

Intracolony patterns of reproduction in the queen-size dimorphic ant *Leptothorax rugatulus*

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Abstract:

Animals that live in genetically heterogeneous groups are faced with a combination of cooperation and conflict. Reproductive skew theory aims to provide a unified theory of the partitioning of reproduction in animal societies by combining genetic, demographic, and environmental factors. Although theoretical elaborations abound, empirical data are rare. Specifically, explicit intraspecific tests are scarce and have provided conflicting results. We present data on reproductive partitioning among queens in the ant *Leptothorax rugatulus* with special emphasis on relatedness and body size. Relatedness was negatively correlated to skew in sexual offspring and uncorrelated to skew in total offspring. Body size was not correlated to a queen's reproductive share. Thus, we did not find any support for the classic optimal skew models that are based on concessions. In artificial colonies, composed of randomly selected, unrelated workers and queens, reproductive skew was higher than in natural colonies, which suggested that unequal reproduction among queens could arise without nepotism by workers. Again, a queen's body size was not a good indicator of her reproductive share, but egg laying rate was. In colonies that contained large and small queens, small queens produced proportionally more sexual offspring. Although this result is in accordance with the kin conflict over caste determination hypothesis, it is more plausibly explained by an adaptation of the caste ratios to alternative dispersal tactics.

Key words: body size, kin conflict, queen size dimorphism, reproductive skew, selfish microgynes.

Article:

INTRODCUTION

Cooperative group living has proven very successful in animal evolution (e.g., Hölldobler and Wilson, 1990). However, most groups are genetically heterogeneous, and cooperation and conflict coexist within groups (Emlen, 1997; Hamilton, 1964a,b; Heinze et al., 1994; Keller, 1993b; Maynard Smith and Szathmáry, 1995; Vehrencamp, 1983a,b). The central conflict occurs over reproduction among several potential breeders, and thus reproductive skew theory has been suggested as a general theory to characterize animal groups (Johnstone, 2000; Reeve and

Ratnieks, 1993; Vehrencamp, 1983a,b). Indices of reproductive skew vary between zero, when reproduction is shared equally, and one, when only one potential breeder reproduces.

Recently, there has been tremendous interest in reproductive skew theory (for reviews, see Johnstone, 2000; Reeve and Keller, 2001) due to its broad and unifying taxonomic perspective and its potential for a comprehensive theory of social evolution (Reeve and Keller, 2001). The majority of work has been theoretical, and a large number of models have been proposed. These models fall into two categories: transactional and compromise (Johnstone, 2000). Transactional models assume that the individuals that control reproduction concede reproduction to subordinates to maintain group stability. In contrast, compromise models consider reproductive division among group members to be the outcome of incomplete control by the dominant and differences in the ability of individuals to claim a share of group reproduction (at the cost of group productivity). Both models create sets of predictions that depend on various aspects of the general biology of the focal species (Johnstone and Cant, 1999b; Kokko and Johnstone, 1999; Ragsdale, 1999; Reeve et al., 1998), and on parameters such as intragroup relatedness (Reeve and Keller, 1995; Johnstone, 2000), group size (Reeve and Emlen, 2000), and the benefits of group living (Johnstone et al., 1999). Despite theoretical elaborations that have created an array of models, independent, empirical tests are scarce and provide conflicting results (Reeve and Keller, 2001).

The concession form of transactional skew models (Vehrencamp, 1983a,b) has received most attention. It predicts a positive association of reproductive skew and the four factors intragroup relatedness, power asymmetry, subordinate contribution to colony productivity, and risk associated with independent breeding. Its most crucial assumptions are that the dominant breeder has absolute control over reproduction, individual reproduction can be estimated, subordinates may leave the group for independent reproduction, and that subordinates benefit the dominant. In vertebrates, some support for these classic optimal skew models has been presented (Emlen, 1997; Jamieson, 1997; Keller and Reeve, 1994), but alternative explanations cannot be ruled out (Clutton-Brock, 1998; Cooney and Bennett, 2000). In invertebrates, evidence in favor of transactional models comes mainly from interspecific comparisons in social Hymenoptera and circumstantial evidence (Heinze, 1995; Hoogendoorn and Velthuis, 1999; Keller and Reeve, 1994; Reeve and Keller, 2001). Direct tests at the intraspecific level are less common and have provided conflicting evidence. For example, many aspects of the reproductive biology of several wasp species of the genus *Polistes* might be explained by reproductive transactions (Reeve and Keller, 2001; Reeve et al., 2000), but another study of this genus found no support for these models (Field et al., 1998).

Compromise models (Reeve et al., 1998) have received less attention. Their main assumptions are incomplete control by the dominant breeder and a struggle for reproduction in which every breeder chooses to divert some energy to claiming reproduction and consequently lowering group productivity. Their main predictions are a weak relation (negative or positive) of reproductive skew and relatedness and a strong positive association between skew and the inequality between dominant and subordinate individuals. Furthermore, they predict a negative association between skew and agonistic interactions.

The most prominent compromise model, the tug-of-war model, is supported by a field study in meerkats (Clutton-Brock et al., 2001). It is also supported by the negative correlation between skew and relatedness found in *Polistes* (Field et al., 1998) but is refuted by another study in the same genus (Tibbetts and Reeve, 2000). Circumstantial evidence in other groups sustains arguments for and against compromise models (e.g., Hoogendoorn and Velthuis, 1999). Few studies have reported intraspecific tests within ant species. There was no support for the prediction of transactional models of a positive association between skew and relatedness in *Myrmica tahoensis* (Evans, 1995), and data from *Formica fusca* agrees better with predictions of compromise models (Hannonen MT and Sundström L, personal communication). In contrast, Bourke et al. (1997) provided supportive data for transactional models by comparing two populations of *Leptothorax acervorum*.

The genus *Leptothorax* may prove particularly important for tests of reproductive partitioning in ants because the relatively small colonies can be collected completely and maintained under seminatural condition in the laboratory (Buschinger, 1974a). *Leptothorax* species exhibit a variety of social structures (Bourke and Heinze, 1994; Buschinger, 1974b), and remarkable data sets exist on various aspects of their biology (e.g., Bourke et al., 1997; Herbers, 1990). Social diversity is also common within *Leptothorax* species: in many cases single-queen (monogynous) and multiple-queen (polygynous) colonies coexist (facultative polygyny; Bourke and Franks, 1995), which can be attributed to variation in ecological constraints on independent founding (Bourke and Franks, 1995; Herbers, 1993). In some cases, variation in reproductive strategies has led to alternative queen morphs (Buschinger and Heinze, 1992; Heinze and Tsuji, 1995; Rüppest and Heinze, 1999).

For the North American ant *Leptothorax rugatulus*, a queen size dimorphism has been documented (Rüppest et al., 1998). Besides normal-sized queens (macrogyenes), small queens (microgyenes) exist as a quasi-isometric reduction. Queen morphology and social structure are correlated: while many macrogyenes occur in monogynous colonies, microgyenes are found almost always in polygynous colonies. Queen-size dimorphism is related to alternative reproductive tactics: after mating, microgyenes are adopted into existing colonies, predominantly related ones, while macrogyenes are more likely to start new colonies independently (Rüppest et al., 2001). Presumably, adoption of new queens is followed by colony budding (as in other facultatively polygynous *Leptothorax* species; Bourke and Franks, 1995), though this has not yet been conclusively demonstrated for *L. rugatulus*. Although workers are only capable of male production (in the absence of queens), both macro- and microgyenes possess a fully developed reproductive tract (Rüppest et al., 1998). Mixed colonies with macro- and microgyenes are relatively rare (13% of all colonies), and a slight genetic differentiation ($F_{ST} = 0.09$) between morphs has been found (Rüppest et al., 2001). The resource allocation to offspring (sex ratio) of colonies is influenced by social structure, but not by queen morphology per se (Rüppest et al., unpublished data).

The highly variable queen body size of *Leptothorax rugatulus* makes the investigation of its reproductive pattern in colonies particularly interesting. On the one hand, the strong differences in body size are expected to give an advantage to larger queens in competitive ability (that could include the ability to fight, produce pheromones, or acquire food) and fertility, as these parameters are generally well correlated with body size (Clutton-Brock, 1988). Furthermore, the

small body size of microgynes poses a serious constraint on independent reproduction (Rüppell et al., 2001). It is therefore predicted that reproductive skew and variance in queen body size in a colony are positively correlated and that in mixed colonies, macrogynes claim a disproportionately large reproductive share. Moreover, according to concession models, subordinate microgynes are expected to accept a higher reproductive skew because they have no option of independent reproduction.

On the other hand, it has been suggested that microgynes constitute intra-specific social parasites (Bourke and Franks, 1991; Buschinger, 1990; Rüppell and Heinze, 1999). Thus, microgynes may dominate reproduction despite their smaller body size, as interspecific social parasites do (Hölldobler and Wilson, 1990). Extending this argument, it was proposed that small body size in social insect queens results from a selfish larval strategy to develop into sexuals against the interest of the remaining colony (Bourke and Ratnieks, 1999; Nonacs and Tobin, 1992). This suggests that the relative proportion of sexuals (and specifically gynes) is expected to be higher in offspring of microgynes than in offspring of macrogynes. In this case, skew in the production of sexuals might be high despite similar egg laying rates of queens.

As studies of reproductive distributions in field collected colonies of *Leptothorax* have proven difficult due to potentially missing queens (Bourke et al., 1997), we investigated the reproductive distribution among cohabiting *Leptothorax rugatulus* queens in a controlled laboratory experiment. Specifically, the influence of intracolony relatedness and body size of queens on reproductive partitioning were studied. We used unmanipulated colonies to investigate naturally occurring skew, as well as randomly assembled colonies to confront alien queens with each other and control for potential worker nepotism (i.e., to exclude the possibility that high skews resulted from workers nepotistically favoring the reproduction of their own mother). In addition, we investigated whether queens biased their reproduction toward one particular offspring type, relative to their nest-mate queens.

METHODS

Leptothorax rugatulus is widespread in the western United States (Creighton, 1950). In our study area (southern Arizona and New Mexico), it mainly nests under stones or in rock crevices in mixed coniferous forests on mountain ranges. To investigate the pattern of reproduction among *Leptothorax rugatulus* queens, two experimental groups of colonies were established in the laboratory: unmanipulated colonies and artificially mixed groups. Both groups comprised colonies with only macrogynes, only microgynes, or both (mixed colonies).

Experiments

After collection of colonies in August 1996 from various populations in the Chiricuhua Mountains (Arizona), Huachuca Mountains (Arizona), and Manzano Mountains (New Mexico), colonies were maintained under standard conditions in three-chamber nest-boxes and kept in incubators (Buschinger, 1974a). In June 1997 the following experimental groups were set up: the first group consisted of 34 original colonies (11 macrogynous, 9 microgynous, and 14 mixed) with 2-7 queens. The second group consisted of 30 artificial colonies (10 macrogynous, 10 microgynous, and 10 mixed) with 4 queens each. These colonies were composed randomly from a pool of workers and queens from approximately 50 original colonies. This pool was set up 2 months before the start of the experiment by mixing the original colonies in one large laboratory

arena that contained only a single nesting cavity. After initial fighting, the colonies settled together in the nest, and 2 months later no aggression could be observed. Physically intact individuals were chosen at random from this "super-colony" to assemble the artificial colonies.

At the start of the experiment, all brood (eggs, larvae and pupae) was completely removed from all colonies in both experimental groups to avoid including brood from past queens. Present queens were randomly paint-marked (Edding 780 paint marker). We determined the primary egg-laying rates of queens 2 weeks later by isolating single queens for 16 h with some workers. The isolation procedure was repeated five times, with queens being returned to their colonies for 32 h of recovery between each period of isolation. For the next 2 years, until the end of the reproductive phase in August 1999, we censused colonies to count the numbers of queens and workers. Direct counts were performed under a Wild stereomicroscope every 3 days during the reproductive season and every 4 weeks during hibernation. All newly emerging off-spring were collected (twice per week in summer, the main reproductive season) and kept frozen at -20°C until DNA extraction. To estimate group productivity, we estimated dry mass production of every colony as a per-year, per-worker value. At the end of the experiment, all queens were killed by freezing, their insemination status was checked by ovarian dissection, and their maximum head and thorax widths were measured. We averaged head and thorax width to give one measure of body size for each individual. Heads and thoraces of queens were then used for DNA extraction.

Genetic analyses

DNA was extracted from individuals by a modification of the Chelex® protocol (Altschmied et al., 1997) and seven micro-satellites were adopted from different species using different polymerase chain reaction (PCR) amplification protocols (Table 1). The internally α -³³P-labeled PCR products were separated on sequencing gels, and their allelic sizes determined by comparison to the Sequamark® size standard. We calculated queen—queen relatedness in all experimental colonies using the computer program Relatedness 5.02 (Goodnight and Queller, 1998) based on the principles of regression relatedness (Queller and Goodnight, 1989). Allele frequencies were estimated (with bias correction and weighting by colony; see Goodnight and Queller, 1998) from an extended data set that comprised 360 additional queens from colonies not included in this experiment.

Table 1 Seven microsatellites adopted from other ant species used to assign offspring to potential mothers

Locus	Original citation	Mastermix	Temperature profile	No. of alleles
L4	Foitzik et al. (1997)	200 μ M dNTPs	Annealing: 56/51°C (60 s)	5
		2.5 mM MgCl ₂	Elongation: 70°C (90 s)	
		0.63 μ M Primer	Cycles: 10/30	
L18	Foitzik et al. (1997)	200 μ M dNTPs	Annealing: 51°C (60 s)	24

		2.5 mM MgCl ₂	Elongation: 70°C (90 s)	
		0.63 μM Primer	Cycles: 36	
LXGT104	Hamaguchi et al. (1993)	310 μM dNTPs	Annealing: 51°C (60 s)	5
		2.5 mM MgCl ₂	Elongation: 70°C (90 s)	
		0.63 μM Primer	Cycles: 36	
LXGT218	Hamaguchi et al. (1993)	200 μM dNTPs	Annealing: 57-48/47°C (90 s)	5
		1.25 mM MgCl ₂	Elongation: 70°C (90 s)	
		0.56 μM Primer	Cycles: 10 x 2/20	
LXGT228	Hamaguchi et al. (1993)	200 μM dNTPs	Annealing: 57-48/47°C (90 s)	21
		1.8 mM MgCl ₂	Elongation: 70°C (120 s)	
		0.41 μM Primer	Cycles: 10 x 2/20	
MYRT3	Evans (1993);	200 μM dNTPs	Annealing: 60/55°C (60 s)	6
	Bourke et al. (1997)	2.5 mM MgCl ₂	Elongation: 70°C (120 s)	
		0.63 μM Primer	Cycles: 10/20	
LXAGT1	Bourke et al. (1997)	200 μM dNTPs	Annealing: 60/55°C (60 s)	27
		2.5 mM MgCl ₂	Elongation: 70°C (120 s)	
		0.63 μM Primer	Cycles: 10/20	

Taq was used at 0.04 U/μl concentration. Initial denaturation was performed for 180 s at 94°C, subsequent denaturation for 60 s at 92°C, and the final elongation step for 300 s at 70°C. The number of alleles for each locus gives an approximate idea about its discriminatory power in maternity assignment.

From the initial genotyping of queens, the power of individual microsatellite loci to discriminate between queens as potential mothers was known. On this basis, colonies were chosen that provided, in addition to a relatively high reproductive output, good prospects for unambiguously assigning offspring to mothers. However, analyzed colonies did not have a significantly lower overall relatedness [$r_{\text{analysed}} = 0.36 \pm 0.27$ (SD), $r_{\text{omitted}} = 0.48 \pm 0.36$, $t = -0.93$, $df = 25$, $p = .363$]. Colonies with queens dying during the course of the experiment after the initial 2 weeks were also excluded. We analyzed 12 artificial and 11 unmanipulated colonies. Neither queen number [median_{original} = 3, 25-75% quartile (Q₂₅₋₇₅) = 2-3, median_{artificial} = 3, Q₂₅₋₇₅ = 3-4; Mann-Whitney $U_{11,12} = 48.0$, $p = .270$], nor worker number (median_{original} = 44, Q₂₅₋₇₅ = 33-84; median_{artificial} = 46, Q₂₅₋₇₅ = 43-49; $U_{11,12} = 66.0$, $p = 1.000$) differed significantly between groups.

Starting with the most informative locus, mothers and offspring were genotyped at as many microsatellite loci as necessary for unambiguous mother—offspring assignment. Offspring that could not be assigned unambiguously (overall, 28 males in 4 colonies) were omitted from the subsequent analysis. On average, 27 (range 16-38) offspring per colony were successfully assigned to one of the potential mothers.

We calculated the overall skew index by combining the worker, male, and gyne offspring, and the sexual skew index was similarly calculated from male and gyne offspring. The desirable calculation of separate skew indices for worker, male, and queen offspring was precluded by small sample sizes. Of the skew indices available (Kokko et al., 1999), we report here the corrected skew index proposed by Keller and Krieger (1996). The hypothesis that queens within groups contribute differentially to worker, male, and female offspring was tested by chi-square tests with subsequent Bonferroni correction. At the individual level, relative offspring production (reproductive share) was investigated as a function of relative queen body size (individual queen size/average queen size in any given colony) and relative egg-laying rate (individual egg laying rate/average egg laying rate in any given colony). We determined significance levels of the correlations by randomization tests (Manly, 1997) because the relative values were not independent of each other within colonies. Furthermore, we investigated the influence of relative body size on reproductive bias toward sexuals. A relative index was calculated to express this bias with the formula $(x_q/n_q) / (x_c/n_c)$, where x is the number of sexuals (gyenes and males) and n is the total offspring number. The subscripts q and c refer to a particular queen and her colony, respectively.

RESULTS

Colony level

Across all colonies, the expected correlation (due to non-independence of the two calculated indices) between skew in overall and sexual offspring production was found ($r_s = .65$, $n = 22$, $p = .001$). The two skew indices and the productivity index are summarized in Table 2, and the raw data for all colonies are given in the Appendix. On average, overall skew and sexual skew were higher in artificial than in original colonies, but only the difference in overall skew was statistically significant (overall skew: median_{artificial} = 0.15, Q₂₅₋₇₅ = 0.06-0.56; median_{original} = 0.04, Q₂₅₋₇₅ = 0.00-0.11; $U_{11,12} = 34.0$, $p = .049$; sexual skew: median_{artificial} = 0.29, Q₂₅₋₇₅ = 0.04-0.47; median_{original} = 0.05, Q₂₅₋₇₅ = -0.07-0.29; $U_{11,11} = 39.0$, $p = .158$). Average productivity was significantly lower in artificial colonies (median_{artificial} = 0.83, Q₂₅₋₇₅ = 0.65-0.98; median_{original} = 2.24, Q₂₅₋₇₅ = 1.47-3.65; $U_{11,12} = 7.5$, $p < .001$).

Table 2 Skew indices and productivity ($\mu\text{g}/\text{worker}$) of the artificial groups (sk1-sk29) and the original colonies (ors4-ors34)

Artificial groups	Overall skew	Sexual skew	Productivity	Original colonies	Overall skew	Sexual skew	Productivity
sk1	0.79	0.30	0.16	ors4	0.18	0.18	0.21
sk2	0.09	-0.01	0.23	ors7	-0.03	-0.20	0.54
sk7	0.21	0.47	0.32	ors8	0.10	0.45	0.23
sk10	0.07	0.05	0.43	ors13	-0.01	0.29	0.56
sk12	0.01	1.00	0.11	ors16	0.03	-0.02	0.36
sk13	0.75	—	0.17	ors18	0.01	-0.01	0.30
sk16	0.44	0.31	0.15	ors22	0.11	0.24	0.13
sk18	0.10	0.24	0.21	ors26	0.11	0.84	0.56
sk20	0.68	1.00	0.13	ors27	0.04	0.05	0.62
sk25	-0.06	-0.03	0.07	ors28	0.00	-0.07	0.64
sk26	0.04	0.11	0.14	ors34	0.04	-0.13	0.64
sk29	0.40	0.29	0.13				

Overall skew was calculated from worker, male and gyne offspring, sexual skew from male and gyne offspring. No skew was calculated when less than four offspring were produced in a group; the sample sizes are given in the Appendix.

As expected, queens were unrelated in all artificially assembled colonies, and consequently no association between relatedness and skew could be tested in this experimental group. Within the sample of original colonies, relatedness was correlated to neither overall skew ($r_s = -.20$, $n = 11$, $p = .555$), nor productivity ($r_s = .08$, $n = 11$, $p = .821$). Sexual skew was negatively correlated with relatedness ($r_s = -0.69$, $n = 11$, $p = .019$; Figure 1). However, the power of the estimation of the underlying within-colony relatedness values was not sufficient for the differences among colonies to be statistically significant. In both experimental groups, neither overall skew nor sexual skew was significantly correlated to variability in queen body size (Figure 2).

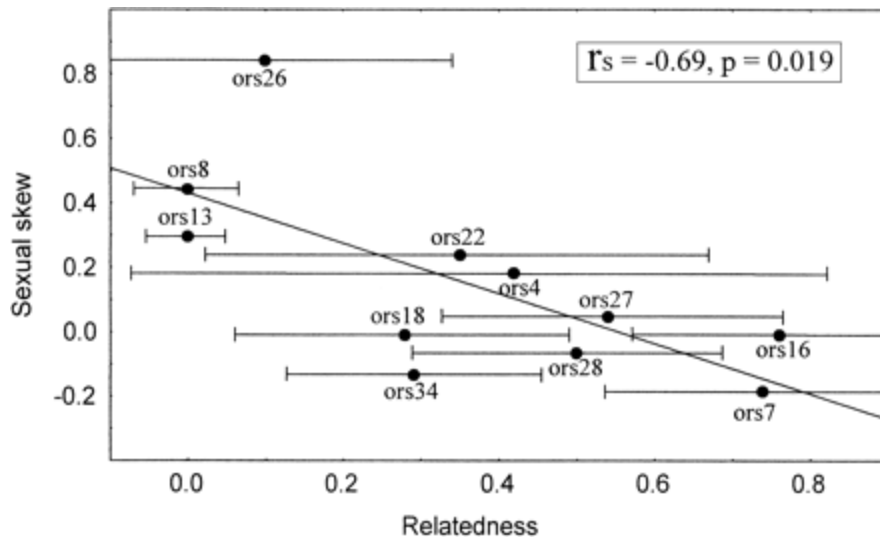


Figure 1 In the unmanipulated colonies, a negative correlation between relatedness and reproductive skew existed. Sexual skew is calculated only from male and gyne offspring and is reported here as Krieger and Keller's (1996) corrected skew index. Relatedness refers to mean regression relatedness between the mature queens coexisting as reproductives in a colony, and the SE of its estimate is given as horizontal lines with each colony value. For all sample sizes, see the Appendix.

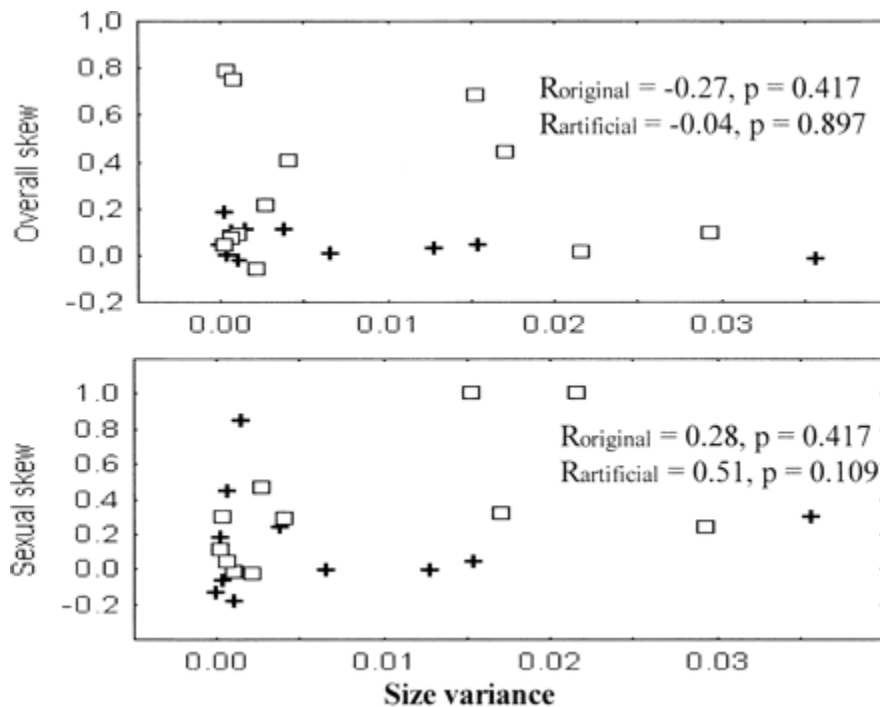


Figure 2 No significant correlations existed between overall or sexual skew and the variance in body size of cohabiting queens. +, original colonies; □, artificial groups.

Because predictions of the basic models strictly apply only to two-member groups (assuming nonidentical subordinates), the analyses were repeated with colonies containing only two queens. This reduced the sample size of original colonies to four and the sample size of artificial colonies

to three. Although separate analyses of these samples were not statistically meaningful, the pooled analyses led to the following results. Queen relatedness was negatively correlated to overall skew ($r_s = -.85$, $n = 7$, $p = .015$) but not significantly to sexual skew ($r_s = -.58$, $n = 6$, $p = .228$). There was a nonsignificant, positive association between queen size variability and overall skew ($r_s = .25$, $n = 7$, $p = .589$) and sexual skew ($r_s = .77$, $n = 6$, $p = .072$).

Colony density and the degree of polygyny in *Leptothorax* populations are believed to reflect ecological constraints on independent colony founding (Bourke and Franks, 1995; Herbers, 1993). Under this assumption, the original colonies were grouped based on whether they were collected from a population with high or low ecological constraint, and the skew indices were compared between the two groups. Populations with >50% polygynous colonies and high colony density were classified as "high ecological constraint," and the remaining <50% polygynous colonies and/or low colony density were classified as "low ecological constraint." The population origin of colonies in this sample had no significant effect on overall skew ($U_{6,5} = 13$, $p = .715$) or on sexual skew ($U_{6,5} = 14$, $p = .855$).

Individual level

The relative size of queens did not significantly affect their reproductive share in the original colonies ($r = .21$, $n = 31$, $p = .061$) or in artificial groups ($r = -.04$, $n = 37$, $p = .389$). The same result was obtained when micro- and macrogynes were compared in mixed colonies (median_{macro} = 0.35, Q₂₅₋₇₅ = 0.14-0.63; median_{micro} = 0.25, Q₂₅₋₇₅ = 0.22-0.38; $U_{11,11} = 72$, $p = .450$). This is corroborated by the absence of a correlation between relative body size and relative egg-laying rate (original colonies: $r = .13$, $n = 31$, $p = .255$; artificial groups: $r = .09$, $n = 36$, $p = .180$) and the lack of a difference in relative egg laying rates between micro- and macrogynes in mixed colonies (median_{macro} = 0.40, Q₂₅₋₇₅ = 0.33-0.46; median_{micro} = 0.29, Q₂₅₋₇₅ = 0.14-0.44; $U_{11,11} = 74.5$, $p = .358$). A positive trend between primary egg-laying rate and reproductive share existed (Figure 3) in original colonies ($r = .41$, $n = 31$, $p = .132$), and a significant correlation was found in artificial groups ($r = .53$, $n = 38$, $p = .001$).

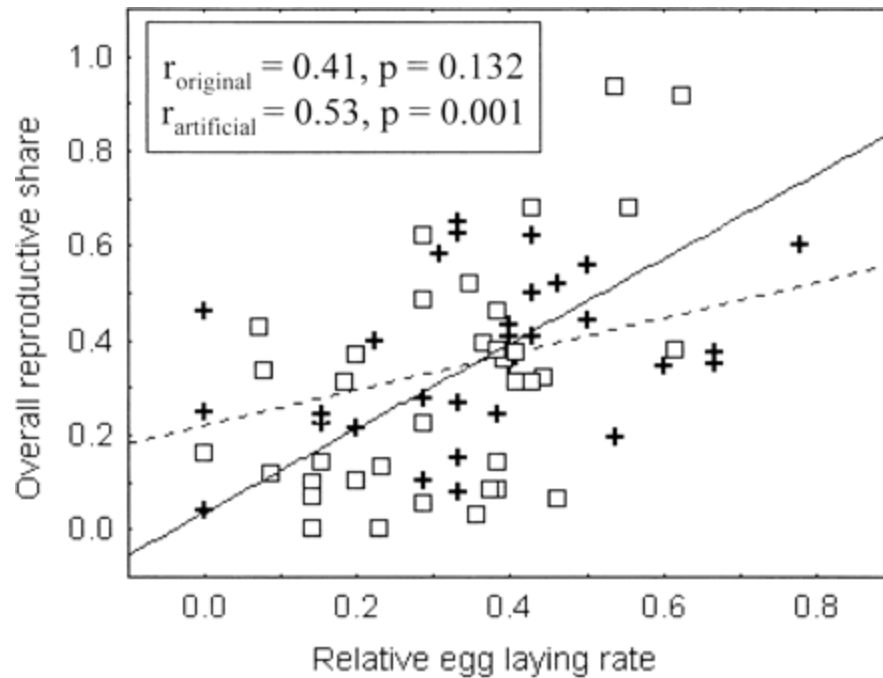


Figure 3 Overall reproductive share (proportion of male, female, and worker offspring) and relative egg-laying rate of individual queens were significantly correlated in artificial groups, and a similar positive trend existed in original colonies, regardless of the queens' body size. +, original colonies; □, artificial groups.

Across both experimental groups, most queens (27) produced workers and gynes; some produced only workers (13), workers and males (10), or all three types of offspring (8); and few specialized in gynes (5) or males only (3), or did not contribute to the investigated offspring at all (3). Thus, co-habiting queens did not contribute evenly to male, female, and worker offspring. The degree of differentiation in offspring production among queens was significant in 8 of the 23 colonies (Table 3). Chi-square values did not differ between original colonies and artificial groups ($U_{12,11} = 72, p = .712$), but were significantly higher in groups with microgynes (microgynous and mixed) than purely macrogynous ones (Kruskal-Wallis ANOVA: $H_{2,23} = 6.75, p = 0.34$). All groups that were significantly differentiated were either microgynous (three) or mixed (five).

Table 3 Queen reproductive specialization

Artificial groups	χ^2	<i>P</i>	Original colonies	χ^2	<i>P</i>
sk1	1.75	.186	ors4	2.34	.310
sk2	6.09	.048	ors7	0.04	.842
sk7	3.63	.304	ors8	4.66	.097
sk10	6.15	.105	ors13	20.9	.000
sk12	21.0	.000	ors16	28.1	.000

sk13	0.07	.791	ors18	20.3	.000
sk16	31.5	.000	ors22	6.08	.193
sk18	1.75	.186	ors26	21.2	.000
sk20	3.05	.218	ors27	5.42	.067
sk25	5.78	.216	ors28	0.69	.406
sk26	17.0	.000	ors34	0.36	.549
sk29	21.5	.000			

In four original colonies and four artificial colonies (all containing microgynes), queens did not produce equal proportions of male, worker, and gyne offspring. Due to multiple testing, only p values $<.002$ indicate a significant differentiation among queens within colonies (colonies in boldface).

The differentiation was caused in three colonies by different queen/worker caste ratios, in two colonies by different sex ratios of the individual queen offspring, and in three cases a mixture of both effects. In mixed colonies, microgynes produced relatively more sexuals than macrogynes ($U_{12,11} = 18.0, p = .003$; Figure 4). Although this is mainly attributable to the significant difference in gyne production ($U_{12,9} = 17.0, p = .009$), microgynes also exhibited a higher relative male production (Figure 4), but this difference was not significant.

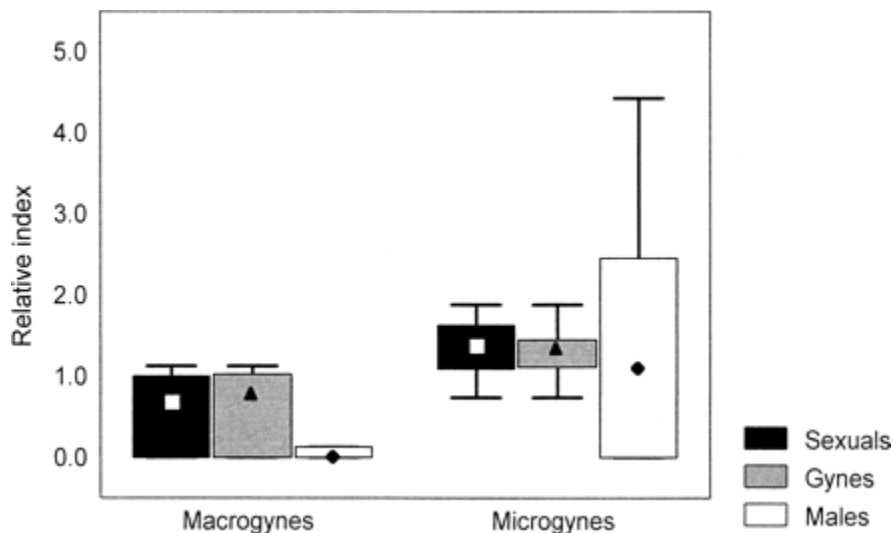


Figure 4 Microgynes significantly biased their offspring production toward sexual production relative to macrogynes in mixed colonies. The relative index (for calculation, see Methods) is a measure of the proportion of sexual offspring of a queen divided by the colony's proportion.

DISCUSSION

Reproductive skew

Johnstone (2000) called for comprehensive tests of reproductive skew theory which account for

all model assumptions and study the complete set of predictions generated by any particular model. Empirical progress, including this study, still falls short of this demand. Nevertheless, our study is an important contribution as one of the few explicit tests of skew models within social insects species (Reeve and Keller, 2001). All our results contradict predictions of transactional skew models (Keller and Reeve, 1994; Reeve and Ratnieks, 1993; Vehrencamp, 1983a, b), which are generally regarded as most applicable to social insects (Reeve et al., 1998, 2000; Reeve and Keller, 2001).

The distinction between sexual skew and overall skew is crucial because skew in worker production does not translate into fitness effects in the absence of worker reproduction (Bourke, 1988) and within-colony kin discrimination (Carlin et al., 1993; DeHeer and Ross, 1997; Keller, 1997). In contrast, differential production of sexuals directly translates into fitness differences among cohabiting queens. Although mechanisms are unknown, some evidence exists that ant queens differ in the caste ratios that they produce (Pamilo and Seppä, 1994; Ross, 1988). Consequently, skew in overall offspring production is not necessarily equivalent to skew in sexual offspring. This has also been found in our study, even though both skew indices were reasonably correlated across all colonies.

A further subdivision of overall skew into male, queen, and worker skew would have been appropriate in our analysis but was precluded by the small sample size. However, the cues that ant queens use for determining their relative reproductive share and for adjusting their behavior accordingly are currently unknown. Until egg laying as a probable cue is ruled out, it is important to report the skew over all offspring classes because it might reflect the queens' reproductive decision rules.

Multiple-queen groups in *Leptothorax* ants seem to fulfill the assumptions of the classic, transactional skew model and consequently have served as key evidence for their support (Reeve and Keller, 1995, 2001; Reeve et al., 1998). The existence of functionally monogynous species, in which only one of several potential queens reproduces in any given colony and linear dominance hierarchies are formed, demonstrates that recognition of individual status is possible and that one queen can fully inhibit the reproduction of remaining queens (Heinze and Lipski, 1990; Ortius and Heinze, 1999). It has also been demonstrated that assessment of individual reproductive output is possible (Ortius and Heinze, 1999). Although the assumption of a direct increase in reproduction by the presence of additional queens is not necessarily fulfilled [Bourke, 1991; Evans and Pierce, 1995; in *L. rugatulus*, queen number had a positive effect on productivity of field colonies in only three out of six studied populations (Rüppell et al., unpublished data)], there may be other cooperative benefits, such as increased genetic diversity in colonies (Cole and Wiernasz, 1999) or "assured fitness returns" due to prolonged colony longevity (Gadagkar, 1990; for a further discussion of benefits, see Bourke and Heinze, 1994). Furthermore, the cost of a subordinate queen departing is high when workers accompany her during colony budding (Bourke and Franks, 1995).

In clear contrast to the prediction of the transactional skew model, our study found a negative association between relatedness and sexual skew and no relation between relatedness and overall skew. However, it is important to note that the differences among colonies of the underlying relatedness estimates were not significant, and, thus, our conclusion is weakened by the possible

inaccuracy of these estimates. Given this provision, our results comply with the prediction of compromise models, although it is at present uncertain how well *L. rugatulus* meets the assumptions of these models.

On one hand, we have argued above that full control in *Leptothorax* colonies is at least theoretically possible. On the other hand, the correlation between relative egg-laying rate and relative reproduction found in this study suggests that high egg-laying rates are perhaps linked to indiscriminate egg cannibalism (Bourke, 1994). This behavioral scenario is a plausible mechanism for reproductive sharing according to compromise models. The artificially mixed colonies display higher reproductive skew, coupled with lower productivity, than natural colonies. This might also be interpreted as support for compromise models because they predict more conflict among less related individuals, which leads to lowered overall group productivity (Reeve et al., 1998). However, the low productivity might also be due to the mixing of the workers, which could result in inefficient division of labor or less cooperation among the workers.

Both transactional and compromise models of reproductive skew predict that macrogynes should dominate reproduction for various reasons. Body size is regarded as a good indicator of power across taxa (Andersson, 1994; Roff, 1992), and it also determines the outcome of conflicts among queens in some ant species (e.g., Nonacs, 1990). Therefore, microgynes should receive smaller peace incentives (Reeve and Ratnieks, 1993), provided that there is the risk of physical conflict. Additionally, they should receive smaller staying incentives because they are less likely to leave the colony and reproduce independently. Compromise models predict body size to be important for different reasons: body size is crucial for parameters that influence resource access and usage (nutrient storage, metabolism, pheromone production, and physical power). However, size did not influence the division of overall reproduction in *L. rugatulus*, and macrogynes actually incurred a fitness disadvantage in mixed colonies (see below). Body size did not influence the reproductive skew in the wasp *Polistes bellicosus* either (Field et al., 1998). Yet for both species, the significance of body size for competitive ability remains to be demonstrated. Provided that subordinates differ in features such as competitive ability, relatedness to dominants, and so on, predictions of skew models can become more complex in multi-member groups (Johnstone et al., 1999; Reeve and Emlen, 1999) than in groups with only two members. For this reason, we reduced our sample for additional analyses to colonies that only contained two queens. However, no new conclusions emerged from these analyses, perhaps due to our small sample size.

With an average skew index of 0.19 in sexual offspring and 0.06 in overall offspring, natural colonies of *Leptothorax rugatulus* display low reproductive skew compared to other social Hymenoptera. We found that reproductive skew was significantly higher in the randomly composed colonies. This suggests that strong reproductive biasing (relative to naturally occurring skew) is possible in *L. rugatulus* when individuals perceive others as alien. It is unlikely that workers were responsible for the reproductive skew by favoring one related queen (nepotism) because all queens were equally unrelated to the workers in this setup. Thus, our results favor the view that queens could be directly responsible for differences in their reproduction and not workers. Although we presented the ants with an extreme, artificial situation and mortality was high during the initial 2 weeks after their mixing, colonies seem to function during the

experimental period: they successfully raised sexual and worker brood, and mortality after the establishment phase was comparable to unmanipulated colonies. However, we cannot exclude the possibility that the treatment itself affected the reproductive patterns in these randomly composed colonies.

To realistically explain reproductive (sexual) skew in *Leptothorax rugatulus*, one could conceivably build a more complex model to integrate the species' general biology and our present results. Species-specific skew models would be the next logical step from the proliferating refinements and variations of skew models in the literature. However, with an increasing number of models, skew theory risks losing its general predictive power and thus its main scientific appeal (Keller and Reeve, 1994). Johnstone (2000) claimed that the different skew models were only variants of the same underlying principle, but the question arises what generality skew theory conveys beyond the theory of kin selection (Hamilton, 1964a,b).

Sexual bias

In 30% of all colonies, individual queens differed significantly in their tendency to produce male, worker, and young queen (gyne) offspring. This was partly explained by microgynes producing relatively fewer workers and more gynes than macrogynes, although some microgynes also overproduced males. No significant differentiation among macrogynes was observed.

Exclusive male production by inseminated queens could be due to unviable sperm or mate incompatibility (Godfray, 1990). Alternatively, queens may refrain from fertilizing their eggs to increase their relative sexual production and thus their contribution to the future gene pool. So far, this potential form of selfishness in the social Hymenoptera has received little attention.

A differential contribution of individual queens to gyne and worker production has only been reported for the ants *Solenopsis invicta* (Ross, 1988, 1993) and *Formica sanguinea* (Pamilo and Seppä, 1994). However, in neither case could these differences be related to proximate or ultimate reasons. In *L. rugatulus*, different caste ratios of macro- and microgyne off-spring were mainly responsible for differential queen contributions. A reduced body size to favor development as gynes has been found in socially parasitic ant species (Aron et al., 1999; Nonacs and Tobin, 1992), and it has also been predicted in the intraspecific context of kin conflict over caste determination (Bourke and Ratnieks, 1999).

However, in our laboratory-reared, mixed colonies, daughter queens of macrogynes were not significantly larger than daughter queens of microgynes (data not shown). Even though similar in size, female larvae derived from microgynes developed more often into gynes than female larvae from macrogynes in the same physical and social environment. This suggests that caste determination in *Leptothorax rugatulus* is, at least partly, determined by mother queens. The influence could be maternal (blastogenic) or genetic. Furthermore, the size similarity between offspring of macrogynes and microgynes suggests that the higher gyne/worker ratio produced by microgynes is not due to selfish caste determination of the larvae facilitated by their smaller body size (Bourke and Ratnieks, 1999). Rather, the difference in caste ratios between macro- and microgynes could be an adaptation to their different reproductive tactics. Macrogyne, mainly founding independently, have to produce workers for colony growth before producing sexuals.

Microgynes, in contrast, mainly readopting into their mother colonies, can produce sexuals from the onset of their reproductive life.

Thus, microgynes have a fitness advantage over macrogynes in mixed colonies, and their usage of the workforce provided by the macrogynes amounts to intraspecific social parasitism.

However, because mixed colonies are rare in natural populations (Rüppell et al., 1998), we interpret the evolution of microgynes in *L. rugatulus* not as an adaptation to intraspecific social parasitism but rather as an adaptation to dependent reproduction (Rüppell et al., 2001). Overall, colonies with microgynous queens do not produce higher gyne/worker ratios than colonies with macrogynes (Rüppell et al., unpublished data). At the colony level, *L. rugatulus* produces caste ratios that are comparable to other *Leptothorax* species (e.g., data in Chan et al., 1999) and that are probably worker controlled.

In conclusion, we have to caution that our results might present artifacts of our experiments because we did not measure lifetime reproduction of queens, which is a practical problem in social insects in general due to the long life spans of reproductives (Keller, 1993a; Ross, 1988).

APPENDIX

Raw data of offspring produced and genotyped in the unmanipulated colonies (ors4-ors34) and the artificial groups (sk1-sk29)

Colony	Old queens	Workers produced	Males produced	New queens produced	Workers genotyped	Males genotyped	New queens genotyped
ors4	3	154	30	0	23	6	0
ors7	2	143	114	0	20	5	0
ors8	3	64	5	0	17	5	0
ors13	3	32	4	38	15	0	17
ors16	2	60	20	12	12	13	7
ors18	3	42	5	26	10	5	22
ors22	5	25	48	1	18	8	0
ors26	3	28	22	12	14	11	6
ors27	3	42	0	34	9	0	20
ors28	2	228	30	62	10	0	15
ors34	2	106	58	4	13	5	2
sk1	4	30	4	5	20	0	4
sk2	4	36	0	11	18	0	7

sk7	4	17	0	21	17	0	18
sk10	4	56	0	23	19	0	19
sk12	4	24	1	7	14	0	7
sk13	2	40	0	4	15	0	1
sk16	4	13	7	12	12	7	12
sk18	2	24	0	21	8	0	17
sk20	2	11	4	10	10	4	10
sk25	3	10	3	6	8	2	6
sk26	3	17	2	25	15	0	22
sk29	4	18	6	13	12	6	11

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