

Colony structure and reproduction in the ant, *Leptothorax acervorum*

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We analyzed the sociogenetic organization of the ant (*Leptothorax acervorum*) from Nürnberger Reichswald in Southern Germany. According to relatedness estimates from allozyme analyses, virgin female sexuals produced in polygynous colonies were on average full sisters, whereas workers in a pooled sample of polygynous colonies were significantly less closely related. Rather than attributing this to reproductive hierarchies among nest mate queens, we show how this phenomenon could result from seasonal fluctuations of colony composition and a decline of the production of female sexuals in polygynous colonies. We suggest that by queen adoption and emigration or budding, colonies easily switch from monogyny to polygyny and vice versa. Due to the long developmental time of sexual larvae, colonies that have become polygynous only recently will still produce the female sexual progeny of a single queen. In older polygynous nests, fewer and fewer female sexuals are produced, but colonies may fragment into monogynous buds in which the production of female sexuals may begin again. Relatedness estimates, dissection results, and field observations support this suggestion. This pattern of cyclical monogyny and polygyny keeps nest mate relatedness high and probably facilitates colony founding in boreal habitats. Preliminary data suggest that the pattern of the production of sexuals in colonies of *L. acervorum* fits the expectations of sex allocation theory. **Key words:** Formicidae, Hymenoptera, *Leptothorax*, polygyny, population structure, relatedness, reproductive success. [*Behav Ecol* 6:359–367 (1995)]

Cooperative breeding in animals raises conflicts among group members about the partitioning of resources and the chances to reproduce. In many social vertebrates, reproductive conflicts are resolved by the formation of social hierarchies in which rank is correlated with reproductive success (Wilson, 1975). In contrast, many societies of eusocial insects, e.g., ants or termites, are free of overt antagonism among nest mate reproductives (Heinze et al., 1994; Hölldobler and Wilson, 1990; Wilson, 1971). Several fertile queens can coexist within the colonies of approximately half of all ant species (Buschinger, 1974a; Hölldobler and Wilson, 1977, 1990). In such “polygynous” colonies, egg-laying rates may differ to some extent among nest mate queens (e.g., Bourke, 1991, 1993; Keller, 1988; Mercier et al., 1985), but uneven reproduction appears to be caused mainly by intrinsic physiological factors rather than dominance hierarchies. Indeed, the queens typically ignore each other or interact amicably, and aggressive interactions are limited to a small number of species (Heinze, 1993a; Heinze and Smith, 1990).

Egg-laying rate, however, is not a good indicator of actual reproductive success (Keller, 1993). Not only may eggs be eaten by other queens, workers, or larvae (Bourke, 1994; Crespi, 1992), they may also develop into nonreproductive workers rather than sexual offspring. In the fire ant (*Solenopsis invicta*), studies employing genetical markers demonstrated that some queens had a larger share than others in the female sexuals produced in polygynous societies (Ross, 1988, 1993). The proximate cause of this reproductive variance remained obscure.

We present data that suggest inequities in reproduction among queens in polygynous colonies of the ant *Leptothorax acervorum*. These appear to result from seasonal changes in colony structure and an inverse correlation between individual reproductive success and the number of queens per colony rather than reproductive hierarchies.

Due to their local abundance and small colony size, *Leptothorax* ants are attractive subjects for population level studies (Buschinger, 1968; Herbers, 1984, 1986b, 1990; Talbot, 1957; Wilson, 1974). *L. (s.str.) acervorum* is an inconspicuous, facultatively polygynous ant common in boreal and alpine coniferous forests throughout the northern hemisphere (Buschinger, 1968; Heinze and Ortius, 1991; Ito, 1990). Researchers using allozyme markers have previously shown that in polygynous colonies several queens contribute to the colony's workforce (Douwes et al., 1987; Lipski et al., 1992; Stille et al., 1991). Nevertheless, the relatedness among both nest mate queens and nest mate workers was high with respect to the number of queens present in polygynous colonies. We extend the relatedness studies to female sexuals produced in polygynous and monogynous colonies of *L. acervorum* in the population of Nürnberger Reichswald. Furthermore, based on relatedness estimates, population censuses, and behavioral observations, we suggest that discrepancies between observed queen numbers and nest mate relatedness can be explained by a life history with a cyclical pattern of polygyny and monogyny.

MATERIALS AND METHODS

Between 1990 and 1993, about 400 complete colonies of *L. acervorum* were aspirated from their nests in rotting pine sticks in Nürnberger Reichswald in southern Germany. The ants were transferred to the laboratory in glass vials or plastic tubes (Buschinger and Heinze, 1988) and censused immediately after collecting. We dissected dealate queens to check their reproductive status following a method described by Buschinger and Alloway (1978). For allozyme analyses, we crushed live or frozen (−20°C) individual adults, pupae, and larvae in 40 µl homogenization buffer (either 0.5 M Tris/HCl pH 8.0, 10% glycerine, 0.01% bromothymol blue, or 0.1 M Tris/HCl pH 8.0, 1 mM EDTA, 0.05 mM NADP, 2 mM β-Mercaptoethanol, 10% glycerine, 0.01% bromothymol blue, which both gave similar results). We applied five to 10 µl of the crude homogenate to 12.5-cm long vertical 7.5% polyacrylamide slab gels (gel buffer: 0.125 M Tris/HCl pH 8.0;

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Table 1

Electromorph frequencies in two enzymes in subsamples of colonies of *Leptothorax acervorum* from Nürnberger Reichswald

	GPI					PGM-1			
	v	s	m	f	x	v	s	m	f
Monogynous colonies									
Female sexuals	0.000	0.046	0.842	0.096	0.016	0.000	0.548	0.424	0.028
Workers	0.005	0.067	0.819	0.104	0.006	0.019	0.474	0.404	0.102
Males	0.000	0.049	0.822	0.118	0.010	0.011	0.390	0.424	0.175
Polygynous colonies									
Female sexuals	0.000	0.103	0.784	0.113	0.000	0.000	0.422	0.441	0.137
Queens	0.000	0.042	0.785	0.166	0.007	0.000	0.433	0.385	0.182
Workers	0.002	0.049	0.822	0.118	0.010	0.011	0.390	0.424	0.175
Males	0.000	0.000	0.963	0.037	0.000	0.000	0.143	0.571	0.286

The different electromorphs are named according to their relative migration velocity in 7.5% polyacrylamide gels (pH 8.0), v (very slow), s (slow), m (medium), f (fast), and x (very fast). Colonies with different sample size were weighted according to Pamilo (1990).

running buffer 0.16 M glycine, 0.025 M Tris, pH 8.3). Proteins were separated at 10°C with 10 mA per gel for 1.5 h. Eighteen enzymes were stained using slightly varied standard recipes (Murphy et al., 1990). Phosphoglucomutase-1 (*PGM-1*, EC 5.4.2.2; four electromorphs) and Glucose-6-phosphate isomerase (*GPI*, EC 5.3.1.9; five electromorphs) showed enough variation to be suitable for the calculation of average nest mate relatedness. In most colonies, *GPI* and *PGM-1* phenotypes of at least 10 workers were analyzed. The number of queens and virgin female sexuals analyzed per colony ranged from two to 20. To exclude any effects of laboratory rearing, we analyzed only individuals already present as adults or late instar larvae when collected. A total of 231 males from 35 colonies (between one and 11 males per colony) were analyzed to detect diploid males and to confirm that enzyme polymorphism is genetically based.

We calculated average relatedness, the regression coefficient between the genotypic values of the individuals concerned, from allozyme frequencies with reference to allozyme frequencies in the respective sample of colonies. For calculation, we used modified versions of computer programs by P. Pamilo, based on the algorithm by Pamilo (Equation 4 in Pamilo, 1984, 1990). Colonies were weighted for unequal sample size (Pamilo, 1990), and standard errors were obtained by jackknifing (Pamilo, 1990). Deviation from panmixis was calculated from the difference between observed and expected heterozygosity (fixation index $F = (h_{exp} - h_{obs})/h_{exp}$) and test-

ed separately for loci according to Seppä (1992, 1994), i.e., using $\chi^2 = (k - 1) N \bar{p}^2$ with $df = k(k - 1)/2$, in which k is the number of alleles, and N is the sample size, i.e., the total number of individuals in the sample of queens from polygynous colonies and the number of colonies multiplied by the "effective number of mothers" in the samples of female sexuals and workers. The effective number of mothers was calculated from relatedness estimates as described by Queller et al. (1988), Ross (1993), and Seppä (1994). Standard errors of the F_{ST} value were calculated by jackknifing over subsamples (Queller et al., 1992). Between-group relatedness was calculated with a computer program by P. Pamilo, based on the algorithm by Pamilo (Equation 10 in Pamilo, 1984).

Differences between relatedness estimates were tested using a two-tailed t test, based on the assumption that jackknifed means are normally distributed. Differences between worker, queen, or male numbers were tested using the Mann-Whitney U test (Sachs, 1992).

We determined the dry weight of male and female sexuals with an electronic microbalance after drying individuals in an oven at 60°C for 24 h (Chan and Bourke, 1994).

RESULTS

Mating structure

In total, we analyzed 490 queens and 1420 workers from field colonies of *L. acervorum* in this study. Electromorph frequencies are given in Table 1. The fixation index (F) was lower than zero in nine out of 10 groups, though not significantly so (Table 2). A significant deviation was observed in *PGM-1* in queens from polygynous colonies, which appears to result from an excess of *PGM-1^{ms}* heterozygotes and a deficiency of *PGM-1^m* queens. Similarly, seven of eight fertile queens from monogynous colonies had the *PGM-1^{ms}* phenotype. This might result from differences in allele frequencies between females and males (Table 1; see also Pamilo, 1993). Allele frequencies did not differ strongly between castes or colonies of different social organization. The F_{ST} value obtained for female castes was $0.01 \pm SE 0.019$ (95% confidence interval -0.04 - 0.06).

Of 231 males, which were analyzed electrophoretically, none had a heterozygous banding pattern. Hence, there was no evidence of male diploidy which might have arisen due to inbreeding (e.g., Cook, 1993). To analyze the mating frequency of queens, we isolated 19 queens in laboratory nests with 10 workers but without brood. From the worker pupae reared in these colonies (five to 12 pupae per queen), we

Table 2

Inbreeding coefficients (F) and chi-square values (χ^2) for subsamples of *L. acervorum* from Nürnberger Reichswald

	GPI		PGM-1	
	F	χ^2	F	χ^2
Monogynous colonies				
Female sexuals	-0.130	1.01	-0.341	6.98
Workers	-0.035	0.45	-0.071	1.38
Polygynous colonies				
Female sexuals	-0.024	0.08	0.032	0.09
Workers	-0.025	0.40	-0.154	11.24
Queens	-0.116	5.77	-0.387	42.83

Deviation from random mating is statistically significant only in *PGM-1* from queens in polygynous colonies ($df = 3$, $p < .001$). The number of alleles (and thus also the degrees of freedom in the chi-square test) varied between the subsamples.

Table 3
Allozyme phenotypes of workers produced by single queens in the laboratory

Colony	GPI	PGM-1
A	10mm	6ss 4sf
B	7mm 2mf	5ss 5sf
C	8sm	5sm 2mf
9a	4mm 3mf	7sm
9d	6mm	6mf
9e	4mm 6mf	10sm
9f	8mf	8mf
9g	7mm 1mf	8mf
9h	5sm 2mm	7sm
9i	1mm 6mf	7sm
9j	7sm 1mm	8sm
45a	9mm	9mf
45b	8mm	8sf
45c	8mm	8sf
45d	7sm 5mm	5sf
45e	6mm	6sf
X52	4sm 4sf	8ss
X54	11mm	2ss 8sm
XI48	1ss 8mm	4mm 5mf

estimated a relatedness of 0.79, SE 0.041 (95% confidence limits 0.70–0.88), suggesting single mating. In each case, the enzyme phenotypes of the offspring workers were consistent with single mating (Table 3). The enzyme phenotypes of males and females from the same colonies also corroborated the assumption that the observed enzyme variation is genetically based and heritable.

Relatedness estimates of workers and fertile queens

Relatedness estimates are given in Table 4. In the following, we compare the combined relatedness estimates from GPI and PGM-1 between different subsets of colonies.

The relatedness estimate for nest mate workers in polygynous colonies (0.46 ± 0.040) was significantly lower than that for nest mate workers in monogynous colonies (0.55 ± 0.040 ; two-tailed *t* test, *df* = 138, *t* = 2.05, *p* < .05). The mean relatedness estimates fell off with increasing queen number (Figure 1); however, the estimate for workers from two-queened colonies ($n = 21$, $r = 0.51 \pm 0.089$) was not significantly different from that of workers from colonies with five

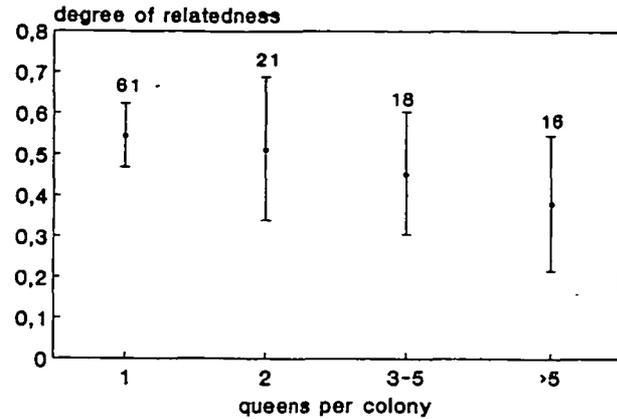


Figure 1
Mean estimate (\pm SE) of regression relatedness among nest mate workers in relation to queen number in colonies of the ant (*Leptothorax acervorum*) from Nürnberger Reichswald. Numbers above each point represent the number of colonies sampled.

and more queens ($n = 16$, $r = 0.38 \pm 0.084$; *df* 35, *t* = 1.52, *p* > .1). Despite presumed single mating, the relatedness estimate for nest mate workers in monogynous colonies was distinctly lower than the value expected for Hymenopteran full sibs (0.75; *df* = 60, *t* = 5.1, *p* < .0001).

The relatedness among fertile nest mate queens (0.63 ± 0.057) was significantly higher than that among workers in the total sample of polygynous colonies (0.46 ± 0.040 ; *df* 110, *t* = 3.53, *p* < .001).

As queens mate singly, the actual number of matriline (the "effective number of mothers," N_e) can be calculated from the relatedness estimates of workers and queens using

$$N_e = (3 - r_q) / (4r_w - r_q)$$

where r_q is the relatedness among mother queens and r_w is the relatedness among nest mate workers.

Assuming that all queens belong to a single generation and that matriline are equally represented within colonies, the formula gives the minimum number of matriline actually present per colony. In monogynous colonies of *L. acervorum*, this number was considerably higher than one (ranging between 1.2 and 2.0, depending on whether the lowest or highest values in the 95% confidence intervals of r_q and r_w were used for calculation), indicating that not all workers in mo-

Table 4

Relatedness estimates (jackknifed mean, standard errors, and 95% confidence limits) obtained from electromorph frequencies for workers, fertile queens and female sexuals in colonies of *L. acervorum* from Nürnberger Reichswald

	Relatedness \pm SE			
	GPI	PGM-1	Total	95% CI
Monogynous colonies				
Workers ($N = 61$, $n = 661$)	0.47 \pm 0.075	0.59 \pm 0.056	0.55 \pm 0.040	0.47–0.63
Female sexuals ($N = 20$, $n = 194$)	0.50 \pm 0.154	0.60 \pm 0.108	0.58 \pm 0.076	0.42–0.74
Polygynous colonies				
Workers, total ($N = 79$, $n = 759$)	0.41 \pm 0.056	0.49 \pm 0.040	0.46 \pm 0.040	0.38–0.54
Workers, prod. col. ($N = 21$, $n = 232$)	0.58 \pm 0.085	0.61 \pm 0.082	0.60 \pm 0.060	0.47–0.72
Workers, nonprod. col. ($N = 12$, $n = 105$)	0.37 \pm 0.100	0.39 \pm 0.124	0.38 \pm 0.095	0.17–0.59
Female sexuals ($N = 22$, $n = 145$)	0.52 \pm 0.171	0.75 \pm 0.092	0.66 \pm 0.085	0.49–0.84
Fertile queens ($N = 33$, $n = 143$)	0.55 \pm 0.091	0.68 \pm 0.080	0.63 \pm 0.057	0.51–0.74

N and n stand for the number of analyzed colonies and individuals, respectively. "Prod." and "nonprod. col." are colonies that either produced or did not produce female sexuals in the rearing period after collection.

Table 5

Colony composition, brood sex ratio, and allozyme phenotypes of queens, workers, and male and female sexuals in colonies of *L. acrororum*

Colony	Queens	Workers	Sex ratio	Queens	Workers	Males	Female sexuals
I 10	12	91	3:1	2sm 6mm 6sf	1vm 3sm mm 2mf 1sm 6sf mm	1m 1f	3mf 3sm
I 27	1	116	3:9	1mm 1sm	1mm 6ss 11sm	9m 5s 4m	3mm 1ss 2sm
I 38	20	181	4:1		10mm 6ss 4sm	1m 1f	4mm 3ss 1sm
I 90	3	97			9sm 1mm 9sf 1mf		2sm 2sf
I 93	2	106			2sm 8mm 9sm 1mm	7m 1f 8m	8sm 8sm
I 107	5	21		5mm	6sf 2mm		2mm
I 121	53	269		1ss 1sm 1mm 2mf	4sm 1mm 3mf 4sm 11mm		2sm 1sm 8mm
VI 35	1	230	19:9	1mm 1sm	1mm 13mf 8ss 6sm	9m 1s 8m	19mf 9ss 10sm
VI 38	20	181	4:1		10mm 6ss 4sm	1m 1m	4mm 4sf
VI 44	2	86		1mf 1ff 1sf 1mf	11mf 3ff 7sf 5mf		9mf 9mf
VI 51	2	78		2mm 2sf	17mm 14sf		2mm 2sf
VI 63	1	226	19:9	1mm 1sm	15mm 1ss 7sm 5mm	9m 7s 2m	17mm 2mf 8sm 11mm
VI 64	1	272	12:11	1mf 1sm	13mm 7mf 20ss	6m 5f 10s 1m	mm 5mf 7ss 5sm
VI 66	6	119		6mm 3mf 3ff	15mm 1sf 17mf	3m 3m	2sm 10mm 2sm 1sf 3mm 3ff
VI 67	1	170	26:11	1mm 1sm	12mm 12sm	11m 7s 4m	26mm 26sm
VI 68	1	92	2:1	1sm 1mf	7sm 10mm 6ss 4sm	1m 1m	1sm 1mm 1sf
VII 34	4	81	0:0	3mf 3ms	5mm 2mf 1ss 4sm 1mm		
X 7	2	91	18:0		8mm 2mf 9sm		2sm 2sf 7mm 9mf 16sm 1mm
X33	3	57	0:0	2ms 1mm	2smm 2mm 1sm 1mf		
X34	3	57	0:0		9mm 3sm 3sf 1ff		
X46	3	16	0:0		1mm 5mf 3sm 2mm		
X 57	2	221	0:0		1sm 11mm 2ss 6sm 4mm		
X70	3	113	0:0	2sm 1ss 1ff	7mm 6mf 3sm 3sf 4mm		
X 78	4	66	3:5	1mm 1sm	10mm 1mf 6sm 4mm 1mf		2mf 2mm
X 79	3	147	7:19		7mf 1ff 5mm 5mf	1m 1m	2ss 4mm 7mm
X 81	3	84	0:0		1sm 9mm 6sm 2mm		
X 82	4	93	0:0		15mm 7vm 3sm 1mm		
X 89	2	34	0:0		6mm 3sm 1sf 1mm 1mf		
X 92	8	67	2:0				2sm 2ss
X 100	3	386	0:5		4mm 5mf 6ss 4sf		
X 101	4	137	3:1	1mm 2mf 3mm	16mm 12sm		
X 105	2	38	12:1	1mm 1mf 1ss	10mm 2smm 2sf 5mm		12mm 8ss 1sf
XI 2	2	52	3:0		1sm 12mm 1sm 2sf 3mm 1mf 1ff		
XI 3	4	56					3mm 2sf 1mm
XI 5	4	121	7:0		7mm 7mmm		7mm 5sm 1mf

Table 5,
Continued

Colony	Queens	Workers	Sex ratio	Queens	Workers	Males	Female sexuals
XI 7	2	64	1:0				
XI 19	8	84	0:0		6mm 4sm 2mm		
XI 22	3	100	4:0		9mm 1mf 10sf		4mm 4sf
XI 23	6	141	10:0		3mm 5mf 1sm 2sf 2mf 4ff		3mm 7mf 9sm 1mm
XI 24	3	21	5:28		6mm 4mf 1sm 9mm	1m 1m	3mm 2mf 5mm
XI 26	4						1sf 1mm 2sm
XI 27	7	9	10:0	3mm 1ss 1mm	9mm 9mm		10mm 4sm 6mm
XI 35	2		11:0		1sm 9mm 1mf 3ss 2sm 4sf		11mm 4ss 2sm 1mm
XI 50	5		0:0		1sm 2mm 5mf 3sf 1mf 4ff		

Only those colonies are listed which were used to calculate the relatedness of workers in those colonies that definitively produced or did not produce female sexuals during the first breeding period following collection or that were used to calculate relatedness asymmetries and sex ratios (females:males). GPI phenotypes are listed in the first line, PGM-I phenotypes in the second line of each colony.

nogynous colonies were offspring of the single present queen. In polygynous colonies, the effective number of mothers (1.5 to 2.9) was lower than the observed queen number ($q_a = 3.2$). However, it must be noted that polygynous nests of *L. acervorum* probably contain queens belonging to several generations, i.e., mothers and daughters. Thus, worker offspring of queens may be related through the mate of the original queen (Queller, 1993), and the real number of mothers might be larger than the calculated effective number.

Relatedness estimates of virgin female sexuals

Female sexuals were available for electrophoresis only from a smaller subset of our population sample (for relatedness estimates and electromorph phenotypes see Tables 4 and 5). The relatedness estimate for female sexuals produced in monogynous colonies (0.55 ± 0.040) was not different from that for workers in the total of monogynous colonies (0.58 ± 0.076 ; $df = 79$, $t = 0.65$, $p > .2$).

Female sexual offspring produced in polygynous colonies were closely related (0.66 ± 0.085). Their relatedness estimate did not differ from the value for female sexuals in monogynous colonies ($df = 40$, $t = 1.04$, $p > .2$), but was significantly higher than the estimate for worker relatedness in the total sample of polygynous colonies (0.46 ± 0.040 ; $df = 99$, $t = 3.76$, $p < .001$, but see below).

The actual number of inseminated and fertile queens present in those polygynous colonies that produced female sexuals varied between two and 43 (22 colonies: arithmetic mean $q_a = 6.4$, harmonic mean $q_a = 3.3$); the average queen number did not yield female sexuals (two to six queens, 12 colonies: $q_a = 3.3$, $q_a = 3.0$; U test, $z = 0.57$, $p \gg .2$). Similarly, worker numbers did not differ significantly between the two samples (producers: 21 colonies, arithmetic mean $w_a = 91.4$; nonproducers: 11 colonies, $w_a = 110.5$; $z = 0.159$, $p \gg .2$; worker numbers were not available for all colonies). Interestingly, the mean relatedness of workers was significantly higher in colonies that produced female sexuals than in nonproducer colonies ($df = 31$, $t = 2.86$, $p < .01$). In producer colonies, the relatedness estimates did not differ significantly between workers and female sexuals ($df = 41$, $t = 0.91$, $p > .2$).

Compared to monogynous colonies, very few males were produced in our polygynous colonies. The number of males produced in those polygynous colonies that also reared female sexuals was not significantly different from the number of males produced in colonies without female sexuals (producers: 14 colonies, arithmetic mean $m_a = 3.9$; nonproducers: 12 colonies, $m_a = 1.0$; U test, $z = 0.988$, $p > .3$; for comparison: 10 monogynous colonies $m_a = 9.3$). The data do not suffice for a detailed study of male parentage.

Though our study was not initially devised to test sex allocation theory and we therefore did not count or analyze all sexuals electrophoretically, a referee (J. J. Boomsma, Århus) pointed out that even with a limited sample relatedness asymmetries that might be underlying different sex allocation ratios could be calculated. We therefore divided all colonies for which sex ratios and the genotypes of workers, virgin queens, and males were known in two groups with female-biased or male-biased sex ratio, regardless of queen number. For these groups, we calculated the average regression relatedness of workers toward virgin queens and males, mean relatedness

Table 6

Between-group relatedness (jackknifed mean and standard errors) between workers and virgin queens (r_{wv}) and workers and males (r_{wm}), relatedness asymmetry (Boomsma and Grafen, 1990), and the mean sex ratio in colonies with female- or male-biased sex ratio

	Female-biased sex ratio	n	Male-biased sex ratio	n
Relatedness workers to virgin queens (r_{wv})	0.48 ± 0.132	6	0.46 ± 0.401	3
Relatedness workers to males (r_{wm})	0.03 ± 0.287	6	0.33 ± 0.287	3
Relatedness asymmetry	14.9		1.4	
Numerical sex ratio (females/males)	2.8 ± 0.96	6	0.3 ± 0.10	

asymmetries, and mean sex allocation ratios (Table 6). Male and female sexuals were similar in dry weight, hence numerical sex ratios were used to estimate sex allocation ratios (68 males: 0.47 ± 0.093 mg; 46 females: 0.46 ± 0.03 mg). The relatedness of workers toward female sexuals was slightly higher than that of workers toward males in colonies with a female-biased sex ratio (two-tailed t test, $df = 10$, $t = 1.99$, $.05 < p < .1$), but not so in colonies with a male-biased sex ratio ($df = 4$, $t = 0.37$, $p > .2$). This trend is probably caused by the highly limited number of colonies with a male-biased sex ratio for which relatedness data were known.

Ovarian development of queens

We dissected all dealate queens before electrophoresis to evaluate the presence of sperm in their spermatheca and the development of their ovaries. However, the color of the fat body, which reflects the age of the queens (Buschinger, 1968), was noted only in the few queens dissected toward the end of our experiments. Of 15 fertile queens in polygynous colonies that had produced female sexuals, seven had a whitish fat body and only few corpora lutea (remnants of eggs already laid), suggesting they were adopted only the year before. In contrast, of 21 queens in polygynous colonies that had not produced female sexuals, only one was a young queen; the fat body of 20 queens was yellow (2×2 test of independence, $df = 1$, $G_{adj} = 8.78$, $p < .005$).

Seasonal fluctuations of the colony structure

A census of 348 colonies suggested that the Reichswald population undergoes slight seasonal changes in average worker and queen numbers. Colonies collected in spring and summer (winter: 195 colonies, arithmetic mean $w_s = 73.9$; summer: 153 colonies, $w_s = 51.1$; $z = 0.0258$, $p < .03$, Figure 2a). Furthermore, colonies collected in fall and winter had more queens than colonies collected the following spring and early summer (Figure 2b). The frequency distribution of queen numbers differed significantly between seasons (winter; 195 colonies, $q_s = 3.3$; summer: 153 colonies, $q_s = 1.8$; chi-square test, $df = 4$, $\chi^2 = 30.73$, $p < .001$).

DISCUSSION

We analyzed the sociogenetic patterns of the facultatively polygynous ant *Leptothorax acervorum* in a population in southern Germany. The following conclusions can be drawn from censuses and relatedness estimates for workers, fertile queens, and female sexuals. Queens apparently mate only with a single male. Nevertheless, workers in singly queened field colonies were not all full sisters. The number of fertile queens actually present in multiply queened colonies was higher than the effective number of mothers calculated from relatedness estimates of nest mate workers. Surprisingly, female sexuals produced in polygynous colonies were on average full sisters, but nest mate workers in a pooled sample of polygynous colonies were on average offspring of a minimum of two mothers.

A first examination of our data seems to indicate an unequal contribution of nest mate queens in polygynous colonies to queen- and worker-destined brood. Such reproductive skew has been reported from the fire ant (*Solenopsis invicta*; Ross, 1988, 1993), where it was suggested that either some queens lay eggs with a larger innate queen-bias or workers preferentially rear female sexuals from the eggs of some mothers rather than others. The pattern in *L. acervorum*, however, is probably better explained by an interplay of seasonal

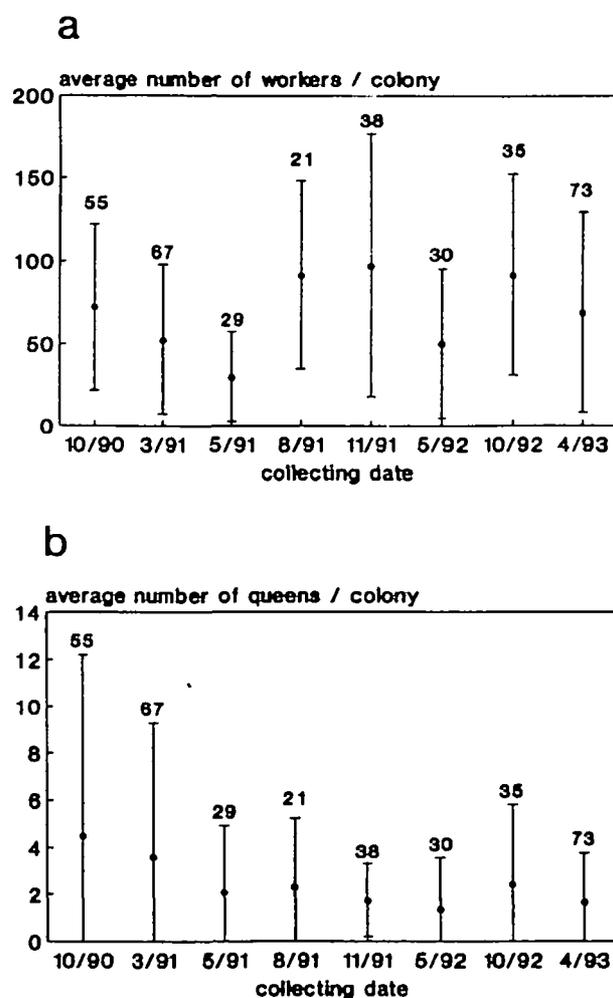


Figure 2
Average number (\pm SD) of workers (a) and queens (b) in colonies of *Leptothorax acervorum* in different collecting seasons. Colonies collected in winter and fall are significantly larger than colonies collected in summer. In (a), numbers above each point represent the number of colonies sampled; sample sizes for (b) are the same as in (a).

changes in colony composition and an inverse correlation between queen number and the production of female sexuals.

In particular, we suggest, firstly, that colonies of *L. acervorum* may easily switch from monogyny to polygyny by the adoption of young queens and budding or queen emigration. Secondly, we suggest that the production of female sexuals is strongly reduced in polygynous colonies and the majority of female sexuals are reared in monogynous colonies. In *L. acervorum*, female sexuals develop from larvae that hibernate at least once, whereas workers may develop within a single summer (Buschinger, 1967, 1974b). Therefore, when a first generation of young female sexuals is readopted in a monogynous colony, the second generation will already be in a late larval instar. During the next summer, female sexuals will eclose in the now polygynous colony, which nevertheless are all progeny of a single mother queen. Older polygynous colonies produce few female sexual offspring but may eventually fragment into smaller, monogynous buds, in which the cycle begins anew. Alternatively, queens could emigrate solitarily to found new colonies. This life history of cyclical monogyny and polygyny

keeps nest mate relatedness on a high level and probably facilitates colony founding by budding.

The suggested seasonal fluctuations in colony structure are well substantiated. According to field studies and laboratory observations, colony composition is unstable in many *Leptothorax* species (Alloway et al., 1982; Heinze, 1993b; Heinze et al., 1992; Herbers, 1989; Herbers and Tucker, 1986; Lipski et al., 1994). Young queens are regularly adopted in their colonies of origin after mating in late summer, and some emigrate from the colony after hibernation, probably attempting to found their own colonies solitarily or by budding. Though budding in *L. acervorum* has never been observed directly in the field, its occurrence is strongly suggested by the distribution of mitochondrial DNA lineages in a Swedish population (Stille and Stille, 1993), well-ordered nest-moving behavior (Möglich, 1978), and occasionally observed spontaneous budding in laboratory colonies (unpublished). Furthermore, the seasonal changes of colony size in the Reichswald population reported here lend additional support to the budding hypothesis: the emigration of workers and queens lowers colony size in spring, whereas colony growth and the adoption of new queens during summer increases colony size before hibernation. Seasonal fluctuations of queen numbers might also explain why the relatedness estimate for workers in monogynous colonies was significantly lower than expected from the presence of a single queen. As already proposed by Stille et al. (1991) for Swedish *L. acervorum*, some monogynous colonies must recently have been polygynous. On the other hand, colonies that were polygynous when collected might have been monogynous the year before. In several northern temperate *Leptothorax* (*Myrafant*) (Alloway et al., 1982; Herbers, 1989; Herbers and Tucker, 1986), colonies may divide into subunits in spring and fuse again for hibernation instead of forming permanently separate buds. Behavioral experiments (Heinze, unpublished) showed that buds of *L. acervorum* that had been separated in the laboratory for five months merged after initial weak aggression. Thus, colony fusion for hibernation appears possible, though little other evidence exists to support this.

We also assume that polygynous colonies rear significantly fewer female sexuals than monogynous colonies. Buschinger (1967, 1974b) analyzed the production of female sexuals in colonies from Nürnberger Reichswald and found that polygynous colonies yielded about five times fewer young female sexuals per mother than monogynous colonies. In a more detailed analysis, Hülsen (1990) observed a decline of per capita production of female sexuals with increasing queen number.

According to the suggested life history, adult queens in polygynous colonies are mainly offspring of a single mother queen. In fact, our analysis yielded a high relatedness estimate for nest mate queens (0.63). Furthermore, if significant numbers of female sexuals are produced only in colonies that recently have become polygynous, then workers in these colonies should mainly be offspring of a single queen. The relatedness estimate for workers from polygynous colonies that produced female sexuals was indeed significantly higher than for workers from those polygynous colonies that did not produce female sexual offspring. Finally, the finding that more young fertile queens were present in polygynous colonies that produced female sexual offspring than in colonies that did not additionally supports the life history we propose.

We therefore conclude that in a large percentage of colonies of *L. acervorum* from Reichswald, coexistence among queens does not extend to the production of female sexuals, but is a transient stage probably preceding budding. As our results show, an analysis of the sociogenetic consequences and the ecological significance of queen number of social insects remains incomplete as long as the origin of sexuals is un-

known. The traditional view of a dichotomy between monogyny and polygyny may be misleading; in many polygynous colonies of *L. acervorum* from Nürnberger Reichswald, female sexuals are offspring of only one queen, while in some monogynous colonies sexuals might be reared that are nevertheless offspring of several queens. On the other hand, in functionally monogynous colonies of *L. gredleri*, nest mate queens form a hierarchy in which at a given time only one single top-ranking queen reproduces. Nevertheless, due to usurpation of the alpha position, female sexuals in *L. gredleri* may be offspring of several queens (Heinze et al., 1992).

High nest mate relatedness might be maintained by similar cyclic patterns of sexual production also in other populations of *L. acervorum* (Douwes et al., 1987; Heinze and Lipski, unpublished; Stille et al., 1991) and other leptothoracines, such as *L. (M.) longispinusus* (Herbers and Stuart, 1990) and *L. (s.str.) cf. canadensis* (Heinze, 1994). Nest mate relatedness appears low in polygynous colonies of *L. (s.str.) muscorum* (Lipski et al., 1994) and some populations of *L. acervorum* (Heinze et al., 1995), suggesting a more equal contribution of nest mate queens to the colony's offspring. As our study yielded average relatedness estimates from limited samples, we cannot exclude that in some cases several queens contribute to a colony's female sexual progeny also in the Reichswald population. According to dissection data, several old, fertile queens may occasionally co-occur in colonies that produce female sexual offspring (unpublished research). Whether queens contribute equally to the female sexual progeny of these colonies is not known. A model by Bourke and Heinze (1994) predicts low reproductive skew for species like *L. acervorum*, but testing this prediction will need to take account of the complexity of life histories demonstrated in the present study.

The colony cycle of *L. acervorum* in the Reichswald population somewhat resembles that of neotropical wasps, in which colonies start with many queens but new queens are reared only when queen number has been reduced to almost one (Gastreich et al., 1993; Queller et al., 1988, 1993; Strassmann et al., 1991, 1992). This pattern, "cyclical oligogyny," was explained by worker control of sex ratios (Queller et al., 1993). Split sex ratio theory (Boomsma, 1993; Boomsma and Grafen, 1990, 1991) predicts that the effective number of mothers per colony and colony sex ratio under worker control covary. In particular, in facultatively polygynous species with on-average low numbers of closely related queens, workers should favor more male-biased sex ratios with increasing queen number. Chan and Bourke (1994) recently demonstrated that in an English population of *L. acervorum*, monogynous colonies produce a female-biased sex ratio, whereas polygynous colonies produce a male-biased sex ratio. According to preliminary data from our study, worker-to-virgin-queen relatedness is higher than worker-to-male relatedness in colonies with a female-biased sex ratio but not so in colonies with a male-biased sex ratio. Sex allocation theory might ultimately explain the decrease in the number of female sexuals produced in older polygynous colonies.

The peculiar reproductive pattern of *L. acervorum* probably facilitates life in boreal habitats. Winter mortality was higher in isolated *L. cf. canadensis* workers than in workers overwintering in the hibernation cluster (Heinze and Hölldobler, 1994). By seeking adoption in established colonies, young queens thus might avoid long solitary hibernation. Furthermore, Herbers (1986a, 1993) found winter mortality to be lower in polygynous than in monogynous colonies of *L. longispinusus*. Budding generally appears a safer strategy in boreal habitats (Heinze and Hölldobler, 1994), and temporary polygyny, during which colony size is increased, might facilitate colony fragmentation. As suggested by Rosengren et al.

(1993) for boreal *Formica* wood ants, a strategy of storing inseminated daughters for later short-range dispersal can be considered as prolonged parental care.

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