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# Dioecy and the evolution of sex ratios in ants

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Split sex ratios, when some colonies produce only male and others only female reproductives, is a common feature of social insects, especially ants. The most widely accepted explanation for split sex ratios was proposed by Boomsma and Grafen, and is driven by conflicts of interest among colonies that vary in relatedness. The predictions of the Boomsma–Grafen model have been confirmed in many cases, but contradicted in several others. We adapt a model for the evolution of dioecy in plants to make predictions about the evolution of split sex ratios in social insects. Reproductive specialization results from the instability of the evolutionarily stable strategy (ESS) sex ratio, and is independent of variation in relatedness. We test predictions of the model with data from a long-term study of harvester ants, and show that it correctly predicts the intermediate sex ratios we observe in our study species. The dioecy model provides a comprehensive framework for sex allocation that is based on the pay-offs to the colony via production of males and females, and is independent of the genetic variation among colonies. However, in populations where the conditions for the Boomsma–Grafen model hold, kin selection will still lead to an association between sex ratio and relatedness.

**Keywords:** split sex ratios; dioecy; ants; sex allocation

## 1. INTRODUCTION

Sex ratios in social insects have been the subject of intense study since [Trivers & Hare \(1976\)](#) first modified [Fisher's \(1930\)](#) sex ratio theory to include kin selection, particularly in species with haplodiploid sex determination. Their argument made specific quantitative predictions about the investment sex ratio and predicted that it would be affected by several aspects of colony structure (mating frequency, worker reproduction, queen number, etc.). In the simplest case, a single queen that mates once, workers are predicted to bias investment in a ratio of 3 : 1 in favour of their sisters, with whom they share higher relatedness. Female-biased sex ratios have subsequently been confirmed in many social Hymenoptera (e.g. [Queller & Strassmann 1998](#); [Ratnieks \*et al.\* 2006](#)).

When colonies are founded by multiple queens, by a multiply mated queen or when workers reproduce, the predictions about sex ratio also change. Many studies have explored the effect of mating frequency (e.g. [Starr 1979](#); [Nonacs 1986](#); [Roisin & Aron 2003](#)), queen number ([Nonacs 1986](#); [Kümmerli \*et al.\* 2005](#); [Bargum \*et al.\* 2007](#)) and worker reproduction ([Benford 1978](#); [Bourke 1988](#); [Pamilo 1991](#)) on the sex ratio. Characteristics of the breeding system, such as the amount of inbreeding, were among the first factors shown to affect sex ratios ([Hamilton 1967](#); [Charnov 1982](#)). A number of investigators have predicted that there should be a correlation between relatedness and colony-level sex ratio. Colonies in which the queen mates a greater number of times, or which have more queens, typically have lower relatedness. In polyandrous colonies, or polygynous ones where the queens are relatives, workers will be more equally related to males and females. If workers control sexual investment, they should favour the production of more males

than that will occur in colonies with a higher relatedness. Reviews of the evidence across many species largely support this generalization, and the interpretation that sex ratios are modified adaptively by workers ([Bourke & Franks 1995](#); [Bourke 1997, 2001](#); [Queller & Strassmann 1998](#); [Meunier \*et al.\* 2008](#)).

A logical extension of these ideas is split sex ratio theory. While adaptive modification of sex ratios can result in differences among colonies that have different genetic structure, it does not require sex ratios to fall into two discrete categories. However, a frequent observation in ant species is that some colonies specialize on producing gynes (new reproductive females) while others produce males (e.g. [Talbot 1945](#); [Scherba 1961](#); [Pamilo & Rosengren 1983](#); synthesized by [Nonacs 1986](#)). Regardless of the average sex ratio in the population, the frequency distribution for single colonies is U-shaped. [Boomsma & Grafen \(1990, 1991\)](#) explained this phenomenon as a consequence of how selection on sex ratio is altered by the variation in relatedness among colonies. If colonies vary in relatedness, and the workers can control the colony sex ratio, then colonies will differ in their ESS sex ratio. Workers from colonies that have a relatively high relatedness will prefer a more female-biased sex ratio while those from colonies with lower relatedness will prefer sex ratios that are biased towards males. These conflicts of interest shift the colony sex ratios until the population reaches an equilibrium where colonies specialize in the production of one sex or the other.

The Boomsma–Grafen model predicts that sex ratios will be split when the following three conditions hold: (i) relatedness varies among colonies, (ii) workers control investment, and (iii) workers can determine whether they are in a high or low relatedness colony. If these conditions are not met, colony sex ratios should approach the average ESS for the population. Queens lay eggs with a particular primary sex ratio, and workers may modify the secondary

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sex ratio by either killing male eggs or increasing the proportion of female eggs that become gynes. A queen can respond to worker actions by altering the primary sex ratio, leading to a coevolutionary arms race (e.g. Helms *et al.* 2005). Models that incorporate costs for modifying the primary or secondary sex ratio, make the detection of colony state very expensive or assume constraints on egg availability can make it uneconomical for workers to split the sex ratio (Ratnieks & Boomsma 1996; Reuter & Keller 2001; Helms *et al.* 2005).

The pattern of sex allocation is consistent with split sex ratio predictions in many species. The test of this theory by Sundström (1994) in *Formica* ants made split sex ratio theory literally a textbook case of the ability of evolutionary theories in general, and ESS theories in particular, to make quantitative predictions (e.g. Alcock (2005)). The ability of the Boomsma–Grafen model to explain the variation of sex ratios in ant species is considered a strong validation of both kin selection and the frequent resolution of queen–worker conflict in the workers’ favour. Although the theory has had considerable success, it does not predict some patterns of sex allocation, which are relatively frequent in social insects. Split sex ratios have been found in species where there is no variation in relatedness. In some cases, the queen universally mates a single time (Vargo 1996; Helms 1999; Foitzik & Heinze 2000), but sex ratios are split. In other cases, split sex ratios are associated with variation in queen number, but because the queens are unrelated to one another, split sex ratios are not expected (Fournier *et al.* 2003; Helms *et al.* 2005). Additionally, split sex ratios may be absent when there is variation among colonies in relatedness due to mating frequency (e.g. MacKay 1981; van der Have *et al.* 1988) or the number of queens (Chan *et al.* 1999). Finally, split sex ratios are sometimes observed in cases where there is variation among colonies in relatedness; however, the variation in relatedness is opposite to the expected direction (Pamilo & Seppä 1994). These counterexamples have been explained either by assuming that workers do not control sex ratios or that they cannot tell the type of colony they are in. Observations of split sex ratios in colonies without variation in relatedness have been interpreted as evidence that the queen controls the sex ratio (Mehdiabadi *et al.* 2003).

In the Boomsma–Grafen theory, the fitness pay-off to colonies is assumed to be proportional to investment, which makes the conversion of investment in females into pay-off from female investment straightforward. Under this assumption, biomass is used instead of simply the numbers to correct for differences in body size (and may be corrected for the different energetic costs of producing males and females as a function of body size, e.g. Boomsma 1989; Boomsma *et al.* 1995) as the measure of pay-off. The Boomsma–Grafen theory assumes that investment, and the pay-off from investing in one sex, directly affects the investment, and therefore the pay-off, from the other sex (e.g. Charnov 1982). The assumed relationship is completely proportional—25 per cent greater investment in one sex increases the pay-off from that sex by 25 per cent and causes a corresponding decrease in investment and pay-off in the other sex. If the size of a reproductive alters the pay-off in a nonlinear way (e.g. Wiernasz *et al.* 2001; Wiernasz & Cole 2003), then investment no longer accurately predicts the pay-off. The goal of this paper is to examine how nonlinear

relationships between investment and pay-off can influence the stability of the ESS and the distribution of colony sex ratios in a population.

Recent approaches (Helms *et al.* 2005) that consider the coevolution of worker and queen sex ratio strategies have shown that split sex ratios can evolve without variation in relatedness within populations. The underlying reason for the evolution of split sex ratios remains a conflict about the optimal sex ratio, but now it is between the strategies of queens and workers. Whether or not split sex ratios evolve depends on the relative magnitude of the costs incurred by workers and queens for biasing the sex ratio, the size of mutational effects and the particular worker strategy.

We adapt a model from plant life-history evolution (Lloyd 1984) that makes explicit the relationship between investment and fitness to explore the evolution of split sex ratios, independent of the effects of genetic relatedness. This model is not based on a specific mechanism, such as conflicts of interest between colonies of high and low relatedness, or between queens and workers within colonies. Instead, we focus on how the stability of the ESS sex ratio leads to either split or mixed sex ratios. Because the model is based on fitness pay-offs through male and female functions, we are not constrained to assume that investment is directly proportional to fitness. We shall explore the effect of control of investment and changes to the social structure on the predictions of the model, and test the same with the data collected from a long-term study of reproduction in the western harvester ant, *Pogonomyrmex occidentalis*.

## 2. THE DIOECY MODEL

Our model is a modification of that proposed in Lloyd (1984). One can express the fitness that a colony acquires through male or female offspring as a function of the proportion of investment in males or females. Although the usual assumption is that the relationship is linear, it is more general to allow male or female fitness to accrue to a colony in a nonlinear manner. The formulation used by Charlesworth & Charlesworth (1981), Charnov (1982), Lloyd (1984) and Campbell (1998, 2000) has been to assume a power function, so that the fitness achieved through male and female functions is

$$w_{\delta} \propto a^y, w_{\sigma} \propto (1-a)^z, \quad \text{where } y, z > 0 \quad (2.1)$$

with  $a$  being the proportion of investment in males (figure 1). If both the exponents  $y, z = 1$ , the relationships are linear and the assumption of most work on sexual investment in social insects is recaptured.

To solve for the ESS sex ratio and find the conditions for split or mixed sex ratios, we first note that the fitness of a colony that has an alternative sex ratio can be written as

$$w_2 = (1-a_2)^z + \frac{(1-a_1)^z a_2^y}{a_1^y},$$

where  $a_1$  is the sex ratio in the population and  $a_2$  is the sex ratio of a ‘mutant’ colony. The first term is the fitness through females and the second is the fitness through males. Female fitness is given by the pay-off function (2.1). Male fitness is achieved by mating and sperm transfer, and is a function of the proportion of females with which

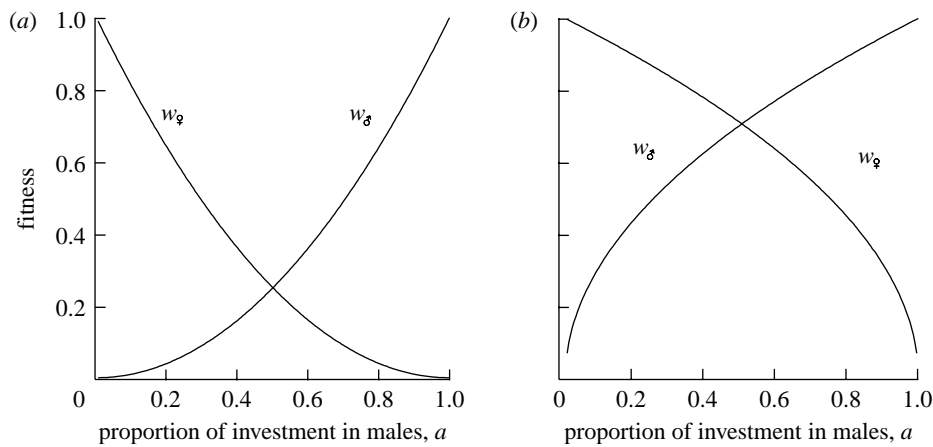


Figure 1. The total fitness through males or females as a function of the investment sex ratio. The maximum fitness for each function is normalized to a value of 1 for ease of presentation. In (a) exponents describing the fitness function from relationship 1 are both  $y$ ,  $z=2$ , while in (b) the exponents are both  $y$ ,  $z=0.5$ .

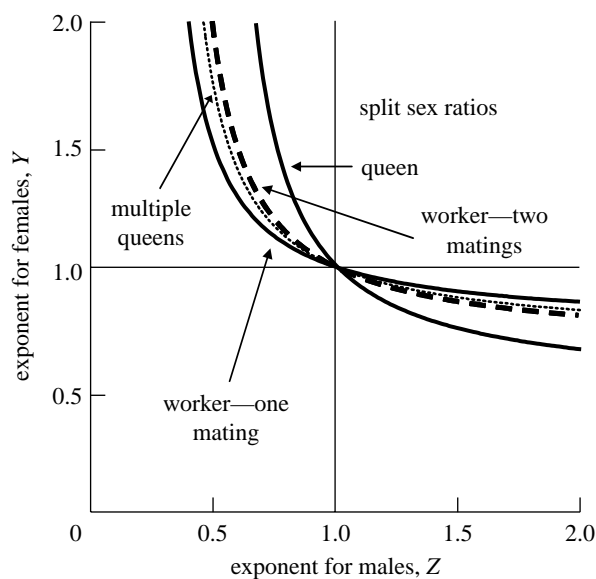


Figure 2. Parameter space for the evolution of split sex ratios under the dioecy model. The solid curves are for queen control and worker control of investment in a colony with a single queen who mates a single time. The dashed curve gives the conditions for the evolution of split sex ratios for worker control in a colony with a doubly mated queen. The dotted curve is the condition for split sex ratios with worker control of investment and two gynes related by  $r=1/2$ .

males can mate. This function gives fitness from the perspective of the queen. We will derive the relationship for the worker point of view below. By solving  $(dw_2/da_2)=0$  for  $a_1=a_2=\hat{a}$ , we find the ESS:  $\hat{a}=y/(z+y)$ . When the functional relationship between investing in males or females is the same ( $y=z$ ), the ESS sex ratio is 1/2. To investigate the stability of the equilibrium, we obtain the second derivative evaluated at the ESS

$$\frac{d^2w_2}{da_2^2} = z(z-1)(1-a_2)^{z-2} + \frac{(1-a_1)^z}{a_1^z} y(y-1)a_2^{y-2}$$

at  $a_1=a_2=\hat{a}$ .

When  $(d^2w_2/da_2^2) < 0$  at  $\hat{a}$ , the ESS sex ratio is stable; sex ratios will tend to an intermediate value. Alternatively, when  $(d^2w_2/da_2^2) > 0$  at  $\hat{a}$ , the ESS is unstable and colonies will become specialists, producing only males- or only females-split sex ratios. The ESS is stable, i.e. mixed sex

ratios are expected, when  $2zy < z+y$  (figure 2). Our formulation is identical to that of Lloyd (1984) and Campbell (2000) for diploid plants. The sex ratio is determined by the functional relationship between investment and fitness pay-off. Split sex ratios are expected, even from the point of view of the queen, when there is a disproportional pay-off to specializing on males or females ( $y, z > 1$ ), and they are not expected when there is a less than proportional pay-off to specializing on a single sex ( $y, z < 1$ ). As the exponents increase significantly above 1, there is a disproportionate pay-off to increasing investment in one sex compared with the fitness penalty for underproducing the other sex—the ESS becomes unstable. The stability of mixed sex ratios refers to the population sex ratio rather than that of individual colonies. In the region where mixed sex ratios are expected, there may be little selection on a colony to have a particular sex ratio, resulting in a large variation in the distribution of colony sex ratio, a pattern that characterizes studies of sex allocation in cosexual plants (e.g. Fox 1993; Masaka & Takada 2006).

From the point of view of a worker in a colony where the queen has mated a single time,

$$w_2 = 3(1-a_2)^z + \frac{(1-a_1)^z a_2^y}{a_1^y}.$$

The difference between the queen and worker fitness measures is that workers value the production of queens more than that of males. Solving for the equilibrium sex ratio:  $\hat{a} = y/(3z+y)$ , which when  $y=z=1$  leads to  $\hat{a}=1/4$ , in other words a 3 : 1 female-biased sex ratio. Solving for the conditions of instability,  $(d^2w_2/da_2^2) > 0$  at  $\hat{a}$ , so that split sex ratios will occur with worker control yields  $2zy > (3z+y)/2$  (figure 2). There is a substantial portion of parameter space in which both the workers and the queen agree either that sex ratios will be split (above both curves) or that sex ratios should be mixed (area below both curves). The areas between the curves correspond to conditions where either worker control or queen control of sex ratio can produce split sex ratios. This is not a conflict between sex ratio strategies in the usual sense, rather that mixed sex ratios are stable under queen control and unstable under worker control. However, in certain portions of the parameter space, split sex ratios are predicted under queen control even when they are not predicted under worker control.



Because variation in relatedness drives split sex ratio evolution in the Boomsma–Grafen model, we next consider the effect of variation in relatedness in the dioecy model. Relatedness can vary among colonies because of differences in the mating frequency of the queen or differences in the queen number, and thus changes conditions from the worker's perspective.

When the queen mates with  $n$  males who contribute equally to the reproductive output of a colony, fitness becomes

$$w_2 = \left(1 + \frac{2}{n}\right)(1 - a_2)^z + \frac{(1 - a_1)^z a_2^y}{a_1^y}.$$

Females, while still more valuable to workers than males, are relatively less valuable when the queen mates multiple times because the workers are not equally related to all females. Because the males are produced by unfertilized eggs, the number of matings by the queen is irrelevant. The population equilibrium sex ratio becomes

$$\hat{a} = \frac{y}{z + \frac{2z}{n} + y},$$

and the condition for the development of split sex ratios becomes  $y(z + k) > k + y$ , where  $k = z(1 + (2/n))$ . As the number of matings increases ( $n \rightarrow \infty$ ), the relationship becomes identical to that of the queen or of a diploid, and the worker curve is warped into that of the queen (figure 2). When the queen is highly polyandrous, the region of parameter space in which the predictions based on worker control and queen control differ decreases.

The effects of queen number (polygyny) are very similar to the effects of multiple mating. If the queens are not related, workers of one queen do not value the offspring of the other queens, regardless of sex. Workers still overvalue females by a 3 : 1 ratio, and no change in sex ratio is expected. Although colonies vary in relatedness, from the viewpoint of the workers, relatedness to the reproductives that matter has not changed. Consequently, split sex ratios are not expected under the Boomsma–Grafen hypothesis. In many cases, however, multiple queens are related. The relatedness of workers to the reproductive offspring of a queen that is not their mother is  $r/4$ , where  $r$  is the relatedness of their mother to the other queen. Because the only pathway of relatedness from workers to the offspring of another queen is through the queen, both her male and female offspring are equally valuable. When a colony has multiple equivalent gynes, the workers value the production of females more than they value the production of males by a factor,

$$F = \frac{\frac{3}{4} + \frac{(N-1)r}{4}}{\frac{1}{4} + \frac{(N-1)r}{4}} = \frac{3 + (N-1)r}{1 + (N-1)r},$$

where  $N$  is the number of gynes and  $r$  is the relatedness among them. When there is a single queen, the workers value females three times more than they value males, and as the number of gynes becomes large,  $F$  approaches 1. When the relatedness among gynes is zero, the relative value of females is unchanged. The equilibrium sex ratio,  $\hat{a} = y/(zF + y)$ , and the conditions for the production of split sex ratios are changed, with split sex ratios expected when  $yz(1 + F) > y + zF$  (figure 2).

Over most of the parameter space, the conditions for the production of split sex ratios by either party are the

same, and are independent of mating frequency, queen number or queen versus worker control. The dioecy model does not require variation in relatedness, although details about the control of sex ratio and intracolony relatedness influence the boundaries between parameter regions where split sex ratios are expected. It is consistent with split sex ratios arising in colonies whose single queens mate a single time or with multiple queens that are unrelated, as well as the absence of split sex ratios even when they are predicted by the Boomsma–Grafen model.

### 3. THE HARVESTER ANT STUDY SYSTEM

As part of our study on the life history of *P. occidentalis*, we have measured the reproductive output of approximately 200 colonies beginning in 1994 (for a description of the study site, and sampling methods see Wiernasz & Cole 1995). We obtained the total reproductive output for colonies by watering them, which stimulates the flight of all reproductives (for details of the methods for inducing mating flights and capturing reproductives, see Cole & Wiernasz 2000). Each colony has been genotyped using highly variable microsatellites (Wiernasz *et al.* 2004). Colonies vary substantially in relatedness, with the number of patriline ranging from 3 to 10 (mean = 6.2).

We obtained sexual investment ratios for colonies in 1994, 1995, 1996, 1997, 1998, 2001 and 2003 by collecting all reproductives produced. In 1999, 2000, 2005 and 2006, early rainfall produced mating flights before we could measure the reproductive output. In 2002, although we measured the reproductive output, only one colony reproduced, due to severe drought. When calculating the sex ratios, we used only the data from colonies that produced at least 20 reproductives. This minimizes the over-representation of apparent split sex ratios due to binomial sampling. The mean number of reproductives in our study colonies was 244. We estimated the investment in each sex by individually weighing the reproductives. During 1994–1998, we used a randomly chosen sample of 30 males and 30 females (or all, if fewer than 30) from each colony; in 2001 and 2003, we weighed all the reproductives produced by a colony. Colonies vary in the sizes of both males and females that they produce, hence we used colony- and year-specific sizes for males and females. To measure male fitness, we estimated the number of sperm transferred as a function of body mass. Both the total sperm count of a male and the number of sperm transferred to females during mating are functions of body mass, and larger males transfer disproportionately more of their sperm than small males (Wiernasz *et al.* 2001). We used the published relationship to calculate the total number of sperm transferred by all of the males that were produced by each colony in each year. For 2001 and 2003, we summed the expected number of sperm transferred by all males. For the period 1994–1998, we calculated the mean male fitness based on the sample of 30 and estimated the total fitness for the entire male output. Fitness through female function is based on the size-specific probability of survival of queens (Wiernasz & Cole 2003). We used the published relationship between body mass and survival probability to estimate the probability of survival of each queen collected from the colonies. We summed the probability of survival over all females giving the expected number of queens that survive colony

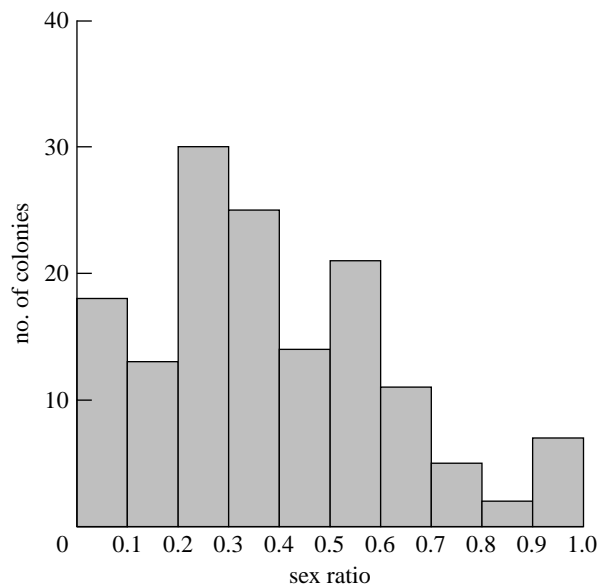


Figure 3. Sex ratio (proportion of investment in males) distribution for all colonies combined across years. Sex ratio distributions within a year have the same overall pattern.

founding. For both the sexes, although individual fitness increases as a function of body size, the pay-off to colonies for making reproductives of increasing size is a decelerating function (Wiernasz *et al.* 2001; Wiernasz & Cole 2003). Although these measures are correlates of total fitness, they represent the best estimates that can be obtained for this species. They are also equivalent to the fitness estimates used for plant sex ratio studies (pollen transfer and seed set/survival, e.g. Campbell 2000). For each year, we plotted the fitness of a colony through male and female functions against the proportional investment in males. We fitted a power function to the data in order to obtain the exponents  $y$  (for females) and  $z$  (for males) that are important for determining the stability of the sexual investment ratios.

We tested whether sex ratio was consistent within a colony using a two-way ANOVA with year and colony as classification variables. We sought further evidence of consistency by testing whether a colony's sex ratio was correlated across years. Because colonies do not reproduce in each year, we cannot use a repeated-measures ANOVA. To avoid multiple comparisons, we calculated the correlation between sex ratios for two pairs of years (1994–1995 and 2001–2003). These two years have the greatest time separation that mitigates the potential non-independence of the results.

For each year, we tested for a correlation between colony sexual investment ratio and the within-colony relatedness. The correlation was not significant in any year or for the overall dataset ( $p > 0.2$  for each year).

For the entire dataset, the colony investment ratios were biased towards females. There was no evidence of split sex ratios. A few colonies produce highly male- or female-biased broods, but overall the distribution is not U-shaped (figure 3; mean  $a = 0.383$  ( $0.34$ – $0.42$  = 95% confidence limits)). If we use the Boomsma (1989) correction for investment ratios based on probable energetic differences in the production of males and females, the investment ratio is slightly different (mean  $a = 0.432$ , 95% confidence limits =  $0.39$ – $0.47$ ), but still

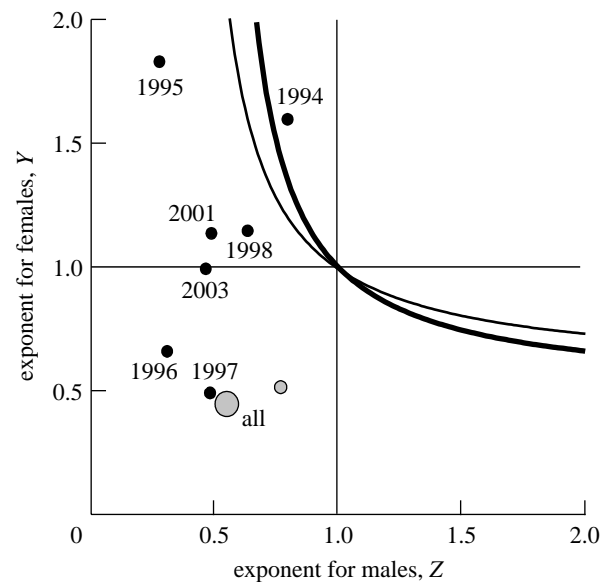


Figure 4. The relationship of exponents for the male and female fitness functions. The points are labelled by year. The shaded points are the exponents using the total investment and fitness for a colony (smaller grey circle with the correction of Boomsma 1989). The curves show the critical values for the evolution of split sex ratios under queen and worker control (for a population where queens mate an average of 6.2 times).

significantly different from the 0.5 expected with queen control of investment. The predicted sexual investment ratio under worker control of investment based on the effective number of patriline is 0.427, which is not different from the observed sex ratio.

Although the population sex ratio varied over time, a given colony's sex ratio was stable. Both year ( $F_{6,225} = 5.55$ ,  $p < 0.001$ ) and colony ( $F_{151,255} = 2.82$ ,  $p < 0.001$ ) effects were highly significant. Both of the across-year comparisons of sex ratios were significant (1994–1995:  $r = 0.48$ ,  $p = 0.008$ ; 2001–2003:  $r = 0.30$ ,  $p = 0.028$ ).

For each year except 1994, the year with the least data (33 colonies), the combination of exponents falls outside of the split sex ratio region of parameter space (figure 4). The overall exponent (large shaded circle), from the summed fitness over eight years, predicts that sex ratios should not be split. Using the Boomsma (1989) correction (smaller shaded circle), we calculate slightly different exponents, but the conclusion is unaltered.

Under the dioecy model, the expectation is that colonies in this population, even though they vary in relatedness, will not have split sex ratios. While the exponent for females is on average near unity (mean = 1.03 (95% confidence limits =  $0.62$ – $1.45$ )), the exponent relating male fitness to sex ratio is significantly less than 1 (mean = 0.49 (95% confidence limits =  $0.36$ – $0.62$ )). Although female fitness is proportional to investment, male fitness decelerates substantially with increasing investment.

#### 4. DISCUSSION

Sex ratio is a colony characteristic in *P. occidentalis*; sexual investment patterns were stable over more than a decade. While sex allocation was consistent, it did not reflect intracolony relatedness. If sex ratios were uncorrelated with relatedness, and also inconsistent across years, we

could argue that they resulted from factors that we did not measure (e.g. a function of local environmental conditions that were expressed in particular colonies in particular years). The population sex ratio did differ among years, which may have been a function of reproduction by different subsets of colonies, and differences in environmental conditions among years. However, because the sex ratio for a particular colony remained consistent, it should be considered an aspect of the colony's phenotype.

In contrast to the predictions of Boomsma–Grafen model, the sex ratios of harvester ant colonies were not split. The absence of split sex ratios could reflect the inability of workers to influence the sex ratio or to determine the genetic diversity within colonies. Two lines of evidence suggest that this is not true. First, the population sex ratio is female biased. It is near the value expected from worker control and different from that expected under queen control—workers appear to control sex ratio in this species. Second, colonies that differ in genetic diversity are very different in their properties, including patterns of colony growth (Cole & Wiernasz 1999; Wiernasz *et al.* 2004) and activity (Wiernasz *et al.* 2008). Workers should have a number of cues that enable them to determine the genetic diversity of their colony.

The dioecy model correctly predicted that harvester ant colonies should not specialize on one sex of reproductives. While female fitness is an approximately linear function of investment, the pay-off through males is decidedly non-linear. Increasing investment in males yields a smaller than proportional pay-off to fitness. Similar differences in the form of the relationship between investment and pay-off have been shown in plants (Campbell 1998, 2000).

In a recent simulation study, Helms *et al.* (2005) have explored the effects of queen–worker conflict on sex ratio evolution in social insect colonies with no variation in genetic relatedness (one singly mated queen). Their model found that the effect of sex ratio biasing in both castes typically was an accelerating function. Split sex ratios evolved when biasing in one caste opposed the biasing in the other. The degree to which opposing strategies evolved was a function of the relative cost of biasing for each caste and the rate of evolution in each caste. These results are concordant with the general conclusion of the dioecy model—that accelerating returns for specializing in one sex (e.g. figure 1a) lead to an unstable ESS sex ratio and split sex ratios. Our approach made no assumptions about the specific mechanism(s) that could produce accelerating functions, in fact we did not permit conflict (either workers or queens had control over the sex ratio) to emphasize what factors are responsible for split sex ratio generation.

The magnitude of the differences in the exponents for males and females suggests that the processes of investing in males and females differ. The first possibility is that colonies invest different resources in each sex. If resources are removed from females, they are not automatically available for male investment. In harvester ants, supplements of non-storable food (insects) increased male, but not female, size. Supplementation with storable food (seeds) increased the number of both males and females. Because resource addition increases the output of both the sexes, it is unlikely that male and female reproductions depend on entirely different pools of resources. However,

the effect of insect supplements suggests that provisioning of male brood is regulated differently from that of female brood, perhaps reflecting decreased efficiencies in male production. Colonies that invest disproportionately in males usually produce only small males, which lower colony fitness pay-offs. The cause of this is unknown—production of numerous small males may reflect extreme queen–worker conflict or local variation in resources (a high proportion of insects relative to seeds).

While the dioecy model can make specific predictions about the occurrence of split sex ratios, it requires considerable data—fitness must be measured through male and female functions. Applying this method to other species will require knowledge about the relationship between body size and fitness in both the sexes, in order to convert investment in terms of either numbers or biomass into fitness estimates. In *P. occidentalis*, fitness of individual reproductives increases with body size (Wiernasz *et al.* 2001; Wiernasz & Cole 2003), justifying the use of biomass of males or females as a fitness measure for the colony. In other species, numbers of males or females may more accurately reflect fitness, even if biomass gives a better indication of investment.

The dioecy model provides a comprehensive framework for sex allocation that is based on the pay-offs to the colony via production of males and females, and independent of the genetic variation among colonies. Colonies produce one sex exclusively when the ESS sex ratio is unstable due to nonlinear pay-offs for investment; however, the model does not predict which colonies should specialize on males versus females. Variation in intracolony relatedness, in local resource availability, or in colony age could influence a colony's propensity to favour one sex over the other. The mechanism of the dioecy model does not preclude a correlation between mating frequency and the sex ratio. When the conditions for the Boomsma–Grafen model hold (relatedness varies, workers control investment and workers can determine relative relatedness), then workers in colonies with higher relatedness will achieve higher fitness if they bias reproduction towards females as predicted by Nonacs (1986). In such populations, kin selection will still lead to an association between sex ratio and relatedness.

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