

Sex investment ratios in monogyne and polygyne populations of the fire ant *Solenopsis invicta*

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Abstract

Both monogyne (single queen per colony) and polygyne (multiple queens per colony) populations of the fire ant *Solenopsis invicta* are good subjects for tests of kin selection theory because their genetic and reproductive attributes are well-characterized, permitting quantitative predictions about the degree to which sex investment ratios should be female-biased if workers and not queens control reproductive allocation. In the study populations, an investment ratio of 3 females:1 male is predicted (a proportional investment in females of 0.75) in the monogyne form, whereas a proportional investment in females between 0.637 and 0.740 is expected in the polygyne form. To test these predictions, colonies from a single population of each social form were collected and censused during three different seasons. Consistent with their alternative modes of colony founding, monogyne colonies invested more in reproduction (sexual production) and less in growth/maintenance (worker production) than did the polygyne colonies. Overall, the sex investment ratios were female-biased in both forms, although there was considerable seasonal variation. After adjusting for sex-specific energetic costs, the proportional investment in females was 0.607 in the monogyne population, a value in between those expected under complete control by either the queen or the workers. However, when combined with data from four other previously studied monogyne populations in the U.S.A., the mean investment ratio did not differ significantly from the value predicted if workers have exclusive control. In the polygyne population, the proportional investment in females of 0.616 was consistent with the level of female bias expected under partial to complete worker control, although the potential influence of two confounding factors – possible contact with monogyne colonies and the preponderance of sterile diploid males – weakens this conclusion some-

what. Taken as a whole, the sex investment ratios of monogyne and polygyne populations of *S. invicta* are consistent with at least partial worker control. Of several ultimate and proximate explanations that have been proposed to explain inter-colonial variation in the sex investment ratio, only the effect of the primary sex ratio (female-determined eggs:male-determined eggs) laid by the queen appears to account for the observed variation among monogyne colonies. In the polygyne population, there is limited support for the hypothesis that greater resource abundance favors investment in females.

Introduction

Like solitary organisms, colonies of social insects must decide how to divide resources between growth/maintenance (worker production) and reproduction (production of sexuals). However, the eusocial Hymenoptera are unique in that the decision involves two parties, workers and queens, that have conflicting interests over the allocation of colony resources between maintenance and reproduction (Pamilo, 1991) and in the relative investment in male and female reproductives (sex allocation; Trivers and Hare, 1976; Nonacs, 1986a). This conflict stems from the relatedness asymmetries that arise from the haplodiploid system of sex determination (Hamilton, 1964a, b), which causes females to be more closely related to sisters ($r = 0.75$) than to daughters ($r = 0.5$), sons ($r = 0.5$) or brothers ($r = 0.25$). Trivers and Hare (1976) were the first to point out that as a consequence of this relatedness asymmetry, workers should prefer a female-biased population sex ratio (3 females:1 male in the case of a single, once mated queen and no reproduction by workers), whereas queens should prefer to invest equally in males and females. Following Trivers and Hare's (1976) seminal paper, sex investment ratios have assumed a central role in tests of inclusive fitness theory (Hamilton, 1964a, b), because they can indicate whether the queen-worker conflict has been resolved in favor of the workers as predicted by the theory.

The general prediction of inclusive fitness theory as expanded by Trivers and Hare (1976), that worker control of the sex allocation should lead to female-biased sex investment ratios, has been supported and extended on theoretical grounds (e.g., Oster et al., 1977; Benford, 1978; Macnair, 1978; Bulmer, 1981; Bulmer and Taylor, 1981; Boomsma and Grafen, 1990; Pamilo, 1990, 1991), and generally has been corroborated by empirical studies, primarily concerning ants (Ward, 1983; Herbers, 1984; Nonacs, 1986a; Have et al., 1988; Boomsma, 1989; Mueller, 1991; Chan and Bourke, 1994; Hasegawa, 1994; Sundström, 1994, 1995; Evans, 1995; Boomsma et al., 1995).

Although essential in testing the worker control hypothesis, field data often can be difficult to interpret because of several factors that may influence the sex investment ratio either by altering the asymmetries of relatedness that underlie queen-worker conflict or by affecting the reproductive values of males and females independently of asymmetries of relatedness. Both multiple insemination of queens and reproduction by workers generally will dilute the asymmetries of relatedness

(Alexander and Sherman, 1977). The occurrence of either or both of these phenomena will favor a more male-biased investment ratio under worker control, the former by deflating relatedness among nestmate workers and the female reproductive brood they rear, and the latter by inflating the relatedness between workers and the males they rear. The number of reproducing queens in a colony also can affect relatedness asymmetries (Trivers and Hare, 1976; Nonacs, 1986a; Boomsma and Grafen, 1991; Boomsma, 1993).

The preferred sex investment ratio of queens and workers also can be influenced by the dispersal patterns of reproductives. Local mate competition (Hamilton, 1967; Alexander and Sherman, 1977; Frank, 1987), in which brothers compete for mates, will decrease the value of males to a colony, leading to more female-biased sex ratios irrespective of queen-worker conflict. Local resource competition (Clark, 1978; Frank, 1987; Pamilo, 1990, 1991) can occur if related females are forced to compete for resources. This will decrease the value of females, favoring greater investment in males. On the other hand, if related females cooperate after dispersal, as in the case of large polydomous (multi-nest) colonies (Pamilo, 1991), then local resource enhancement can occur, resulting in increased reproductive value of females and greater female bias (Clark, 1978; Schwarz, 1988). In sum, the most powerful tests of inclusive fitness theory involving sex investment ratios at the population level require that the species or populations under study be well-characterized regarding colony genetic structure and dispersal pattern of reproductives.

Ant colonies often produce heavily biased or unisexual brood (Nonacs, 1986a), and this variation in sex investment ratios among colonies in a population has also been used to test the worker control hypothesis. Boomsma and Grafen (1990, 1991) introduced the notion that variation among colonies in levels of multiple mating, worker reproduction and polygyny will lead to intercolonial differences in the relative relatedness asymmetry. In such cases workers are expected to specialize in rearing reproductives of the sex to which they are more related than is the average worker in the population. Evidence for split sex ratios in accordance with relative relatedness asymmetries recently has been obtained by several researchers (Chan and Bourke, 1994; Sundström, 1994; Evans, 1995). In addition to relative relatedness asymmetry, differential dispersal of reproductives and variation in local resource abundance have been proposed to account for split sex ratios in ants (reviewed in Nonacs, 1986a; Crozier and Pamilo, 1992). Thus, as in studies of population sex investment ratios, the utility of investigations of colony-level variation in sex allocation as tests of inclusive fitness theory depends on the degree to which the colony genetic structure, dispersal habits of reproductives and distribution of local resources are known for the populations in question.

One of the best studied social insects with respect to colony genetic structure, reproductive biology and ecology is the fire ant *Solenopsis invicta*, especially within its range in the U.S.A. where it was inadvertently introduced from its native South America some 60 years ago (Lofgren et al., 1975). Although the wealth of relevant information on this species makes it a good candidate for sex ratio studies, it should be kept in mind that populations in the U.S.A. may not yet have reached equilibrium with respect to their new environment and/or genetic changes resulting

from the population bottleneck that occurred during its introduction to North America (Ross et al., 1993, 1996).

S. invicta occurs in two distinct social forms that differ dramatically in queen number and colony genetic structure. The social and genetic structures of these colonies have been well-documented (Fletcher et al., 1980; Lofgren and Williams, 1984; Greenberg et al., 1985; Ross and Fletcher, 1985a; Ross et al., 1987; Ross, 1988, 1993; Vargo and Fletcher, 1989; Vargo and Ross, 1989; Vargo, 1992). The predominant social form of *S. invicta* consists of monogyne colonies containing a single, once-mated queen (Ross and Fletcher, 1985a; Ross et al., 1996). Workers of this species are sterile and therefore all individuals in the colony are produced by queens. Consequently, asymmetries of relatedness between workers and brood of each sex in these colonies are maximized, because workers are related to female brood by 0.75 and to males by 0.25 (Ross and Fletcher, 1985a). Reproduction in monogyne colonies involves large simultaneous mating flights and relatively long distance dispersal (Markin and Dillier, 1971; Morrill, 1974; Bass and Hays, 1979), making it unlikely that limited dispersal of reproductives plays a significant role in affecting sex allocation patterns. Thus if workers have complete control over sex allocation, we expect monogyne populations of *S. invicta* to favor investment in female sexuals 3:1 over investment in males.

In contrast, the polygyne form, which usually occurs in localized populations (Ross et al., 1987; Porter, 1992; but see Porter et al., 1991 for the distribution of polygyne colonies in Texas), consists of colonies containing a few to several hundred queens (Glancey et al., 1973; Fletcher et al., 1980; Fletcher, 1983; Lofgren and Williams, 1984; Vargo and Fletcher, 1987). Like their monogyne counterparts, queens in polygyne colonies are inseminated by a single male, so that the female offspring within a matriline are full sisters (Ross and Fletcher, 1985a; Ross et al., 1996). However, the average relatedness among cohabiting queens and among their female offspring in polygyne colonies is very low and is statistically indistinguishable from 0 (Ross and Fletcher, 1985a; Ross, 1993; Ross et al., 1996).

Reproduction in the polygyne form occurs by budding, in which queens accompanied by workers leave their resident mound and establish a new nest a short distance away (Vargo and Porter, 1989). Queens in these colonies appear to originate from two sources: (1) recruitment of female sexuals which remain in or return to the nest after mating (Porter, 1991), and (2) adoption of newly mated queens from foreign nests following large mating flights (Ross and Fletcher, 1985b; Glancey and Lofgren, 1988; Porter, 1991). As discussed above, colony reproduction by budding may influence the sex allocation pattern when limited dispersal of females leads to the residents of daughter nests being more related to the inhabitants of the mother nest than to individuals in other nests. The very low degree of genetic relatedness among nestmates makes such population substructuring unlikely in polygyne *S. invicta*, and recent empirical studies confirm this (K. Ross and M. Goodisman, unpublished data). Thus neither local resource competition nor local resource enhancement are likely to exert a strong influence on the sex investment ratio in the polygyne study population.

In polygyne ant colonies the worker relatedness asymmetry (RA) is given by the following equation of Boomsma (1993):

$$RA = 0.5 + \frac{0.5(r'_{fw} - r'_{mw})}{r'_{fw} + r'_{mw} + 0.5r_{qq}(n_q - 1)}$$

Where r_{qq} is the relatedness among mated resident queens and n_q is the effective number of mated resident queens. The variables r'_{fw} and r'_{mw} refer to the average relatedness of workers to new queens and males within a single matriline, which in *S. invicta* are 0.75 and 0.25, respectively. All relatedness coefficients are so called life-for-life relatednesses (Hamilton, 1972). Obtaining values for the other two variables, r_{qq} and n_q , is not as clear-cut. There are two estimates of r_{qq} for the study population, both based on allozyme markers: Ross and Fletcher (1985a) estimated that nestmate queens were related by 0.011, whereas Ross (1993) subsequently arrived at a value of 0.048. For the colonies sampled in the present study, an average of 35.4 mated queens was recovered (Vargo and Fletcher, 1987). However, the effective queen number, the number of reproducing queens adjusted for variation in reproductive output, may be the more appropriate parameter to use. Provided that all queens reproduce equally, the harmonic mean number of queens per nest will equal the effective queen number per nest in the population (Ross, 1993). Two separate samples of the study population (Ross, 1993; Ross et al., 1996) give harmonic mean queen numbers of 8.74 (1985) and 9.54 (1990). Simultaneously varying r_{qq} and n_q in Boomsma's (1993) equation to include all estimated values gives a range of RAs for the study population from 0.637 for the highest estimates of queen-queen relatedness and queen number ($r_{qq} = 0.048$, $n_q = 35.4$) to 0.740 for the lowest estimates ($r_{qq} = 0.011$, $n_q = 8.7$). These values define a range over which worker relatedness asymmetry is likely to vary in the Georgia polygyne population in the absence of confounding influences. However, there are some factors that potentially complicate the expected ratio, including the proximity of the polygyne study population to monogyne colonies (Ross and Keller, 1995a) and the production of large numbers of sterile diploid males by polygyne colonies (Ross and Fletcher, 1985b).

In this study, I collected monogyne and polygyne colonies in northern Georgia throughout a single year. The colonies in this study originally were sampled to examine the effects of colony queen number on the numbers of sexuals produced under field conditions. These results have been reported previously (Vargo and Fletcher, 1987). Here I examine the data, at both the population and colony levels, to see if they are consistent with the worker control hypothesis and to assess the ability of various genetic and ecological hypotheses to account for intercolonial variation in sex investment ratio patterns in *S. invicta*.

Methods

Collection of samples

To determine sex allocation in monogyne and polygyne populations of *S. invicta*, 25 colonies of each form were collected in the spring (4 and 5 May), summer

(11 and 12 August) and fall (25 October and 6 November) of 1985. The polygyne colonies were from Walton Co., Georgia, U.S.A. and the monogyne colonies were from three nearby counties (Jasper, Putnam, and Newton) where the monogyne form consists of one large unbroken population.

Sexuals are not produced in the study region in winter (Markin and Dillier, 1971; E. Vargo, unpublished data); therefore, this season was excluded from the sample. Whereas colonies of the monogyne form generally occupy a single mound (Wilson et al., 1971; Bhatkar and Vinson, 1987), colonies of the polygyne form appear to be polydomous, i.e., they occupy more than one mound (Greenberg et al., 1985; Bhatkar and Vinson, 1987; Vargo and Porter, 1989). However, for convenience, the term *colony* is used here to refer to the inhabitants of a single mound of either form. To ensure the collection of the maximum number of individuals from each mound, the ants were collected by digging soon after rainfall on warm, sunny mornings following relatively cool nights, under which conditions they accumulate near the top of the mound. Colonies were chosen haphazardly, except that only large mounds (ca. 45 × 40 × 15 cm) were selected to ensure maturity, i.e., a size large enough to permit the production of sexuals. Selection of larger mounds in this case probably did not bias the sample (see e.g., Boomsma, 1989) because colonies of the monogyne form, which produce more sexuals than polygyne colonies (Vargo and Fletcher, 1987), do not begin producing sexuals until they have some 25 000 workers (Markin et al., 1973; Vargo, 1988; Tschinkel, 1993) and weigh about 50 g. The range of colonies chosen included colonies of this and smaller size. Moreover, Boomsma (1989) considers a sample size of 20 or more colonies sufficient to avoid a major bias from unplanned selection of larger colonies.

Contact between the study populations and colonies of the alternate social form

It is unlikely that the monogyne population was in contact with any conspecific polygyne colonies, including those sampled in the present study. This is because (1) the Walton County polygyne population is the only polygyne population known from Georgia, (2) the polygyne population was located at least 30 km from the monogyne study population, a distance outside the normal dispersal range of about 2 km for *S. invicta* sexuals (Markin et al., 1971), and (3) colonies of the monogyne form were located to the southeast of the polygyne population, whereas the prevailing winds during the times of mating flights in this area are from the southwest (Ross and Keller, 1995). Thus possible influences on sex allocation patterns, such as gene flow or sex ratio compensation (Ward, 1983), through contact with polygyne colonies can be ruled out.

On the other hand, the polygyne study population was not as isolated from the alternate social type during the study period. Presently, this population is embedded within the larger, continuous monogyne population, and some 80% of the matings in the polygyne population are attributable to males from surrounding monogyne colonies (Ross and Keller, 1995a). However, when the polygyne site was first discovered (Fletcher, 1983), it was located about 15 km north of the advancing

front of the monogyne form, and was therefore isolated from monogyne colonies. When the present samples were collected in 1985, the monogyne population was closer but was still separated by several kilometers (unpublished data), and therefore outside the normal dispersal range of sexuals. Thus some mating with monogyne males may have been occurring, but most likely it was at a far lower frequency than Ross and Keller (1995a) recently reported.

Determination of sex investment ratios

After collection, ants were taken to the laboratory and separated from the soil within 24 h by flooding with water (Jouvenaz et al., 1977). Within 6 days of collection, each colony was weighed to the nearest 0.1 g and then sieved (1.4 mm mesh) to remove sexual forms (alates, sexual pupae and sexualized larvae; Vargo and Fletcher, 1986), which were frozen for later examination. Numerical sex ratios were determined by counting the numbers of male and female sexuals (alates and pupae) present in each colony. The proportional investment in females was determined for each colony by adjusting the numbers of males and females by their respective dry weights, corrected for sex-specific differences in energetic cost (see below).

Because there was large seasonal variation in the production of sexuals in both social forms (see Tab. 2), I used a weighted average (Sokal and Rohlf, 1969) to obtain an overall mean investment ratio for each form. This involved weighting the mean for each season by its proportional contribution to the total sexual investment. For example, in the monogyne population the mean for the spring colonies contributed about 10 times more to the overall mean than did the mean for the summer colonies, because the total investment in sexuals after energetic cost conversion (see below) in the monogyne form was about 10 times greater in spring than in summer.

Female sexuals of *S. invicta*, like those of many ant species, store large amounts of body reserves before departing on mating flights (Keller and Passera, 1989; Keller and Ross, 1993). An accurate estimate of the total resources invested in female sexuals should be based on the amount of resources, usually measured by dry weight, that will normally be allotted each individual up to the mating flight. In the present study, alates departing on mating flights were not collected from the sampled colonies. To obtain estimates of the final weights of female sexuals, I combined data on fresh weights of departing alates from other authors with dry weight determinations from the present study. Keller and Ross (1993) obtained weights of virgin queens departing on mating flights from 10 monogyne colonies in northern Georgia in 1990 and 1991 ($n = 100$; mean fresh weight = 15.75 mg) and mating flights from 10 colonies in the Walton Co. polygyne population in the same years ($n = 99$; mean fresh weight = 10.6 mg). The corresponding dry weights were determined by matching these mean fresh weights with individuals of the same fresh weight in the present data set whose dry weights had been determined. The values ($\bar{X} \pm S.D.$) obtained were 8.12 ± 0.68 mg ($n = 16$) and 4.67 ± 0.29 mg ($n = 15$) for

females from monogyne and polygyne colonies, respectively. The weights of males do not change noticeably from the pupal stage to the time of the mating flight for *S. invicta* (E. Vargo, unpublished data), as has been noted for other ant species (Boomsma and Isaaks, 1985). Therefore, the mean dry weights for males in the colonies were used to estimate investment in males. These values were 2.6 ± 0.3 mg for males from monogyne colonies and 3.0 ± 0.6 mg for those from polygyne colonies.

Sex investment ratios based only on dry weight differences tend to be biased in favor of females, because males generally have a higher metabolic rate and therefore, on a per weight basis, males cost more to produce and maintain than do females (Boomsma, 1989). To correct for differences in metabolic rates, Boomsma (1989) suggests converting female/male dry weight ratios (D) to energetic cost ratios (C) by the formula $C = D^{0.7}$.

To test the resource availability hypothesis of Nonacs (1986b), which predicts a negative correlation between total sexual biomass and investment in males, I performed partial correlation analysis on these two variables. In the monogyne form colony weight was held constant, whereas in the polygyne form colony weight and queen number were held constant, because queen number is negatively related to the production of sexuals in polygyne *S. invicta* (Vargo and Fletcher, 1986, 1987, 1989).

In all statistical tests involving proportions, the angular (arcsin square-root) transformation was used. All significance levels refer to two-tailed tests.

Results

Monogyne colonies allocated considerably more resources in reproduction (production of winged male and female sexuals) than did polygyne colonies (Tab. 1). Whereas reproductive allocation varied considerably with season in both forms, it was 3 to 11 times higher in the monogyne colonies, and about 4 times greater overall.

The numerical sex ratios of both social forms exhibited large seasonal variation (Tab. 2) and differed substantially between the two forms. However, the weighted

Table 1. Relative investment in sexuals (mg dry weight sexuals/g colony fresh weight) by colonies of the two social forms of *S. invicta*. The weighted average for each form was obtained by weighting the mean for each season by its proportional contribution to the total investment in sexuals.

Season	Investment in sexuals ($\bar{X} \pm S.D.$)	
	Monogyne colonies	Polygyne colonies
Spring	92.8 ± 49.1	28.6 ± 14.5
Summer	35.6 ± 38.1	3.2 ± 5.7
Fall	102.7 ± 58.1	11.1 ± 16.0
Weighted mean	89.8 ± 52.7	22.8 ± 14.4

Table 2. Sex investment ratios in a monogyne and a polygyne population of the fire ant *S. invicta*. The dry weight investment ratio was determined by weighting the numbers of sexuals of each sex by the respective average dry weights of mature sexuals of each social form. To correct for sex-specific differences in metabolic rate, I used the energetic cost conversion of Boomsma (1989) where energetic cost = female:male dry weight ratio^{0.7}. The totals given for each form are weighted averages, reflecting the contribution of each season to the yearly total production of sexuals.

Form	Season	No. males	No. females	Proportional investment in females ($\bar{X} \pm \text{S.D.}$)	
				Dry weight	Energetic cost
Monogyne	Spring	41,076	15,642	0.492 \pm 0.276	0.452 \pm 0.280
	Summer	4,722	10,411	0.869 \pm 0.160	0.842 \pm 0.179
	Fall	35,361	32,460	0.670 \pm 0.213	0.645 \pm 0.229
	Total	81,159	58,513	0.653 \pm 0.207	0.607 \pm 0.241
Polygyne	Spring	6,657	14,922	0.755 \pm 0.035	0.732 \pm 0.037
	Summer	1,696	915	0.379 \pm 0.131	0.355 \pm 0.127
	Fall	5,153	2,719	0.349 \pm 0.302	0.336 \pm 0.301
	Total	13,506	18,556	0.642 \pm 0.106	0.616 \pm 0.108
	Total excluding diploid males			0.879 \pm 0.070	0.858 \pm 0.079
	Total counting diploid males as females			0.938 \pm 0.018	0.931 \pm 0.018

averages of the proportional investment in females, based on both dry weight and the energetic cost conversion, was very similar in the two forms, with no significant difference between them (both $t_{122} \leq 1.3$, $P > 0.09$). Figure 1 shows a comparison between the estimated sex investment ratios in the two populations and the ratios predicted by either exclusive worker control or exclusive queen control. In the monogyne population the weighted average of 0.607 fell between the 0.75 value expected under worker control and the 0.5 value predicted under queen control and differed significantly from both of these values (both $t_{71} \geq 3.8$, $P < 0.001$). Similarly, the weighted average of 0.616 for the polygyne population (all males included) was in the area between the worker and queen control levels. The polygyne population invested significantly more in females than expected under queen control ($t_{51} = 7.7$, $P < 0.001$), whereas the level of female bias was consistent with the lowest part of the range predicted under exclusive worker control (v. 0.637, $t_{51} = 1.4$, $P > 0.2$).

A complicating factor that must be taken into account in polygyne *S. invicta* is that the preponderance of males produced are diploid (Ross and Fletcher, 1985b). Because they are sterile (Hung et al., 1974), these diploid males are not active members of the breeding population. Ross and Fletcher (1985b) estimated that 77.7% of the males produced in the present study polygyne population are diploid. It is not clear how the diploid males should be considered in estimates of the sex investment ratio. This depends on how the workers who rear the diploid males perceive them. Whether the workers perceive them as sexuals at all, and if so, whether they perceive them as males or females, will greatly affect estimates of the

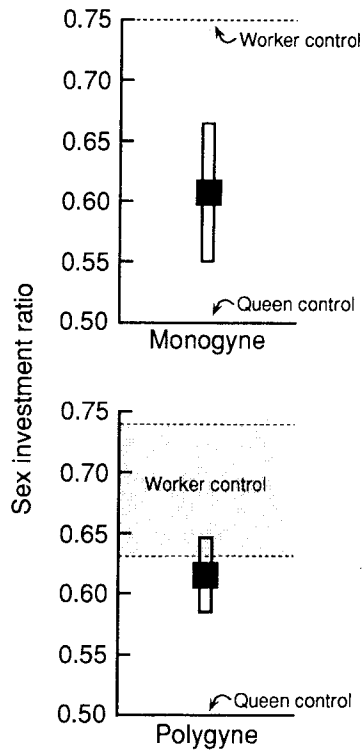


Fig. 1. Estimated proportional investment in females in a monogyne and a polygyne population of *S. invicta*. Shown are the means bounded by the 95% confidence limits. These are compared with the values expected under exclusive control of workers or queens. The worker control levels are based on relatedness asymmetries between workers and the male and female brood they rear. In the polygyne population, the shaded region shows the range over which worker relatedness asymmetry is likely to vary.

sex investment ratio (Tab. 2). In any event, diploid males should be excluded from consideration of the operational sex ratio, leading to a highly biased sex ratio in the polygyne population, with females outnumbering viable males about 6:1.

As reported by Vargo and Fletcher (1987), there was considerable variation in colony sex ratio, especially in the monogyne population where most colonies contained sexuals of predominantly one sex (Fig. 2). In the monogyne form there was a strong negative correlation between the numbers of males and females in individual colonies in spring ($r_{24} = -0.901$, $P < 0.0001$) and in fall ($r_{24} = -0.663$, $P < 0.0005$), the two seasons that when combined accounted for 89% of the total sexual production; in summer there was a positive but non significant correlation ($r_{24} = 0.289$, $P > 0.05$). The only significant relationship between the numbers of males and females present in colonies of the polygyne form was a positive correlation in summer ($r_{24} = 0.868$, $P < 0.0001$).

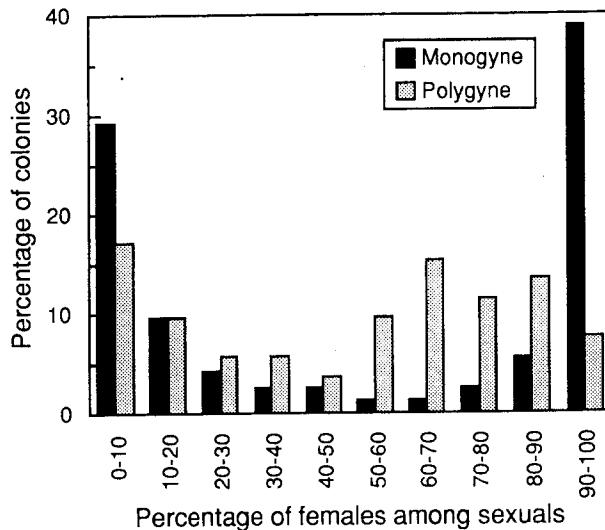


Fig. 2. Sex ratios within individual colonies of monogyne and polygyne *S. invicta*.

The results of the partial correlation analysis give mixed support to the resource availability hypothesis of Nonacs (1986b): for the monogyne colonies the correlation between total sexual biomass and investment in males was negative for the spring ($r_{22} = -0.54$, $P < 0.01$) but positive in both summer ($r_{19} = 0.11$, $P > 0.5$) and fall ($r_{22} = 0.45$, $P < 0.05$); and for the polygyne colonies proportional investment in males was negatively correlated with total sexual biomass in all three samples, but significantly so only in spring ($r_{21} = -0.54$, $P < 0.01$).

Discussion

As predicted by the worker control hypothesis, the sex investment ratios in both the monogyne and the polygyne populations were female-biased. Support for some measure of worker control is strongest in the monogyne population because of the absence of confounding factors that can influence sex allocation patterns. The fact that the degree of female bias generally was lower than expected under complete worker control suggests that queens of *S. invicta* also have some say in decisions concerning reproductive allocation. These findings are consistent with a recent population genetic model (Matessi and Eshel, 1992) in which compromise between the interests of the queen and workers can lead to an evolutionary stable state with a level of female bias between 0.5 and 0.75.

The present data set is based on a large number of samples, but it is limited because it comes from a single population of each social form taken during one year, and such a "snapshot" may not be representative of general sex allocation patterns in either social form. A more accurate assessment of the sex investment

ratio in each form would involve samples from several populations (see e.g., Herbers, 1990). For example, in an exceptionally thorough study, Ward (1983) found that the mean sex investment ratio in 10 populations of monogyne *Rhytidoponera confusa* was close to the expected 3 females:1 male, but the ratios in individual populations varied by as much as 40%.

Although data on other polygyne populations of *S. invicta* have not been published, the female-biased investment found in the Georgia monogyne population of the present study is similar to those found in three other monogyne populations of this ant in the U.S.A. At a site near Gainseville, Florida, Morrill (1974) reported a numerical sex ratio (males/females) of 0.923, which, using the weight ratios for monogyne sexuals in the present study, corresponds to a proportional investment in females of 0.772 (0.706 with the energetic cost correction). In Mississippi, Markin and Dillier (1971) documented a sex ratio of 0.917, which converts to a proportional investment in females of 0.773 (0.708 with the energetic cost correction). In the Tallahassee, Florida, area S. Porter (unpublished data) found a sex ratio of 0.996, giving a proportional investment in females of 0.758 (0.690 with the energetic cost correction). An interesting exception to this consistently large female bias in sex allocation was reported by Bass and Hays (1979) working in South Carolina, who found a sex ratio of 2.6 males:1 female, corresponding to a proportional investment in females of only 0.545 (0.461 with the energetic cost correction).

Averaging the values (arcsin transformed data) for all five available studies of sex ratios in monogyne *S. invicta*, including the present one, gives a mean \pm S.D. (re-transformed) proportional investment in females of 0.703 ± 0.011 for the dry weight ratios and 0.636 ± 0.012 after the energetic cost correction. Both of these means were significantly higher than 0.5 as expected under complete queen control (both $t_4 \geq 2.9$, $P < 0.05$), but neither value differed significantly from 0.75 as predicted under exclusive worker control (both $t_4 \leq 2.6$, $P > 0.06$). Thus, taken as a whole, I conclude that the existing data for monogyne populations of *S. invicta* are consistent with some measure of worker control over the sex investment ratio.

The strong female bias in the polygyne study population would appear to provide support for the worker control hypothesis, but the presence of potentially confounding factors should not go without mention. First, colony reproduction by budding in polygyne *S. invicta* raises the question of whether the workers that accompany queens during the budding process should be included as investment in female reproductives. The answer depends on how independent the daughter nests are from the mother nest (Pamilo, 1991). Some species (e.g., honey bees and army ants) undergo colony fission, in which the mother nest splits into two independent nests, one nest headed by the mother queen and the other headed by a newly-mated daughter queen. Several authors have argued that in such cases workers should be counted as part of the female investment (Hamilton, 1975; Macevicz, 1979; Ward, 1983; but see Pamilo, 1991). Alternatively, other species reproduce by budding in which strong connections between mother and daughter mounds are retained and workers and food flow freely among them. In this case the different mounds can be viewed as cooperating subnests within one large polydomous supercolony. Consequently, the process of budding is equivalent to nest expansion in a monodomous

colony, so that the workers involved in the budding process are an investment in colony growth and maintenance rather than part of reproductive investment (see also Pamilo, 1991). In polygyne *S. invicta* there appears to be widespread movement of both workers (Bhatkar and Vinson, 1987) and food (Drees et al., 1992) among neighboring mounds, suggesting a supercolonial nest structure. Thus, in my view, the worker force participating in the establishment of daughter nests in polygyne *S. invicta* does not constitute investment in female sexuals and should not be included in calculations of the sex investment ratio.

Another potentially complicating factor concerning the sex investment ratio in polygyne *S. invicta* is the problem of diploid males. Should these sterile alates be counted as males, as females, or not counted as sexuals at all? This will depend on whether the workers who rear diploid males perceive them as viable males, as female sexuals, or as neither. Workers may not be able to distinguish diploid males from normal haploid males and may count them among the males in their decisions about the sex investment ratio. Alternatively, diploid males are larger than the normal haploid males and they lack sperm (Hung et al., 1974; Ross and Fletcher, 1985b), possible signs of feminization which may lead workers to perceive and treat diploid male larvae as developing female sexuals. If so, energetic investment in females would exceed that in males by 13.5:1 in the present study population. A third possibility is that workers are not able to distinguish diploid males from either haploid males or sexually-determined females. In this case diploid males should be excluded from calculations of the sex investment ratio, which in the present study would yield a heavily biased female investment ratio (6:1). Because we are currently unable to determine how workers view diploid male larvae, and because there is a lack of any compelling evidence to the contrary, the safest assumption is that workers recognize and treat diploid males as normal males. In fact, the extreme female bias that results either from regarding diploid males as females or from disregarding them altogether, coupled with the close match between the investment ratio predicted under worker control and that observed when diploid males are included as male investment would suggest that workers do treat males as normal males. By contrast, Mueller et al. (1994) considered the small number of diploid males they found as part of the female investment in their study of colony genetic structure in the primitively social bee, *Augochlorella striata*, concluding that these large sterile individuals developed on provisions "designated" for females.

With regard to the diploid male problem, it would be of interest to examine the sex investment ratios in native polygyne populations in South America. Because many of the alleles at the sex determining locus were lost during the introduction of *S. invicta* to North America (Ross and Fletcher, 1985b; Ross et al., 1993), the frequency of diploid males is much lower in Argentina (10–20%) than in the U.S.A. (70–100%). A recent study by Ross et al. (1996) provides the necessary data on social and genetic structure in two Argentine polygyne populations to predict the proportional investment in females under exclusive worker control in these native populations (ignoring any confounding effects due to limited dispersal of sexuals). Effective queen number is much lower in Argentina (about 5 queens per colony) than in the U.S.A. (ca. 13), whereas relatedness among queens in the native

populations is much higher ($r = 0.45$ v. $0.011-0.048$). Plugging into Boomsma's (1993) relatedness asymmetry equation the range of values observed in the South American populations gives a range of worker relatedness asymmetries between 0.684 and 0.712, a much narrower range than that calculated above for the introduced Georgia population. Studies of sex allocation patterns in native populations should help elucidate the general rules by which decisions concerning reproductive investment are made in polygyne *S. invicta*, as well as indicate the extent to which relatedness asymmetry, as predicted by the worker control hypothesis, can account for sex investment ratios generally in this species.

The high frequency of diploid males in the Georgia polygyne population leads to a very female biased operational sex ratio, the relative numbers of individuals of each sex available for mating. Because the members of both sexes mate only once (Ross, 1993), the operational sex ratio is equivalent to the numerical sex ratio for fertile sexuals. Assuming that 77.7% of the males produced in the Georgia polygyne population are sterile, as was reported previously for this same population (Ross and Fletcher, 1985b), the operational sex ratio is heavily female-biased (6.2:1), whereas it is slightly male biased (0.7 females:1 male) in the Georgia monogyne population. The relative shortage of fertile males in the polygyne form but not in the monogyne form may help explain some peculiar features of the reproductive biology of the introduced populations (Ross and Keller, 1995b). First, a large proportion (ca. 30%) of reproductively active queens in polygyne colonies remain permanently unmated (Vargo and Fletcher, 1989; Ross and Keller, 1995a; Ross et al., 1996), whereas nearly all monogyne queens become mated (Glancey and Lofgren, 1985; Ross and Fletcher, 1986). Second, among the polygyne queens that do mate, at least in the Georgia polygyne population, most are inseminated by males issuing from monogyne colonies (Ross and Keller, 1995a).

Extensive contact between the polygyne study population and monogyne colonies could influence the sex allocation ratios in the polygyne population in two opposing ways. First, female bias in the much larger surrounding monogyne population may raise the value of males in the polygyne form, leading the polygyne population to compensate by investing more in males than they would otherwise (Ward, 1983). Second, gene flow from monogyne colonies to the polygyne population may provide a conduit for genes favoring greater female investment in the polygyne population than expected from worker relatedness asymmetries alone. A recent study by Ross and Keller (1995a) conducted in 1993 shows that the Georgia polygyne population currently is embedded within a large continuous monogyne population and that 80–90% of the queens in the polygyne colonies are inseminated by males from monogyne colonies. Thus at the present time sex ratio compensation and gene flow could be influencing the sex investment ratio in the polygyne study population, although the relative strength of these two influences is unknown and would be difficult to determine. However, at the time of the present study (1985) the polygyne population was not surrounded by monogyne colonies, and contact between this population and the approaching monogyne front was undoubtedly more limited than it is currently. In conclusion, although proximity of monogyne colonies may have influenced sex allocation patterns in the polygyne study popula-

tion, the extent of this influence is difficult to quantify and was almost certainly less than it is at the present time.

In the present study monogyne colonies invested considerably more in reproduction (sexuals) and less in maintenance/growth (worker production) than did polygyne colonies. These differences in allocation patterns reflect the alternative reproductive strategies employed by the two social forms of *S. invicta*. The monogyne form reproduces by independent colony founding, in which newly-mated queens initiate new colonies by themselves or in groups without the aid of workers (Markin et al., 1972; Tschinkel and Howard, 1983), whereas the polygyne form reproduces by budding, where queens are dependent on workers to aid them in establishing new mounds (Vargo and Porter, 1989). A similar difference in reproductive allocation between monogyne and polygyne colonies also has been reported for the wood ant *Formica truncorum* (Sundström, 1995), and is probably a general phenomenon among ants, in which monogyne and polygyne colonies commonly exhibit alternative modes of reproduction (Rosengren and Pamilo, 1983; Keller, 1993; Ross and Keller, 1995b).

In the present study there was large variation in the sex investment ratios of individual colonies, especially in the monogyne form. Such split sex investment ratios appear to be common among ants (Nonacs, 1986a; Boomsma and Grafen, 1990, 1991). Although several genetic and ecological hypotheses have been proposed to explain the bimodal distribution of colony sex ratios, none of the explanations appears to account for the split sex ratios found here. First, differences in relatedness structure between male and female producing colonies (Boomsma and Grafen, 1990, 1991) can be ruled out, because monogyne colonies of *S. invicta* do not appear to vary in genetic structure; queens invariably mate with a single male and workers do not reproduce (Ross and Fletcher, 1985a). Second, local mate competition (Hamilton 1967; Frank, 1987) is unlikely, because *S. invicta* engages in large simultaneous mating flights from many colonies (Markin and Dillier, 1971; Morrill, 1974; Bass and Hays, 1979) and there is no evidence of inbreeding in several well-studied populations (Ross et al., 1987, 1993; Ross, 1993). Third, the present data set did not lend strong support to the resource availability hypothesis of Nonacs (1986a); although total sexual biomass, a presumed indicator of resource abundance, was significantly correlated with proportional investment in males in spring and fall, the two seasons of greatest sexual production, only in spring was the correlation negative as predicted by the hypothesis.

Aron et al. (1995) recently found evidence of a proximate mechanism determining colony-level sex ratio in monogyne *S. invicta*. These authors investigated the relationship between the primary sex ratio (ratio of male/female determined eggs) produced by the queen to the secondary sex ratio (ratio of male/female adult reproductives) produced by the colony. They found evidence that workers exert some control over the secondary sex ratio by eliminating many male eggs laid by queens. However, the extent to which workers eliminate male larvae depends on the primary sex ratio. When few male eggs were present ($\leq 11\%$), nearly all were destroyed resulting in a strongly female-biased secondary sex ratio. When the primary sex ratio consisted of at least 19% male eggs, then the workers reared many

male alates leading to a heavily male-biased secondary sex ratio. The authors concluded that the results were consistent with the primary sex ratio and subsequent destruction of male larvae being a staging ground for queen-worker conflict over sex allocation in monogyne *S. invicta*. If so, then the intermediate level of female bias found at the population level in the present study suggests that queens and workers may have reached an ergonomic compromise giving each party some influence over the sex investment ratio.

Do the hypotheses concerning intercolonial variation in the sex investment ratio help explain the observed variation in the polygyne colonies studied here? At the present time we do not have sufficient data on colony genetic structure to test for a possible role for variation in relative relatedness asymmetry in determining colony-level sex investment ratios; although the average degree of relatedness among nestmate queens in the polygyne study population has been well-characterized (Ross and Fletcher, 1985a; Ross, 1993), there has been no detailed study of intercolonial variation in queen relatedness structure. The results of the present study were consistent with the resource availability hypothesis, as evidenced by the negative partial correlation between total sexual biomass and proportional investment in males. However, support for the resource availability hypothesis was not strong, because the correlation was significant in only one of three seasons. The factors influencing colony-level sex investment ratio certainly deserve further study, including a possible effect of the primary sex ratio on the secondary sex ratio, which has not yet been investigated in polygyne colonies of *S. invicta*.

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