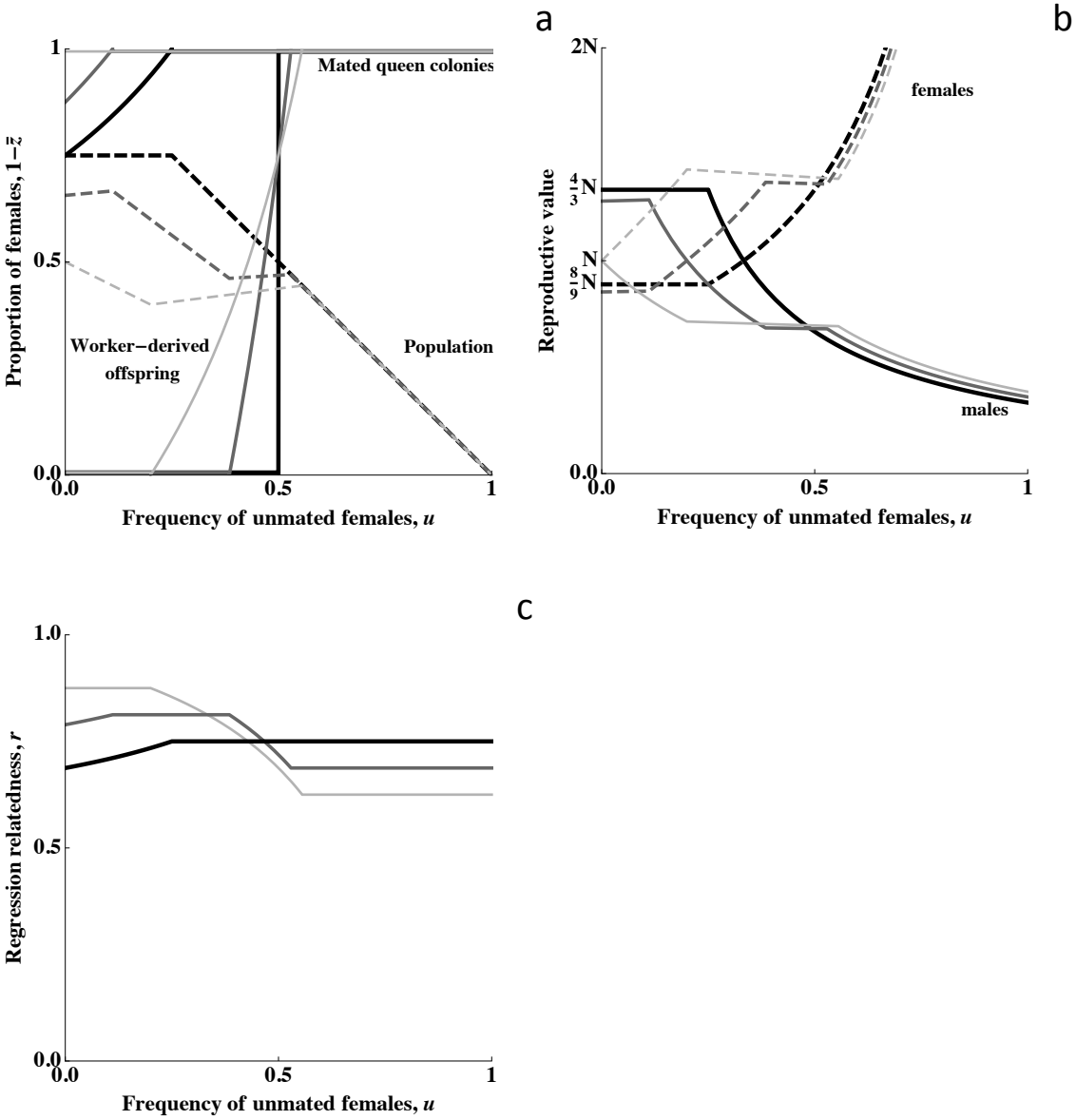


Figure 4.S4. Queen virginity and worker reproduction – workers produce their convergence stable sex ratio strategy. (a) As mated-queen offspring is produced with a female bias, workers are favoured to produce a male-biased sex ratio if the frequency of unmatedness is low and a female-biased sex ratio as the frequency of unmatedness increases in the

population. The population sex ratio becomes increasingly male biased with the increase of unmatedness in the population. (b) Worker reproduction increases the reproductive value of females and decreases the reproductive value of males. (c) Leads to an increase of the colony relatedness if the proportion of unmatedness is low and to a decrease of the colony relatedness if the proportion of unmatedness is higher.

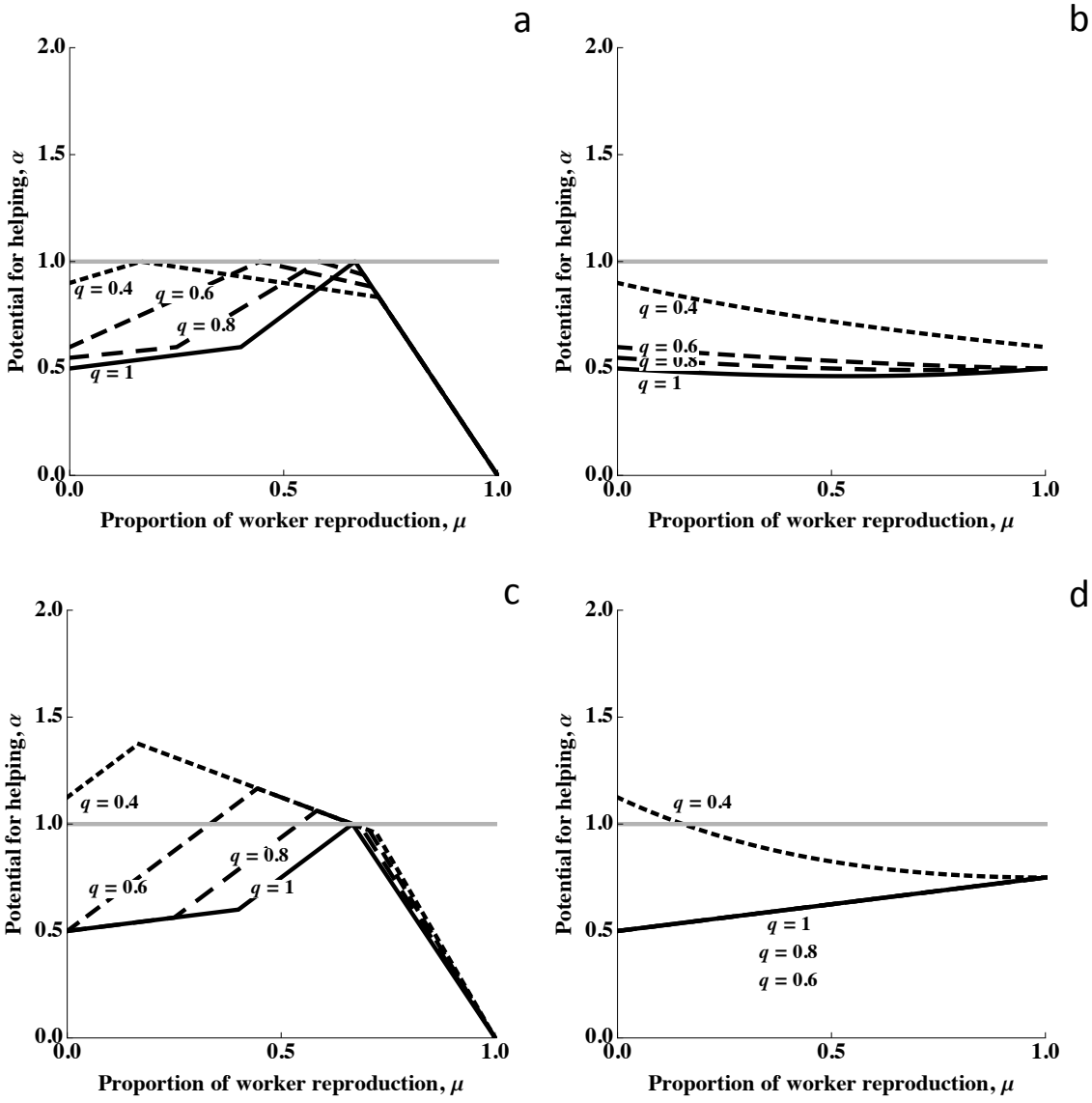


Figure 4.S5. Queen replacement and worker reproduction If workers produce a proportion $\mu$ of the colony offspring and the focal worker produces her convergence stable sex ratio strategy, then: Obligate helping is never favoured, either if (a) If workers produce sons, or (b) workers produce the same sex ratio as their colonies. (c) If workers produce sons, facultative

helping is favoured for lower values of unmatedness ($\alpha > 1$ if $q < (1+2\phi)/(5-2\mu+2\phi(1-2\mu))$) in the population. (d) If workers produce the same sex ratio as their colonies, facultative helping may be favoured for low frequency of queen survival, but never within its empirical range ($\alpha \leq 1$ for $0.6 \leq q \leq 0.8$).

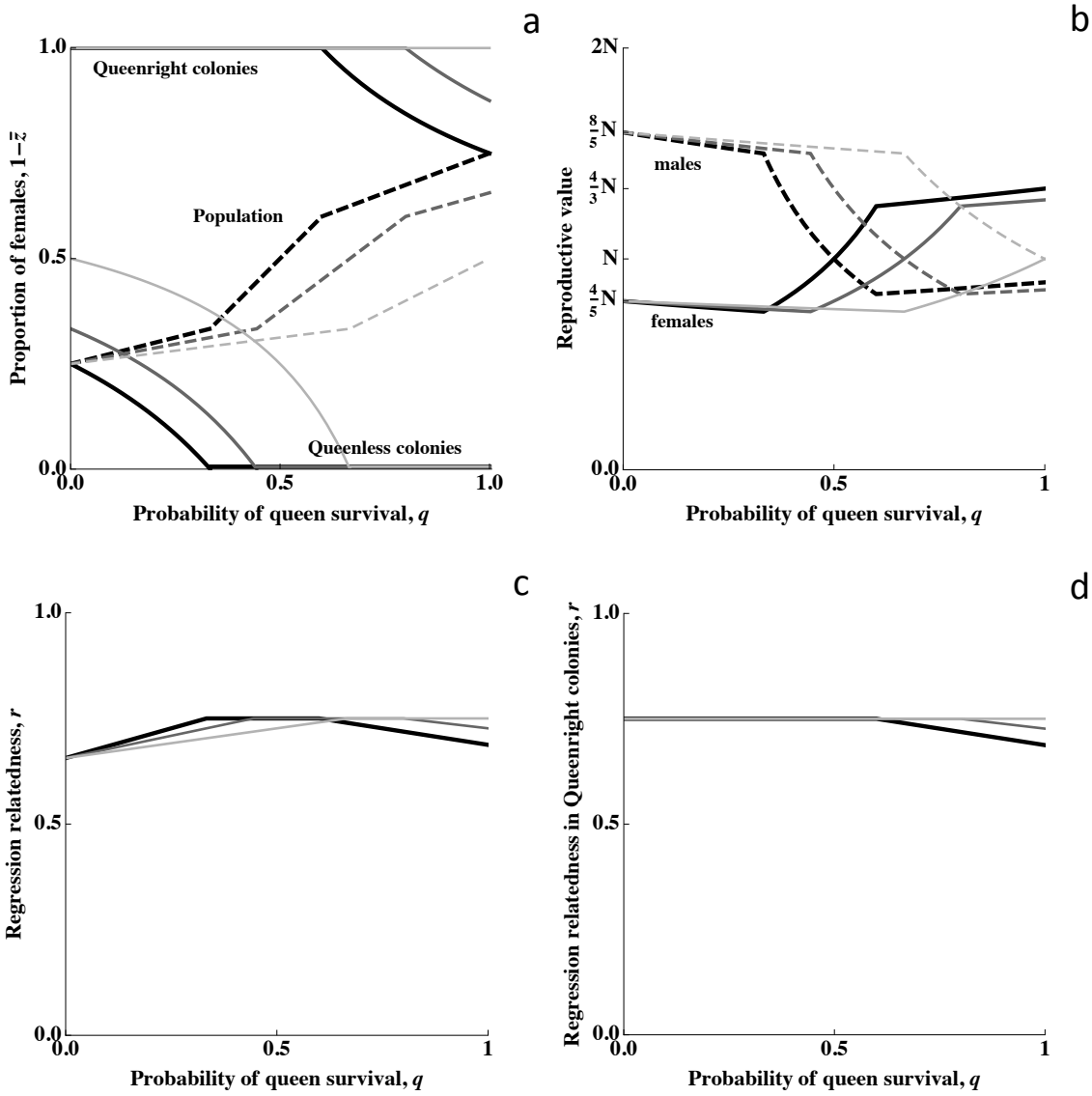


Figure 4.S6. Queen replacement and worker control: workers produce sons. (a) Queen replacement leads to split sex ratios with queenright colonies producing a female-biased sex ratio and queenless colonies producing a male-biased sex ratio. The population sex ratio depends on the frequency of the two types of colonies; (b) Queen replacement leads to an increase of female reproductive value, as queenless colonies produce a male-biased sex ratio and decrease the female bias of the population sex ratio. (c) The average relatedness increases

initially with queen replacement as queenless colonies produce nephews. As queen replacement increases, queenless colonies produce nieces as well, decreasing the average relatedness. Worker reproduction leads to an increase of the average relatedness if there is no queen replacement but it decreases the average relatedness if queen replacement is common. (d) Worker reproduction leads to an increase of the relatedness within queenright colonies, when the proportion of queenright colonies in the population is high. Worker production of sons leads to a male bias in the population which in turn favour queenright colonies to produce a more female-biased sex ratio and thus increasing the within colony relatedness.

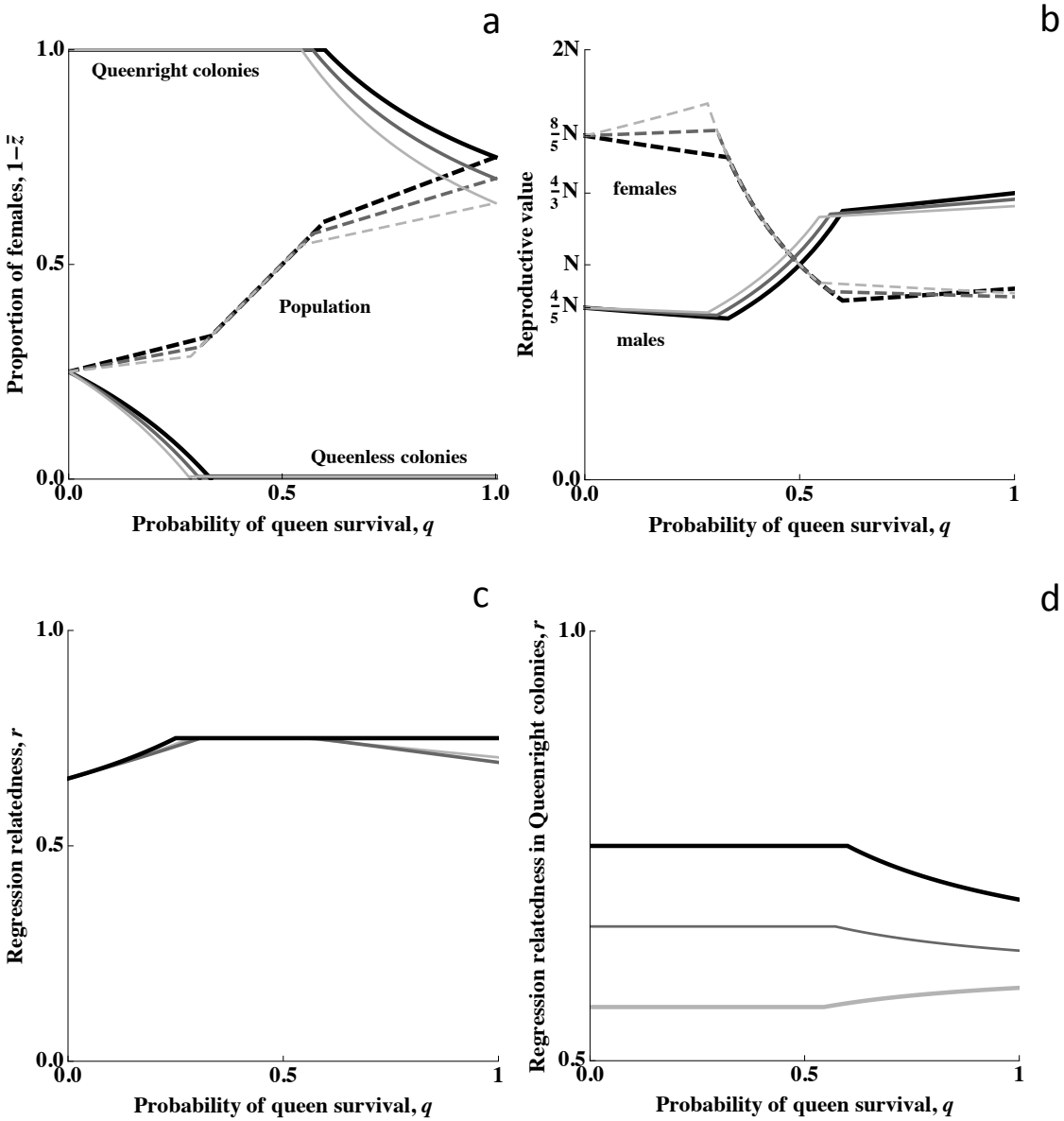


Figure 4.S7. Queen replacement and worker control: workers produce the same sex ratio as their colony. (a) Worker replacement leads to an male bias of the sex ratio strategy in queenright colonies and to a slight male bias in the sex ratio strategy of queenless colonies; (b) Decreases male reproductive value and increases female reproductive value if queenright colonies are common; (c) It leads to a decrease of the average colony relatedness. (d) Leads to a decrease in the relatedness within queenright colonies.

# 5. Co-evolution of soldier production and brood allocation in polyembryonic parasitoid wasps


## Abstract

Parasitoid wasps from the genus *Copidosoma* embryos are characterized by a particular embryological development, where each egg undergoes clonal proliferation and develops multiple embryos. Also, in some species of this genus, there is differentiation into casts, with most embryos developing into reproductive adults, but with a fraction of embryos developing into sterile soldiers. These individuals develop earlier and influence the survival of reproductive adults. One hypothesis for the function of these soldiers is that they mediate the resolution of a sex ratio conflict in mixed-sex broods. Previous work considered soldier production in a population composed of mixed-sex broods only, showing that female broods are expected to produce soldiers as a mechanism to mediate a sex ratio conflict (Gardner et al. 2007). However, in natural populations females produce both mixed-sex and single-sex broods and this brood allocation may influence both the sex ratio conflict in mixed-sex broods and even the proportion of mixed-sex broods produced. Here, I study this hypothesis considering a population where females produce both single-sex and mixed-sex broods. I find that the same conflict showed by Gardner et al. (2007) exists in mixed-sex broods when single-sex broods are also produced, with its intensity changing with the proportion of single-sex broods. Also, under the assumption of a "sex ratio conflict function", soldier production is costly and I show that producing mixed-sex broods is no longer a stable strategy if mated females are given the opportunity to produce single-sex broods. Finally, I examine the co-evolution of

soldier and brood allocation, and find that only females are selected to produce soldiers, under the assumption of a sex ratio conflict function.

## Introduction

Spite is defined as a behaviour which is both costly to the actor and the recipient (Hamilton 1970; West et al. 2007). A spiteful trait or behaviour is favoured by natural selection if it is directed to individuals sharing negative relatedness with the actor – i.e., the recipient of the action is less related to the actor than the population average (Hamilton 1970; Grafen 1985). This requires either a very particular population structure or the ability of the actor to identify negative relatedness, making a spiteful trait difficult to evolve. The development of a soldier caste in polyembryonic wasps from the family *Encyrtidae* constitutes one of the best-known and studied examples of spite. In wasps from this family, females oviposit eggs into the eggs of *Lepidoptera*, with the wasp eggs proliferating clonally within the developing caterpillar. Depending on the species, each egg can give rise to either from a few embryos to hundreds of embryos. Whilst most of the embryos develop into regular wasps, some develop into precocious sterile "soldiers". Female soldiers from *Copidosoma floridanum* have been shown to exhibit an aggressive behaviour towards conspecifics. A series of experiments have shown that, in this species, soldier attacks are negative correlated with relatedness (Giron et al. 2004), female broods produce more soldiers that emerge earlier than male broods (Giron et al. 2007b), and that, unlike male soldiers, female soldiers are aggressive and reduce the number of brothers within the brood (Grbic et al. 1992; Giron et al. 2007a; Giron et al. 2007b).

The adaptive function for the production of sterile soldiers is still an open question, with two main hypothesis considered. Soldier function may be related with the resolution of a sex ratio conflict within mixed-sex broods. According with this idea, soldiers are produced in order to kill opposite-sex embryos and in this way to bias the sex ratio in favour of their sex (Grbic et al. 1992; Giron et al. 2004; Gardner et al. 2007). Another hypothesis is that soldiers exhibit an altruistic behaviour favouring the development of siblings by defending and facilitating the availability of the host's resources to their siblings (Cruz 1981).

Gardner et al. (2007) developed a kin selection model based on *Copidosoma floridanum* life cycle, showing a scope for a sex ratio conflict between males and females, and that the female bias in soldier production is consistent with the sex ratio conflict function, whereas males would be favoured in producing soldiers with the facilitation function. The model developed by Gardner et al. (2007) assumed that females always lay two opposite sex eggs in each host and, as a consequence, that the population is composed entirely of mixed-sex broods. However, in *C. floridanum* females produce about 60% of mixed-sex broods, with the remaining being equally allocated between male and female single-sex broods (Ode and Strand 1995). Although the high proportion of mixed-sex broods in *C. floridanum* may explain the aggressive behaviour of female soldiers against brothers and support the role of soldiers in the sex-ratio conflict hypothesis, the significant presence of single-sex broods may change the outcome of the model presented by Gardner et al. (2007). For example, under a sex ratio conflict hypothesis, the production of single-sex broods may be favoured by natural selection as a mean to avoid the conflict among kin.

Also, soldier function in the context of sex ratio conflict is not found in other related species from the genus *Copidosoma*, sharing similar developmental pathways. In *C. koehleri*, female eggs also develop a soldier caste but in this species females only produce single-sex broods (Keasar et al. 2006). Also, *C. bakeri* produces clutch sizes similar to *C. floridanum* but produces mainly single-sex broods (92% of the broods), with male and female eggs giving rise to similar numbers of soldiers and these being agressive towards heterospecific competitors (Smith et al. 2010).

Here, I extend the model presented by Gardner et al. (2007) to consider how the presence of single-sex broods mediates the evolution and adaptive function of soldiers. I approach this question firstly by determining whether there is still a sex ratio conflict in mixed-sex broods when single-sex broods are also present in the population. I follow by determining whether the observed female bias in soldier activity is still consistent with a sex ratio conflict function when there are single-sex broods in the population. Finally, I investigate how the frequency of single-sex broods may evolve in response to soldier activity. I present a general model considering the co-evolution of brood allocation, sex allocation in single and mixed-sex broods, and soldier production.

## Model & Analysis

I develop a kin selection approach to model the evolution of a soldier cast in polyembryonic wasps. I first examine the existence and extent of a sex ratio conflict within mixed-sex broods. For that, I determine the optimal sex ratio for mixed-sex broods, from the point of view of a reproductive emerging female (sister), her

reproductive emerging brother and her mother. I then determine under which conditions will there be a sex ratio bias in the production of soldiers, considering a sex ratio conflict function. Finally, I examine soldier allocation showing how this influences the production of single-sex versus mixed-sex broods and develop a model where soldier and brood allocation co-evolve. Model notation is summarized in Table 5.A1.

Table 5.1: Summary of model notation used in this chapter.

| Symbol | Definition |
|---|---|
| $\sigma$ | Proportion of single-sex broods |
| $\mu$ | Sex ratio of single-sex broods as the proportion of male broods |
| $z$ | Sex ratio of mixed-sex broods as the proportion of males in the brood |
| $a$ | Soldier allocation |
| $d_{\mathrm{m}}$ | Premating dispersal rate of males |
| $d_{\mathrm{f}}$ | Premating dispersal rate of females |
| $p_{\mathrm{ij}}$ | Consanguinity between individuals i and j. |
| $r_{\mathrm{ji}}$ | Relatedness of individual j to individual i |
| $c_{\mathrm{m}}$ | Class reproductive value of males, 1/3 |
| $c_{\mathrm{f}}$ | Class reproductive value of females, 2/3 |
| $x$ | Soldier strategy followed by a focal female |
| $y$ | Soldier strategy followed by a focal male |
| $\xi$ | Non-developing males strategy, followed by a focal male |
| $G$ | Genetic locus controlling female trait |
| $g$ | Genic value of a random gene from locus $G$ in a focal individual, male or female |
| $H$ | Genetic locus controlling male trait |

| | |
|---|---|
| $h$ | Genic value of a random gene from locus $H$ in a focal individual, male or female |
| $J$ | Genetic locus controlling female single-sex brood allocation, $\sigma$ |
| $j$ | Genic value of a random gene from locus $j$ in a focal individual, male or female |
| $U$ | Genetic locus controlling female single-sex brood sex allocation, $\mu$ |
| $u$ | Genic value of a random gene from locus $u$ in a focal individual, male or female |
| $k$ | Shape parameter for soldier action |
| $s$ | Degree of soft selection at the host |
| $N$ | Number of embryos formed from a local host, prior to development into reproductive adults or soldiers |
| $N_e$ | Number of emerging individuals from a local host |
| $L$ | Number of host larvae |

*Population model*

I assume an island model (Wright 1931), in which the population of wasps is structured into a very large number $L$ of patches. Each patch contains a single host individual, which is parasitized by a single mated wasp foundress. With probability $\sigma$ the foundress oviposits a single egg into the host, producing a single-sex brood. From these, with probability $\mu$ the female lays an unfertilized egg, producing a male brood, and with probability 1-$\mu$ she lays a fertilized egg, producing a female brood. With probability 1-$\sigma$ the foundress oviposits two eggs, one unfertilized (male) and one fertilized (female) into the host, therefore producing a mixed-sex brood.

Each egg proliferates clonally to give rise to a large number of embryos, some of which survive to pupation and emerge from the host as adult wasps. I assume that

single-sex broods yield a large number $N$ of adult wasps of the appropriate sex, and mixed-sex broods yield a large number $N(1-a)$ of adult wasps, of which a proportion $z$ are male and a proportion $1-z$ are female. The parameter $0 \leq a \leq 1$ reflects that there is scope for wasteful conflict within mixed-sex broods (which are non-clonal) but not within single-sex broods (which are clonal, and hence expected to maximize the number of emergent adults (e.g. Gardner and Grafen 2009). After emergence, I assume that a proportion $d_f$ of mixed-sex-brood females, a proportion $d_m$ of mixed-sex brood males, and all single-sex brood individuals, independently migrate to randomly chosen patches elsewhere in the population, where all individuals present in that patch mate at random. Finally, mated females migrate to parasitize a new generation of host individuals, returning the model the beginning of the life cycle. Note that the life cycle described here includes two dispersal stages, first to mate and after that to find a new host. Also, in the special case of $\sigma = 0$, the model reduces to the scenario analyzed by Gardner et al (2007). For $\sigma > 0$, the model generalizes to allow for single-sex broods, and hence more effectively captures the biology of *Copidosoma* species.

*Natural selection*

I use a neighbor-modulated (personal) fitness approach to model sex allocation. I follow Taylor and Frank (1996) and considering sex allocation as a differential mortality of potential individuals. I assign to each host a male and a female egg and then consider that the female laying the eggs may effectively kill one or neither of these eggs, giving either a single-sex male brood, a single-sex female brood or a mixed-sex brood. I consider that each egg can potentially originate $N$ individuals but

each host has resources only enough for the survival and development of $N$ individuals. I assume that, while in mixed-sex broods $aN$ individuals develop as soldiers and do not leave offspring, in single-sex broods there is no production of soldiers and thus, all $N$ individuals develop into regular adults. Hence, the expected fitness of a female is given the proportion of females that will develop to reproductive maturity, and is given by the expected number of females emerging after clonal proliferation and development within the host:

$$w_f = \sigma(1-\mu)N + (1-\sigma)N(1-a)(1-z). \quad (5.1)$$

For males, fitness is given by the expected number of emerging males weighted by the expected mating success, which depends not only on the number of emergent adults in a given patch but also on the number of emigrants and on the ratio of females to males in the mating groups. Male fitness will then be given by the expression:

$$w_m = \sigma\mu N(\sigma M_S + (1-\sigma)M_M) + (1-\sigma)Nz(d_m(\sigma M_S + (1-\sigma)M_M) + (1-d_m)M'_M), \quad (5.2)$$

where $M_S$ is a given male's mating success expectation in a single-sex brood patch, $M_M$ is a given male's mating success expectation in a mixed-sex brood patch and $M'_M$ is mating success expectation of a non-disperser male, in his own mixed-sex brood patch.

I write explicitly these three types of fecundity functions:

$$M_{\mathrm{S}} = \frac{\sigma(1-\mu)+(1-\sigma)(1-a)(1-\bar{z})d_{\mathrm{f}}}{\sigma\mu+(1-\sigma)(1-a)\bar{z}d_{\mathrm{m}}} \tag{5.3}$$

$$M_{\mathrm{M}} = \frac{\sigma(1-\mu)+(1-\sigma)(1-a)(1-\bar{z})d_{\mathrm{f}}+(1-a)(1-\bar{z})(1-d_{\mathrm{f}})}{\sigma\mu+(1-\sigma)(1-a)\bar{z}d_{\mathrm{m}}+(1-a)\bar{z}(1-d_{\mathrm{m}})}, \tag{5.4}$$

$$M'_{\mathrm{M}} = \frac{\sigma(1-\mu)+(1-\sigma)(1-a)(1-\bar{z})d_{\mathrm{f}}+(1-a)(1-z)(1-d_{\mathrm{f}})}{\sigma\mu+(1-\sigma)(1-a)\bar{z}d_{\mathrm{m}}+(1-a)z(1-d_{\mathrm{m}})}. \tag{5.5}$$

*Conflict over the sex ratio*

I determine the scope for a sex ratio conflict between sisters and brothers, considering the full range of brood allocation ($0 \leq \sigma \leq 1$). The potential for conflict is demonstrated if sisters and brothers from the same mixed-sex brood have different sex-ratio optima. For completeness, I also consider the mother's preferred sex ratio strategy. Within each mixed-sex brood, in order to find the optimal sex ratio for a given family member, I grant her/him full control over the sex ratio. I also assume that the total number of emergent offspring, *N*, is the same and constant among single-sex and mixed-sex broods. I determine the convergence sex ratio strategies (Taylor 1996; Taylor and Frank 1996) from the point of view of each one of the family members: the sister ($z_{\mathrm{S}}^{*}$), brother, ($z_{\mathrm{B}}^{*}$), and mother ($z_{\mathrm{M}}^{*}$).

**Result 1:** The convergence sex ratio strategies of the mother, brother and sister differ, with the sister preferring a higher female bias than the brother and the mother preferring an intermediate sex ratio ($z_{\mathrm{B}}^{*} \geq z_{\mathrm{M}}^{*} \geq z_{\mathrm{S}}^{*}$). An increase of the proportion of

single-sex broods (with an unbiased sex ratio, $\mu = 0.5$) results in an increase of this difference: brothers are favoured to produce an increasingly male biased sex ratio ( $[\mathrm{d}z_{\mathrm{B}}^{*} / \mathrm{d}\sigma]\big|_{\mu=1/2} > 0$ ) while sisters are favoured to produce an increasingly female biased sex ratio ($[\mathrm{d}z_{\mathrm{S}}^{*} / \mathrm{d}\sigma]\big|_{\mu=1/2} < 0$).

Local mate competition theory predicts that, if siblings from the same sex compete for mating, then natural selection can favour a reduction of investment into that sex (Hamilton 1967). If there are no single-sex colonies ($\sigma = 0$) and no male dispersal from mixed-sex broods, brothers and sisters agree in the production of a female-biased sex ratio, as this reduces the mating competition between brothers (Fig. 5.1a). However, both an increase of male dispersal in mixed-sex brood as the allocation to single-sex broods allow for males to outbreed and thus lead a male bias in the preferred sex ratio of brothers (Fig. 5.1b-1e). At the same time, the production of single-sex broods may increase the number of males present at mating leading females to prefer the production of an even more female-biased sex ratio. In Appendix 5B, I show that sisters always favour a higher female bias than brothers and that the mother prefers an intermediate sex ratio.

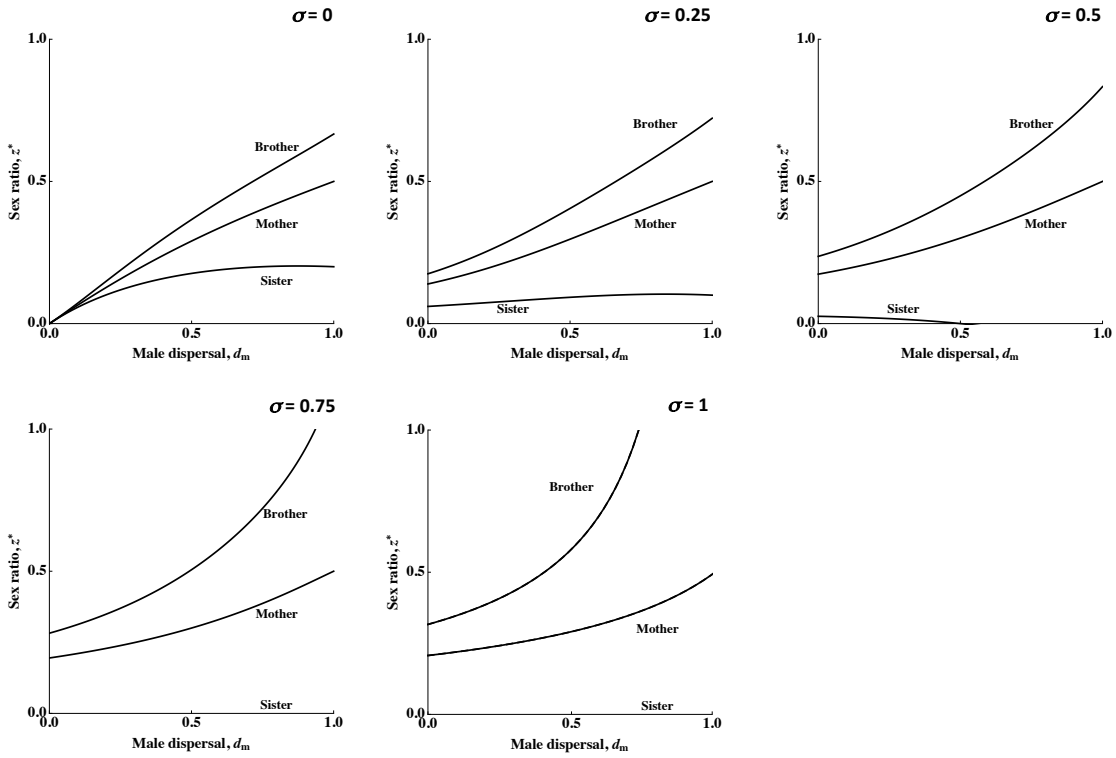


Figure 5.1. Convergence stable sex ratio strategies for mixed-sex brood colonies, $z^*$, from the point of view of the mother, brother and sister, and for the range of male dispersal ($d_m$). Pannels a-e: $\sigma = 0$; $\sigma = 0.25$; $\sigma = 0.5$; $\sigma = 0.75$ and $\sigma = 1$; $\mu = 0.5$; $d_f = 0$; $a = 0$.

## *Soldier production*

In this section I investigate the adaptive function of soldier production. In the previous section, I showed the existence of a sex ratio conflict between sisters and brothers from the same mixed-sex brood. Gardner et al. (2007) have shown that, in a population consisting entirely of mixed-sex broods, females produce more soldiers than males if soldier production leads to a decline in the output of adults from the host – consistent with the sex ratio conflict function – and that males produce more soldiers than females if soldier production leads to an increase of the output of adults from the host – consistent with the defence and facilitation function. I now investigate

in this more general model, under which conditions will male or female broods produce more soldiers.

In order to determine soldier allocation, I define $X$ and $Y$ as the probability of a male or a female of developing as soldiers. I am primarily interested in determining the function of soldiers in mixed-sex broods and therefore only consider the allocation decision for soldiers present in these broods. I use a similar approach to Gardner et al. (2007) and define the number of adults in mixed-sex broods and the sex ratio in mixed-sex broods to be a function of male and female soldier action. Thus, the number of emergent adults, $N_a=N(1-a(X,Y))$, and the sex ratio in mixed-sex broods, $z(X,Y)$, are functions of soldier allocation, where $X=x$ and $Y=y$' if the focal individual is a female and $X=x'$ and $Y=y$ if the focal individual is a male. I start by setting males and females to invest equally into soldiers, such that the sex ratio in mixed-sex broods is unbiased ($z = 1/2$). As a result, as both sexes are allocation equally to the production of soldiers total number of emergent individuals is the same ($\partial N_a/\partial x = \partial N_a/\partial y$). We also assume that an increase in female soldier allocation has the same impact on the total number of emergent adults as an increase in male soldier allocation ($\partial a/\partial x = \partial a/\partial y = A > 0$). This means that a slight increase in female soldier allocation has the same negative impact on the number of emergent adults as the same slight increase in male soldier allocation ($\partial N_a/\partial x = \partial N_a/\partial y < 0$). A small increase in female soldier allocation decreases the sex ratio by a magnitude equal to the increase in sex ratio that would happen if male soldiers were to increase by the same amount ($\partial z/\partial y = -\partial z/\partial x$). With this in mind, I find the marginal fitness of the female trait and find the convergence stable sex ratio as a function of female soldier production, $\partial z/\partial x$, at equilibrium. I then use this level of investment in the marginal fitness function for

the soldier trait of males by replacing $\partial z/\partial y = -\partial z/\partial x$. Finally, I determine when is the marginal fitness of the male trait ($\partial W/\partial h$) positive, meaning that at those points males are favoured to produce a higher amount of soldiers than females. As the expression obtained is not amenable to be analytically solved, I constructed a numerical procedure to find when is male marginal fitness positive, by first determining numerically the optimal single-sex brood sex ratio strategy ($\mu$; using Eq. 5.D1) for fixed single-sex brood allocation ($\sigma$, varying between 0.01 and 0.99 in 0.01 intervals), male dispersal ($d_m$, varying between 0 and 1, in 0.01 intervals), female dispersal ($d_f$, varying between 0 and 1, in 0.01 intervals), and soldier allocation ($a$, varying between 0 and 1, in 0.01 intervals) and then used these values to determine the signal of the marginal fitness function for the male trait. Through the range of parameters studied, the marginal fitness for the male trait was found to be always negative ($dW/dy<0$) when the production of soldiers decreases the output of adults from the host, suggesting a female-biased soldier production ($x^* > y^*$).

**Result 2:** In a population composed of single-sex and mixed-sex broods, females are always favoured to produce more soldiers under a sex ratio conflict function $x^* > y$ If I consider a defence and facilitation function, males are favoured to produce more soldiers than females. I recovered the result of Gardner et al. (2007), now extended for a population composed of both mixed and single-sex broods ($\sigma > 0$).

*Explicit model of soldier function*

In the previous sections, I found arguments supporting the function of soldiers in mixed-sex broods to be correlated with the resolution of a sex ratio conflict in mixed-sex broods. Here, I illustrate the resolution of this conflict by explicitly defining the

effect of soldier action on the number of emergent adults and their sex ratio in mixed-sex broods. I follow Gardner et al. (2007) model for the sex ratio conflict hypothesis and consider soldier action in the context of a sex ratio conflict and thus the soldier cast evolves only in mixed-sex broods. I defined in the previous section $x$ as the proportion of female embryos developing as soldiers. I assume that a proportion $x^k$ non-soldier males are killed by soldier females, such that there are diminishing returns on soldier action ($0 < k < 1$). I also use $y$ as the proportion of male embryos developing as soldiers and consider that a proportion $y^k$ non-soldier females are killed by soldier males. Thus, the proportion of surviving females is $(1-x)(1-y^k)$, the proportion of surviving males is $(1-y)(1-x^k)$, and the total number of emergent adults from mixed-sex broods is:

$$N \propto \left( \frac{(1-y)(1-x^k)+(1-x)(1-y^k)}{2} \right)^{1-s} \qquad (5.6)$$

where $s$ describes the degree of soft selection at the host. At $s = 0$, the extreme of hard selection, the number of emergent adults is proportional to the number of surviving embryos. At $s = 1$, the extreme of soft selection, the number of emergents is constant and doesn't vary with the degree of soldier action. Soldier action impacts in the sex ratio of emerging adults as well. In this case, the sex ratio is given by:

$$z = \frac{(1-y)(1-x^k)}{(1-y)(1-x^k)+(1-x)(1-y^k)}. \qquad (5.7)$$

In an alternative version of this model, I consider that, instead of producing soldiers, a proportion $\zeta$ of the potential regular male individuals stops developing in order to

save resources for the females. This is an extreme case of the defense and facilitation model where males may facilitate female proliferation just by giving up their own development. Because of that it is not feasible to have male soldiers and male limitation evolving at the same time (Gardner et al. 2007).

*Co-evolution of soldier and brood allocation*

I use the expressions determined in the previous section to follow the co-evolution of brood allocation between single-sex broods and mixed-sex broods ($\sigma$), the sex ratio among single-sex broods ($\mu$), the production of female soldiers in mixed-sex broods ($x$), and either the production of male soldiers in mixed-sex broods ($y$) or the production of non-developing male embryos in mixed-sex broods ($\zeta$; Appendix E). I assume that the mother controls egg allocation and the sex ratio of single-sex broods, sisters control the allocation to female soldiers and brothers control the allocation to male soldiers. I find that:

**Result 3:** Females evolve to produce either only single-sex broods, with an even sex ratio, or to produce a mixture of single-sex and mixed-sex broods ($\sigma = 1$ or $\sigma \approx 0.83$, with s=0.5 and $k$=0.5), with equal production of male and female broods ($\mu = 0.5$). The mixed strategy is only observed if there is no female dispersal in mixed-sex broods ($d_f = 0$, Fig. 5.3).

**Result 4:** Female but not male broods are selected to produce soldiers (if $x \geq 0$, $y = 0$; Fig. 5.5; Fig. S.A3). As a result of soldier action, mixed-sex broods produce a female biased sex ratio ($z < 0.5$; Fig. 5.4, Fig. 5.S2). These results are consistent holding $k$ = 0.1, 0.2 ,.., 0.8, 0.9, and holding $s$ = 0.1, 0.2, ..., 0.8, 0.9.

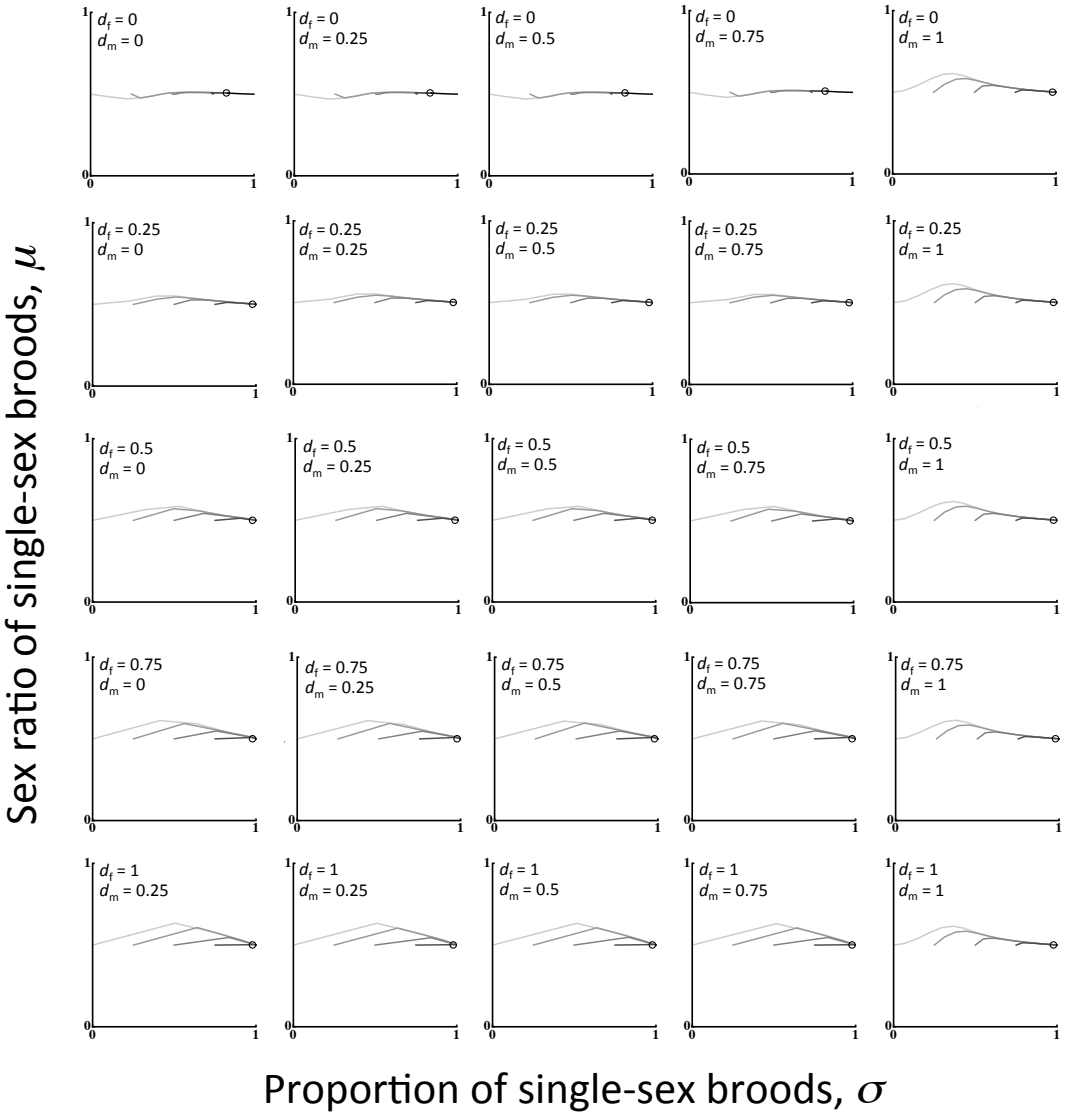


Figure 5.2. Co-evolution of brood allocation and soldier production – female and male broods may produce soldiers: evolution of brood allocation $\sigma$ and of the sex ratio of single-sex broods, $\mu$. Single-sex broods evolve to fixation if there are females dispersing from mixed-sex broods ($\mu > 0$). Otherwise, females evolve to produce a mixed strategy ($\sigma \approx 0.83$). Single-sex broods evolve to produce an even sex ratio. Different lines represent different initial values for brood allocation, that changes from $\sigma_I = 0.0001$ (light grey) to $\sigma_I = 0.9999$ (black), with $\sigma_I = 0.25$, $\sigma_I = 0.5$ and $\sigma_I = 0.75$ as intermediate values. In this figure, the shape parameter for soldier killing curve $k = 0.5$ and the degree of soft selection $s = 0.5$.

Due to the cost of soldier action, females evolve to allocate preferentially or fully to the production of single-sex broods (Fig. 5.3). This result is expected under the assumption that there is either no soldier production in single-sex broods or that there is no dispersal cost. In the alternative version of the model, I found that males may evolve to limit their own developmental proliferation at the same time as females produce soldiers, resulting in an even more female biased sex ratio in mixed-sex broods (Fig. 5.S1-5.S4). In this case, single-sex broods always evolve to fixation (Fig. 5.S1).

In the Appendix 5.D I show that, if I consider soldier production in mixed-sex broods to be related with a sex ratio conflict, then the costs of producing soldiers will make a mixed-sex brood strategy unstable and prone to the invasion of a single-sex brood strategy. This means that the model presented by (Gardner et al. 2007) is inherently unstable, as the proportion of single-sex broods $\sigma$ will increase from zero, if I allow brood allocation to evolve.

## Discussion

In this paper, I have studied the evolution a soldier caste on polyembryonic parasitoid wasps, as a response to a sex ratio conflict in mixed-sex broods. I have extended previous work by incorporating the impact of single-sex broods production, and I found that: 1) There is a sex-ratio conflict in mixed-sex broods, in a population with both single-sex and mixed-sex broods; 2) Females are consistently the gender selected to produce more soldiers as a mechanism to bias the mixed-broods sex ratio towards

the production of females; 3) Soldier production is costly and, in my framework, it will drive the evolution of brood allocation towards an increasing production of single-sex broods.

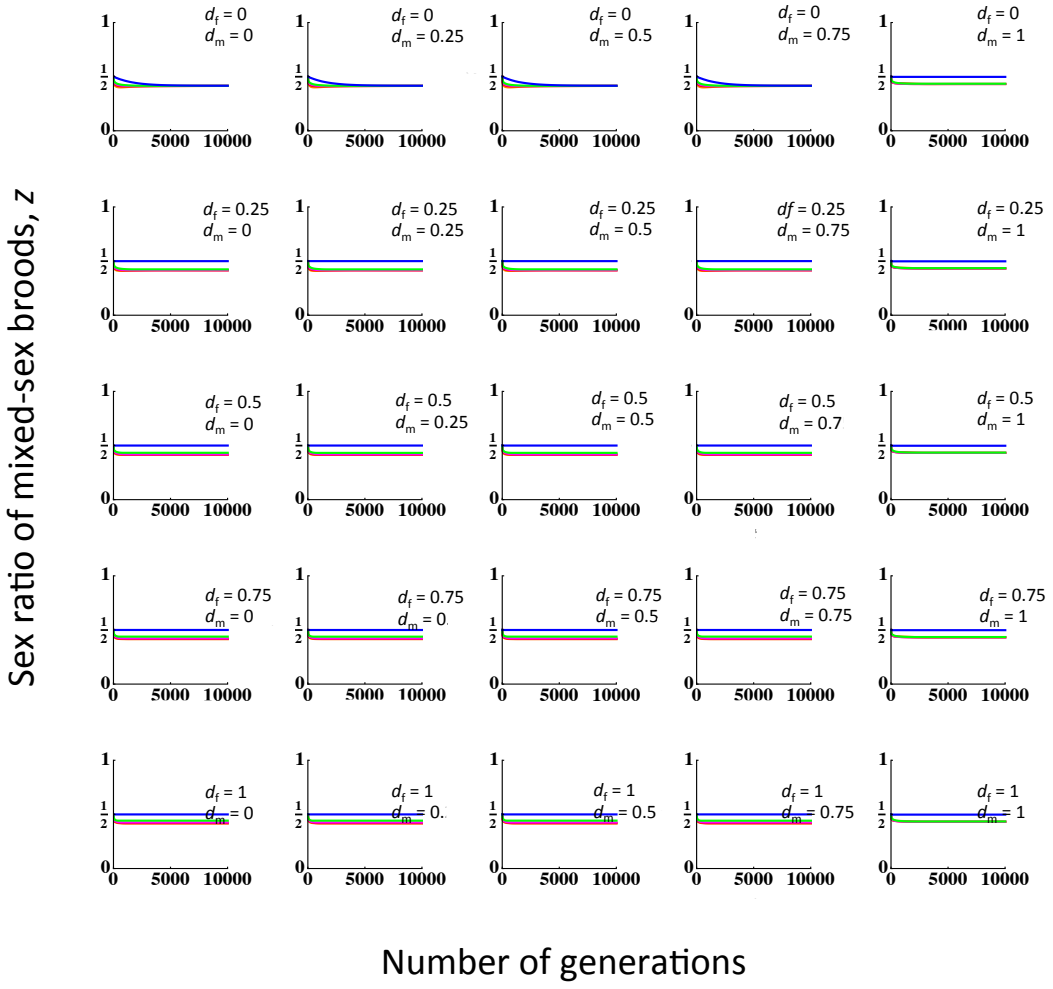


Figure 5.3. Co-evolution of brood allocation and soldier production – female and male broods may produce soldiers: Mixed-sex broods evolve to produce a female-biased sex ratio, that decreases with the initial value of single-sex brood allocation ($\sigma_I$), and with the increase of male ($d_m$) and female ($d_f$) dispersal in mixed-sex broods. Different lines represent different initial values for brood allocation: $\sigma_I = 0.0001$ (orange), $\sigma_I = 0.25$ (red), $\sigma_I = 0.5$ (pink), $\sigma_I = 0.75$ (green) and $\sigma_I = 0.9999$ (blue). . In this figure, the shape parameter for soldier killing curve $k = 0.5$ and the degree of soft selection $s = 0.5$.

I have shown that the existence of single-sex broods don't help solving the sex ratio conflict in mixed-sex broods, but can actually increase its intensity. Gardner et al. (2007) have shown the scope for the evolution of soldiers as a response to a sex ratio conflict in a population composed of mixed-sex broods. Here, I have extended that model by allowing females to produce single-sex broods as well. In a population composed of mixed-sex broods only, male and female dispersal define the degree of sex-ratio conflict between males and females. If there is no dispersal before mating, there is total inbreeding and both males and females agree in producing an extremely female-biased sex ratio – complete local mate competition. As male dispersal increases, there is competition for mating between non-related males and also an opportunity to increase mating success through dispersion and in this case, males and female disagree on the brood sex ratio. As these individuals also disperse to mixed-sex broods, this means that competition for mating will happen between unrelated males, favouring males to prefer a more male-biased sex ratio. The increase of unrelated males originated from single-sex broods leads females to prefer to produce a more female-biased sex ratio. As a result, the production of single-sex broods increases the sex ratio conflict in mixed-sex broods.

My results also suggest that the production of singles-sex broods doesn't change the prediction that females will be favoured to produce more soldiers if these act to solve a sex ratio conflict. The haplodiploid genetic system leads to the existence of a relatedness asymmetry between brothers and sisters, where the relatedness of a sister to her brother is higher than the relatedness of a brother to her sister. Hence, unless females are favoured to produce a male bias in single-sex broods, such that the reproductive value of males increases in relation to the reproductive value of females,

a brother will value more his sister than a sister values her brother. My co-evolution models show that this is not the case, and single-sex broods sex ratio evolves to an equal share of males and females. In *C. floridanum,* both field observations and laboratory experiments have shown that female eggs develop more soldiers than male eggs, that these soldiers develop earlier than male soldiers and that female soldiers attack brothers as well as unrelated individuals (Grbic et al. 1992; Ode and Strand 1995; Giron et al. 2007a; Giron et al. 2007b). My results are in accordance to these findings but don't explain either brood allocation or soldier function, such as *C. khoeleri* or *C. bakeri*, where soldier appear to be less aggressive towards siblings (Keasar et al. 2006; Smith et al. 2010).

My model focused on the scope for a sex ratio conflict on mixed-sex broods, considering the impact of having females producing both single-sex and mixed-sex broods. Because of that, I don't consider soldier production, costs of dispersal or other costs related with the production of single-sex broods (as, for instance egg limitation). As a result, it is not surprising that my model predicts that a single-sex strategy is more successful than the production of both single-sex and mixed-sex broods, as single-sex broods don't incur in costs related with soldier production. This result shows that the model presented by (Gardner et al. 2007) is inherently unstable, as the proportion of single-sex broods $\sigma$ will increase from zero if I allow brood allocation to evolve.

By showing the costs of soldier production and in extension, of producing mixed-sex broods, I have exposed the necessity of taking into account factors that account for costs or risks of producing singles-sex broods. I assumed that all individuals from

single-sex broods disperse, which is reasonable considering that they need to find an opposite sex individual to mate. However, I didn't consider any costs of dispersal. These can be either survival costs, or a decrease in the probability of mating. Hence, producing mixed-sex broods may be a form of fertility insurance. In accordance with this, Ode and Strand (1995) have shown that females lay more mixed-sex broods when host encounter rates were low. Another biological detail that may explain the existence of singles-sex broods is host limitation. In this case, a mated female might be driven to allocate two eggs in each host instead of one. Finally, factors as host size and condition have been shown to influence egg allocation in *Copidosoma* species (Ode and Strand 1995). The age of host-egg when parasitized seems to influence both soldier behaviour and brood allocation in *C. floridanum*. Ode and Strand (1995) have shown in *C. floridanum* that female soldiers are more abundant in young host-egg, whereas in older host-eggs male and female soldiers numbers were similar. Also, mated females lay more single-sex female eggs in younger hosts and mixed broods in older hosts. It is feasible that these differences of oviposition behaviour with host-egg age may be related with the condition offered for the development of the wasp embryos, such as availability of nutrients and probability of super-parasitism.

Equally, I didn't address the role of soldier production in single-sex broods. In *C. khoeleri* and *C. bakeri*, two species from the same genus as *C. floridanum*, soldiers are produced but females from these species rarely produce mixed-sex broods. Also, the decision of producing single-sex or mixed-sex broods, as well as the outcome of soldier action in mixed-sex broods appears to be influenced by the type of host and her condition. It is then expected that the condition of the host or other parasitic events influence both the outcome of soldier action as well as the evolution of soldier

function. Hence, polyembryonic parasitoid wasps present an ideal model to further investigate social interactions and their co-evolution with the ecological factors presented above.

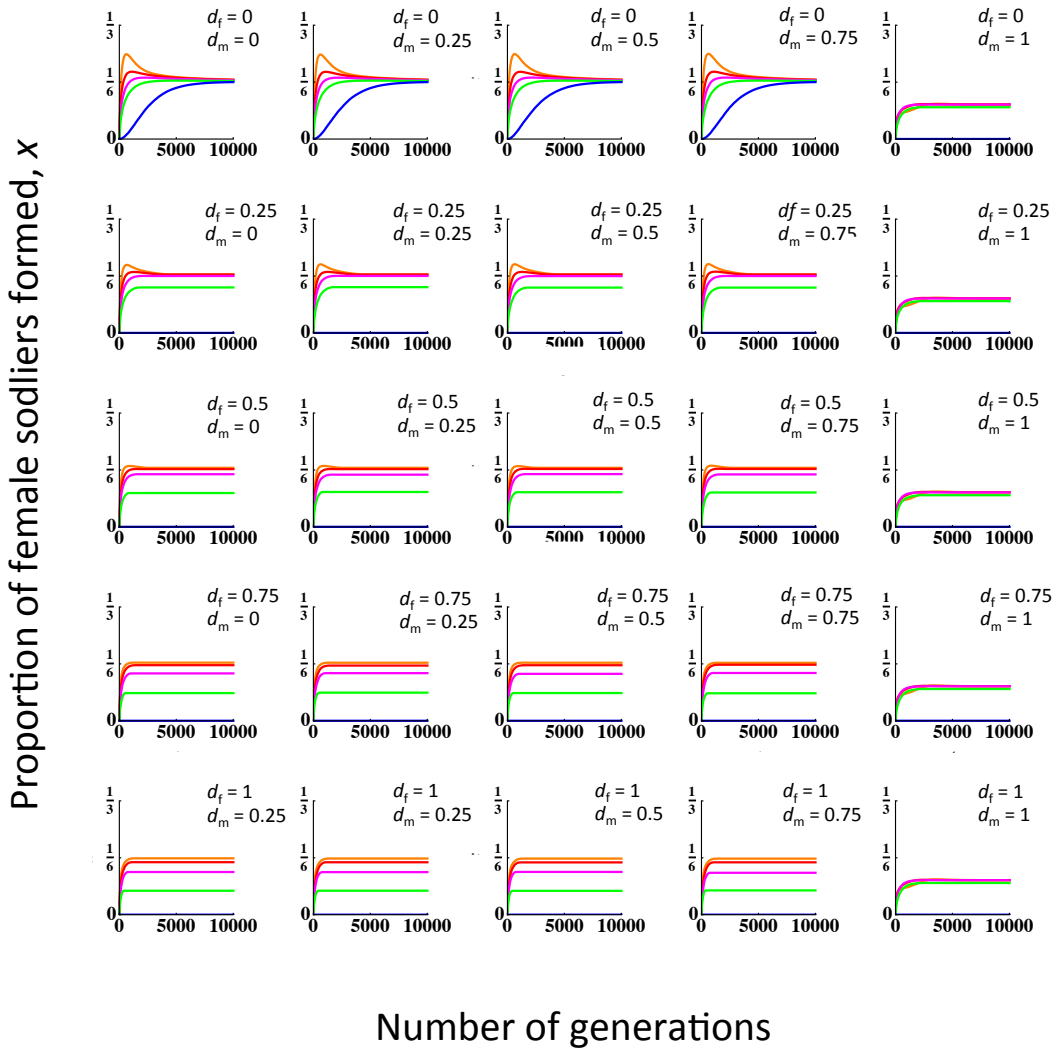


Figure 5.4. Co-evolution of brood allocation ($\sigma$) and soldier production ($a$) – female and male broods may produce soldiers: The value of female soldier allocation increases with male and female dispersal from mixed-sex broods ($d_m$ and $d_f$), and decreases with the initial frequency of single-sex brood allocation ($\sigma_I$). Different lines represent different initial values for brood allocation: $\sigma_I = 0.0001$ (orange), $\sigma_I = 0.25$ (red), $\sigma_I = 0.5$ (pink), $\sigma_I = 0.75$ (green) and $\sigma_I = 0.9999$ (blue). In this figure, the shape parameter for soldier killing curve $k = 0.5$ and the degree of soft selection $s = 0.5$.

## Appendix 5A – Genetic associations

Here I determine the genetical associations between members of each family at equilibrium. For each family unit, I focus on the egg stage, i.e. before clonal proliferation. Hence I can have four individuals: mother, father, sister and brother. I define the coefficients of consanguinity between individuals *i* and *j*, $p_{ij}$ as the probability of picking from individual *i* and from individual *j* a given allele and both are identical by descent (Bulmer 1994). I can then define the relatedness of individual *i* to individual *j* as $r_{ij} = p_{ij}/p_{ii}$ (Bulmer 1994). With this in mind, I first determine the consanguinity coefficients between family members and use them to determine four coefficients of relatedness $r_{BS}$, relatedness of brother to sister, $r_{SB}$ relatedness of sister to brother, relatedness of son to mother $r_{BM}$ and relatedness of daughter to mother, $r_{SM}$.

All coefficients of relatedness arise as a function of the probability of brother-sister mating (inbeeding; Ω), as summarised in Table 5.A1. This is the probability that a female herself has developed in a mixed-sex brood, times the probability that she remains in the patch, times the probability that her mate was a local mate, i.e. a brother. In terms of the model parameters:

$$\Omega = \frac{(1-\sigma)N(1-a)(1-z)}{(1-\sigma)N(1-a)(1-z)+\sigma(1-\mu)N}(1-d_f)\frac{(1-d_m)(1-\sigma)N(1-a)z}{(1-d_m)(1-\sigma)N(1-a)z+\sigma\mu N+d_m(1-\sigma)N(1-a)z}$$

(5.A1)

Table 5.A1 presents a summary of the genetic associations used in this paper.

Table 5.A1: Genetic associations.

| Relationship | Relatedness coefficient | Consanguinity ratio | In terms of inbreeding |
|---|---|---|---|
| Brother to sister | $r_{BS}$ | $p_{BS}/\,p_{SS}$ | $\frac{1}{2-\Omega}$ |
| Sister to brother | $r_{SB}$ | $p_{BS}/\,p_{BB}$ | $\frac{1}{4-3\Omega}$ |
| Son to mother | $r_{SM}$ | $p_{BM}/\,p_{SS}$ | 1 |
| Daughter to mother | $r_{BM}$ | $p_{SM}/\,p_{SS}$ | $\frac{1}{2-\Omega}$ |

## Appendix 5B – Sex allocation strategies

Here I describe a general approach for modelling the action of natural selection on the traits of interest. I use the neighbour modulated (personal) fitness methodology (Hamilton 1964a, b; Taylor 1996; Taylor and Frank 1996; Frank 1997b, 1998; Taylor et al. 2007), a form of kin selection analysis that is particularly useful for solving problems that involve class structure (such as separate sexes). In contrast to the more familiar inclusive fitness approach, which focuses attention on a single actor and examines the fitness consequences of its actions on multiple recipients, the neighbour modulated fitness approach considers the impact of the action of multiple actors upon the fitness of a single recipient (Hamilton 1964a, b). If, on average, individuals carrying a gene of interest are fitter than the other individuals of their class, then natural selection will act to increase the frequency of the gene in the population (Fisher 1930; Hamilton 1964a, b; Price 1970). The appropriate average of fitness across different classes is obtained by weighting each class by its reproductive value, which is the relative asymptotic contribution of genes made by this class to future generations. Hence, the actor's personal fitness is:

$$W = c_{\mathrm{f}} \frac{w_{\mathrm{f}}}{\bar{w}_{\mathrm{f}}} + c_{\mathrm{m}} \frac{w_{\mathrm{m}}}{\bar{w}_{\mathrm{m}}}, \qquad (5.B1)$$

where: $w_{\mathrm{f}}$ is the fitness of an individual female (calculated below); $\bar{w}_{\mathrm{f}}$ is the average fitness of all females in the population; $w_{\mathrm{m}}$ is the fitness of an individual male (calculated below); $\bar{w}_{\mathrm{m}}$ is the average fitness of all males in the population; $c_{\mathrm{f}}$ is the class reproductive value of females, and is given by 2/3 for haplodiploids; and $c_{\mathrm{m}}$ is the class reproductive value of males, and is given by 1/3 for haplodiploids (Price 1970; Taylor and Frank 1996).

Consider a given locus $G$, with genic value $g$, controlling for a phenotypic trait expressed by females. Natural selection acts to increase the average genetic value when $\mathrm{d}W/\mathrm{d}g>0$. As I am working with a class-structured population it is important to use a standardized measure of fitness. Hence, $W_{\mathrm{f}} = w_{\mathrm{f}} / \bar{w}_{\mathrm{f}}$, and $\mathrm{W}_{\mathrm{m}} = w_{\mathrm{m}} / \bar{w}_{\mathrm{m}}$. I use a neighbour-modulated (personal) fitness approach (Taylor and Frank 1996) and define the marginal fitness of locus $G$ as:

$$\frac{\mathrm{d}W}{\mathrm{d}g} = c_{\mathrm{f}} \frac{\mathrm{d}W_{\mathrm{f}}}{\mathrm{d}g} + c_{\mathrm{m}} \frac{\mathrm{d}W_{\mathrm{m}}}{\mathrm{d}g}. \qquad (5.B2)$$

I can extend this definition, by making explicit the link between phenotype variation, locus genetic value and the class's relative fitness. I define $g$ as the individual's average genetic value of for the phenotype. I denote this $\hat{g}$ for the focal individual and

$\hat{g}'$ for the focal individual's sibling. Expanding the derivatives in equation (5.B1) I obtain:

$$\frac{\mathrm{d}W}{\mathrm{d}g} \propto c_{\mathrm{f}} \frac{\partial W_{\mathrm{f}}}{\partial x} \frac{\partial x}{\partial \hat{g}} \frac{\mathrm{d}\hat{g}}{\mathrm{d}g} + c_{\mathrm{m}} \frac{\partial W_{\mathrm{m}}}{\partial x'} \frac{\partial x'}{\partial \hat{g}'} \frac{\mathrm{d}\hat{g}'}{\mathrm{d}g}. \qquad (5.B3)$$

I assume that the breeding value maps to phenotypic trait in the same way for all females. This allows us to treat $\partial x / \partial \hat{g} = \partial x / \partial \hat{x}' = 1$. Also, I can substitute the derivatives of breeding value with respect to genic value by the respective coefficients of consanguinity: $d\hat{g} / dg = p_{\mathrm{SS}}$ and $d\hat{g}' / dg = p_{\mathrm{BS}}$. Thus, I may write:

$$\frac{\mathrm{d}W}{\mathrm{d}g} \propto c_{\mathrm{f}} \frac{\partial W_{\mathrm{f}}}{\partial x} + c_{\mathrm{m}} \frac{\partial W_{\mathrm{m}}}{\partial x'} r_{\mathrm{BS}}. \qquad (5.B4)$$

The right hand side must be bigger than zero for the trait to increase its frequency I assume that this trait presents a vanishing variation, about its population average value. This leads selection to be sufficiently weak allowing us to use relatedness coefficients calculated in a neutral population.

If I assume that the number of adults emerging from each host is constant *N*, such that there are *Nz* males and *N*(1-*z*) females emerging, I can define $z_{\mathrm{S}}^{*} = \bar{z}_{\mathrm{S}}$ as the convergence stable strategy if the female juveniles have complete control over the sex ratio of their mixed-sex brood. In this $z_{\mathrm{S}}^{*}$ satisfies:

$$\frac{\mathrm{d}W}{\mathrm{d}z}\Big|_{z=\bar{z}=z_{\mathrm{S}}^{*}} = c_{\mathrm{f}} \frac{\partial W_{\mathrm{f}}}{\partial z_S} + c_{\mathrm{m}} \frac{\partial W_{\mathrm{m}}}{\partial z_S} r_{\mathrm{BS}} = 0 \qquad (5.B5)$$

Similarly, for the males I describe a locus *H* with genic value *h*, controlling for a trait *y* only expressed by juvenile males. I use the above approach to write the marginal fitness expression for locus *H*:

$$\frac{\mathrm{d}W}{\mathrm{d}h} \propto c_{\mathrm{f}} \frac{\partial W_{\mathrm{f}}}{\partial y'} r_{\mathrm{SB}} + c_{\mathrm{m}} \frac{\partial W_{\mathrm{m}}}{\partial y} . \qquad (5.B6)$$

*y* and *y*' are, respectively , the phenotypes of a focal male and the brother of a focal female. Similar to the previous case, if we define $z_{\mathrm{B}}^{*}$ as the convergence stable sex ratio strategy if male juveniles have full control over the sex ratio in their mixed-sex brood patches, then $z_{\mathrm{B}}^{*}$ satisfies the following condition:

$$\frac{\mathrm{d}W}{\mathrm{d}z}\Big|_{z=\bar{z}=z_{\mathrm{B}}^{*}} = c_{\mathrm{f}} \frac{\partial W_{\mathrm{f}}}{\partial z} r_{\mathrm{SB}} + c_{\mathrm{m}} \frac{\partial W_{\mathrm{m}}}{\partial z} = 0 \qquad (5.B7)$$

Similarly, if $z_{\mathrm{M}}^{*}$ is the convergence stable sex ratio strategy for mixed-sex broods if the laying female has full control over the sex ratio of her mixed-sex brood, then $z_{\mathrm{M}}^{*}$ satisfies the following condition:

$$\frac{\mathrm{d}W}{\mathrm{d}z}\Big|_{z=\bar{z}=z_{\mathrm{M}}^{*}} = c_{\mathrm{f}} \frac{\partial W_{\mathrm{f}}}{\partial z} r_{\mathrm{SM}} + c_{\mathrm{m}} \frac{\partial W_{\mathrm{m}}}{\partial z} r_{\mathrm{BM}} = 0 , \qquad (5.B8)$$

Equations 5.B5, 5.B7 and 5.B8 are not amenable to be analytically solved. Figure 5.1 from the main text gives numerical examples showing how the convergent stable sex ratio strategies from the different family members diverge and give scope to a sex

ratio conflict. I present an analytical argument showing that the convergence sex ratio strategy of sisters is always more female biased than the one from the mother, and this one more female biased than the one from the brother. Following equation 5.B2, and assuming that the breeding values map to the phenotypic trait in the same way for all females, I can rearrange the marginal fitness expression as:

$$\frac{\mathrm{d}W}{\mathrm{d}h} = \frac{\partial z_{\mathrm{S}}}{\partial \hat{g}} \frac{\mathrm{d}\hat{g}}{\mathrm{d}g} \left( c_{\mathrm{f}} \frac{\partial W_{\mathrm{f}}}{\partial z_{\mathrm{S}}} + c_{\mathrm{m}} \frac{\partial W_{\mathrm{m}}}{\partial z_{\mathrm{S}}^{'}} \right). \qquad (5.B9)$$

I can define $F(z_{\mathrm{S}}) = c_{\mathrm{f}} (\partial \mathrm{W_f}/\partial z)|z{=}z_{\mathrm{S}}$ and $m(z_{\mathrm{S}}) = c_{\mathrm{m}} (\partial \mathrm{W_m}/\partial z)|z{=}z_{\mathrm{S}}$. This way, the condition for the an increase in the sex ratio, if it is controlled by the sisters is:

$$F(z_{\mathrm{S}}) + m(z_{\mathrm{S}})\, r_{\mathrm{BS}} \geq 0. \qquad (5.B10)$$

Using the same argument, a condition for an increase of the sex ratio, if controlled by the brothers is:

$$F(\mathrm{z_B})\, r_{\mathrm{SB}} + m(z_{\mathrm{B}}) \geq 0. \qquad (5.B10)$$

Let $z_{\mathrm{S}}^{*}$ be the female's convergence stable sex ratio strategy. Then $F(z_{\mathrm{S}}^{*}) + m(z_{\mathrm{S}}^{*})\mathrm{r_{BS}} = 0$. It is easy to see that $F(z_{\mathrm{S}}^{*}) = - m(z_{\mathrm{S}}^{*}) r_{\mathrm{BS}}$. If I replace $\mathrm{z_B}$ with $z_{\mathrm{S}}^{*}$ in the marginal fitness of brothers, I can write condition 5.B10 as:

$$- m(z_{\mathrm{S}}^{*}) r_{\mathrm{BS}}\, r_{\mathrm{SB}} + m(z_{\mathrm{S}}^{*}) \geq 0\ , \qquad (5.B11)$$

which is only true if $r_{BS}\, r_{SB} \leq 1$. As both $r_{BS}$ and $r_{SB}$ are smaller that one, this condition holds, meaning that, at $z_B = z_S^*$, the marginal fitness of males is positive thus showing that brothers are favoured to produce a more male biased sex ratio than sisters ($z_B^* \geq z_S^*$). I can use the same reasoning to prove that $z_M^* \geq z_S^*$ and that $z_B^* \geq z_M^*$.

## Appendix C – Sex ratio of mixed-sex broods

Here I study how brood allocation changes with soldier activity. I first determine the marginal fitness function of single-sex broods sex ratio, $\mu$. As in Appendix B, I use a neighbour-modulated (personal) fitness approach to determine the marginal fitness function of a trait $J$ with genic value $j$, expressed by laying females, that controls female single-sex brood sex ratio, $\mu$. Natural selection favours the increase of the value of the trait if $\mathrm{d}W/\mathrm{d}h > 0$. Using a similar approach to the one described in Appendix B (expressions B1-B3), and using male and female relative fitness expressions (equations 5.2-5.5), I find that the marginal fitness expression is:

$$\left.\frac{\mathrm{d}W}{\mathrm{d}\mu}\right|_{\mu=\mu^*=\mu} = \frac{\sigma\left(\sigma M_S + (1-\sigma)M_M - \frac{2}{2-\Omega}\right)}{3\left(1-z-a(1-z)(1-\sigma(\mu-z))\right)}, \qquad (5.C1)$$

where $M_S$ and $M_M$ are given by equations 5.3 and 5.4. I now focus on the extremes of brood allocation. In a population composed only of single-sex broods population, the convergence stable sex ratio is to produce an unbiased sex ratio ($\mu^* = 1/2$). In this

case, if a single female produces instead single-sex broods, the sex ratio of her offspring won't be intermediate ($\mu$= 0 or $\mu$ = 1), The convergence stable single-sex brood sex ratio, $\mu$, is given by:

$$\mu^* = \begin{cases} 0 & z > \dfrac{1 + d_{\mathrm{f}}(1 - d_{\mathrm{m}}) + d_{\mathrm{m}}}{3 + d_{\mathrm{f}}(1 - d_{\mathrm{m}}) + d_{\mathrm{m}}} \\ 1 & z < \dfrac{1 + d_{\mathrm{f}}(1 - d_{\mathrm{m}}) + d_{\mathrm{m}}}{3 + d_{\mathrm{f}}(1 - d_{\mathrm{m}}) + d_{\mathrm{m}}} \end{cases} . \qquad (5.C2)$$

I use these to determine the direction of selection for brood allocation. If I consider a trait *U*, with genic value *u*, that controls brood allocation ($\sigma$), the value of *U* increases if d*W*/d*u* > 0.

If I consider a population composed only of single-sex broods ( $\bar{\sigma}$ = 1 and $\bar{\mu}$ = ½), then, the marginal fitness for brood allocation d*W*/d$\sigma$ is given by:

$$\left.\frac{\mathrm{d}W}{\mathrm{d}\sigma}\right|_{\sigma=\bar{\sigma}=\sigma^*} = \frac{2(a - 2(1-a)(1 - a - (2 - d_{\mathrm{f}}) - d_{\mathrm{f}})(1 - d_{\mathrm{m}})z2(1-a)^2(1 - d_{\mathrm{m}})(2 - d_{\mathrm{f}} - d_{\mathrm{m}})z^2)}{3 + 6(1-a)(1 - d_{\mathrm{m}})z} \qquad (5.C3)$$

And an increase in the soldier allocation in a rare mixed-sex brood colony is favoured when:

$$a > \frac{1+2(1-d_{\mathrm{m}})z(3-2d_{\mathrm{f}}-2(2-d_{\mathrm{f}}-d_{\mathrm{m}})z)-\sqrt{1+4(1-d_{\mathrm{m}})z(3-2d_{\mathrm{f}}-(3-2d_{\mathrm{f}}-d_{\mathrm{m}})z)}}{4(1-d_{\mathrm{m}})z(2-d_{\mathrm{f}}-(2-d_{\mathrm{f}}-d_{\mathrm{m}})z)}.$$

(5.C4)

This means that if soldier allocation $a$ is higher than the threshold expressed by (5.C3), then marginal fitness for brood allocation $\mathrm{d}W/\mathrm{d}\sigma > 0$ and, in this case, a population composed of single-sex broods is stable against invasion by a mixed-sex brood strategy.

The change in the marginal fitness of brood allocation in relation to soldier production in mixed-sex broods, is given by:

$$\frac{\mathrm{d}}{\mathrm{d}a}\frac{\mathrm{d}W}{\mathrm{d}u}\Big|_{\sigma=1,\mu=1/2} = \frac{2+8(1-a)(1-d_{\mathrm{m}})z(1+(1-a)(1-d_{\mathrm{m}})z)(2-d_{\mathrm{f}}-z(2-d_{\mathrm{f}}-d_{\mathrm{m}})}{3(1+2z(1-a)(1-d_{\mathrm{m}}))^2} > 0,$$

(5.C5)

meaning that brood allocation to the production of single-sex broods increases with soldier cost. Figure 5.D1 shows this boundary line, separating allocation to single-sex and to mixed-sex broods. Mixed-sex broods may invade a single-sex brood population, only if the cost of soldier activity $a$ is low

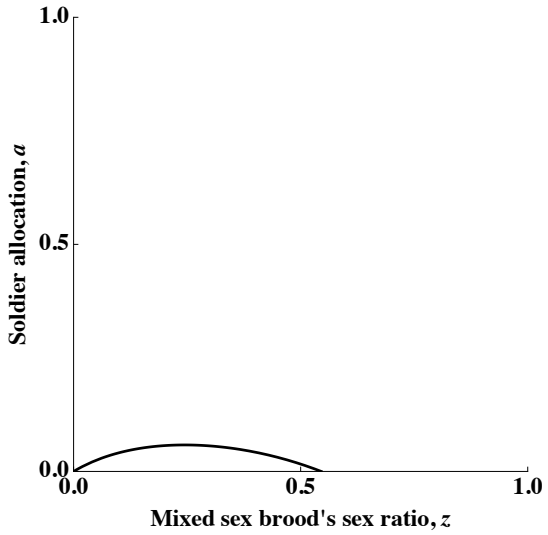


Figure 5.D1. Boundary condition for the increase in the allocation to single-sex broods ($\sigma$), in a population composed only of single-sex broods with the emergence of a mixed-sex brood strategy. A mixed-sex brood strategy may invade a population of single-sex brood strategists if the cost of soldier allocation *a* is bellow the threshold line. Above that line, the production of single-sex broods is an evolutionary stable strategy. For this figure, $d_m$=0.5 and $d_f$=0.4.

Now I consider a population of mixed-sex broods ( $\bar{\sigma} = 0$) and a female is allowed to produce a mutant strategy composed of single-sex and mixed-sex broods ($\sigma > 0$). In this case, the convergence stable sex ratio strategy for single-sex broods is either $\mu =$ 0 or $\mu = 1$. If $\bar{\mu} = 0$, the convergence sex ratio strategy increases with soldier allocation *a* if:

$$a > 1 - \frac{2}{(3 + d_f(1 - d_m) + d_m)(1 - z)}, \qquad (5.C6)$$

and,

$$\frac{\mathrm{d}}{\mathrm{d}a}\frac{\mathrm{d}W}{\mathrm{d}u}\Big|_{\sigma=0,\mu=0} = \frac{2}{3((1-a)^2(1+d_\mathrm{f}(1-d_\mathrm{m})+d_\mathrm{m})(1-z)} > 0\,, \tag{5.C7}$$

which shows us that single-sex brood allocation increases as soldier allocation increases. If $\mu = 1$, then the convergence sex ratio strategy increases with soldier action $a$ if:

$$a > 1 - \frac{1+d_\mathrm{f}+d_\mathrm{m}-d_\mathrm{f}d_\mathrm{m}}{(3+d_\mathrm{f}(1-d_\mathrm{m})-d_\mathrm{m})z}\,, \tag{5.C8}$$

and,

$$\frac{\mathrm{d}}{\mathrm{d}a}\frac{\mathrm{d}W}{\mathrm{d}u}\Big|_{\sigma=0,\mu=0} = \frac{1}{3((1-a)^2 z} > 0\,, \tag{5.C9}$$

meaning that also in this case, single-sex brood allocation increases with soldier action.

If I compare expressions D5 and D7 at the threshold condition found in D2, I find that $a^* = 0$ at this threshold. By finding $\mathrm{d}a/\mathrm{d}z$ at the threshold condition found in D5 and D7, I find that:

$$\frac{\mathrm{d}a}{\mathrm{d}z}\Big|_{\sigma=0,\mu=0} = -\frac{2}{(3+d_\mathrm{f}(1-d_\mathrm{m})+d_\mathrm{m})(1-z)^2} < 0\,, \tag{5.C10}$$

and,

$$\frac{\mathrm{d}a}{\mathrm{d}z}\Big|_{\sigma=0,u=1} = \frac{1+d_\mathrm{f}+d_\mathrm{m}(1-d_\mathrm{f})}{(3+d_\mathrm{f}(1-d_\mathrm{m})+d_\mathrm{m})z^2} > 0\ . \qquad (5.C11)$$

This means that the only possible condition for mixed-sex broods to invade single-sex broods is if $a = 0$. This means that $\sigma = 0$ is not a stable condition and it is only neutrally stable if soldier cost is zero ($a = 0$).

## Appendix D – Co-evolution of male and female soldier and brood allocation

Here I study the joint convergence stable strategies for brood allocation, sex allocation in both single and mixed-sex broods and soldier allocation in mixed-sex broods. I use a neighbor-modulated (personal) fitness approach to describe the laying female's brood allocation strategy, in a similar fashion to the methodology described in Appendix 5B. I define a gene $U$ with genic value $u$, controlling for the brood allocation decision ($\sigma$) made by a laying female. Natural selection will act to increase the average genic value when $\mathrm{d}W/\mathrm{d}u > 0$, and following similar steps as equations 5B2-5B4, I can write the marginal fitness for brood allocation as:

$$\frac{\mathrm{d}W}{\mathrm{d}u} \propto c_\mathrm{f}\frac{\partial W_\mathrm{f}}{\partial u}r_\mathrm{SM} + c_\mathrm{m}\frac{\partial W_\mathrm{m}}{\partial u}r_\mathrm{BM}\,, \qquad (D1)$$

where $r_{SM}$ and $r_{BM}$ are the relatedness of a daughter to her mother and of a son to his mother, respectively. We define the laying female's preferred brood allocation strategy ($\sigma^*$) as satisfying the condition:

$$\frac{\mathrm{d}W}{\mathrm{d}u}\Big|_{\sigma^*=\bar{\sigma}=\sigma} = c_{\mathrm{f}} \frac{\partial W_{\mathrm{f}}}{\partial \sigma} r_{SM} + c_{\mathrm{m}} \frac{\partial W_{\mathrm{m}}}{\partial \sigma} r_{BM} . \quad \text{(D2)}$$

The sex ratio of single-sex broods ($\mu$), defined as the proportion of male-only broods, is also determined by the laying female. In this case, we define a gene *J* with a genic value *j*, controlling for the sex allocation strategy if producing a single-sex brood. In this case, the marginal fitness for single-sex broods' sex allocation decision is given by:

$$\frac{\mathrm{d}W}{\mathrm{d}j} \propto c_{\mathrm{f}} \frac{\partial W_{\mathrm{f}}}{\partial j} r_{SM} + c_{\mathrm{m}} \frac{\partial W_{\mathrm{m}}}{\partial j} r_{BM} , \quad \text{(D3)}$$

and the laying female's preferred sex allocation strategy ($\mu^*$) satisfies the condition:

$$\frac{\mathrm{d}W}{\mathrm{d}j}\Big|_{\mu^*=\bar{\mu}=\varpi} = c_{\mathrm{f}} \frac{\partial W_{\mathrm{f}}}{\partial \mu} r_{SM} + c_{\mathrm{m}} \frac{\partial W_{\mathrm{m}}}{\partial \mu} r_{BM} \geq 0 . \quad \text{(D4)}$$

I consider that female soldier allocation *a* in mixed-sex broods is controlled by a gene *G* with genic value, *g* expressed in the female embryo. In this case, the marginal fitness for female soldier allocation is given by:

$$\frac{\mathrm{d}W}{\mathrm{d}g} \propto c_{\mathrm{f}} \frac{\partial W_{\mathrm{f}}}{\partial a} + c_{\mathrm{m}} \frac{\partial W_{\mathrm{m}}}{\partial a} r_{\mathrm{BS}}, \tag{D5}$$

and the preferred female soldier allocation strategy ($a^*$) satisfies the condition:

$$\frac{\mathrm{d}W}{\mathrm{d}x}\Big|_{a^*=\bar{a}=a} = c_{\mathrm{f}} \frac{\partial W_{\mathrm{f}}}{\partial a} + c_{\mathrm{m}} \frac{\partial W_{\mathrm{m}}}{\partial a} r_{\mathrm{BS}} \geq 0. \tag{D6}$$

Similarly, if a gene $H$ with genic value $h$ controls male soldier allocation in mixed-sex broods, the marginal fitness for male soldier allocation ($a^*$) is given by:

$$\frac{\mathrm{d}W}{\mathrm{d}h} \propto c_{\mathrm{f}} \frac{\partial W_{\mathrm{f}}}{\partial a} r_{\mathrm{SB}} + c_{\mathrm{m}} \frac{\partial W_{\mathrm{m}}}{\partial a}, \tag{D7}$$

and the preferred female soldier allocation strategy ($a^*$) satisfies the condition:

$$\frac{\mathrm{d}W}{\mathrm{d}h}\Big|_{a^*=\bar{a}=a} = c_{\mathrm{f}} \frac{\partial W_{\mathrm{f}}}{\partial a} r_{\mathrm{SB}} + c_{\mathrm{m}} \frac{\partial W_{\mathrm{m}}}{\partial a} \geq 0. \tag{D8}$$

I also consider that males may be favoured to produce a proportion $\zeta$ of non-developing embryos. In this case, $H$ controls the allocation to non-developing male embryos in mixed-sex broods. Thus, $\zeta^*$ is the convergence stable strategy for the production of non-developing embryos if:

$$\frac{\mathrm{d}W}{\mathrm{d}h}\Big|_{\zeta^*=\bar{\zeta}=\zeta} = c_{\mathrm{f}} \frac{\partial W_{\mathrm{f}}}{\partial \zeta} r_{\mathrm{SB}} + c_{\mathrm{m}} \frac{\partial W_{\mathrm{m}}}{\partial \zeta} \geq 0$$

To define explicitly the effect of soldier and non-developing embryos allocation on the number of emergent reproductives ($N$) in mixed-sex broods and in their sex ratio ($z$), I use equations 5.6 and 5.7, generalized to include the production of non-developing individuals. Thus, the number of emergent reproductive is given by:

$$N \propto \left( \frac{(1-y)(1-\zeta)(1-x^k)+(1-x)(1-y^k)}{2} \right)^{1-s}, \qquad \text{(D9)}$$

and the sex ratio $z$ is:

$$z = \frac{(1-y)(1-\zeta)(1-x^k)}{(1-y)(1-\zeta)(1-x^k)+(1-x)(1-y^k)}, \qquad \text{(D10)}$$

where $x$ is the proportion of female soldiers produced, $y$ is the proportion of male soldiers produced and $\zeta$ is the proportion of male suiciders produced. The parameter $k$ gives the shape for soldier action curve and $s$ describes the degree of soft selection within the host.

The resolution of this set of equations, for the joint convergence stable strategies, is not amenable to be solved analytically. I use a numerical approach where I first define the initial conditions for the proportion of female and male soldiers, male non-developing embryos, brood allocation and single-sex brood sex ratio. I also define male and female mixed-sex broods' dispersal rate, the shape of soldier action $k$ and the degree of soft selection $s$ as fixed parameters. I then follow by inserting these initial conditions in the above set of marginal fitness functions and use a computational routine to find the variable values that maximize the set of marginal

fitness functions. I use the initial conditions to determine the sign of each marginal fitness function. If that sign is positive, that variable increases its value by a small random amount. If it is negative, the variable decreases its sign by a small random amount. If the trait value decreases bellow 0.9999 I set it to 0.9999, and if it increases above 1, I set it to 1. I use the new set of variable values and repeat the routine, for at least 50000 generations using Wolfram's Mathematica (Wolfram, 2011). I consider two particular scenarios: i) a population where females may produce soldiers and males may limit own development and ii) a population where both males and females may produce soldiers. The following routine code considers the evolution of female ($x$) and male ($y$) soldier allocation, brood allocation ($\sigma$) and single-sex brood's sex allocation ($\mu$):

```
CoEvo[σstart_, ξ_, dm_, df_, k_, s_, its_Integer] :=
  Module[{it}, (μydata = Table[{, }, {its}]; μxdata = Table[{, }, {its}];
    μdata = Table[{, }, {its}]; xdata = Table[{, }, {its}]; ydata = Table[{, }, {its}];
    μ = 0.5; y = 0.0001; x = 0.0001; σ = σstart;
    Do[
     μnew = μ + 0.01 (2 CDF[NormalDistribution[0, 1], wμ[x, y, ξ, σ, μ, dm, df, k, s]] - 1) RandomReal[];
     σnew = σ + 0.01 (2 CDF[NormalDistribution[0, 1], wσ[x, y, ξ, σ, μ, dm, df, k, s]] - 1) RandomReal[];
     ynew = y + 0.01 (2 CDF[NormalDistribution[0, 1], wm[x, y, ξ, σ, μ, dm, df, k, s]] - 1) RandomReal[];
     xnew = x + 0.01 (2 CDF[NormalDistribution[0, 1], wf[x, y, ξ, σ, μ, dm, df, k, s]] - 1) RandomReal[];
     If[μnew < 0.00001, μnew = 0.00001, If[μnew > 1, μnew = 1,]];
     If[σnew < 0.00001, σnew = 0.00001, If[σnew > 1, σnew = 1,]];
     If[ynew < 0.00001, ynew = 0.00001, If[ynew > 1, ynew = 1,]];
     If[xnew < 0.00001, xnew = 0.00001, If[xnew > 1, xnew = 1,]];
     μ = μnew;
     σ = σnew;
     y = ynew;
     x = xnew;
     μydata[[it]] = {μ, y}; μxdata[[it]] = {μ, x};
     μdata[[it]] = {it, μ}; ydata[[it]] = {it, y}; xdata[[it]] = {it, x},
     {it, its}])];
RunCoEvo[k_, s_, its_Integer] :=
  Module[{σstart, dm, df, σ}, (listμy = Table[, {5}, {5}, {5}]; listμx = Table[, {5}, {5}, {5}];
    listy = Table[, {5}, {5}, {5}]; listμ = Table[, {5}, {5}, {5}]; listx = Table[, {5}, {5}, {5}];
    Do[
     Do[
      Do[
       If[dmcount == 1, dm = 0, If[dmcount == 5, dm = 1, (dmcount - 1)/4]];
       If[dfcount == 1, df = 0, If[dfcount == 5, df = 1, df = (dfcount - 1)/4]];
       If[σcount == 1, σstart = 0.0001, If[σcount == 5, σstart = 0.9999, σstart = (σcount - 1)/4]];
       CoEvo[σstart, 0, dm, df, k, s, its];
       listμ[[dfcount, dmcount, σcount]] = μdata;
       listy[[dfcount, dmcount, σcount]] = ydata;
       listx[[dfcount, dmcount, σcount]] = xdata;
       listμy[[dfcount, dmcount, σcount]] = μydata;
       listμx[[dfcount, dmcount, σcount]] = μxdata,
       {σcount, 5}], {dmcount, 5}], {dfcount, 5}])];
Run3[0.5, 0.8, 50000]
```

## Supporting material

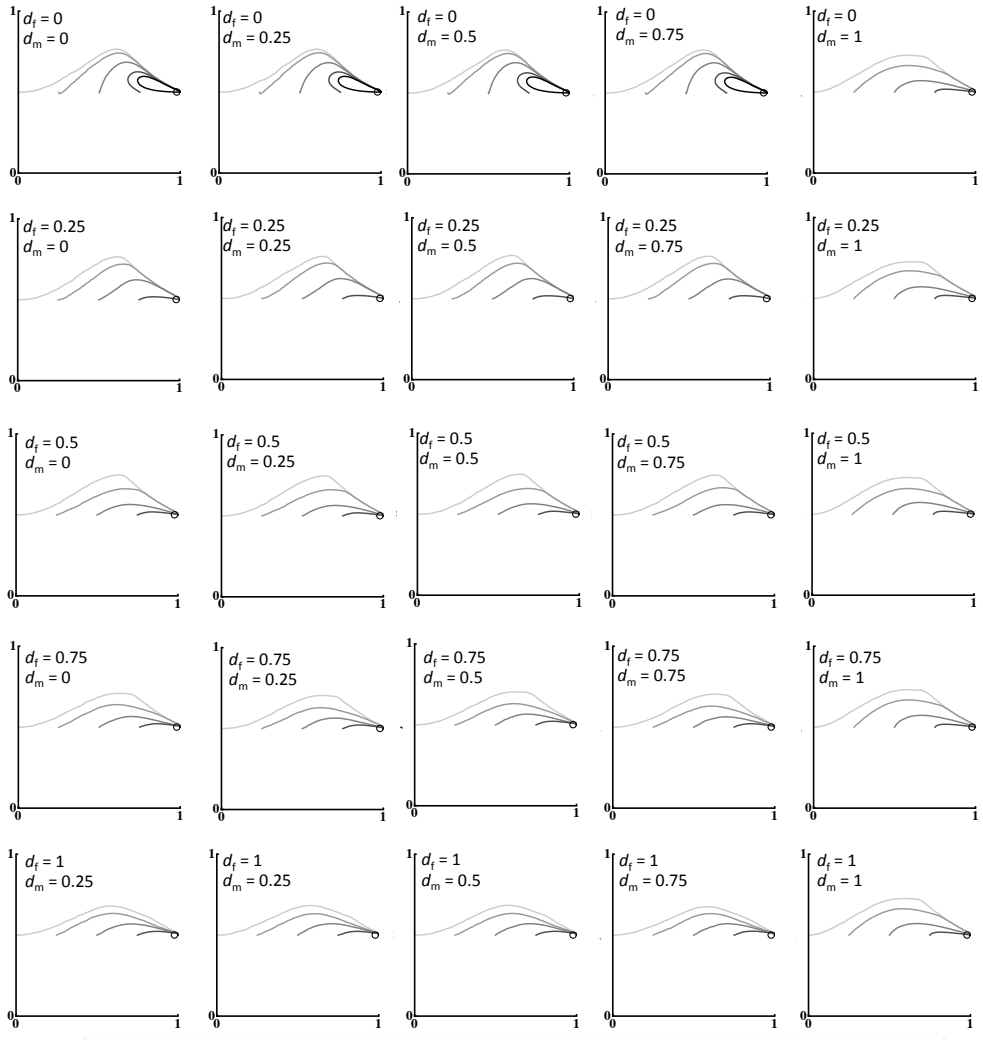


Figure 5.S1. Co-evolution of brood allocation and soldier production – female may produce soldiers and males may limit own development: Single-sex broods always evolve to fixation ($\sigma = 1$) and to produce an unbiased sex ratio ($\mu = 0.5$). Different values of male and female dispersal from mixed-sex broods ($d_m$ and $d_f$) or initial brood allocation ($\sigma$) lead to different evolutionary trajectories but with the same outcome. Different lines represent different initial values for brood allocation, that changes from $\sigma_I = 0.0001$ (light grey) to $\sigma_I = 0.9999$ (black), with $\sigma_I = 0.25$, $\sigma_I = 0.5$ and $\sigma_I = 0.75$ as intermediate values.

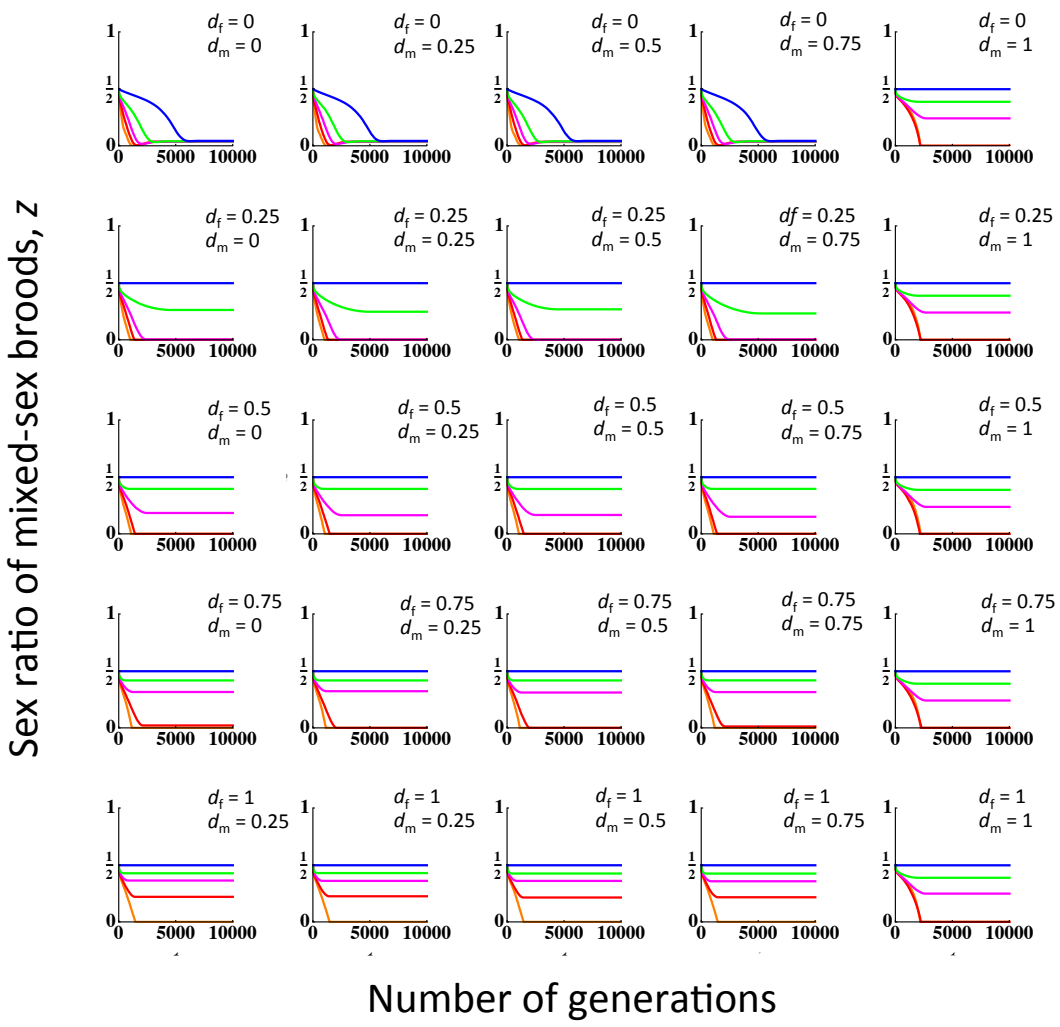


Figure 5.S2. Co-evolution of brood allocation and soldier production – female may produce soldiers and males may limit own development: Mixed-sex broods evolve to produce a female biased sex ratio. This bias decreases with male and female dispersal from mixed-sex broods ($d_m$ and $d_f$), and with an increase in single-sex brood allocation ($\sigma$).Different lines represent different initial values for brood allocation: $\sigma_I = 0.0001$ (orange), $\sigma_I = 0.25$ (red), $\sigma_I = 0.5$ (pink), $\sigma_I = 0.75$ (green) and $\sigma_I = 0.9999$ (blue).

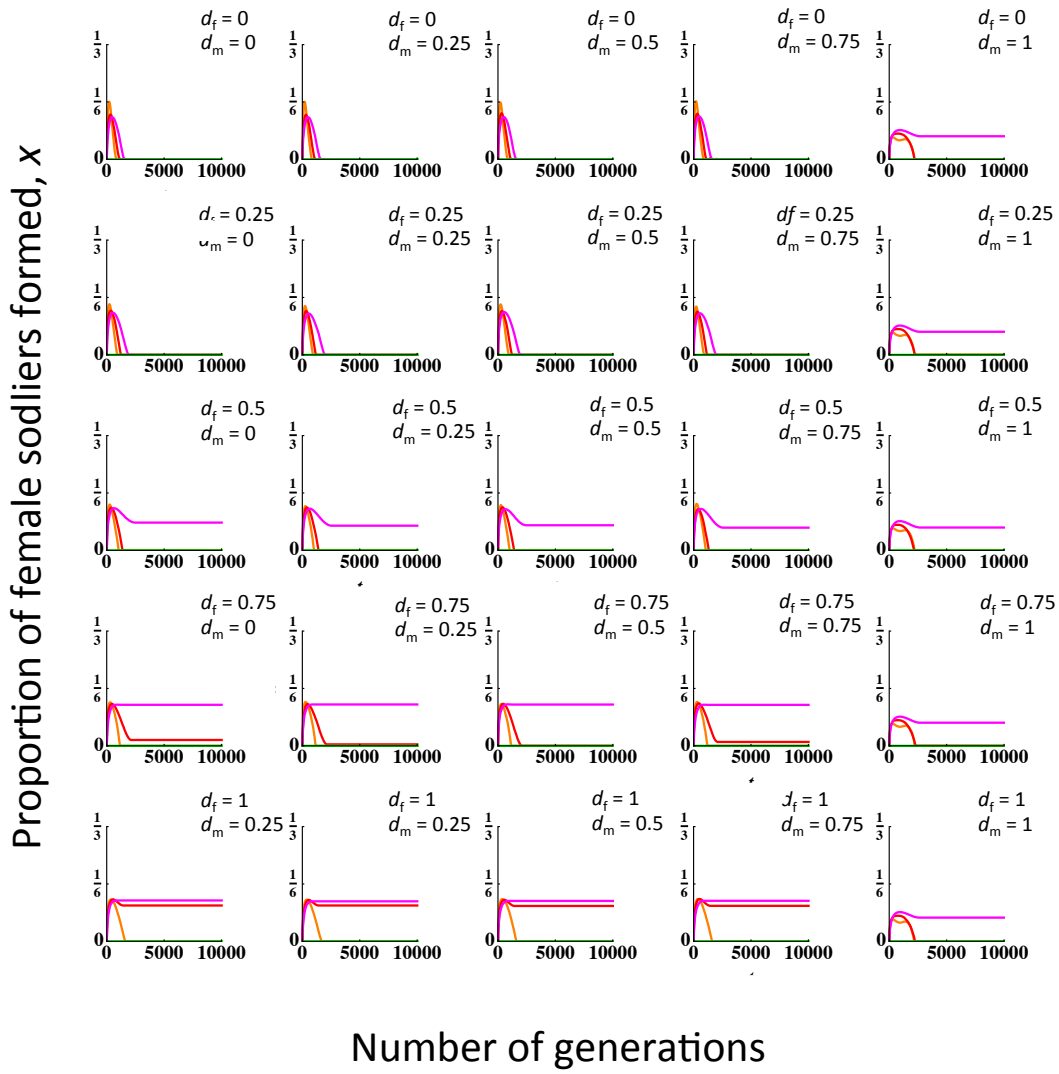


Figure 5.S3. Co-evolution of brood allocation and soldier production – female may produce soldiers and males may limit own development: Female soldier allocation increases with male ($d_m$) and female ($d_f$) dispersal in mixed-sex broods, and decreases with the initial value of single-sex brood allocation ($\sigma_I$). Males do not evolve to produce soldiers. Different lines represent different initial values for brood allocation: $\sigma_I = 0.0001$ (orange), $\sigma_I = 0.25$ (red), $\sigma_I = 0.5$ (pink), $\sigma_I = 0.75$ (green) and $\sigma_I = 0.9999$ (blue).

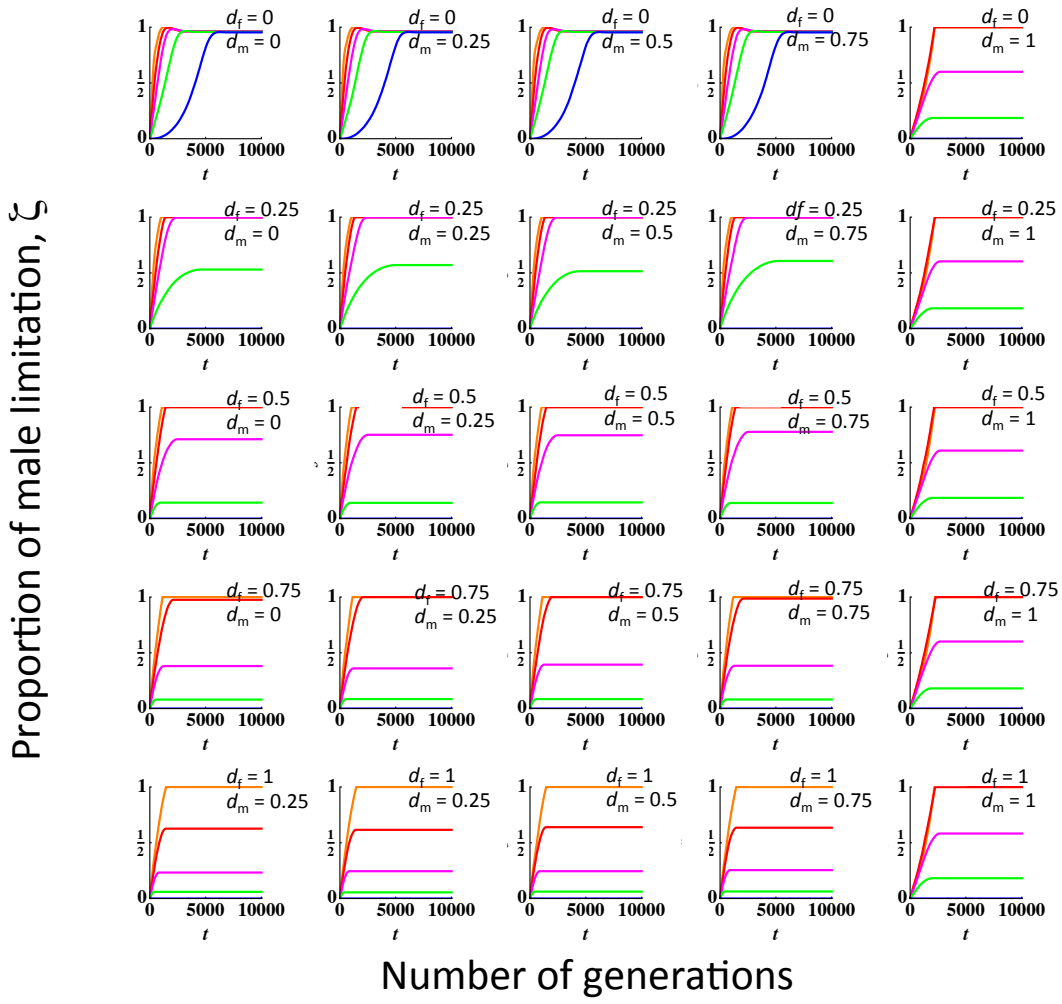


Figure 5.S4. Co-evolution of brood allocation and soldier production: female broods may produce soldiers and male brood may limit own development. The proportion of males limiting their development increases with the initial value of single-sex brood allocation ($\sigma_I$) and decreases with male ($d_m$) and female ($d_f$) dispersal in mixed-sex broods. Different lines represent different initial values for brood allocation: $\sigma_I = 0.0001$ (orange), $\sigma_I = 0.25$ (red), $\sigma_I = 0.5$ (pink), $\sigma_I = 0.75$ (green) and $\sigma_I = 0.9999$ (blue).

# 6. Discussion

Each of the preceding chapters of this thesis has included its own extensive discussion. In this chapter I briefly review the main findings of each one of the preceding chapters and discuss emerging general points and implications arising from this work.

### Chapter 2. Sex and death in malaria parasites

In chapter 2, I used fertility insurance theory to examine the consequences of fertility limiting therapeutic interventions in malaria infections, and consequently provide an assessment of how robust they will be to evolutionary counter-adaptations by the parasite. The two-host life cycle characteristic of these parasites, where the sexual stages are transmitted from the vertebrate host to the insect vector, opens a window of opportunity for the development of interventions affecting the development of the sexual stages and as, a consequence, the transmission of the parasite to the vector (Saul 2008). Accordingly, much work has been done in the identification of potential transmission-blocking factors, as well as on their mechanism of action (Paul et al. 2003a; Saul 2008). However, the efficacy of these interventions may be eroded if the parasites adjust their sex allocation. The purpose of the model I present in Chapter 2 was to understand the impact of such interventions in the sex ratio strategy of the parasites.

I developed a sex allocation model considering the trade-off between the production of male and female gametocytes. I focused on an extreme situation, of having a single

parasite lineage in each host and have shown how the number of male and female gametocytes reaching mating maturity affects the expected sex ratio strategy of the parasites. Two important results emerged from this analysis: first, that differences in mortality through the development stages of male and female mating types affect the expected sex ratio outcomeand second, that the production of unviable gametes doesn't drive the evolution of the sex ratio strategies of the parasites. The model helped to understand the experimental results obtained in this project showing, on one hand, the power of taking an approach integrating both experimental and theoretical approaches and also, on the other hand, the power of sex allocation theory when used taking in consideration relevant biological details. In this context, additional work can be made on testing some of the assumptions taken in this model. For instance, I assumed that the parasite is selected to maximize the number of zygotes produced. However, recent work has found a huge bottleneck that occurs between developmental stages of mosquito (L. Pollitt, T. Churcher, E. Dawes, N Colegrave, S. Reece, et al., unpublished data), suggesting that this assumption may not be necessarily true. Programmed cell death has been shown to occur in a broad range of protozoan parasites (Al-Olayan et al. 2002; Zangger et al. 2002), suggesting that this might be a self-regulating mechanism (Duszenko et al. 2006; van Zandbergen et al. 2010; Reece et al. 2011). It is also relevant to understand what is the epidemiological impact of variations in the transmission rates in particular, how it affects the prevalence and the virulence of the parasite (Reece et al. 2011).

## Chapter 3. Haplodiploidy and the evolution of eusociality: worker revolution

In Chapter 3, I studied the relevance of haplodiploidy for the evolution of eusociality. In a haplodiploid genetic system, males are produced from unfertilized haploid eggs, while females are produced from fertilized diploid eggs. Due to this genetic asymmetry a female is more related with her sister than with her own offspring. Hamilton pointed this genetic asymmetry as a potentially important fact in the evolution of eusociality, in what has become known as the "haplodiploidy hypothesis". Trivers and Hare (1976) proposed that haplodiploidy may favour eusociality through two scenarios: first, workers driving a female bias in the queen's offspring sex ratio; and second, workers replacing brothers with their own sons. However, the work presented by Trivers and Hare (1976) doesn't consider formally the consequences of the gradual change in the control over the sex ratio of the colony or the consequences of having worker reproduction.

In Chapter 3, I formalized these two scenarios and showed that haplodiploidy tends to disfavor rather than favour a helping trait. This result is due to the complex changes in relatedness and in reproductive value caused by the workers' action, both by changing the sex ratio of the queen's offspring and by replacing that offspring with their own. Several papers have shown that haplodiploidy could favour the evolution of eusociality for other reasons, including maternal effects, asymmetrical relatedness in structured populations or synergistic interactions between genes for helping (Wade 2001; Lehmann et al. 2008; Fromhage and Kokko 2011; Johnstone et al. 2012). However, these ideas require restrictive assumptions and end up showing that

haplodiploidy may work instead of showing if it would work in realistic biological scenarios (Gardner et al. 2012) . Instead, my results support the suggestion that monogamy has played a more important role in the evolution of eusociality, by maximizing the relatedness between a female and her siblings. This is consistent with empirical findings showing that eusocial lines emerge from strictly monogamous ancestors and that the evolution of cooperative breeding is more common in species with lower rates of promiscuity (Boomsma 2007; Hughes et al. 2008; Boomsma 2009; Cornwallis et al. 2010).

## Chapter 4: Haplodiploidy and the evolution of eusociality: worker reproduction

In Chapter 3, I extended the analysis presented in (Gardner et al. 2012) on the role of split sex ratios due to queen virginity and queen replacement, by focusing on the influence of worker reproduction and variation on the sex ratio produced by workers. Split sex ratios have been suggested as a mechanism whereby workers at colonies producing relative female-biased broods would gain the relatedness benefits of rearing sisters without these being offset by a reduced reproductive value of the females. This is because colonies producing a relative male-biased offspring would help to maintain an even sex ratio at the population level. In the previous chapter, we addressed one mechanism for split sex ratios, queen versus worker control for the sex ratio of queen-derived offspring. In this chapter we focused on the two other mechanisms that are supported by empirical evidence and are also likely to have had occurred during the transition to eusocial living: queen virginity and queen replacement (Gardner et al. 2012).

My analysis shows first that, if a focal worker is able to produce offspring with a different sex ratio than her colony, this may lead the worker to value her own offspring over her siblings and in this way inhibit the evolution of helping. We also found that, in general, worker reproduction decreases the value of the colony offspring. This because it means that a worker will be faced with the possibility of helping to raise not only siblings, but also nephews and nieces. As in the previous chapter, I found that monogamy has a more consistent role in increasing the value of the colony offspring and in this way contribute for helping to be promoted. The approach used to study the evolution of eusociality, in this Chapter and in Chapter 2, shows the potential of a kin selection approach in pinpointing how key quantities as relatedness and reproductive value change and influence the expected outcome of natural selection under different biological assumptions.

### Chapter 5: Co-evolution of soldier production and brood allocation in polyembryonic parasitoid wasps

In this chapter I examine the existence of a sex ratio conflict in polyembryonic parasitoid wasps and its importance on the evolution and function of a sterile soldier cast soldier. In species from the genus *Copidosoma*, females may lay one or two opposite sex eggs, that develop into multiple embryos. These may give rise either to reproductive individuals or to soldiers. Two main hypotheses explain the evolution of soldiers: to mediate a sex ratio conflict, or to provide defense and facilitate the development of siblings. Gardner et al. (2007) developed a model considering sex ratio conflict and the development of a soldier caste in a population where females

produce mixed-sex broods. However, this model did not include important biological details, as the posssibility of females producing single-sex broods.

I extended that approach by allowing females to produce both types of brood. I demonstrated that this conflict still holds in a population composed of both types of broods, and that allocation to single-sex broods may even increase the difference between the preferred sex ratio of sisters and brothers. After showing the scope for a sex ratio conflict, I demonstrate that females are in the majority of cases favoured to produce more soldiers than males. This result was illustrated with a numerical simulation, where I found that females but not males are selected to produce soldiers, to mediate the sex ratio conflict. I have shown as well that males may be selected to limit their developmental proliferation. Also, due to the cost of producing soldiers, females are selected to increase the production of singles-ex broods, to the extent in some cases of allocation all eggs to single-sex broods. This work demonstrates the importance of incorporating relevant biological details, as the production of both types of brood, on the outcome of soldier production. This work also exposed the need of incorporating other factors to fully understand soldier allocation and function. Soldier production is costly, making mixed-sex broods more expensive to produce than single-sex broods. It is therefore necessary to extend this work to take into account possible costs of producing only single-sex broods. Such costs may be related for instance with a limitation of hosts, leading females to allocate two eggs per host instead of one. Also, mixed-sex broods may be produced in order to assure that all females in a brood are mated. Also, it is necessary to account for soldier production in single-sex broods, which may be related with a conflict over resources with other parasites present in the host.

## General remarks

*Biological details are important*

My work highlights how important biological assumptions are in order to develop models that explain the variation found in natural populations. For example, in Chapter 5 I extend (Gardner et al. 2007) analysis of function and soldier production in polyembryonic wasps. By allowing females to produce both mixed-sex and single-sex broods, I show that producing mixed-sex broods only is not a stable strategy if females have the opportunity of producing single-sex broods as well. My results agree with (Gardner et al. 2007) in relating soldier function with a sex ratio conflict, but in my model, I show that mated females evolve to produce mainly or only single-sex broods. This result shows the limitations of my model and that of Gardner et al. (2007), as mixed-sex broods are predominant in natural populations. Factors as fertility insurance, host limitation, unmatedness or costs of dispersing may important in maintaining mixed-sex broods. Hence, to have a better understanding of soldier and brood allocation, these models have to be extended in order to encompass costs of producing single-sex broods.

*Simple is beautiful but not always true*

Biological details are also relevant, even when simple explanations seem to be powerful enough to provide answers. The haplodiploid hypothesis is an example of how, an apparently simple and powerful insight – the higher relatedness of a worker to her sister than to her own offspring explains why eusociality evolves in haplodiploid populations – can be misleading. First, Trivers and Hare (1976) showed that relatedness to siblings is only higher than relatedness to own offspring if workers

direct helping preferencially towards sisters instead of brothers. However, Craig (1979) showed that the resulting female-bias in the population increases male reproductive value offsetting the effect of relatedness.

In Chapter 3, I followed Trivers and Hare's (1976) hypothesis for the evolution of eusociality and found that, in order to fully capture their argument, several different models could be constructed. Objective assumptions are necessary to build a model, as colony sex ratio evolution, as an association between helping and colony type, as variation on the potential sex ratio produced by the worker or as the number of workers in the colony, are important as they will influence the value for a focal worker of own offspring versus colony offspring. For example, it seems reasonable to hypothesize as Trivers and Hare (1976) do, that worker production of males would promote helping and be important for the evolution of altruism. However, that means that a worker will be allocation help towards siblings and towards nephews and nieces, much more than to own offspring. This detail makes worker reproduction to inhibit helping more often than to promote helping.

*Sex allocation is (still) an important area of research*

Sex allocation is one of the most successful areas of evolutionary biology ,in particular ,of social evolution theory. The remarkable predictive power of this body of theory to explain biological variation in sex ratios doesn't mean that there are no more interesting sex allocation problems to be tacked. In Chapter 2 I used a sex allocation model to predict how the malaria parasite sex ratio will change with different male and female mortality and viability in the sexual stages of the parasite. This question is crucial for the development of drugs affecting the transmission of the parasite from

the host to the vector. I have shown that an intervention increasing the mortality of gametocytes or gametes may not be evolution-proof, as the parasite may be selected to change sex-allocation towards the production of the limiting sex. However an intervention that decreases the ability of a gamete to produce a viable zygote will not change the sex allocation strategy and thus will potentially be more efficient than an intervention that increases gamete mortality.

In Chapter 3, I addressed the conflict between queen and workers over the optimal sex ratio of the colony reproductive offspring. Most of the models developed in this area assume that either the queen or the colony workers are in control of the offspring and data supports both scenarios (however, see Reuter and Keller 2001), but the question on which biological details are responsible for each one of the cases is still to be answered (West 2009). This question is probably related with the amount of information the queen and the workers have on the sex ratio manipulation done in the colony. Pen and Taylor (2005) presented a model showing that information flow influences the outcome of the colony's sex ratio. Models focusing in similar situations but including ecological complications has relatedness asymmetry variation, partial control of caste determination, cost of manipulation or the possibility of split sex ratios, for instance, would be useful and comparable with empirical data (West 2009).

*Relatedness is important but not the only thing important*

One of the misconceptions still prevalent in evolutionary ecology textbooks is that the haplodiploidy hypothesis works due to the higher relatedness of workers to the female biased offspring produced by the queen (Krebs and Davies 1993; Alcock 2005), leading to the idea that relatedness alone explains the evolution of eusociality. Part of

my work in this thesis concerns making clear the factors that may affect the evaluation made by a focal worker of producing her own offspring versus helping to raise the colony offspring. In particular, the change in the reproductive value of males and females is essential to fully understand why haplodiploidy alone doesn't explain the evolution of eusociality. Hamilton's rule is a simple but powerful conceptualization of the forces that shape social adaptation and adaptation in general. Adaptation depends on three measures of value: cost/benefits, relatedness and reproductive value (Frank 1998). Focusing on any of these, to the exclusion of others, will provide us an incomplete picture of evolution.

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# Appendix

This Appendix contains the other publications resulting from my D.Phil.:

**Section 1:** Gardner A, Arce A & Alpedrinha J (2009) Budding dispersal and the sex ratio. Journal of Evolutionary Biology 22, 1036-1045.

*This is a theoretical paper examining budding dispersal as a mechanism to avoid local competition.*

**Section 2:** Gardner A, Alpedrinha J & West SA (2012) Haplodiploidy and the evolution of eusociality: split sex ratios. American Naturalist 179, 240-256.

*This is the first of a series of papers examining the role of haplodiploidy on the evolution of eusociality.*

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# Budding dispersal and the sex ratio

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scale of competition;
sex allocation;
viscous population.

## Abstract

There is much interest in understanding how population demography impacts upon social evolution. Here, we consider the impact of rate and pattern of dispersal upon a classic social evolutionary trait – the sex ratio. We recover existing analytical results for individual dispersal, and we extend these to allow for budding dispersal. In particular, while a cancelling of relatedness and kin competition effects means that the sex ratio is unaffected by the rate of individual dispersal, we find that a decoupling of relatedness and kin competition means that budding dispersal favours increasingly female-biased sex ratios. More generally, our analysis illustrates the relative ease with which biological problems involving class structure can be solved using a kin selection approach to social evolution theory.

## Introduction

In recent years there has been much interest in the impact of population demography on social evolution. Of particular interest has been the problem of social evolution in 'viscous' populations (Queller, 1992a; West *et al.*, 2002). Hamilton (1964, 1971) suggested that cooperative behaviours should be especially favoured in those populations where individuals do not disperse far during the course of their lifetime, owing to the high relatedness that is expected to build up between neighbours. However, limited dispersal leads to intensified kin competition, and in the simplest possible scenario – Wright's (1931) infinite island model – this exactly cancels the effect of increased relatedness, such that cooperation evolves no more readily in a viscous population than in a fully mixing population (Taylor, 1992). This result holds for sexual and asexual populations, under haploid, diploid and haplodiploid modes of inheritance.

This surprising dispersal-independence result appears to have been first reported by Bulmer (1986), in the context of a reanalysis of Hamilton's (1967) local mate competition (LMC) model that allowed for incomplete dispersal of diploid individuals after mating. Bulmer's numerical results suggested that individual dispersal had no or limited impact upon the evolution of the sex ratio – i.e. proportion of offspring that are male. Frank (1986a) recovered the same results from an analytical examination of Bulmer's model, and explained these on the basis of opposing relatedness and kin competition effects. Later, Taylor (1988) extended the analysis to haplodiploids revealing that, while the cancellation of relatedness and kin competition was no longer exact, the impact of individual dispersal on the sex ratio remained negligible.

A huge amount of literature has been generated in pursuit of simple mechanisms that could allow population viscosity to promote social behaviours. One mechanism that has received recent attention is 'budding' dispersal, whereby individuals disperse away from their social groups (relaxing kin competition) but alongside other group mates (ensuring high relatedness; Haldane, 1932; Pollock, 1983; Wilson *et al.*, 1992; Goodnight, 1992; Gardner & West, 2006; Lehmann *et al.*, 2006). The ability for budding dispersal to favour cooperation has been confirmed analytically (Gardner & West, 2006; Lehmann *et al.*, 2006) and experimentally (Kümmerli *et al.*, 2009). Owing to its simplicity, this mechanism has the potential to explain cooperation at all levels of biological organization, from replicating molecules to bacterial cells to social insects.

Although budding dispersal can be important for the evolution of cooperation, its impact upon sex ratio evolution is poorly understood. Avilés (1993) has

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suggested that budding dispersal may be responsible for the strikingly female-biased sex ratios of certain species of social spider, which are on the order of one male to every ten females. Social spider populations are structured into colonies, between which there is little or no migration of individuals. Unlike those of the eusocial insects, social spider colonies are not characterized by a sterile caste, and essentially all individuals in the colony are reproductive. Colonies reproduce by budding once they have grown to a threshold size, which varies between species from a few dozen to several thousand individuals. Avilés (1993) found that this problem was not amenable to mathematical treatment, although support for the idea that budding dispersal promotes a female-biased sex ratio was obtained by numerical simulation. However, the underlying reasons for extreme female bias remain obscure.

In this article, we examine the impact of budding dispersal upon the evolution of the sex ratio. Our aim is to provide an illustrative overview of how and why budding dispersal promotes the evolution of female-biased sex ratios. To this end, we derive solutions for haploid, diploid and haplodiploid modes of inheritance, in a generic model that is not tailored to the specific biology of any particular taxon (such as social spiders). For ease of comparison and in order to better conceptualize our results, we also re-derive the results of Bulmer (1986), Frank (1986a) and Taylor (1988) for individual dispersal, using more recent developments in kin selection methodology to simplify and clarify their original analyses.

## Models and analyses

### Individual dispersal

We assume an infinite island model (Wright, 1931), in which each patch is founded by $n$ mated females. We focus our attention upon a particular adult female, and we denote her proportional allocation of reproductive resources into sons (hereafter, 'sex ratio strategy') by $x$, the average sex ratio strategy of her patch by $y$ and the average sex ratio strategy of all females in the population by $z$. In particular, we assume that each female produces a large number $K$ of juvenile sons and the same large number $K$ of juvenile daughters, but has only sufficient resources to raise half of these offspring; hence, the focal female successfully raises $Kx$ sons and $K(1-x)$ daughters to maturity, at which point she dies. These offspring mate at random within patches, with each female mating only once, but males mating potentially numerous times. Males then die, and each mated female either disperses to a new, randomly chosen patch with probability $d_I$, or else remains in her natal patch with probability $1-d_I$. After dispersal, random density dependent culling leaves $n$ mated females in each patch, returning the population to the beginning of the lifecycle. Table 1 provides a summary of model notation.

**Table 1** A summary of model notation.

| Symbol | Definition |
|---|---|
| $d_I$ | Individual dispersal rate |
| $d_B$ | Budding dispersal rate |
| $K$ | Female fecundity |
| $m$ | Migration rate (after density-dependent regulation) |
| $n$ | Number of adult females per patch |
| $p_X$ | Consanguinity of adult female and individual X |
| D | Juvenile daughter of focal adult female |
| F | Random juvenile female on same patch as focal adult female |
| S | Juvenile son of focal adult female |
| M | Random juvenile male on same patch as focal adult female |
| P | Juvenile sharing patch with focal adult female |
| O | Offspring of focal adult female |
| $G$ | Locus controlling sex ratio |
| $g$ | Genic value |
| $\tilde{g}$ | Genetic breeding value of mother of focal individual |
| $\tilde{g}'$ | Genetic breeding value of adult female on same patch as focal individual |
| $\bar{g}$ | Population average genetic breeding value |
| $\gamma$ | Genotype-phenotype map |
| $c_f$ | Class reproductive value of females |
| $c_m$ | Class reproductive value of males |
| $f$ | Consanguinity of mating partners (inbreeding coefficient) |
| $\phi$ | Consanguinity of two juvenile females from same patch |
| $\mu$ | Consanguinity of two juvenile males from same patch |
| $r_X$ | Relatedness of individual X to focal adult female |
| $r$ | Relatedness of same-patch juvenile, relative to own offspring, from perspective of focal adult female |
| $a$ | Scale of competition |
| $w_f$ | Expected fitness of a daughter of focal adult female |
| $\bar{w}_f$ | Mean fitness of daughters |
| $W_f$ | Relative fitness of a daughter of focal adult female |
| $w_m$ | Expected fitness of a son of focal adult female |
| $\bar{w}_m$ | Mean fitness of sons |
| $W_m$ | Relative fitness of a son of focal adult female |
| $x$ | Sex ratio strategy of focal adult female |
| $y$ | Average sex ratio strategy of adult females in focal patch |
| $z$ | Average sex ratio strategy of adult females in population |
| $z^*$ | Convergence stable sex ratio |

With these assumptions, we may write the fitness of a juvenile daughter of the focal female as the product of her probability of survival to adulthood and her probability of survival through density-dependent regulation:

$$w_f = (1-x)\left\{(1-d_I)\frac{1}{(1-d_I)K(1-y)+d_IK(1-z)} + d_I\frac{1}{K(1-z)}\right\}, \quad (1)$$

and the fitness of a juvenile son of the focal female is given by the product of his survival to adulthood, his expected number of mates and the expected survival of his mates through density-dependent regulation:

$$w_m = x\frac{1-y}{y}\left\{(1-d_I)\frac{1}{(1-d_I)K(1-y)+d_IK(1-z)} + d_I\frac{1}{K(1-z)}\right\}. \quad (2)$$

Analysing these fitness functions using the neighbour-modulated fitness methodology of Taylor & Frank (1996) (see also Taylor, 1996; Frank, 1997, 1998; Taylor *et al.*, 2007), we find that the convergence stable sex ratio ($z^*$; Eshel & Motro, 1981; Christiansen, 1991; Taylor, 1996) is given by:

$$z^* = \frac{c_m(r_S - r_M)}{c_f\left(r_D - (1-d_I)^2 r_F\right) + c_m\left(r_S - (1-d_I)^2 r_M\right)}, \quad (3)$$

where $r_D$ and $r_S$ are the kin selection coefficients of relatedness for a daughter and son, respectively, from the perspective of their mother; $r_F$ and $r_M$ are the relatedness of a random juvenile female and random juvenile male, respectively, from the perspective of a random adult female in the same patch; and $c_f$ and $c_m$ are the class reproductive values for females and males respectively. A full derivation is given in the Appendix. This general equation applies to any genetic system with two sexes and no further class structure – here we are interested in haploidy, diploidy and haplodiploidy, but eqn 3 equally describes the sex ratio for triploidy, haplotriploidy or any other conceivable system of inheritance.

Restricting our attention to haploidy and diploidy for now, we have class reproductive values $c_f = c_m = ½$, relatedness to offspring $r_D = r_S = r_O$ and relatedness to random same-patch juveniles $r_F = r_M = r_P$, and eqn 3 can be rewritten as:

$$z^* = \frac{1-r}{2(1-ar)}, \quad (4)$$

where $r = r_P/r_O$ is the relatedness of a random same-patch juvenile as a fraction of the relatedness of one's own offspring, from the perspective of an adult female; and $a = (1 - d_I)^2$ is the degree of local resource competition, being the probability that two randomly chosen adult females experiencing density-dependent regulation in the same patch are both native to that patch (Frank, 1986a; see also Gardner & West, 2006).

A special case of the above model is Hamilton's (1967) diploidy model of LMC. Owing to complete dispersal of females after mating ($d_I = 1$) in Hamilton's model, here we have $r = 1/n$ and $a = 0$, and eqn 4 gives the usual result $z^* = (n - 1)/2n$. More generally, eqn 4 reveals the impact of relatedness and local resource competition on the sex ratio in viscous populations: the RHS of eqn 4 is a decreasing function of $r$ and an increasing function of $a$, hence higher within-patch relatedness favours more female-biased sex ratios (Fig. 1a; Frank, 1985), and higher local resource competition favours less female-biased sex ratios (Fig. 1b; Frank, 1986a).

Because the relatedness and competition effects of population viscosity impact upon the sex ratio in opposing ways, inspection of eqn 4 does not reveal the overall impact of population viscosity upon the sex ratio. In order to make a concrete prediction, we are required to specify the precise relationship between individual dispersal, within-patch relatedness, and local resource competition. For both haploidy and diploidy, we find that the within-patch relatedness is given by:

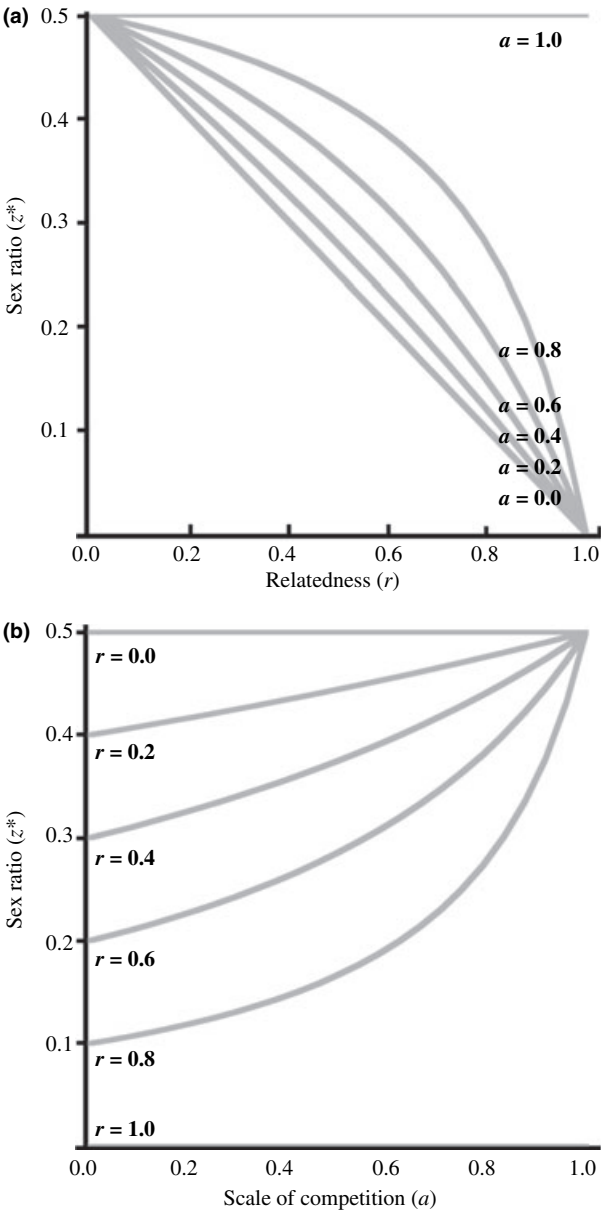


**Fig. 1** Impact of relatedness and scale of competition upon the sex ratio. (a) The convergence stable sex ratio ($z^*$) as a function of within-group relatedness ($r$), for varying degrees of competition ($a$ = 0.0, 0.2, 0.4, 0.6, 0.8 & 1.0). (b) The convergence stable sex ratio ($z^*$) as a function of scale of competition ($a$), for varying degrees of relatedness ($r$ = 0.0, 0.2, 0.4, 0.6, 0.8 & 1.0).

$$r = \frac{1}{n - (n-1)(1-d_I)^2}, \quad (5)$$

(mathematical details are given in the Appendix; see also Frank, 1986a). Hence, we find that relatedness decreases with increasing individual dispersal, and is also mediated

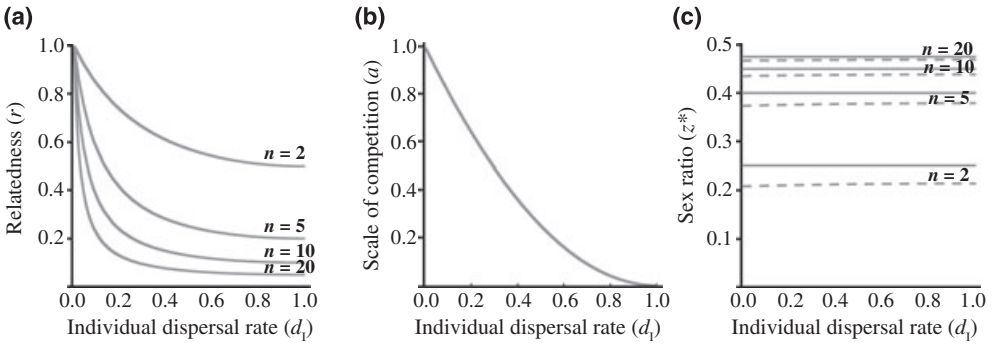


**Fig. 2** Individual dispersal and the sex ratio. (a) Within-group relatedness ($r$) as a function of individual dispersal rate ($d_I$), for varying patch size ($n$ = 2, 5, 10 & 20). (b) Scale of competition ($a$) as a function of individual dispersal rate ($d_I$). (c) The convergence stable sex ratio ($z^*$) as a function of individual dispersal rate ($d_I$), for varying patch size ($n$ = 2, 5, 10 & 20). Bold lines represent the convergence stable sex ratio for haploids and diploids, dashed lines represent the convergence stable sex ratio for haplodiploids.

by patch size (Fig. 2a). Above, we showed that the local resource competition is given by $a = (1 - d_I)^2$. Hence, competition also decreases with increasing individual dispersal (Fig. 2b). Having made relatedness and competition explicit functions of individual dispersal, we may substitute these expressions into eqn 4, giving us:

$$z^* = \frac{n-1}{2n}. \tag{6}$$

We have recovered the surprising result – shown numerically by Bulmer (1986) and derived analytically by Frank (1986a) – that individual dispersal does not impact upon the sex ratio, the latter taking the same value that it would in a fully dispersing population (Fig. 2c, solid lines). Although population viscosity impacts upon relatedness and competition, and both of these mediate sex ratio, the two effects exactly cancel so that there is no overall impact of population viscosity upon the sex ratio. This was previously shown for the diploid case, and here we have shown that the result also extends to haploids.

For haplodiploids, $c_f = 2/3$ and $c_m = 1/3$, and $r_D \neq r_S$ and $r_F \neq r_M$, so eqn 3 cannot be reduced to the simple form of eqn 4. In this case, the convergence stable sex ratio is given by:

$$z^* = \frac{n-1}{2n}\frac{4n-(n-1)(1-d_I)^2-2}{4n-(n-1)(1-d_I)^2-1}, \tag{7}$$

(mathematical details are given in the Appendix; see also Taylor, 1988). Here, the sex ratio is no longer independent of the individual dispersal rate. However, numerical analysis reveals that the impact of individual dispersal is negligible (Fig. 2c, dashed lines). In particular, although a more female-biased sex ratio is predicted in a more viscous population, the discrepancy between this and the sex ratio for a fully mixing population is always less than 0.007. In contrast, the sex ratio is strongly affected by the number of foundresses, with a less female-biased sex ratio being favoured as the patch size increases (Fig. 2c, dashed lines).

## Budding dispersal

We now consider that, when females disperse to new patches, they do so with patch mates ('budding dispersal'; Haldane, 1932; Pollock, 1983; Goodnight, 1992; Gardner & West, 2006; Lehmann *et al.*, 2006). We assume that, after mating, females form groups (buds) of size $n$ at random within their patch, and that each group either remains on the focal patch with probability $1 - d_B$ or else it disperses to a random patch elsewhere in the population with probability $d_B$. After dispersal, density-dependent regulation leaves one randomly chosen group on each patch. We also allow for some random exchange of females between patches after density-dependent regulation: with probability $m$ a female migrates, in which case she is repositioned in a random space vacated by another migratory individual, and with probability $1 - m$ she remains in her patch. Incorporating this migration into the model facilitates synthesis with the computer simulations of Avilés (1993) and the mathematical analysis of Gardner & West (2006). With these assumptions, the fitness of juvenile males and females can be written as:

$$w_f = (1-x)\left\{(1-d_B)\frac{1}{(1-d_B)K(1-y)+d_BK(1-z)} + d_B\frac{1}{K(1-z)}\right\}, \tag{8}$$

and

$$w_m = x\frac{1-y}{y}\left\{(1-d_B)\frac{1}{(1-d_B)K(1-y)+d_BK(1-z)} + d_B\frac{1}{K(1-z)}\right\}. \tag{9}$$

Note that the fitness functions are identical to those derived for the individual dispersal model, except that the individual dispersal parameter $d_I$ has been replaced with the budding dispersal parameter $d_B$. Thus, following the same procedure as before, we obtain a convergence stable sex ratio of:

$$z^* = \frac{c_m(r_S - r_M)}{c_f\left(r_D - (1-d_B)^2 r_F\right) + c_m\left(r_S - (1-d_B)^2 r_M\right)}, \quad (10)$$

where, again, the only difference from the model of the previous section is the replacement of $d_I$ with $d_B$. Restricting our attention to haploidy and diploidy, so that the class reproductive values are $c_f = c_m = ½$, relatedness to offspring is $r_D = r_S = r_O$ and relatedness to random same-patch juveniles is $r_F = r_M = r_P$, eqn 10 again reduces to eqn 4, which we reproduce here as eqn 11 for ease of reference:

$$z^* = \frac{1-r}{2(1-ar)}, \quad (11)$$

where relatedness is $r = r_P/r_O$ and the intensity of local resource competition is $a = (1 - d_B)^2$. Because eqn 11 is identical – as it is written – to eqn 4, we see that relatedness and localized resource competition impact upon the sex ratio in the same way in both the individual dispersal and budding dispersal models: higher relatedness favours a more female-biased sex ratio (Fig. 1a), and stronger competition favours a less female-biased sex ratio (Fig. 1b).

The difference between the two models lies in how relatedness and competition emerge (and become associated) as a consequence of the rate and pattern of dispersal. For the purposes of calculating within-patch relatedness, the migration of females after density-dependent regulation in the budding dispersal model plays the same role as dispersal prior to density-dependent regulation in the individual dispersal model, hence we can simply write $m$ where we see $d_I$ in eqn 5 to yield relatedness for the budding dispersal model:

$$r = \frac{1}{n - (n-1)(1-m)^2}. \quad (12)$$

Hence, relatedness is a decreasing function of female migration after density-dependent regulation, but is unaffected by the rate of budding dispersal (Fig. 3a). Above, we found that the intensity of localized competition in the budding dispersal model is given by the same expression as for the individual dispersal model, but with individual dispersal rate being replaced by the budding dispersal rate: $a = (1 - d_B)^2$. Hence, competition is a decreasing function of budding dispersal rate, and is unaffected by migration after density-dependent regulation (Fig. 3b).

Although relatedness and competition are both determined by the individual dispersal rate in the first model, they are decoupled in the second model, with relatedness being determined by female migration after density-dependent regulation and competition being determined by budding dispersal before density-dependent regulation. Expressing the within-patch relatedness ($r$) and intensity of local resource competition ($a$) in terms of model parameters ($n$, $d_B$ and $m$), and substituting into eqn 11, obtains a convergence stable sex ratio of:

$$z^* = \frac{n-1}{2n} \frac{n\left(1-(1-m)^2\right)}{(n-1)\left(1-(1-m)^2\right) + 1 - (1-d_B)^2}, \quad (13)$$

which is a decreasing function of the budding dispersal rate – i.e. budding dispersal promotes a female bias. Furthermore, the sex ratio is an increasing function of the migration rate of individuals after density-dependent regulation and an increasing function of the number of foundresses (Fig. 3c, solid lines; Avilés, 1993). Assuming full budding dispersal ($d_B = 1$), we find that a vanishingly small allocation to males is favoured in the limit of low migration ($z^* \to 0$ as $m \to 0$) and also that the sex ratio asymptotes towards one half with increasingly large numbers of foundresses ($z^* \to ½$ as $n \to \infty$). However, we also recover Avilés' (1993) simulation result that substantial female bias is favoured, even with very large numbers of foundresses, provided that between patch migration is sufficiently low [$z^* \approx mn/(1 + 2mn)$ if $m \ll 1$, $n \gg 1$ and $mn$ is on the order of 1] – for example, one male for every two females if there is an average of one migrant foundress per patch. Note that,

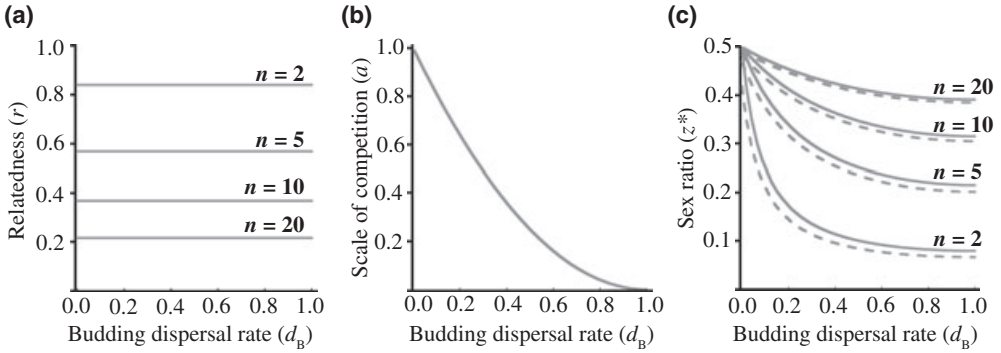


**Fig. 3** Budding dispersal and the sex ratio. (a) Within-group relatedness ($r$) as a function of budding dispersal rate ($d_B$), for varying patch size ($n$ = 2, 5, 10 & 20). (b) Scale of competition ($a$) as a function of budding dispersal rate ($d_B$). (c) The convergence stable sex ratio ($z^*$) as a function of budding dispersal rate ($d_B$), for varying patch size ($n$ = 2, 5, 10 & 20) and a fixed rate of migrant exchange ($m$ = 0.1). Bold lines represent the convergence stable sex ratio for haploids and diploids, dashed lines represent the convergence stable sex ratio for haplodiploids.

evaluating eqn 13 at $d_B = m = d_I$ exactly recovers eqn 6 of the individual dispersal model.

Again, the situation is somewhat more complicated for haplodiploids, whose peculiar biology ($c_f \neq c_m$, $r_D \neq r_S$ and $r_F \neq r_M$) means that the sex ratio of eqn 10 cannot be reduced to the more simple form of eqn 11. In this case, the convergence stable sex ratio is given by:

$$z^* = \frac{n-1}{2n}\,\frac{4n-(n-1)(1-m)^2-2}{4n-(n-1)(1-m)^2-1} \times \frac{n\left(1-(1-m)^2\right)}{(n-1)\left(1-(1-m)^2\right)+1-(1-d_B)^2}, \qquad (14)$$

(mathematical details are given in the Appendix). Here, the convergence stable sex ratio is strongly affected by the rate of budding dispersal, a higher budding dispersal rate favouring a more female-biased sex ratio (Fig. 3c, dashed lines). Conversely, the sex ratio is predicted to be less female biased as the rate of migration after density-dependent regulation increases, and as the number of foundresses increases (Fig. 3c, dashed lines; Avilés, 1993). Again, for complete budding dispersal ($d_B = 1$) we find that a vanishingly low proportion of males is favoured as the migration rate approaches zero ($z^* \to 0$ as $m \to 0$), that a sex ratio of one half is favoured in the limit of a large number of foundresses ($z^* \to ½$ as $n \to \infty$), and that even if patches are founded by large numbers of foundresses a substantial female bias can be favoured provided that migration is sufficiently rare [$z^* \approx mn/(1 + 2mn)$ if $m \ll 1$, $n \gg 1$ and $mn$ is on the order of 1]. Finally, note that eqn 7 of the individual dispersal model is recovered by evaluating eqn 13 at $d_B = m = d_I$.

## Discussion

We have derived analytical results for a model of sex ratio evolution in a group-structured population characterized by budding dispersal. Previously, theoretical understanding of this problem has been based upon the results of numerical simulations (Avilés, 1993). In line with Avilés' (1993) simulation study, we have found that budding dispersal favours female-biased sex allocation, with the convergence stable sex ratio (proportion male) decreasing as the rate of budding dispersal increases. This is in contrast to the astonishing result that the sex ratio is unaffected by the rate of individual dispersal in a purely viscous population (Bulmer, 1986; Frank, 1986a; Taylor, 1988). A low rate of individual dispersal results in a high within-patch relatedness, which favours a more female-biased sex ratio (Frank, 1985), but it also results in a high degree of kin competition, which favours a less female-biased sex ratio (Frank, 1986a,b), and these effects cancel exactly or approximately, giving no net impact of individual dispersal upon sex ratio evolution (Bulmer, 1986; Frank, 1986a; Taylor, 1988). The difference with budding dispersal is that it reduces the extent to which the offspring of patch-mates compete for resources, which promotes a more female-biased sex ratio, and by keeping relatives together as they disperse, it allows a high relatedness to be maintained, which also promotes a more female-biased sex ratio. In other words, budding decouples the competition and relatedness consequences of dispersal (Gardner & West, 2006).

We have also examined the impact of other demographic parameters upon sex allocation. We have found that an increased number of adult females reproducing on a patch favours a reduced female-biased sex ratio, owing to a corresponding reduction in within-patch relatedness (Hamilton, 1967; Avilés, 1993). Following Avilés (1993; see also Gardner & West, 2006), we have also examined the impact of exchange of individuals after dispersal and density-dependent regulation, and have found that an increased exchange of individuals also favours a reduced female-biased sex ratio, again owing to a reduction in within-patch relatedness. Importantly, we have recovered Avilés' (1993) interesting result that even very large groups can be expected to exhibit strongly female-biased sex ratios, provided that the rate of exchange of individuals between groups is sufficiently low – as is the case in social spiders. For example, in the limit of large group size, and only one migrant per group per generation, the sex ratio corresponds to two females for every male.

We have taken a kin selection approach to the problem of budding dispersal and the sex ratio. Previously, this problem has been tackled using a multilevel selection approach. Indeed, Avilés (1993) suggested that the female-biased sex ratios of social spiders could not be explained by kin selection, and emphasized the need for selection between groups. However, at a formal level, the multilevel selection approach to social evolution is equivalent to the kin selection approach; any analysis of multilevel selection can always be translated into an equivalent kin selection analysis and, provided that the analyses are performed correctly, these will not lead to conflicting predictions (Hamilton, 1975; Grafen, 1984; Wade, 1985; Frank, 1986b, 1998; Queller, 1992b; Bourke & Franks, 1995; West *et al.*, 2007). Despite this equivalence, it is often easier to solve biological problems using kin selection methodology, for instance because multilevel selection theory is difficult to apply to class-structured populations (West *et al.*, 2008). This is the situation for the present problem of budding dispersal and the sex ratio, which appears complicated when viewed from a multilevel selection perspective – and hence has previously been tackled using numerical simulation methods, which provide only limited insight – but which is easily resolved if approached from a kin selection perspective.

This study has clarified the use (and, in particular, the complementarity) of 'open' model vs. 'closed'

model approaches to social evolution theory (Gardner & West, 2006). Eqn 4, repeated as eqn 11, describes the dependence of the convergence stable sex ratio ($z^*$) upon within-group relatedness ($r$) and local resource competition ($a$), without specifying how these two determinants are themselves associated and how they emerge as a consequence of population demographic assumptions. This open model approach allows a general overview – here it applies equally to the individual dispersal and budding dispersal models – but it lacks in concreteness. In contrast, eqns 6 and 13 show explicitly how sex ratio depends upon more tangible population parameters, such as number of foundresses per patch ($n$), and dispersal/migration rates ($d_I$, $d_B$ and $m$). This closed model approach allows for more specific, quantitative predictions, but lacks in generality as it is dependent on particular model assumptions. Put another way, whereas the closed model approach tells us precisely what sex ratio to expect, the open model approach helps us conceptualize why the sex ratio takes the value that it does, and how to generalise from the findings of specific models to yield deeper insights about the fundamental processes driving sex ratio evolution.

Our aim has been to provide an illustrative overview of how and why budding dispersal impacts upon sex ratio, and to form conceptual links with the theory of social evolution in viscous populations more generally. For this reason, we have considered a generic model with relatively broad applicability, and a range of inheritance modes, whilst explicitly neglecting biological details particular to individual taxa. For example, Avilés' (1993) simulation study allowed for multiple generations between social spider dispersal events (see also Frank, 1987), which has not been considered in the present study. We also assumed a system of monandry in order to simplify relatedness calculations, and we highlight the possibility that other mating systems might give rise to quantitatively different predictions. Finally, we have assumed complete maternal control of sex, yet there may be scope for evolutionary conflicts of interests between (and even within) individual family members in this respect (Beukeboom *et al.*, 2001; Wild & West, 2009). Sex allocation is one of the few topics in evolutionary biology where researchers can reasonably aim for a quantitative – rather than just a qualitative – fit between theoretical prediction and empirical data (West, 2009), so extension of the basic theory to allow for more complex models, tailored to the particular biology of specific taxa, represents an important avenue for future research.

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## Appendix

### Convergence stable sex ratio

Here we determine the convergence stable sex ratio for haploid, diploid and haplodiploid populations. We use notation consistent with the individual dispersal model, but the derivation applies equally to the budding dispersal model if $d_I$ is replaced with $d_B$ throughout. We begin by noting that the average fitness of all juvenile females is found by evaluating eqn 1 at $x = y = z$, to obtain $\bar{w}_f = 1/K$. Hence, the relative fitness of the focal female's daughter, expressed as a fraction of the average for her class, is $w_f/\bar{w}_f$, or:

$$W_f = (1-x)\left\{(1-d_I)\frac{1}{(1-d_I)(1-y)+d_I(1-z)} + d_I\frac{1}{1-z}\right\}. \quad \text{(A1)}$$

Similarly, the average fitness of juvenile males is found by evaluating eqn 2 at $x = y = z$, to obtain $\bar{w}_m = 1/K$, and the relative fitness of the focal female's son, expressed as a fraction of the average for his class, is $w_m/\bar{w}_m$, or:

$$W_m = x\frac{1-y}{y}\left\{(1-d_I)\frac{1}{(1-d_I)(1-y)+d_I(1-z)} + d_I\frac{1}{1-z}\right\}. \quad \text{(A2)}$$

Consider a locus $G$ that controls sex ratio, and denote the genic value of a gene drawn from this locus from a focal juvenile by $g$. Further, denote the additive genetic 'breeding' value (Price, 1970; Falconer, 1981; Grafen, 1985) for the sex ratio strategy of this individual's mother by $\tilde{g}$, the average breeding value of all the adult females in the juveniles patch by $\tilde{g}'$ and the average breeding value of the population by $\bar{g}$. Natural selection favours those genes that are associated with increased fitness; the appropriate definition of fitness in a sex structured population is $W = c_f W_f + c_m W_m$, where $W_f$ and $W_m$ are the fitness of females and males, respectively, expressed relative to the average for their sex; and $c_f$ and $c_m$ are the class reproductive values of females and males respectively (Taylor, 1996; Taylor & Frank, 1996; Frank, 1997, 1998; Taylor *et al.*, 2007). Assuming vanishing genetic variation, the direction of natural selection acting upon the average breeding value of the population is given by the sign of:

$$\frac{dW}{dg} = c_f\frac{dW_f}{dg_f} + c_m\frac{dW_m}{dg_m}, \quad \text{(A3)}$$

where, for clarity, we have subscripted an individual's genic value $g$ with f or m if it is female or male, respectively; and all derivatives are evaluated at the population average $g = g_f = g_m = \bar{g}$ (Taylor, 1996; Taylor & Frank, 1996; Frank, 1997, 1998; Taylor *et al.*, 2007).

The first derivative on the RHS of eqn A3 describes the impact of the value of a gene drawn from a juvenile female on that individual's relative fitness, which is mediated by (i) the correlation between her genic value and her mother's breeding value, the correlation between her mother's breeding value and her mother's sex ratio strategy, and the correlation between her mother's sex ratio and her own relative fitness; and (ii) the correlation between her genic value and the average breeding value of adult females on her patch, the correlation between the average breeding value of adult females on her patch and the average sex ratio strategy of her patch, and the correlation between the average sex ratio strategy of her patch and her relative fitness, i.e.:

$$\frac{dW_f}{dg_f} = \frac{\partial W_f}{\partial x}\frac{dx}{d\tilde{g}}\frac{d\tilde{g}}{dg_f} + \frac{\partial W_f}{\partial y}\frac{dy}{d\tilde{g}'}\frac{d\tilde{g}'}{dg_f} = \left(\frac{\partial W_f}{\partial x}p_D + \frac{\partial W_f}{\partial y}p_F\right)\gamma, \quad \text{(A4)}$$

where $p_D$ is the coefficient of consanguinity between an adult female and her juvenile daughter; $p_F$ is the coefficient of consanguinity between an adult female and a random juvenile female on the same patch; and the

mapping between genotype and phenotype is $dx/d\tilde{g} = dy/d\tilde{g}' = \gamma$.

We can perform the analogous expansion for the second derivative on the RHS of eqn A3:

$$\frac{dW_m}{dg_m} = \frac{\partial W_m}{\partial x}\frac{dx}{d\tilde{g}}\frac{d\tilde{g}}{dg_m} + \frac{\partial W_m}{\partial y}\frac{dy}{d\tilde{g}'}\frac{d\tilde{g}'}{dg_m} = \left(\frac{\partial W_m}{\partial x}p_S + \frac{\partial W_m}{\partial y}p_M\right)\gamma, \quad \text{(A5)}$$

where $p_S$ is the coefficient of consanguinity between an adult female and her juvenile son; and $p_M$ is the coefficient of consanguinity between a random adult female and a random juvenile male on the same patch.

Substituting eqns A4 and A5 into eqn A3, the condition for an increase in the population average sex ratio strategy is:

$$c_f\left(\frac{\partial W_f}{\partial x}p_D + \frac{\partial W_f}{\partial y}p_F\right) + c_m\left(\frac{\partial W_m}{\partial x}p_S + \frac{\partial W_m}{\partial y}p_M\right) > 0. \quad \text{(A6)}$$

Finally, using eqns A1 and A2 to calculate the appropriate partial derivatives, and dividing both sides of inequality (A6) by the consanguinity for a focal adult female to herself ($p_I$), the condition for increase becomes:

$$c_f\left(-\frac{1}{1-z}r_D + \frac{(1-d_I)^2}{1-z}r_F\right) + c_m\left(\frac{1}{z}r_S - \frac{1-z(1-d_I)^2}{z(1-z)}r_M\right) > 0, \quad \text{(A7)}$$

where $r_D = p_D/p_I$ is the relatedness of a mother and daughter from the perspective of the mother; $r_F = p_F/p_I$ is the relatedness of a random adult female and random juvenile female from the same patch, from the perspective of the former; $r_S = p_S/p_I$ is the relatedness of a mother and son from the perspective of the mother; and $r_M = p_M/p_I$ is the relatedness of a random adult female and a random juvenile male from the same patch, from the perspective of the former. Setting the LHS of condition (A7) to zero, and solving for $z = z^*$, yields an equilibrium sex ratio given by eqn 3 in the main text. The derivative of the LHS of condition (A7) with respect to $z$ is negative for all $r_F < r_D$ and $r_M < r_S$, hence this equilibrium sex ratio is globally convergence stable (Eshel & Motro, 1981; Christiansen, 1991; Taylor, 1996).

## Relatedness

Here we derive the consanguinity and relatedness coefficients for haploid, diploid and haplodiploid populations. We use notation appropriate to the individual dispersal model, but the calculations apply equally to the budding dispersal model if $d_I$ is replaced with $m$ throughout.

### *Haploidy*

We focus on the inbreeding coefficient $f$, which is the average consanguinity of mating partners, and hence it is also the average consanguinity of opposite and same sex juveniles within a patch. At equilibrium, in a neutral population, the consanguinity of two juveniles randomly chosen from the same patch is given by the probability that they have the same mother ($1/n$) times the consanguinity of full siblings ($\frac{1}{2} + \frac{1}{2}f$), plus the probability that they have different mothers [$(n-1)/n$] times the probability that the mothers (and hence also the fathers) derive from the same patch [$(1-d_I)^2$] times the consanguinity for two juveniles randomly drawn from the same patch ($f$), i.e.:

$$f = \frac{1}{n}\left(\frac{1}{2} + \frac{1}{2}f\right) + \frac{n-1}{n}(1-d_I)^2 f. \quad \text{(A8)}$$

Solving eqn A8 for $f$ obtains:

$$f = \frac{1}{2n - 1 - 2(n-1)(1-d_I)^2}. \quad \text{(A9)}$$

The consanguinity of an adult female with a random juvenile sharing her patch is $p_P = f$, and the consanguinity of an adult female with one of her offspring is given by $p_O = \frac{1}{2} + \frac{1}{2}f$. Using the solution (A9), the within-patch relatedness $r = p_P/p_O$ is given by eqn 5 in the main text.

### *Diploidy*

Again we focus on the coefficient of inbreeding, $f$. At equilibrium in a neutral population this is equal to the probability that two random juveniles on the same patch share the same mother ($1/n$) times the consanguinity for full siblings ($\frac{1}{2}(\frac{1}{2} + \frac{1}{2}f) + \frac{1}{2}f$) plus the probability that they do not share the same mother [$(n-1)/n$] times the probability that their mothers (and hence also their fathers) derive from the same patch [$(1-d_I)^2$] times the consanguinity of two random juveniles drawn from the same patch ($f$), i.e.:

$$f = \frac{1}{n}\left(\frac{1}{2}\left(\frac{1}{2} + \frac{1}{2}f\right) + \frac{1}{2}f\right) + \frac{n-1}{n}(1-d_I)^2 f. \quad \text{(A10)}$$

Solving eqn A10 for $f$ obtains:

$$f = \frac{1}{4n - 3 - 4(n-1)(1-d_I)^2}. \quad \text{(A11)}$$

As before, the consanguinity of an adult female with a random juvenile sharing her patch is $p_P = f$, and the consanguinity of an adult female with one of her offspring is given by $p_O = \frac{1}{2}(\frac{1}{2} + \frac{1}{2}f) + \frac{1}{2}f$. Using the solution (A11), the within-patch relatedness $r = p_P/p_O$ is once again given by eqn 5 in the main text.

### *Haplodiploidy*

We continue to denote the consanguinity of mating partners by $f$, and this is also the average consanguinity of opposite-sex juveniles sharing the same patch. In addition, we now denote the average consanguinity of two juvenile females sharing the same patch by $\phi$, and the average consanguinity of two juvenile males sharing the same patch by $\mu$. The consanguinity of two opposite-sex juvenile patch mates is given by the probability that they share the same mother ($1/n$) times the consanguinity of

full sibs (with probability ½ the juvenile female derives her gene from her mother, as does the juvenile male, in which case the consanguinity is ½ + ½$f$, and with probability ½ the juvenile female derives her gene from her father, and the juvenile male, from his mother, in which case the consanguinity is $f$) plus the probability that they do not share the same mother [$(n - 1)/n$] times the probability that their mothers (and hence also the juvenile female's father) derive from the same patch [$(1 - d_I)^2$] times the consanguinity of the parents from which their genes originated (with probability ½ this is the juvenile female's mother and the juvenile male's mother, in which case consanguinity is $\phi$, and with probability ½ this is the juvenile female's father and the juvenile male's mother, in which case consanguinity is $f$), i.e.:

$$f = \frac{1}{n}\left(\frac{1}{2}\left(\frac{1}{2}+\frac{1}{2}f\right)+\frac{1}{2}f\right)+\frac{n-1}{n}(1-d_I)^2\left(\frac{1}{2}\phi+\frac{1}{2}f\right). \quad \text{(A12)}$$

The average consanguinity of two juvenile females sharing the same patch is given by the probability that they share the same mother ($1/n$) times the consanguinity of full sisters (¼(½ + ½$f$) + ½$f$ + ¼) plus the probability that they do not share the same mother [$(n - 1)/n$] times the probability that their mothers (and hence also their fathers) derived from the same patch [$(1 - d_I)^2$] times the consanguinity of the parents from which their genes originated (with probability ¼ they both derived their genes from their mothers, so consanguinity is $\phi$; with probability ½ they derived their genes from opposite-sex parents, so consanguinity is $f$; and with probability ¼ they both derived their genes from their fathers, so consanguinity is $\mu$), i.e.:

$$\phi = \frac{1}{n}\left(\frac{1}{4}\left(\frac{1}{2}+\frac{1}{2}f\right)+\frac{1}{2}f+\frac{1}{4}\right) + \frac{n-1}{n}(1-d_I)^2\left(\frac{1}{4}\phi+\frac{1}{2}f+\frac{1}{4}\mu\right). \quad \text{(A13)}$$

The consanguinity of two juvenile males sharing the same patch is given by the probability that they share the same mother ($1/n$) times the consanguinity of full brothers (½ + ½$f$) plus the probability that they do not share the same mother [$(n - 1)/n$] times the probability that their mothers derived from the same patch [$(1-d_I)^2$] times the average consanguinity of two juvenile females on the same patch ($\phi$), i.e.:

$$\mu = \frac{1}{n}\left(\frac{1}{2}+\frac{1}{2}f\right)+\frac{n-1}{n}(1-d_I)^2\phi. \quad \text{(A14)}$$

Simultaneously solving eqns A12, A13 and A14, we obtain:

$$f = \frac{n}{n+(n-1)\left(1-(1-d_I)^2\right)\left(3n+(n-1)\left(1-(1-d_I)^2\right)+1\right)}, \quad \text{(A15)}$$

$$\phi = \frac{2n+(n-1)\left(1-(1-d_I)^2\right)}{2n+2(n-1)\left(1-(1-d_I)^2\right)\left(3n+(n-1)\left(1-(1-d_I)^2\right)+1\right)}, \quad \text{(A16)}$$

and

$$\mu = \frac{n+(n-1)\left(1-(1-d_I)^2\right)}{n+(n-1)\left(1-(1-d_I)^2\right)\left(3n+(n-1)\left(1-(1-d_I)^2\right)+1\right)}. \quad \text{(A17)}$$

From $f$, $\phi$ and $\mu$, we can calculate all of the other coefficients of consanguinity that are required in order to determine the convergence stable sex ratio. The consanguinity of an adult female to (i) herself is $p_I$ = ½ + ½$f$; (ii) her daughter is $p_D$ = ½$p_I$ + ½$f$; (iii) a random juvenile female on her patch is $p_F = (1/n)p_D + ((n - 1)/n)(1 - d_I)^2(½\phi + ½f)$; (iv) her son is $p_S = p$ and (v) a random juvenile male on her patch is $p_M = (1/n)p_S + ((n - 1)/n)(1 - d_I)^2\phi$. Hence, we can calculate coefficients of relatedness, from the perspective of the adult female, for (i) her daughter ($r_D = p_D/p_I$); (ii) a random juvenile female on her patch ($r_F = p_F/p_I$); (iii) her son ($r_S = p_S/p_I$) and (iv) a random juvenile male on her patch ($r_M = p_M/p_I$). Substituting these into eqn 3, and setting $c_f = 2/3$ and $c_m = 1/3$, we obtain eqn 7 of the main text.

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# Haplodiploidy and the Evolution of Eusociality: Split Sex Ratios

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abstract: It is generally accepted that from a theoretical perspective, haplodiploidy should facilitate the evolution of eusociality. However, the "haplodiploidy hypothesis" rests on theoretical arguments that were made before recent advances in our empirical understanding of sex allocation and the route by which eusociality evolved. Here we show that several possible promoters of the haplodiploidy effect would have been unimportant on the route to eusociality, because they involve traits that evolved only after eusociality had become established. We then focus on two biological mechanisms that could have played a role: split sex ratios as a result of either queen virginity or queen replacement. We find that these mechanisms can lead haplodiploidy to facilitating the evolution of helping but that their importance varies from appreciable to negligible, depending on the assumptions. Furthermore, under certain conditions, haplodiploidy can even inhibit the evolution of helping. In contrast, we find that the level of promiscuity has a strong and consistently negative influence on selection for helping. Consequently, from a relatedness perspective, monogamy is likely to have been a more important driver of eusociality than the haplodiploidy effect.

*Keywords:* altruism, helping, inclusive fitness, kin selection, monogamy, sex allocation.

## Introduction

> If a female is fertilized by only one male all the sperm she receives is genetically identical. Thus, although the relationship of a mother to her daughters has the normal value of 1/2, the relationship between daughters is 3/4. Consider a species where the female consecutively provisions and oviposits in cell after cell so that she is still at work when the first of her female offspring ecloses, leaves the nest and mates. Our principle tells us that even if this new adult had a nest ready constructed and vacant for her use she would prefer, other things being equal, returning to her mother's and provisioning a cell for the rearing of an extra sister to provisioning a cell for a daughter of her own. (Hamilton 1964, p. 28–29)

The eusocial societies are dominated by species with haplodiploid genetics, especially the social Hymenoptera—the ants, bees, and wasps. Although eusociality is also found in diploid species, such as termites, its distribution is significantly biased toward haplodiploid families (Crozier 2008). Hamilton (1964, 1972) suggested that this was because haplodiploidy facilitates the evolution of altruistic helping. Altruistic helping behaviors are favored if the benefit of helping relatives outweighs the costs to the altruist and to other relatives, with all costs and benefits weighted by the genetic relatedness of the recipients to the actor (Hamilton 1963, 1964, 1970). Haplodiploidy involves females developing from fertilized (i.e., diploid) eggs and having both a mother and a father, and males developing from unfertilized (i.e., haploid) eggs, and having no father. Hamilton (1964, 1972) suggested that because this leads to a worker being more related to her full sisters (life-for-life relatedness, $R = 3/4$) than to her own daughters ($R = 1/2$), haplodiploidy makes eusociality easier to evolve, even in the absence of efficiency benefits to cooperation.

Trivers and Hare (1976) showed that while Hamilton's "haplodiploidy hypothesis" can work, things are not so simple. Haplodiploidy also leads to a female being less related to her brothers ($R = 1/4$) than to her sons ($R = 1/2$). In the simplest case, with an unbiased sex ratio among reproductives, the relative benefit of rearing sisters is exactly canceled by the relative cost of rearing brothers, and so haplodiploidy has no overall influence (Trivers and Hare 1976). A female-biased sex ratio does not solve this problem, because the benefit of more sisters is exactly counterbalanced by the fact that it increases the average number of mates for each male, making females worth relatively less (Trivers and Hare 1976; Craig 1979). Consequently, in order to make the haplodiploidy hypothesis work, it is required that workers preferentially help sisters but that the population sex ratio is not biased to the same

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**Table 1:** Split sex ratios and the evolution of eusociality

| Reason for split sex ratios | Empirical evidence that this has led to split sex ratios | Could such split sex ratios have occurred on the route to eusociality? |
|---|---|---|
| Partially overlapping generations (Seger 1983) | No (West 2009) | Potentially |
| Worker control of sex allocation in some broods and queen control in others (Trivers and Hare 1976) | No, but would be transient (West 2009) | Potentially (to be analyzed in a companion paper: J. Alpedrinha et al. unpublished manuscript) |
| Variation across colonies in the relative cost of producing males and females (Grafen 1986) | No (West 2009) | Potentially |
| Virginity (or any other factor which constrains some queens to produce only males; Taylor 1981; Godfray and Grafen 1988) | Yes, this occurs in solitary and social hymenopteran species, although usually at low (<6%) frequencies (Godfray and Hardy 1992; West 2009 | Yes |
| Competition for mates between related males (local mate competition, LMC; Frank 1987) | No (West 2009) | Potentially, although LMC is very rare in social Hymenoptera (West et al. 2005) |
| Synergistic benefits of rearing siblings (Frank and Crespi 1989) | No (West 2009) | Potentially |
| Variation in response to whether sibmated or not (Greeff 1996; Reece et al. 2004) | No (West 2009) | No evidence for such sex ratio shifts in any organism, and sibmating is rare in social Hymenoptera |
| Competition between related females for resources (local resource competition; Brown and Keller 2000) | Yes, in ants (West 2009) | No, this occurs in multiple-queen colonies, which only evolved after obligate eusociality was established (Boomsma 2007, 2009; Hughes et al. 2008) |
| Queen control (Passera et al. 2001) | Yes, in ants and bees (West 2009) | No, this occurs only in obligately eusocial colonies |
| Relatedness asymmetry due to variation in queen mating frequency (Boomsma and Grafen 1991) | Yes, in ants (Chapuisat and Keller 1999; Meunier et al. 2008; West 2009) | No, multiple mating only evolved after obligate eusociality was established (Boomsma 2007, 2009; Hughes et al. 2008) |
| Relatedness asymmetry due to variation in queen number (Boomsma and Grafen 1991; Boomsma 1993) | Yes, in ants and wasps (Chapuisat and Keller 1999; Meunier et al. 2008; West 2009) | No, this occurs in multiple-queen colonies, whereas eusociality has only arisen in monogynous species (Boomsma 2007, 2009; Hughes et al. 2008). |
| Relatedness asymmetry due to queen replacement (Boomsma 1991) | Yes, in bees (Chapuisat and Keller 1999; Meunier et al. 2008; West 2009) | Yes |

Note: Although there are many mechanisms that lead to split sex ratios in hymenoptera, only two of these (virginity and queen replacement) are supported by empirical evidence (column 2) and are also likely to have occurred during the transition from solitary to eusocial living (column 3).

extent. Trivers and Hare (1976) suggested that this could happen if workers at some nests gained control of sex allocation and biased this toward sisters. Such sex ratio variation between broods, termed "split sex ratios," can allow helpers at the relatively female-biased broods to gain the relatedness benefit of rearing sisters, without this being exactly canceled by a reduced reproductive value of females, thanks to the relatively male-biased broods leading to a more even sex ratio at the population level (Seger 1983; Grafen 1986). The idea here is that this favors the initial evolution of helping at some broods and hence facilitates the spread of genes that lead to more specialized helping en route to eusociality.

Since Trivers and Hare's (1976) landmark article, a large body of theoretical work has arisen showing that split sex ratios can be favored in response to a multitude of selective forces, with many of these scenarios being supported by the empirical data (table 1). Indeed, work on split sex ratios has even been hailed as one of the most successful and productive areas of evolutionary biology, with a rich interplay between theoretical, observational, and experimental studies (West 2009). Furthermore, subsequent

work has suggested other ways that might allow haplodiploidy to facilitate the evolution of helping, via its impact on relatedness, for example, by selecting for the enforcement of cooperation with worker policing (Ratnieks 1988) or by increasing the benefit of helping siblings in structured populations (Lehmann et al. 2008; Johnstone et al. 2011). Overall, this body of work has led to the general assumption that from a theoretical perspective, the haplodiploidy effect does facilitate the evolution of eusociality (Seger 1991; Krebs and Davies 1993; Bourke and Franks 1995; Crozier and Pamilo 1996; Queller and Strassmann 1998; Alcock 2005).

Here, we reassess the haplodiploidy hypothesis on two grounds. First, empirical progress has clarified the relevant biological scenarios. Eusociality has evolved under specific conditions, with strict lifetime monogamy via the subsocial route, where offspring stay at home to help their parents, and with single queens (Boomsma 2007, 2009; Hughes et al. 2008; Duffy and Macdonald 2010; Bourke 2011). This means that several factors cannot have facilitated the evolution of eusociality, including: (a) the most empirically common causes of split sex ratios, which rely on variation in the number of queens and queen mating frequency and therefore evolved after eusociality was established (table 1); (b) worker policing in response to multiple mating (Ratnieks 1988); and (c) any mechanism that relies on helping siblings to rear offspring (the semisocial route; Lehmann et al 2008; Johnstone et al. 2011). Furthermore, several other suggested mechanisms for producing split sex ratios are unlikely to be important, such as in response to sib mating or partially overlapping generations, as a recent overview of the literature has shown that there is lack of empirical evidence that they occur (West 2009). Consequently, if the haplodipoidy effect has facilitated the evolution of eusociality, it has done so via split sex ratios, and the potentially important causes of split sex ratios are limited to queen virginity, queen replacement, and partial worker control (table 1).

Second, haplodiploidy can have different influences at different stages of the evolution of eusociality and for different types of helping trait. One issue is whether we are considering facultative helping at a fraction of broods or obligate helping in all broods. Previous split sex ratio arguments have been relatively heuristic, showing how selection for facultative helping can be increased at relatively female-biased broods. However, at relatively male-biased broods, potential helpers will be less related to the young that they could help to raise, which disfavors helping. While this will not matter for facultative helping traits, which are only expressed at female-biased broods, it will have to taken into account when considering obligate helping traits, such as those committing the individual to permanent sterility relatively early in her life and before she is able to assess the sex ratio of the brood that she would help to rear. Another issue is whether we are examining the initial evolution (origin) of helping, or the subsequent elaboration (maintenance) of helping (Charnov 1978). In the former, a potential worker must decide whether to stay at her mother's nest and rear siblings or disperse away to found her own nest, whereas in the latter, a potential worker must decide how much to invest in rearing the queen's offspring versus selfishly pursuing her own reproduction within the same nest.

Our aim in this article is to determine, from a theoretical perspective, the extent to which the haplodiploidy effect can facilitate the evolution of eusociality. In order to provide an illustrative overview, we first consider a population divided into colonies that may vary in their sex allocation but not in any other respect. We then construct models for two specific scenarios that the empirical data suggest could have played a role on the route to eusociality: queen virginity and queen replacement (table 1). Our aim with these models is not just to show whether haplodiploidy and split sex ratios can facilitate the evolution of eusociality but also to parameterize the models with empirical data and hence quantify their possible importance. Although it seems reasonable that both mechanisms could facilitate the evolution of helping, we do not know whether the effect is large or negligible. Quantification requires specific models because factors such as queen replacement that lead to split sex ratios can cause variation in the relatedness structure within colonies, which may also mediate selection for helping. For each of the situations that we consider, we examine (when relevant) how the impact of haplodiploidy varies along the route to eusociality (i.e., initial evolution versus later elaboration of helping), and for different types of trait (facultative vs. obligate helping). We consider a third possible cause of split sex ratios, Trivers and Hare's (1976) idea of partial worker control of sex allocation, alongside the role of male production by workers, in a separate article (J. Alpedrinha et al., unpublished manuscript), because it leads to only transient split sex ratios and hence is not amenable to the equilibrium analysis approach that we take in this study.

## Split Sex Ratios and the Evolution of Helping

We examine how haplodiploidy influences selection for helping when the sex ratio (proportion of reproductives that are male) may vary between broods (Grafen 1986). We perform an inclusive fitness analysis (Hamilton 1963, 1964, 1970, 1972), weighing the $b$ extra siblings that the female could rear if she were more helpful against the $c$ extra offspring that she could rear if she were less helpful, with the valuation of each relative being given by the prod-

**Table 2:** A summary of model notation used in the main text

| Symbol | Definition |
|---|---|
| $\alpha$ | Potential for helping |
| $\alpha_{\mathrm{OBL}}$ | Potential for obligate helping |
| $\alpha_{\mathrm{FAC}}$ | Potential for facultative helping |
| $v_{\mathrm{f}}$ | Reproductive value of a juvenile female |
| $v_{\mathrm{m}}$ | Reproductive value of a juvenile male |
| $p_{\mathrm{d}}$ | Consanguinity of a female to her daughter |
| $p_{\mathrm{s}}$ | Consanguinity of a female to her son |
| $p_{\mathrm{f}}$ | Consanguinity of a female to a queen-derived juvenile female |
| $p_{\mathrm{m}}$ | Consanguinity of a female to a queen-derived juvenile male |
| $\phi$ | Degree of monogamy (probability that maternal sisters are paternal sisters) |
| $a$ | Relative productivity of workerless colonies (virginity model) |
| $u$ | Frequency of unmated queens (virginity model) |
| $q$ | Frequency of queenright colonies (queen replacement model) |
| $z$ | Colony sex ratio |
| $\bar{z}$ | Population sex ratio |
| $\bar{z}_{\mathrm{M}}$ | Sex ratio of mated-queen colonies (virginity model) |
| $\bar{z}_{\mathrm{U}}$ | Sex ratio of unmated-queen colonies (virginity model) |
| $\bar{z}_{\mathrm{R}}$ | Sex ratio of queenright colonies (queen replacement model) |
| $\bar{z}_{\mathrm{L}}$ | Sex ratio of queenless colonies (queen replacement model) |

uct of its reproductive value $v$ and its consanguinity $p$ to the focal female (app. A; Hamilton 1964, 1970; Taylor and Frank 1996; Frank 1998; Rousset 2004; Grafen 2006*a*, 2006*b*). We compute the threshold ratio $c/b$, below which helping is favored and above which helping is disfavored, and denote this $\alpha$. This is the "efficiency ratio" of Charnov (1978) and Grafen (1986), and the "potential for altruism" of Gardner (2010). A higher value of $\alpha$ corresponds to a scenario where helping is more readily favored. In particular, $\alpha > 1$ indicates that helping is more readily favored under the given scenario than it is under the assumption of diploidy with full monogamy (Grafen 1986). Model notation is summarized in table 2.

### *Origin of Helping*

We assume an otherwise solitary species, and examine the inclusive fitness consequences for a female (the "worker") who chooses to stay with her mother (the "queen") and rear siblings rather than dispersing and rearing her own brood in her own nest. We allow the sex ratio $z$ of the queen's offspring to depart from the population average $\bar{z}$. The expected sex ratio of the worker's own offspring, should she disperse and raise her own brood, is simply the population average $\bar{z}$. Thus, the inclusive fitness valuation that the worker places on queen-derived offspring is $zv_{\mathrm{m}}p_{\mathrm{m}} + (1 - z)v_{\mathrm{f}}p_{\mathrm{f}}$, and the inclusive fitness valuation that she places on her own offspring is $\bar{z}v_{\mathrm{m}}p_{\mathrm{s}} + (1 - \bar{z})v_{\mathrm{f}}p_{\mathrm{d}}$, where $p_{\mathrm{m}}$ and $p_{\mathrm{f}}$ are the consanguinities of the worker to the queen's sons and queen's daughters, respectively; $p_{\mathrm{s}}$ and $p_{\mathrm{d}}$ are the consanguinities of the worker to her own sons and daughters, respectively; and $v_{\mathrm{f}}$ and $v_{\mathrm{m}}$ are the reproductive values of a female and a male, respectively. If the worker is trading $b$ of the queen's offspring against $c$ of her own offspring, then she increases her inclusive fitness by helping to rear the queen's offspring if $b[zv_{\mathrm{m}}p_{\mathrm{m}} + (1 - z)v_{\mathrm{f}}p_{\mathrm{f}}] > c[\bar{z}v_{\mathrm{m}}p_{\mathrm{s}} + (1 - \bar{z})\,v_{\mathrm{f}}p_{\mathrm{d}}]$. Rearranging this condition into the form $c/b < \alpha$ obtains the potential for helping $\alpha$, and this is given by dividing the valuation of the queen's offspring by the valuation of the worker's own offspring.

If the focal female knows the sex ratio of her mother's brood and adjusts her helping accordingly, we obtain a potential for facultative helping of $\alpha_{\mathrm{FAC}} = [z\,v_{\mathrm{m}}p_{\mathrm{m}} + (1 - z)v_{\mathrm{f}}p_{\mathrm{f}}]/[\bar{z}v_{\mathrm{m}}p_{\mathrm{s}} + (1 - \bar{z})v_{\mathrm{f}}p_{\mathrm{d}}]$. Substituting in the appropriate reproductive values and consanguinity coefficients (app. B), this is

$$\alpha_{\mathrm{FAC}} = \frac{1}{4}\left[\frac{1 - z}{1 - \bar{z}}(1 + 2\phi) + \frac{z}{\bar{z}}\right], \tag{1}$$

where $\phi$ is probability that two given maternal sisters are also paternal sisters. In figures 1–3, we assume $\phi = 1$ because eusociality has evolved only in monogamous species. The corresponding result for diploidy is $\alpha = (1 + \phi)/2$ (app. B), so haplodiploidy promotes facultative helping when the right-hand side (RHS) of equation (1) exceeds $(1 + \phi)/2$, and haplodiploidy inhibits facultative helping when the RHS of equation (1) is less than $(1 + \phi)/2$. If $\bar{z} > 1/[2(1 + \phi)]$ (i.e., $\bar{z} > 1/4$ , under full monogamy), then haplodiploidy promotes the origin of facultative helping in relatively female-biased broods ($z < \bar{z}$)

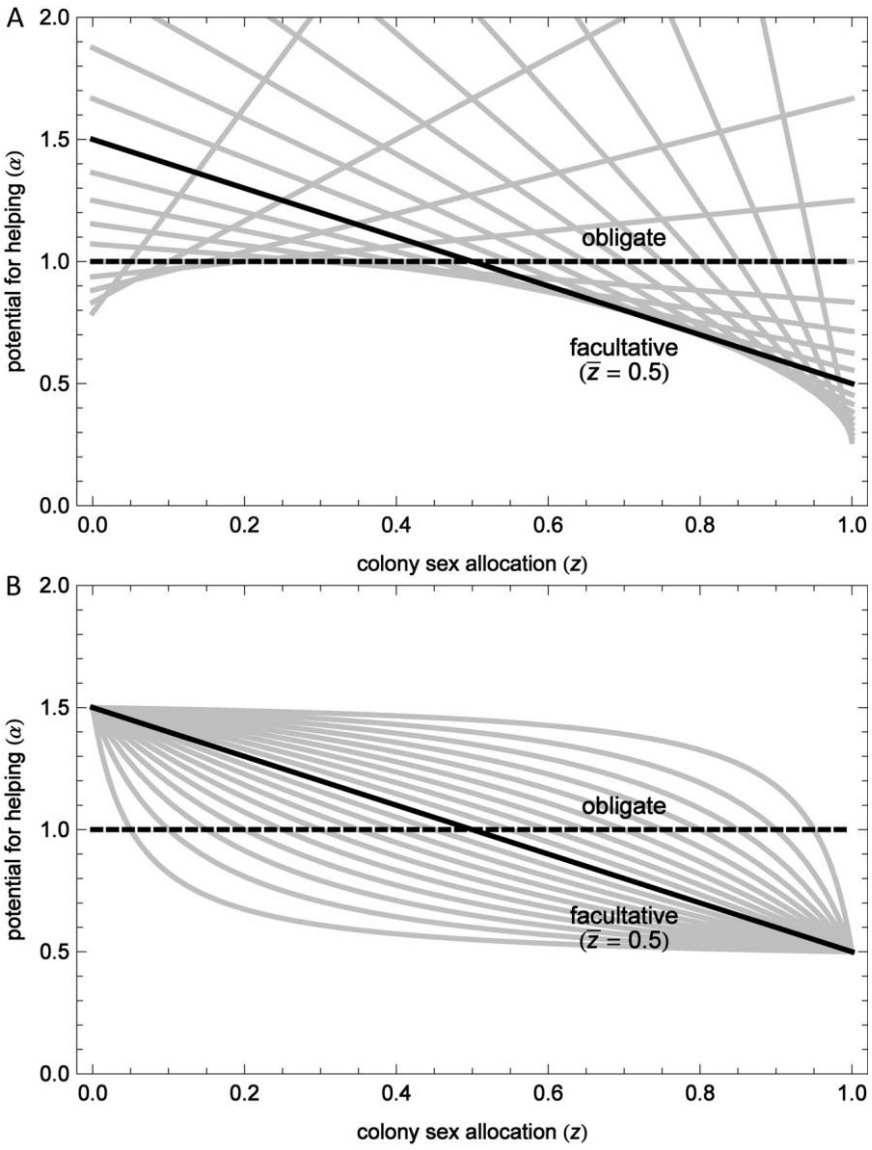


**Figure 1:** Potential for helping in a generic model of split sex ratios, assuming full monogamy ($\phi = 1$). *A*, In the context of the origin of helping, haplodiploidy does not promote obligate helping ($\alpha_{OBL} = 1$, dashed black line), but it promotes facultative helping in colonies that have relatively female-biased sex ratios ($z < \bar{z}$ when $\bar{z} > 1/4$) and inhibits facultative helping in colonies that have relatively male-biased sex ratios ($z > \bar{z}$ when $\bar{z} > 1/4$). The solid black line indicates facultative helping when the population sex ratio is $\bar{z} = 0.5$, and the solid gray lines indicate facultative helping for other population sex ratios, with all lines intersecting $\alpha = 1$ at $z = \bar{z}$. *B*, In the context of the elaboration of helping, haplodiploidy does not promote obligate helping ($\alpha_{OBL} = 1$, dashed black line), but it promotes facultative helping in colonies that have relatively female-biased sex ratios ($z < \bar{z}$) and inhibits facultative helping in colonies that have relatively male-biased sex ratios ($z > \bar{z}$). The solid black line indicates facultative helping when the population sex ratio is $\bar{z} = 0.5$, and the solid gray lines indicate facultative helping for other population sex ratios, with all lines intersecting $\alpha = 1$ at $z = \bar{z}$.

and inhibits the origin of facultative helping in relatively male-biased broods ($z > \bar{z}$). If $\bar{z} < 1/[2(1 + \phi)]$ (i.e., $\bar{z} < 1/4$, under full monogamy), then the opposite is true (fig. 1*A*) because in such strongly female-biased populations, brothers are more valuable than sisters (Trivers and Hare 1976). In the absence of split sex ratios ($z = \bar{z}$ for every brood), haplodiploidy neither promotes nor inhibits the origin of facultative helping, irrespective of the population sex ratio, because the increased relatedness to siblings is exactly balanced by the decreased reproductive value of daughters (Craig 1979).

If the focal female does not know the sex ratio of her

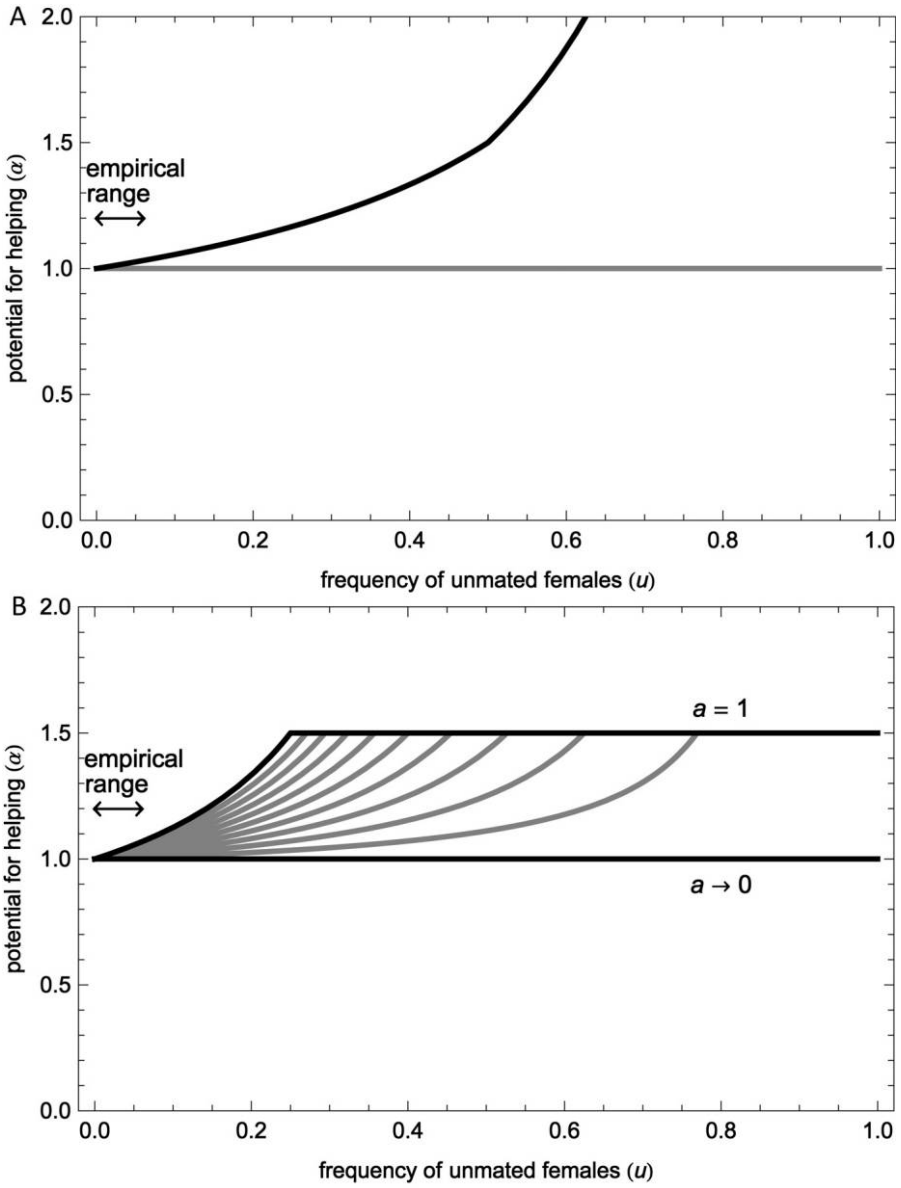


**Figure 2:** Potential for helping in a model of split sex ratios owing to virginity, assuming full monogamy ($\phi = 1$). *A*, In the context of the origin of helping, haplodiploidy always promotes helping. As the rate of unmatedness is generally <6%, this suggests the potential for helping is $\alpha < 1.03$. *B*, In the context of the elaboration of helping, haplodiploidy always promotes helping. Again, as the rate of unmatedness is generally <6%, the potential for helping is $\alpha < 1.07$. Furthermore, it is predicted to be lower if workerless colonies suffer reduced productivity ($a < 1$; with $\alpha \to 1$ as $a \to 0$), as will be the case when helping is selected for.

mother's brood, the decision as to whether or not she should help must be made by taking an average over this uncertainty, and this is equivalent to evaluating the right-hand side of equation (1) at $z = \bar{z}$. This obtains $\alpha_{\text{OBL}} = (1 + \phi)/2$, which is identical to the condition for obligate helping under diploidy, so haplodiploidy neither promotes nor inhibits the origin of obligate helping (fig. 1*A*).

### *Elaboration of Helping*

We next consider the evolutionary elaboration of helping in a social haplodiploid species, by examining the inclusive fitness consequences for a newly eclosed female (the "worker") who chooses to help her mother (the "queen") to rear siblings rather than selfishly rearing her own offspring within the same colony. We imagine that the sex

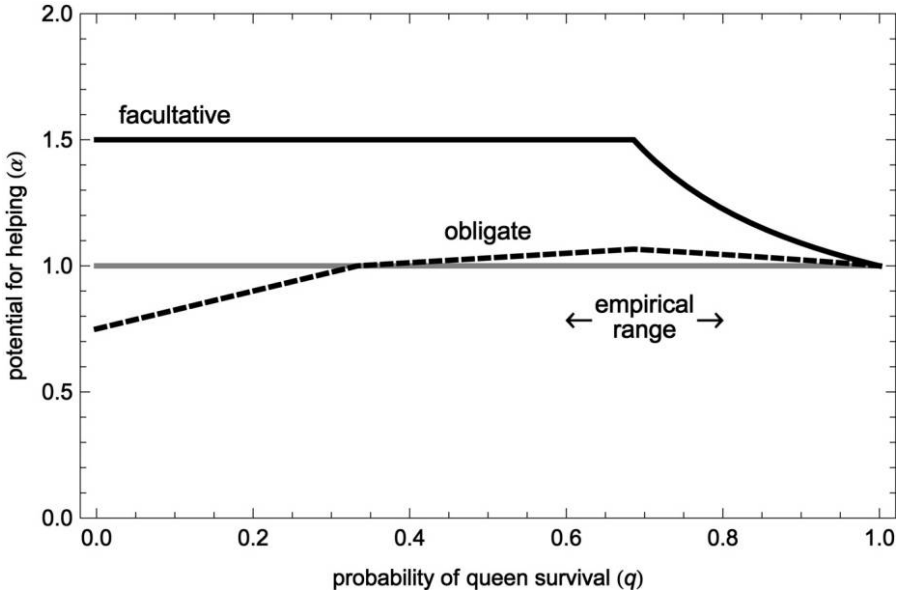


**Figure 3:** Potential for helping in a model of split sex ratios owing to queen replacement, assuming full monogamy ($\phi = 1$). In the context of the elaboration of helping, haplodiploidy can promote or inhibit obligate helping (dashed black line). Over the empirically estimated range of queen survival ($0.6 < q < 0.8$), haplodiploidy has a promoting effect, with maximum potential for obligate helping at $\alpha_{\text{OBL}} \approx 1.07$ when $q \approx 0.69$. Moreover, haplodiploidy always promotes facultative helping in relatively female-biased (i.e., queenright) colonies (solid black line). The maximum potential for facultative helping is at $\alpha_{\text{FAC}} \approx 1.50$, for a range of empirically valid rates of queen survival.

ratio of the colony is controlled by its cohort of workers, and we allow the sex ratio $z$ among the queen's offspring to depart from the population average $\bar{z}$. We assume that the expected sex ratio of the worker's own offspring, should she choose to reproduce, is also $z$ (this assumption is relaxed by J. Alpedrinha et al., unpublished manuscript). Thus, the inclusive fitness valuation that the worker places on queen-derived offspring is $zv_{\text{m}}p_{\text{m}} + (1 - z)v_{\text{f}}p_{\text{f}}$, and the inclusive fitness valuation that she places on her own offspring is $zv_{\text{m}}p_{\text{s}} + (1 - z)v_{\text{f}}p_{\text{d}}$. We assume that worker reproduction is sufficiently rare to be considered negligible for the purpose of calculating reproductive values (this assumption is relaxed by J. Alpedrinha et al., unpublished manuscript).

If the focal female knows the sex ratio of her mother's brood, and adjusts her helping accordingly, we obtain a potential for facultative helping of $\alpha_{\text{FAC}} = [zv_{\text{m}}p_{\text{m}} + (1 - z)v_{\text{f}}p_{\text{f}}]/[zv_{\text{m}}p_{\text{s}} + (1 - z)v_{\text{f}}p_{\text{d}}]$. Substituting in the appropriate reproductive values and consanguinity coefficients (app. B), this is

$$\alpha_{\text{FAC}} = \frac{1}{2} + \phi\frac{\bar{z}(1 - z)}{z(1 - \bar{z}) + \bar{z}(1 - z)}. \qquad (2)$$

Again, haplodiploidy promotes facultative helping when the RHS of equation (2) exceeds $(1 + \phi)/2$ and haplodiploidy inhibits facultative helping when the RHS of equation (2) is less than $(1 + \phi)/2$. That is, haplodiploidy promotes the elaboration of facultative helping in relatively female-biased broods ($z < \bar{z}$) and inhibits the elaboration of facultative helping in relatively male-biased broods ($z > \bar{z}$), irrespective of the actual population sex ratio (fig. 1*B*). In the absence of split sex ratios ($z = \bar{z}$ for every brood), haplodiploidy neither promotes nor inhibits the elaboration of facultative helping, irrespective of the population sex ratio (Craig 1979).

If the focal female does not know the sex ratio of her mother's brood, the decision as to whether she should help must be made by taking an average over this uncertainty, and this is equivalent to evaluating the RHS of equation (2) at $z = \bar{z}$. This obtains $\alpha_{\text{OBL}} = (1 + \phi)/2$, which is identical to the condition for obligate helping under diploidy, so haplodiploidy neither promotes nor inhibits the maintenance of obligate helping (fig. 1*B*).

### Split Sex Ratios Owing to Virginity

Virginity can lead to split sex ratios because unmated females cannot produce daughters but can produce sons (Taylor 1981; Godfray and Grafen 1988). Hence, colonies founded by unmated queens cannot produce workers or female reproductives and so specialize in male reproduc-

tion, whereas colonies founded by mated queens can produce workers and reproductives of either sex.

### *Origin of Helping*

We consider the evolutionary origin of helping in an otherwise solitary haplodiploid species, by examining the inclusive fitness consequences for a newly eclosed female (the "worker") who chooses to stay with her mother (the "queen") and rear siblings rather than dispersing and rearing her own brood. We assume that a fraction $1 - u$ of queens are mated and are able to exhibit any sex allocation strategy $0 \le z_M \le 1$, whereas a fraction $u$ of queens are unmated (or by some other constraint are able to produce only sons; Godfray 1990) and are constrained to exhibit a sex allocation strategy of $z_U = 1$. We assume that a mated queen controls her own sex allocation, and we find that her convergence stable strategy (Taylor 1996) is

$$\bar{z}_M = \begin{cases} \dfrac{1 - 2u}{2(1 - u)} & \text{if } u \le \dfrac{1}{2} \\ 0 & \text{if } u \ge \dfrac{1}{2} \end{cases} \tag{3}$$

(see app. C for derivation). The population sex ratio is given by $\bar{z} = u + (1 - u)\bar{z}_M$, or

$$\bar{z} = \begin{cases} \dfrac{1}{2} & \text{if } u \le \dfrac{1}{2} \\ u & \text{if } u \ge \dfrac{1}{2} \end{cases}. \tag{4}$$

Only mated queens can produce daughters, so the focal worker must be at the nest of a mated queen, and hence, there is no sense in discriminating facultative versus obligate helping. The expected sex ratio of the worker's own offspring, should she disperse and raise her own brood, is simply the population average $\bar{z}$, as she could be either a mated or an unmated queen. Thus, the inclusive fitness valuation that the worker places on queen-derived offspring is $\bar{z}_M \nu_m p_m + (1 - \bar{z}_M)\nu_f p_f$, and the inclusive fitness valuation that she places on her own offspring is $\bar{z}\,\nu_m p_s + (1 - \bar{z})\nu_f p_d$. The potential for helping is $\alpha = [\bar{z}_M \nu_m p_m + (1 - \bar{z}_M)\nu_f p_f]/(\bar{z}\nu_m p_s + (1 - \bar{z})\nu_f p_d]$ and, substituting in the appropriate reproductive values, consanguinity coefficients, and sex ratios, this obtains

$$\alpha = \begin{cases} \dfrac{1 - u + \phi}{2(1 - u)} & \text{if } u \le \dfrac{1}{2} \\ \dfrac{1 + 2\phi}{4(1 - u)} & \text{if } u \ge \dfrac{1}{2} \end{cases}. \tag{5}$$

Again, haplodiploidy promotes helping, relative to diploidy, when $\alpha > (1 + \phi)/2$, which is true for all $u > 0$ (fig. 2*A*). Hence, haplodiploidy always promotes the origin of helping under split sex ratios caused by queen virginity. Empirical estimates of the frequency of unmatedness suggest that is it usually low in outbreeding species, in the range $0.00 < u < 0.06$ (with a mode of 0.00; Godfray and Hardy 1992). Assuming full monogamy ($\phi = 1$), this would lead to the potential for helping in the range $1.00 < \alpha < 1.03$ (with a mode of ~1.00), which even in the best case scenario is only marginally greater than the corresponding value for diploidy ($\alpha = 1$; fig. 2*A*). That is, it is only for species in which the cost/benefit ratio lies within the narrow range $1.00 < c/b < 1.03$ that the haplodiploidy effect can matter. Below this range, helping is disfavored in both diploids and haplodiploids, and above this range, helping is favored in both diploids and haplodiploids. Higher levels of virginity are observed in species with local mate competition, where males do not disperse, but this does not occur on the route to eusociality (West et al. 1997).

### *Elaboration of Helping*

We next consider the evolutionary elaboration of helping in a situation where helping is already common, by examining the inclusive fitness consequences for a newly eclosed female (the "worker") who chooses to help her mother (the "queen") to rear siblings rather than selfishly rearing her own offspring within the same colony. We consider that colonies founded by unmated queens have a fraction $0 < a < 1$ of the productivity (i.e., number of reproductive offspring) enjoyed by colonies founded by mated queens, owing to the absence of workers in the former. Also, we now assume that the workers control sex allocation in the mated-queen colonies, and we find that their convergence stable sex allocation strategy is

$$\bar{z}_M = \begin{cases} \dfrac{1 - [1 + (1 + 2\phi)a]u}{2(1 + \phi)(1 - u)} & \text{if } u \le \dfrac{1}{1 + (1 + 2\phi)a} \\ 0 & \text{if } u \ge \dfrac{1}{1 + (1 + 2\phi)a} \end{cases} \tag{6}$$

(see app. C for derivation). The population sex ratio is given by $\bar{z} = [ua + (1 - u)z_M]/[1 - (1 - a)u]$, or

$$\bar{z} = \begin{cases} \dfrac{1}{2(1 + \phi)} & \text{if } u \le \dfrac{1}{1 + (1 + 2\phi)a} \\ \dfrac{ua}{1 - (1 - a)u} & \text{if } u \ge \dfrac{1}{1 + (1 + 2\phi)a} \end{cases}. \tag{7}$$

The inclusive fitness valuation that the worker places on queen-derived offspring is $\bar{z}_M \nu_m p_m + (1 - \bar{z}_M)\nu_f p_f$, and the inclusive fitness valuation that she places on her own

offspring is $\bar{z}_M \nu_m p_s + (1 - \bar{z}_M)\nu_f p_d$. Assuming that worker reproduction is sufficiently rare to be considered negligible for the purpose of calculating reproductive value, the potential for helping is $\alpha = [\bar{z}_M \nu_m p_m + (1 - \bar{z}_M)\nu_f p_f]/[\bar{z}_M \nu_m p_s + (1 - \bar{z}_M)\nu_f p_d]$ and, substituting in the appropriate reproductive values, consanguinity coefficients, and sex ratios, this obtains

$$\alpha = \begin{cases} \dfrac{(1-u)(1+\phi)}{2[1-u(1+\phi a)]} & \text{if } u \le \dfrac{1}{1+(1+2\phi)a} \\ \dfrac{1+2\phi}{2} & \text{if } u \ge \dfrac{1}{1+(1+2\phi)a} \end{cases}. \tag{8}$$

Haplodiploidy promotes helping, relative to diploidy, when $\alpha > (1 + \phi)/2$, which is true for all $u > 0$ (fig. 2*B*). However, the empirical estimate of $0.00 < u < 0.06$ indicates a maximum potential for helping of only $\alpha \approx 1.07$ under full monogamy ($\phi = 1$), which is only marginally greater than the corresponding value for diploids ($\alpha = 1$; fig. 2*B*). Again, this means that the haplodiploidy hypothesis has explanatory power only insofar as ancestral taxa fell into the cost/benefit range defined by $1.00 < c/b < 1.07$. Moreover, the potential for helping can be substantially lower if workerless colonies suffer a productivity penalty relative to colonies that contain workers ($a < 1$), with the haplodiploidy effect vanishing in the limit of low productivity of workerless colonies ($\alpha \to [1 + \phi]/2$ as $a \to 0$; fig. 2*B*). Overall, this suggests that as helping spreads through the population, and becomes more efficient, the benefit of haplodiploidy will be removed.

### Split Sex Ratios Owing to Queen Replacement

We next consider the scenario where split sex ratios evolve owing to queen replacement, when the queen is lost from some colonies and replaced by a mated daughter. Assuming haplodiploidy then, in colonies where the original queen is still present ("queenright" colonies), the workers are more related to the queen's daughters (sisters, $r = 3/4$) than to her sons (brothers, $r = 1/2$). In contrast, in colonies where the original queen has been replaced by one of her daughters ("queenless" colonies), the workers are more related to the new queen's sons (nephews, $r = 3/4$) than they are to her daughters (nieces, $r = 3/8$). This favors workers to bias the colony sex ratio toward females in queenright colonies, and toward males in queenless colonies, as has been observed and experimentally demonstrated in cooperative bees (Boomsma 1991; Mueller 1991; Packer and Owen 1994). In contrast, under diploidy, queen replacement does not drive split sex ratios, owing to the symmetry of male and female inheritance. This mechanism applies only to the elaboration of helping, in already social species where workers have seized control of the colony sex ratio, as queen-controlled sex allocation does not give rise to split sex ratios.

We consider a model that is identical to the generic model of split sex ratios presented above, except that we now assume that only a proportion $q$ of colonies are headed by their original queen, and that a proportion $1 - q$ of colonies are headed by one the original queen's daughters. One consequence of queen replacement is that males gain extra reproductive value, owing to their ability to father and mate with replacement queens (Trivers and Hare 1976; app. D). We assume that colony sex allocation is controlled by workers, and we find that the convergence stable state of the population, in terms of the sex allocation of queenright ($\bar{z}_R$) and queenless ($\bar{z}_L$) colonies, is given by

$$(\bar{z}_R, \bar{z}_L) = \begin{cases} \left[0, \dfrac{3-q}{4(1-q)}\right] & \text{if } q \le \dfrac{1}{3} \\ (0, 1) & \text{if } \dfrac{1}{3} \le q \le \dfrac{-3+\sqrt{9+8\phi(1+2\phi)}}{4\phi} \\ \left\{\dfrac{3q-1-2(1-q^2)\phi}{2q[2+(1+q)\phi]}, 1\right\} & \text{if } q \ge \dfrac{-3+\sqrt{9+8\phi(1+2\phi)}}{4\phi} \end{cases} \tag{9}$$

(see app. D for derivation). This solution can be used to calculate the population sex ratio $\bar{z} = q\bar{z}_R + (1 - q)\bar{z}_L$.

If the focal female knows the status of her natal colony when deciding whether or not to help, and facultatively adjusts her helping according to this information, then she may be expected to help more in colonies where the sex ratio is more female biased, that is, queenright colonies. Here, the sex ratio among the queen's offspring is $\bar{z}_R$. We assume that the expected sex ratio of the worker's offspring, should she choose to reproduce, is also $\bar{z}_R$. Thus, the inclusive fitness valuation that the worker places on queen-derived offspring is $\bar{z}_R \nu_m p_{mR} + (1 - \bar{z}_R)\nu_f p_{fR}$, and the inclusive fitness valuation that she places on her own offspring is her own offspring is $\bar{z}_R \nu_m p_s + (1 - \bar{z}_R)\nu_f p_d$. Thus, the potential for helping is $\alpha_{FAC} = [\bar{z}_R \nu_m p_{mR} + (1 - \bar{z}_R)\nu_f p_{fR}]/[\bar{z}_R \nu_m p_s + (1 - \bar{z}_R)\nu_f p_d]$. Substituting in the appropriate reproductive values, consanguinity coefficients and sex ratios, we obtain:

$$\alpha_{FAC} = \begin{cases} \dfrac{1+2\phi}{2} & \text{if } q \le \dfrac{-3+\sqrt{9+8\phi(1+2\phi)}}{4\phi} \\ \dfrac{q[2+(1+q)\phi]}{4q-2(1-q^2)\phi} & \text{if } q \ge \dfrac{-3+\sqrt{9+8\phi(1+2\phi)}}{4\phi} \end{cases}. \tag{10}$$

Since $\alpha > (1 + \phi)/2$ is true for all $q < 1$, haplodiploidy promotes the maintenance of facultative helping in queen-right colonies, under split sex ratios caused by queen replacement (fig. 3). This effect of haplodiploidy is substantial: assuming full monogamy ($\phi = 1$), the potential for facultative helping can be as great as $\alpha_{\text{FAC}} = 1.50$. In natural populations, the empirically observed range of queen survival rates is $0.6 < q < 0.8$, which would give $1.23 \leq \alpha_{\text{FAC}} \leq 1.50$ (fig. 3)

If the decision to help rear the queen's offspring versus own offspring is taken without reference to whether the queen is original or a replacement, then the inclusive fitness value of queen offspring and own offspring must be taken as an expectation over this uncertainty. Hence, the potential for obligate helping is given by $\alpha_{\text{OBL}} = \{q[\bar{z}_{\text{R}} v_{\text{m}} p_{\text{mR}} + (1 - \bar{z}_{\text{R}}) v_{\text{f}} p_{\text{fR}}] + (1 - q)[\bar{z}_{\text{L}} v_{\text{m}} p_{\text{mL}} + (1 - \bar{z}_{\text{L}}) v_{\text{f}} p_{\text{fL}}]\}/\{q[\bar{z}_{\text{R}} v_{\text{m}} p_{\text{s}} + (1 - \bar{z}_{\text{R}}) v_{\text{f}} p_{\text{d}}] + (1 - q)[\bar{z}_{\text{L}} v_{\text{m}} p_{\text{s}} + (1 - \bar{z}_{\text{L}}) v_{\text{f}} p_{\text{d}}]\}$. Substituting in the appropriate reproductive values, consanguinity coefficients and sex ratios, this obtains

$$\alpha_{\text{OBL}} = \begin{cases} \dfrac{(1+q)(1+2\phi)}{4} & \text{if } q \leq \dfrac{1}{3} \\ \dfrac{(5+q)(1+2\phi)}{16} & \text{if } \dfrac{1}{3} \leq q \leq \dfrac{-3+\sqrt{9+8\phi(1+2\phi)}}{4\phi} \\ \dfrac{[1+q+2(1-q)\phi][2+(1+q)\phi]}{8} & \text{if } q \geq \dfrac{-3+\sqrt{9+8\phi(1+2\phi)}}{4\phi} \end{cases}. \tag{11}$$

Here, $\alpha_{\text{OBL}} > (1 + \phi)/2$ is not always satisfied. Thus, haplodiploidy sometimes promotes and sometimes inhibits the maintenance of obligate helping if there is queen replacement, relative to the basic model of diploidy (fig. 3). Assuming full monogamy ($\phi = 1$), then $0.75 < \alpha_{\text{OBL}} < 1.07$. Considering the empirically observed range of queen survival rates ($0.6 < q < 0.8$), then $1.05 < \alpha_{\text{OBL}} < 1.07$, with the greatest potential for obligate helping being $\alpha_{\text{OBL}} \approx 1.07$ at $q \approx 0.69$. Note that the potential for obligate helping under diploidy is actually lower than $(1 + \phi)/2$ with queen replacement, owing to the reduced relatedness of a worker to the offspring of her queen. However, it is not meaningful to compare the potential for obligate helping under haplodiploidy with this lower value: all that we learn is that queen replacement inhibits helping more under diploidy than under haplodiploidy.

## Discussion

We have shown that: (1) many of the proposed consequences of haplodipoidy are unlikely to have been important for the evolution of eusociality, because they rely on biological assumptions that the comparative data suggest did not occur en route to eusociality, such as multiple mating or associations between same-generation breeders (the "semisocial route"); (2) the most plausible route by which the haplodiploidy hypothesis could work is with split sex ratios, building on Trivers and Hare (1976); (3) although split sex ratios can be favored for many reasons, there are only two mechanisms that have both been observed empirically and are consistent with the biology of primitively social hymenopterans—virginity and queen replacement; and (4) while these two mechanisms can lead to haplodiploidy favoring eusociality, the overall effect is likely to be small and can even be negative.

### *Split Sex Ratios*

We have examined two specific mechanisms that could have led to evolutionarily stable split sex ratios. First, unmated queens are constrained to produce only sons, whereas mated queens may produce both sons and daughters (Godfray and Grafen 1988). Hence, workers—who are female and therefore necessarily born into mated-queen colonies—have the option of rearing a cohort of siblings with a sex ratio that is female biased relative to the average for the population, which favors helping in haplodiploids. However, under the empirically plausible range of unmatedness rates (0%–6%, with a mode of 0%), the potential for helping is boosted by only 0%–3% when considering the origin of helping and only 0%–7% when considering the subsequent elaboration of helping. Moreover, this effect is predicted to be substantially lower if colonies with mated queens and workers have increased productivity, because this reduces the extent to which their offspring are female biased relative to the population average. That is, the situation that is most conducive to the evolution of helping (i.e., when helping leads to a large increase in the colony's productivity) is precisely the situation that erodes the impact of haplodiploidy on the potential for helping.

Second, split sex ratios may evolve in response to when queens die and are replaced by their daughters (Boomsma 1991). In colonies that retain the original queen (the mother of the workers), the workers are more related to the female (sisters) than the male (brothers) reproductives and so are favored to produce a relatively female-biased sex ratio. In contrast, in colonies where the original queen has died and has been replaced by one of her daughters (sister of the workers), the workers are less related to the female (nieces) than the male (nephews) reproductives and so are favored to produce a relatively male-biased sex ratio. We have found that this can lead to selection for helping being either promoted or inhibited by haplodiploidy, de-

**Table 3:** Empirical objections to the haplodiploidy hypothesis and their current status

| Potential problem | Current status |
|---|---|
| If queens mate multiply, this removes the relatedness advantage of helping to raise siblings (Bourke and Franks 1995; Queller and Strassmann 1998) | Irrelevant, as multiple mating only evolved after obligate eusociality was established (Boomsma 2007, 2009; Hughes et al. 2008) |
| Assumes subsocial route, where offspring help mothers, and does not work with semisocial route where sisters cooperate (Bourke and Franks 1995) | Irrelevant, as phylogentic evidence provides no evidence for eusociality ever evolving by the semisocial route (Boomsma 2007, 2009; Hughes et al. 2008) |
| Requires sex ratio manipulation with worker control (Charnov 1978; Stubblefield and Charnov 1986) | Not a problem, as considerable evidence for worker control of sex allocation, including prior to the evolution of permanent eusociality (Mueller 1991; West 2009) |
| There are alternative explanations for why eusociality is common in the Hymenoptera, such as extended parental care (Stubblefield and Charnov 1986; Queller and Strassmann 1998) and monogamy (Boomsma 2007, 2009) | True, but does not exclude a role of haplodiploidy |
| Given more recent discoveries of eusociality (albeit facultative, not obligate) in other taxa (Aoki 1977; Jarvis 1981; Crespi 1992; Kent and Simpson 1992; Duffy 1996), has haplodiploidy really evolved more often in haplodiploids? | Valid question, but it does not exclude a role of haplodiploidy. Crozier (2008) has shown that eusociality is significantly more common in haplodiploid families, but this does not allow for fact that families are not phylogenetically independent. There has been no phylogenetic study testing whether the rate of transition to eusociality is significantly higher in haplodiploids. |

pending on: (a) the incidence of queenright colonies; and (b) whether the workers can facultatively adjust their helping behavior according to the queenright/queenless status of the colony, or are obliged to help equally in both types of colonies. However, under the empirically supported range of probabilities of queen survival (60%–80%), haplodiploidy always promotes helping, with the potential for facultative helping boosted by up to 50% and the potential for obligate helping boosted to up to 7%. The overall importance of this mechanism will depend on how frequently queen replacement leads to split sex ratios: to date, it has been found only in some cooperative bees, suggesting it is not a general factor on the route to eusociality (Boomsma 1991; Mueller 1991; Packer and Owen 1994).

Furthermore, our analyses are likely to have overestimated the extent to which haplodiploidy favors eusociality via queen replacement. We followed previous analyses in assuming that when workers selfishly produce their own offspring within the queen's nest, these offspring will exhibit the same sex ratio as the queen-derived juveniles (Craig 1979). However, the female bias in the population sex ratio means that males have higher reproductive value than females, so that when workers are reproductive they will be favored to produce sons rather than daughters. This would tend to decrease the potential for facultative helping in queenright colonies and obligate helping in both colony types. We consider this effect, and the consequences of reproduction by workers more generally, elsewhere (J. Alpedrinha et al., unpublished manuscript).

Our emphasis in this article has been different to most previous work on the haplodiploidy hypothesis. Most previous articles have examined whether the haplodiploidy hypothesis can be made to work (e.g., Trivers and Hare 1976; Seger 1983; Grafen 1986; Stubblefield and Charnov 1986; Godfray and Grafen 1988). In contrast, our aim has been to quantify the extent to which the haplodiploidy effect favors the evolution of eusociality. We have focused on those scenarios that are biologically most plausible (table 3), and found that the extent to which haplodiploidy favors eusociality will be either: (a) small (unmated females); or (b) small to medium but not widespread (queen replacement). In addition, we have clarified the distinction between selection on facultative versus obligate helping. The latter is less aided by haplodiploidy, because the increased relatedness to siblings when helping at female-biased colonies is negated by the decreased relatedness when helping at male-biased colonies. This means that haplodiploidy will be less likely to favor helping if females must choose whether to help before they know which type of colony they will be helping in, especially with regard to commitments to expressing helping adaptations that are made relatively early in development; for example, preeclosion. Overall, our results suggest that, for the scenarios we consider here, haplodiploidy would have had only a minor influence on the evolution of eusociality.

### *Manipulation, Maternal Care, and Monogamy*

In this final section, we briefly consider other factors that may have influenced the evolution of eusociality. First,

parental manipulation or parasitism may have helped the evolution of eusociality, by enforcing cooperation on workers (Alexander 1974; Charnov 1978). However, the extent to which workers will be favored to resist versus acquiesce to their queen depends on their relatedness to (and the reproductive value of) the different types of offspring, just as when considering cases with split sex ratios (Crozier 2008). Consequently, it is wrong to think of parental manipulation and kin selection as competing hypotheses for the evolution of eusociality (Crozier 2008). Furthermore, explicit theory has shown that queen manipulation is equally likely to occur in diploids and haplodiploids, and so it will not lead to haplodiploids being predisposed to eusociality (Charnov 1978).

Second, it is possible that haplodiploidy has predisposed certain taxa to eusociality, for reasons that are separate from Hamilton's (1964, 1972) suggestion concerning the asymmetry in relatedness to sisters versus daughters. Wade (2001; Linksvayer and Wade 2005) has suggested that maternal care—a prerequisite for eusociality—evolves more readily in haplodiploids than in diploids. Reeve (1993; Reeve and Shellman-Reeve 1997) has suggested that, even when helping genes experience the same systematic selection pressure under diploidy and haplodiploidy, they may be better protected from stochastic loss under haplodiploidy. Fromhage and Kokko (2011) have suggested that haplodiploidy can enhance synergistic interaction between genes for helping. However, these three ideas require restrictive assumptions, which make them unlikely to be of general importance. Specifically, they require that maternal care genes have particular, deleterious pleiotropic effects (Wade 2001); helping genes are overdominant (Reeve 1993); or that the worker phenotype is controlled by a single allele of large effect (Fromhage and Kokko 2011). More generally, we emphasize the importance of constructing realistic models of specific scenarios that are led by and parameterized with empirical data.

Third, both theory and data suggest that monogamy has played a key role in the evolution of eusociality. Strict lifetime monogamy leads to a worker being equally related to her own offspring and to the offspring of her mother and hence to a potential for helping of $\alpha = 1$ (Boomsma 2007, 2009). In this case, any small efficiency benefit from rearing siblings ($b/c > 1$) would lead to helping being favored by natural selection (i.e., $b/c > 1/\alpha$). Multiple mating reduces the relatedness of siblings and decreases selection for any form of cooperation (Charnov 1981). For example, if females mate with two or three males, this reduces the potential for helping to $\alpha = 3/4$ or 2/3, respectively, and so substantial efficiency benefits to cooperation would be required ($b/c > 4/3$ or 3/2, respectively). Consistent with this, obligate eusociality has evolved only in lineages where strict monogamy is the ancestral state (Boomsma 2007, 2009; Hughes et al. 2008), and the evolution of facultative cooperative breeding is more common in species with lower rates of promiscuity (Cornwallis et al. 2010). Consequently, the hunt to find a way for haplodiploidy to push the potential for helping higher than unity ($\alpha > 1$) may have been misguided. Instead, a more important factor may have been the need for monogamy to keep the potential for helping at unity ($\alpha = 1$), and some small efficiency benefit for rearing siblings over one's own offspring ($b/c > 1$). Efficiency benefits appear to arise from the life insurance of allowing helpers to complete parental care after the death of the mother, or the fortress-defense benefits of protecting a common nest (Hamilton 1964, 1972; Queller 1989, 1994; Foster 1990; Gadagkar 1991; Queller and Strassmann 1998; Field et al. 2000; Strassmann and Queller 2007).

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## APPENDIX A

### Reproductive Value and Relatedness

Inclusive fitness is gained by sending copies of one's genes into future generations. Hence, an actor is predicted to behave as if she values the reproductive success of her relatives, as they may carry copies of her genes and pass them on to their descendants (Hamilton 1964). Specifically, the value that she places on a relative is given by the product of the relative's reproductive value ($v$; i.e., how well they transmit copies of their own genes into future generations; Fisher 1930) and the consanguinity of the relative to the actor ($p$; i.e., the probability the relative's and actor's genes are identical by descent; Bulmer 1994).

Reproductive value describes the expected contribution of genes made by an individual or class of individuals to a generation in the distant future (Fisher 1930; Taylor 1990; Grafen 2006*b*). Typically, reproductive value is first calculated for a class, and then the class's reproductive value is shared equally over all individuals in that class. For example, in diploids, the probability that a gene picked at random from a distant future generation descends from

a male ancestor in the present generation is 1/2. Hence, the class reproductive value of males is $c_m = 1/2$, and the reproductive value of an individual male is $v_m = c_m/N_m$, where $N_m$ is the number of males in the population. Alternatively, reproductive values may be scaled by any arbitrary constant, to make the quantities more manageable. For example, multiplying all individual reproductive values by the total number of individuals in the population, we have $v_m = c_m/\bar{z} = 1/[2(1 - \bar{z})]$ and $v_f = c_f/(1 - \bar{z}) = 1/[2(1 - \bar{z})]$, where $\bar{z}$ is the proportion of reproductive individuals who are male.

Consanguinity is defined as the probability that two genes picked at random from two given individuals are identical by descent (Bulmer 1994). Thus, the probability of drawing a given allele from the recipient given that it has already been drawn from the actor is $x' = p + (1 - p)\bar{x}$, where $p$ is the consanguinity of the actor and recipient and $\bar{x}$ is the frequency of the allele in the whole population. This allows a regression interpretation for consanguinity: rearranging, we have $p = (x' - \bar{x})/(1 - \bar{x})$, that is, consanguinity measures the concentration of the actor's genes in the recipient.

It is often natural to seek a measure of relative genetic similarity such that the similarity to oneself is 1. This is obtained by dividing consanguinity between actor and recipient by the consanguinity of actor to self, that is, $r = p/p_{self}$. This is the regression coefficient of relatedness (Hamilton 1970; Michod and Hamilton 1980; Pamilo and Crozier 1982; Grafen 1985) and, for outbred diploids, this takes the familiar values of $r = 1$ for self, $r = 1/2$ for full sibs, $r = 1/4$ for half sibs, and $r = 1/8$ for cousins. More generally, since throughout our analysis we will assume outbreeding and diploid actors (i.e., females), the consanguinity to self for the actor is always $p_{self} = 1/2$, and hence the regression coefficient of relatedness is $r = 2p$.

The regression relatedness values for a female to her son and to her brother under haplodiploidy are $r = 1$ and $r = 1/2$, respectively. Many readers will be more familiar with values of 1/2 and 1/4, respectively. The latter values refer to "life-for-life" relatedness coefficients, which were commonly used in the kin selection literature on haplodiploids to account for both genetic similarity and reproductive value effects in a single coefficient (Hamilton 1972; Trivers and Hare 1976; Grafen 1986). Assuming an even sex ratio and vanishingly rare worker reproduction, these life-for-life relatedness coefficients can be recovered by simply multiplying the regression coefficient of relatedness by individual reproductive value, that is, $R = r \times v$ (Hamilton 1972; Bulmer 1994), and scaling all reproductive values such that the reproductive value of a female is $v_f = 1$. Thus, the life-for-life relatedness of a female to her son is $R = 1 \times 1/2 = 1/2$ and to her brother is $R = 1/2 \times 1/2 = 1/4$. Sometimes, life-for-life relatedness coefficients have been defined as the probability that a gene picked at random from the actor is identical by descent with any gene in the recipient (Trivers and Hare 1976; Charlesworth 1980). If the recipient is haploid this is simply the consanguinity of the actor and recipient, whereas if the recipient is diploid this is twice the consanguinity of the actor and recipient. This corrects for reproductive value but only owing to a mathematical coincidence: in both diploids and haplodiploids, the ratio of female to male ploidies is equal to the ratio of female to male individual reproductive values (under the assumption of vanishingly rare worker reproduction and an even sex ratio). This correction does not work more generally, for example, under certain hypothetical haplotriploid modes of inheritance (Grafen 1986) or, more importantly, when worker reproduction is common and/or sex ratios are biased (see apps. B–D).

## APPENDIX B

### Split Sex Ratios and the Evolution of Helping

For the purpose of calculating reproductive value, we census the population at the moment of production of offspring. The proportion of genes in female larvae at the time of census that derive from the females of the last census is $\wp_{f\leftarrow f} = 1/2$ and hence the proportion of genes in female larvae that derive from the males of the last census is $\wp_{f\leftarrow m} = 1 - \wp_{f\leftarrow f} = 1/2$. The proportion of genes in male larvae at the time of census that derive from the females of the last census is $\wp_{m\leftarrow f} = 1$, and hence the proportion of genes in male larvae that derive from the males of the last census is $\wp_{m\leftarrow m} = 1 - \wp_{m\leftarrow f} = 0$. These quantities can be summarized in a gene flow matrix:

$$\mathbf{M} = \begin{bmatrix} \wp_{f\leftarrow f} & \wp_{f\leftarrow m} \\ \wp_{m\leftarrow f} & \wp_{m\leftarrow m} \end{bmatrix}. \tag{B1}$$

The class reproductive values are given by the dominant left eigenvector of the gene flow matrix, that is, the solution to $(c_f, c_m) = (c_f, c_m) \cdot \mathbf{M}$ (Taylor 1996). This yields $c_f = 2/3$ for females and $c_m = 1/3$ (Price 1970; Taylor 1996). Individual reproductive values can be expressed as the class reproductive value divided by the proportion of the population that belongs to that class, that is, $v_m = c_m/\bar{z}$ for males and $v_f = c_f/(1 - \bar{z})$ for females (Taylor 1996).

The consanguinity of a female to her brothers and sisters is $p_m = 1/4$ and $p_f = (1 + 2\phi)/8$, respectively, where $\phi$ is the consanguinity of two random sperm that fertilize the eggs of the same female. The consanguinity of a female to her sons and daughters is $p_s = 1/2$ and $p_d = 1/4$, respectively.

## APPENDIX C

### Split Sex Ratios Owing to Virginity

#### *Origin of Helping*

Here we derive the queen's convergence stable sex allocation strategy. We model this by assuming that she produces fertilized (female) and unfertilized (male) eggs in equal numbers but has resources to raise only half of these eggs to maturity, so by choosing which individuals to raise, she determines the sex ratio of her offspring. The expected fitness of a female egg laid by a mated queen, before the enactment of the sex allocation decision, is therefore $1 - z_M$, that is, the probability that she will be reared to reproductive maturity. The average fitness of her class is $1 - \bar{z}_M$, and hence her relative fitness is $W_f = (1 - z_M)/(1 - \bar{z}_M)$. Similarly, the relative fitness of a male egg, expressed relative to the average for all males, is $W_m = [u + (1 - u)z_M]/[u + (1 - u)\bar{z}_M]$. Natural selection favors an increase in the population average value of any trait if individuals carrying genes for this trait are fitter on average. In a class structured population, fitness is averaged using class reproductive values as weights, that is, $W = c_f W_f + c_m W_m$ (Taylor 1996; Taylor and Frank 1996; Frank 1997, 1998; Rousset 2004; Taylor et al. 2007). If a gene affecting the trait of interest has genic value $g$, then the condition for natural selection to favor an increase in the population average value of this trait is $dW/dg > 0$. Hence, the direction of selection acting on the sex ratio of mated queens is given by

$$\frac{dW}{dg} = c_f \frac{dW_f}{dg_f} + c_m \frac{dW_m}{dg_m},$$

$$= c_f \frac{\partial W_f}{\partial z_M} \frac{dz_M}{d\tilde{g}} \frac{d\tilde{g}}{dg_{fM}} + c_m \frac{\partial W_m}{\partial z_M} \frac{dz_M}{d\tilde{g}} \frac{d\tilde{g}}{dg_{mM}}, \quad \text{(C1)}$$

where $\partial W_f/\partial z_M = -1/(1 - \bar{z}_M)$ and $\partial W_m/\partial z_M = (1 - u)/[u + (1 - u)\bar{z}_M]$ are the fitness effects of the queen's sex allocation decision on female and male eggs, respectively; $\tilde{g}$ is the queen's genetic value for her sex allocation trait; $dz_M/d\tilde{g} = 1$ is the genotype-phenotype map; and $d\tilde{g}/dg_{fM} = p_d = 1/4$ and $d\tilde{g}/dg_{mM} = p_s = 1/2$ are the coefficients of consanguinity between mother and daughter and between mother and son, respectively (Taylor 1990, 1996; Taylor and Frank 1996; Frank 1997, 1998; Rousset 2004; Taylor et al. 2007). Substituting in the class reproductive values obtains a condition for increase in sex ratio strategy employed by mated queens $dW/dg > 0$ in terms of model parameters, and we use this to obtain equation (3) in the main text.

#### *Elaboration of Helping*

Here we derive the workers' convergence stable sex allocation strategy. This is modeled by assuming that the queen produces fertilized (female) and unfertilized (male) eggs in equal numbers, and the workers choose which half of these are to be raised to reproductive maturity. The expected fitness of a female egg in a mated-queen colony, before the enactment of the worker sex allocation decision, is therefore $1 - z_M$, that is, the probability that she will be reared to reproductive maturity. The average fitness of her class is $1 - \bar{z}_M$, and hence her relative fitness is $W_f = (1 - z_M)/(1 - \bar{z}_M)$. Similarly, the relative fitness of a male egg, expressed relative to the average for all males, is $W_m = [ua + (1 - u)z_M]/[ua + (1 - u)\bar{z}_M]$. As before, the direction of selection acting on the sex ratio of mated-queen colonies is given by equation (C1), where $\partial W_f/\partial z_M = -1/(1 - \bar{z}_M)$ and $\partial W_m/\partial z_M = (1 - u)/[ua + (1 - u)\bar{z}_M]$ are the fitness effects of the worker sex allocation decision on female and male eggs, respectively; $\tilde{g}$ is the average of the workers' genetic values for their sex allocation trait; $dz_M/d\tilde{g} = 1$ is the genotype-phenotype map; and $d\tilde{g}/dg_{fM} = p_f = 3/8$ and $d\tilde{g}/dg_{mM} = p_m = 1/4$ are the coefficients of consanguinity between worker and queen's female offspring and between worker and queen's male offspring, respectively (Taylor 1990, 1996; Taylor and Frank 1996; Frank 1997, 1998; Rousset 2004; Taylor et al. 2007). Substituting in the class reproductive values obtains a condition for increase in sex ratio of mated-queen colonies $dW/dg > 0$ in terms of model parameters, and we use this to obtain equation (6) in the main text.

## APPENDIX D

### Split Sex Ratios Owing to Queen Replacement

One consequence of queen replacement is that males gain extra reproductive value, owing to their ability to father and mate with replacement queens. Again, for the purpose of calculating reproductive value, we census the population at the moment of production of reproductive offspring. The proportion of genes in female larvae at the time of census that derive from the females of the last census is $\wp_{f\leftarrow f} = q/2 + (1 - q)/4 = (1 + q)/4$, and hence the proportion of genes in female larvae that derive from the males of the last census is $\wp_{f\leftarrow m} = 1 - \wp_{f\leftarrow f} = (3 - q)/4$. The proportion of genes in male larvae at the time of census that derive from the females of the last census is $\wp_{m\leftarrow f} = q + (1 - q)/2 = (1 + q)/2$, and hence the proportion of genes in male larvae that derive from the males of the last census is $\wp_{m\leftarrow m} = 1 - \wp_{m\leftarrow f} = (3 - q)/2$. Using the procedure outlined in appendix B, the class reproductive values are $c_f = (2 + 2q)/(5 + q)$ and $c_m = (3 -$

$q)/(5 + q)$. It is useful to define four classes of young reproductives, according to their sex and the colony type from which they were reared. So, the class reproductive value of queenright females is $c_{fR} = [q(1 - \bar{z}_R)/(1 - \bar{z})]c_f$, that of queenright males is $c_{mR} = [q\bar{z}_R/\bar{z}]c_m$, that of queenless females is $c_{fL} = [(1 - q)(1 - \bar{z}_L)/(1 - \bar{z})]c_f$, and that of queenless males is $c_{mL} = [(1 - q)\bar{z}_L/\bar{z}]c_m$, where $\bar{z}_R$ and $\bar{z}_L$ are the average sex ratios of queenright colonies and queenless colonies, respectively.

The sex allocation decision of the workers is modelled in the usual way. An equal number of male and female larvae are produced, and the workers choose which of these to rear. The expected fitness of a female egg in a queenright colony, prior to the enactment of the worker sex allocation decision, is therefore $1 - z_R$, that is, the probability that she will be reared to reproductive maturity. The average fitness of her class is $1 - \bar{z}_R$, and hence her relative fitness is $W_{fR} = (1 - z_R)/(1 - \bar{z}_R)$. Similarly, the relative fitness of a queenright male is $W_{mR} = z_R/\bar{z}_R$, that of a queenless female is $W_{fL} = (1 - z_L)/(1 - \bar{z}_L)$, and that of a queenless male is $W_{mL} = z_L/\bar{z}_L$. Thus, natural selection maximizes the quantity $W = c_{fR}W_{fR} + c_{mR}W_{mR} + c_{fL}W_{fL} + c_{mL}W_{mL}$ (Taylor 1996; Taylor and Frank 1996; Frank 1997, 1998; Rousset 2004; Taylor et al. 2007). Hence, the direction of selection acting on the sex ratio of queenright colonies is given by

$$\frac{dW}{dg} = c_{fR}\frac{dW_{fR}}{dg_{fR}} + c_{mR}\frac{dW_{mR}}{dg_{mR}},$$

$$= c_{fR}\frac{\partial W_{fR}}{\partial z_R}\frac{dz_R}{d\tilde{g}}\frac{d\tilde{g}}{dg_{fR}} + c_{mR}\frac{\partial W_{mR}}{\partial z_R}\frac{dz_R}{d\tilde{g}}\frac{d\tilde{g}}{dg_{mR}}, \quad \text{(D1)}$$

where $\partial W_{fR}/\partial z_R = -1/(1 - \bar{z}_R)$ and $\partial W_{mR}/\partial z_R = 1/\bar{z}_R$ are the fitness effects of the worker sex allocation decision on female and male eggs, respectively, in a queenright colony; $\tilde{g}$ is the average of the workers' genetic values for their sex allocation trait; $dz_R/d\tilde{g} = 1$ is the genotype-phenotype map; and $d\tilde{g}/dg_{fR} = p_{fR}$ and $d\tilde{g}/dg_{mR} = p_{mR}$ are the coefficients of consanguinity between worker and queen's female offspring and between worker and queen's male offspring, respectively, in a queenright colony (Taylor 1996; Taylor and Frank 1996; Frank 1997, 1998; Rousset 2004; Taylor et al. 2007). Since the queen's offspring are the worker's siblings, these are $p_{fR} = 3/8$ and $p_{mR} = 1/4$. Substituting in the class reproductive values obtains a condition for increase in sex ratio of queenright colonies $dW/dg > 0$ in terms of model parameters. We consider the action of selection on the sex ratio of queenless colonies in exactly the same way, deriving a condition for increase. In this case, the coefficients of consanguinity to female and male eggs are those for nieces and nephews, that is $p_{fL} = 3/16$ and $p_{mL} = 3/8$. We use these conditions to identify the convergence stable state of the population (Taylor 1996). This obtains equation (9) in the main text.

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A queen ant (*Formica truncorum*) preparing for her nuptial flight. Photograph by L. Sundström.

# Correction

In our article "Haplodiploidy and the evolution of eusociality: split sex ratios" (Gardner et al., *American Naturalist* 179:240–256), we neglected the impact of split sex ratios on the class reproductive values of females and males in the context of our model of queen replacement. Correcting this oversight changes some of our quantitative results (see below). In particular, it reduces the potential for both facultative and obligate helping in this model and prevents haplodiploidy from ever promoting the evolution of obligate helping ($\alpha_{\mathrm{OBL}} \leq 1$). This strengthens our general conclusion that the haplodiploidy effect often only weakly promotes—and may even inhibit—the evolution of helping and hence has not been an important factor in driving the evolution of eusociality.

While the elements $\wp$ of the gene-flow matrix appearing on page 253 are correct in the absence of split sex ratios ($\bar{z}_{\mathrm{R}} = \bar{z}_{\mathrm{L}} = \bar{z}$), more generally they are given by $\wp_{\mathrm{f}\leftarrow\mathrm{f}} = 1/2[q(1 - \bar{z}_{\mathrm{R}})]/(1 - \bar{z}) + 1/4[(1 - q)(1 - z_{\mathrm{L}})/(1 - \bar{z})]$, $\wp_{\mathrm{f}\leftarrow\mathrm{m}} = 1 - (1/2[q(1 - \bar{z}_{\mathrm{R}})/(1 - \bar{z})] + 1/4[(1 - q)(1 - \bar{z}_{\mathrm{L}})/(1 - \bar{z})])$, $\wp_{\mathrm{m}\leftarrow\mathrm{f}} = [q\,\bar{z}_{\mathrm{R}}/\bar{z}] + 1/2[(1 - q)\bar{z}_{\mathrm{L}}/\bar{z}]$ and $\wp_{\mathrm{m}\leftarrow\mathrm{m}} = 1 - ([q\,\bar{z}_{\mathrm{R}}/\bar{z}] + 1/2[(1 - q)\,\bar{z}_{\mathrm{L}}/\bar{z}])$. This leads to class reproductive values of $c_{\mathrm{f}} = [2(1 - \bar{z})(qz_{\mathrm{R}} + \bar{z})]/[\bar{z}(5(1 - \bar{z}) - q) + q(2 - \bar{z})z_{\mathrm{R}}]$ and $c_{\mathrm{m}} = [\bar{z}(3(1 - \bar{z}) - q(1 - z_{\mathrm{R}}))]/[\bar{z}(5(1 - \bar{z}) - q) + q(2 - \bar{z})z_{\mathrm{R}}]$. This impacts upon sex allocation, and expression (9) must be replaced by

$$(\bar{z}_{\mathrm{R}},\bar{z}_{L}) = \begin{cases} \left[0, \dfrac{3 - q}{4(1 - q)}\right] & \text{if } q < 1/3 \\ (0, 1) & \text{if } 1/3 \leq q \leq (1 + 2\phi)/(3 + 2\phi) \\ \left[\dfrac{2 - (1 - q)(3 + 2\phi)}{4q(1 + \phi)}, 1\right] & \text{if } q > (1 + 2\phi)/(3 + 2\phi) \end{cases}.$$

It also changes the potential for facultative helping in queenright colonies. Expression (10) must be replaced by

$$\alpha_{\mathrm{FAC}} = \begin{cases} \dfrac{1 + 2\phi}{2} & \text{if } q < (1 + 2\phi)/(3 + 2\phi) \\ \dfrac{q(1 + \phi)}{q(2 + \phi) - \phi} & \text{if } q \geq (1 + 2\phi)/(3 + 2\phi) \end{cases}.$$

Finally, it changes the potential for obligate helping in both queenright and queenless colonies. Expression (11) must be replaced by

$$\alpha_{\mathrm{OBL}} = \begin{cases} \dfrac{(1 + q)(1 + 2\phi)}{4} & \text{if } q < 1/3 \\ \dfrac{1 + 2\phi}{3} & \text{if } 1/3 \leq q \leq (1 + 2\phi)/(3 + 2\phi) \\ \dfrac{(1 + \phi)[1 + q + 2(1 - q)\phi]}{4 + 2(1 - q)\phi} & \text{if } q > (1 + 2\phi)/(3 + 2\phi) \end{cases}.$$

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Accordingly, figure 3 and its accompanying legend should be replaced as shown. We apologize for any confusion caused by this oversight.

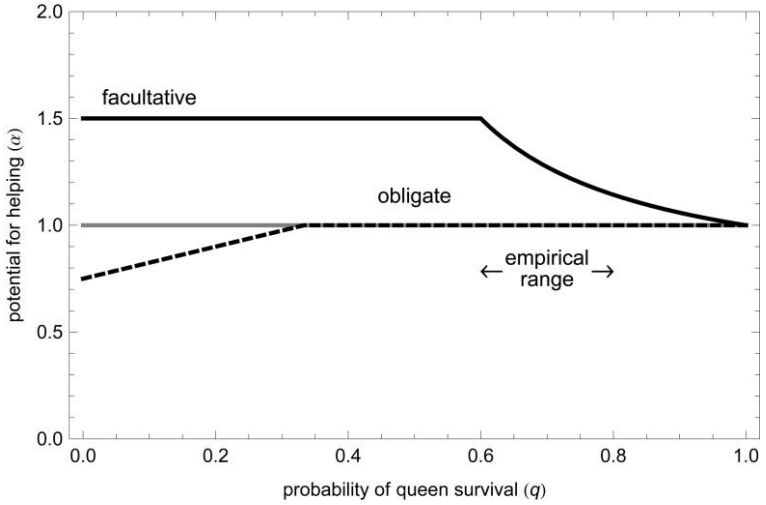


**Figure 3:** Potential for helping in a model of split sex ratios owing to queen replacement, assuming full monogamy ($\phi = 1$). In the context of the elaboration of helping, haplodiploidy either inhibits or has no impact upon obligate helping (dashed black line). Over the empirically estimated range of queen survival ($0.6 < q < 0.8$), haplodiploidy has no impact upon the potential for obligate helping, which remains at $\alpha_{\text{OBL}} = 1.0$. Conversely, haplodiploidy always promotes facultative helping in relatively female-biased (i.e., queenright) colonies (solid black line). The maximum potential for facultative helping is at $\alpha_{\text{FAC}} = 1.50$ at the lower end of the empirically valid rates of queen survival.

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