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Research article

Mating biology and population structure of the ant, *Leptothorax gredleri*

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Summary. Female sexuals of the ant *Leptothorax gredleri* attract males by "sexual calling." In an experimental set-up allowing for competition among males, both female and male sexuals copulated with up to four partners, with the median being one mate in both sexes. Neither male nor female sexuals invariably mated with the first partner they encountered, but we could not find any morphological difference between sexuals that succeeded in mating multiply and those that copulated only once. Males did not aggressively compete for access to the female sexuals. According to microsatellite genotyping, workers produced by multiply mated queens were all offspring of a single father, i.e. queens appear to use sperm from a single mate to fertilize their eggs. Population genetic studies revealed a strong population subdivision, suggesting that both male and female sexuals mate in the vicinity of their maternal nests and that gene flow is strongly restricted even between forest patches isolated only by a few meters of grassland.

Key words: Leptothorax gredleri, mating, reproductive biology, population structure.

Introduction

The variance in reproductive success is more often larger among the males of a given species than among its females. This appears to be strikingly different in social insects. In termites, males and females form stable, monogamous pairs without any chances for extra-pair copulation (Wilson, 1971). In males of social bees, wasps, and ants, testes degenerate by the time males reach sexual maturity (for the only known exception, ergatoid males of *Cardiocondyla* ants, see Heinze and Hölldobler, 1993). The sperm supply of a male is thus limited and suffices only for one or a few inseminations (Hölldobler and Bartz, 1985). Consequently, male social

insects likely differ from other males in their reproductive tactics and might therefore provide interesting systems to test predictions from sexual selection theory. For example, males of social Hymenoptera are expected to be less aggressive and choosier than males of other species (Hölldobler and Bartz, 1985).

Compared with the solitary Hymenoptera (Alcock et al., 1978), surprisingly little is known about the mating biology of the social Hymenoptera. In ants, in particular, aspects other than the queen's mating frequency have rarely been studied in detail. Mating here is often technically difficult to observe, because sexual activity is frequently limited to a short period of a few hours or days per year and may take place somewhere up in the sky (Hölldobler and Bartz, 1985). As a result, for most ant species it is unknown how mating success varies among individual males, whether and how they compete for mating chances, and whether males and females chose among different potential mates.

Overt fighting for access to female sexuals appears to be confined to two ant genera, where males are morphologically highly specialised for combat and mating in the nest (*Hypoponera*, Hamilton, 1979; *Cardiocondyla*, Kinomura and Yamauchi, 1987; Heinze and Hölldobler, 1993). By eliminating all rivals with their strong mandibles, successful *Cardiocondyla* males may monopolise mating with all female sexuals eclosing in the nest over a period of several weeks (Kinomura and Yamauchi, 1987; Heinze et al., 1998; Cremer and Heinze 2002). In contrast, in other species with mating in the nest (e.g., *Linepithema humile,* Keller and Passera, 1992; Passera and Keller, 1994) and also in the vast majority of ant species, in which sexuals mate outside of the nest, males appear to be peaceful and do not establish or defend a harem (Hölldobler and Bartz, 1985; Hölldobler and Wilson, 1990). In species with large colony size, thousands of male and female sexuals from different colonies gather in a dense aggregation for a brief nuptial flight. In such swarms, males may engage in scramble competition for mating chances and female sexuals have been observed to resist the copulation attempts of certain males in the field (Höll-

dobler, 1976; Hölldobler and Bartz, 1985). In *Pogonomyrmex* harvester ants, both sexes apparently prefer to mate with larger partners, leaving small individuals bound with smaller mates (Davidson, 1982). Large males of *P. occidentalis* had on average a higher reproductive success than small males (Wiernasz et al., 1995; Abell et al., 1999). In contrast, in *Myrmica rubra*, female sexuals were seen to indiscriminately mate with the first male encountered (Woyciechowski, 1990).

In ant genera with small colony size, such as *Leptothorax* and other genera of the tribe Formicoxenini, mating typically does not occur in such large nuptial swarms. Instead, young queens leave their maternal nests on their own, climb on grass stems etc., raise the abdomen and attract males by releasing droplets of poison gland secretion from the tip of the extruded sting ("female calling", Buschinger, 1968a, 1976; Buschinger and Alloway, 1979). Males approach these calling queens on the wing, land in their vicinity and quickly walk closer. While rapidly antennating the female's head with their antennae, males attempt to bring the tip of their abdomen close the female's abdomen and to insert their genitalia. Copulation itself is characterised by the male tilting backwards and becoming immobile. After a few seconds, the females turn around and start to bite the male's gaster until the male withdraws. Other than detailed descriptions of the behaviour involved and the chemical analyses of the presumed sex pheromone (Reder et al., 1995), the reproductive biology of ants with female calling has as yet not been studied in depth. In particular, nothing is known on male-male competition and the variation in copulation success of males.

Here we report on the mating biology of the small myrmicine ant *Leptothorax gredleri* Mayr, 1855, one of the few ant species in which sexual behaviour can reliably be observed in flight cages (Heinze et al., 1992). In controlled mating experiments in the laboratory we examined whether both sexes indeed are choosy and vary little in their copulation success as expected from the limited sperm supply of males (Hölldobler and Bartz, 1985). We also investigated whether males show aggressive behaviour towards their rivals in a situation allowing for male-male competition and whether large body size gives them an advantage as observed in *Pogonomyrmex*. Body symmetry has been shown to be important in the females' decisions about accepting or rejecting mates in some non-social insects. For example, fluctuating asymmetry of wings and/or legs was found to be smaller in copulating males than in single males of *Drosophila pseudoobscura* (Markow and Ricker, 1992) and *Scatophaga stercoraria* (Liggett et al., 1993). We therefore measured fluctuating asymmetry of successful and unsuccessful males and females. Finally, we attempted to corroborate our laboratory results by indirectly studying the reproductive biology of *L. gredleri* using DNA markers and examining whether populations are subdivided, whether inbreeding occurs, and how many fathers contribute to a queen's offspring.

Material and methods

Collection and laboratory rearing

Colonies of *Leptothorax gredleri* were collected from their nests in dead branches in small, scattered oak and pine stands on an abandoned army drill ground in Erlangen, Germany. Colonies contained one to six queens, however, as shown in previous studies, only one of these laid eggs ("functional monogyny", Buschinger, 1968b; Heinze et al., 1992).

We focussed on three individual pine stands, each approximately 25 to 40 m² large and separated from each other by 7 to 15 m of sandy grassland. The number of colonies living in two such sites was estimated by re-sampling each for one hour per day over 9 days. The number of newly collected colonies was plotted over the total number of previously found and removed colonies, and from the regression line the total number of colonies present in two of the three stands was estimated (Mühlenberg, 1993). This was 51 in stand 1 and 49 in stand 2. Calculating the number of colonies in each pine stand using the method suggested by Zippin (1956, 1958) gave estimates of $47.8 \pm$ S.E. 4.7 and $47.0 \pm$ S.E. 4.8, respectively. Colonies were kept in the laboratory under standard conditions (Buschinger, 1974; Heinze and Ortius, 1991).

Mating studies

Female sexuals of *L. gredleri* exhibit sexual calling behaviour from around 10.00 h to 12.00 h in the morning (Heinze et al., 1992). Because individual sexuals could not be followed in the field, we examined mating success in 12 trials in flight cages, each with nine or ten males (mean 9.9) and 13 to 16 (mean 14.5) female sexuals originating from a total of 38 colonies, collected shortly before sexuals eclosed. The ratio of male and female sexuals in the flight cages reflected the numerical sex ratio produced by these colonies.

Sexuals that had left their nests were individually marked the day before the experiment, kept in a dark container over night and placed into a perspex flight cage (appr. $15 \times 15 \times 25$ cm³) around 9.30 h in the morning. The flight cage was then exposed to full sunlight on a south-exposed balcony. To prevent the temperature in the cage from increasing over ambient temperatures, the lid of the cage was opened every 15 to 20 min for about 5 s. First sexual calling was observed at approximately 9.45 h to 10.00 h. The observations were stopped when no sexual activity had been observed for 30 min. The observation time for each trial was between 120 and 165 min. Each individual was used in only one experiment.

To determine the fluctuating asymmetry of sexuals, we measured the tibiae of the front legs, the antennae, and, in males, the forewings using a Leica microscope with up to 100× magnification. Wings could not be measured in female sexuals because they were quickly shed after mating. Each measurement was performed twice. To correct for measurement errors, we investigated the data by a body side × individual ANOVA as suggested by Palmer (1994). Subsequent analyses were performed using the index σ^2 ($\sigma^2 = (MS_{aj} - MS_m)/M$, with M = number of measurements, MS_{aj} = variance between sides, MS_m = measurement error). This index is not biased by directional asymmetry. All asymmetries were normally distributed, i. e. there was no evidence for antisymmetry (i.e., of characters being generally asymmetric, with equal frequencies of right- and left-handed asymmetries in a population). As an estimate of body size, we measured thorax length and head width of each individual.

Genetic analyses

DNA was isolated from nine to 12 workers from nine to 11 colonies per pine stand (in total 323 workers). Samples preserved in alcohol were washed three times in water before DNA isolation. Ants were homogenised in 100 µl H₂O and 100 µl 10% Chelex. The homogenate was boiled for 6 min, stirred, and boiled again for additional 6 min. The

samples were centrifuged for 3 min at 12000 rpm and the supernatant was stored for later analysis.

Ants were genotyped at four microsatellite loci, two originally developed for *Leptothorax nylanderi* (L5, L18; Foitzik et al., 1997) and two for *L. acervorum* (LXAGA1, LXAGT1; Bourke et al., 1997). Locus L18 had six alleles, GA1 and GT1 three alleles each, and L5 two alleles. Relatedness was estimated from allele frequencies using the computer program "genrel97" by P. Pamilo, based on algorithms by Pamilo (1984) and Queller and Goodnight (1989). Inbreeding coefficients and estimates for F-statistics were calculated using the program GDA (Lewis and Zaykin, 2001). Confidence intervals for F-values were obtained by bootstrapping over loci.

To determine sperm use by queens that had been observed to mate with multiple males in the laboratory, we determined the microsatellite genotypes of 13 queens that had mated multiply in the flight cage, their mates, and up to ten workers from each of the queens (only three to four daughters were analysed from three queens each; of the remaining ten queens, ten daughters were analysed). Because we knew the genotypes of all putative fathers, we could calculate the non-sampling probabilities for a second patriline as $f = 1 - [1 - c^n - (1 - c^n)]$, where c is the assumed average paternity skew and n is the number of individuals examined (Pedersen and Boomsma, 1999).

Results

Mating studies

We observed a total of 79 copulations. Individual male and female sexuals differed strongly in their copulation frequencies. Of a total of 174 female sexuals used in the experiments, 72 did not show any sexual calling behaviour and where excluded from further analysis. Of the 102 remaining female sexuals, 57 succeeded in copulating with a male at least once. Surprisingly, 18 female sexuals copulated more than once (31.6% of all mating queens; 15 double, 2 triple, and 1 quadruple matings). The average and median mating frequencies for calling female sexuals were 0.77 and 0, those for actually mating females 1.38 and 1.

Of 119 males, 74 showed some sexual activity, but only 48 succeeded in copulation. Twenty males copulated with more than one female sexual (11 double, 7 triple, and 2 quadruple matings). The average and median mating frequencies for mating males were 1.65 and 1.

The duration of copulations (from first direct contact between male and female sexual to separation; measured only in some of the observed copulations) was normally distributed (Kolmogorov-Smirnov test, $D = 0.998$, $P > 0.20$) and ranged from 13 to 141 seconds (44 copulations, mean $66 \pm SD\ 25$ s). In males, the duration did not differ between their first, second, third, or fourth copulation (medians 68.5 s, 69 s, 65 s, 135 s; median test; $\chi^2 = 1.142$, df = 3, $P = 0.767$). Furthermore, the duration of the first copulation did not differ between multiply and singly mating males (medians, singly mating: 60.0 s, multiply mating: 68.5 s; Mann-Whitney U-test, $N_1 = 35$, $N_2 = 18$, $U = 270.5$, $P = 0.40$). Unsuccessful mating attempts lasted between 10 and 257 s (not normally distributed; Kolmogorov-Smirnov test, $D = 0.12$, $P < 0.05$, N = 129, median 78 s, quartiles 54 and 109.5).

In nine of 12 trials, several male and female sexuals from the same colony were present in the same flight cage. From the individual numbers of active sexuals per trial, we calculated that 24 of the 381 possible combinations between male and female sexuals were between individuals from the same colony, i.e., supposedly between relatives. In six of the 79 actually observed copulations, sexuals from the same colony mated. This is not different from the frequency expected assuming random mating ($\chi^2 = 0.214$, $P < 0.644$).

Males occasionally antennated a mating pair but never interfered with the copulation. Furthermore, male-male aggression or scramble competition was never seen. Female sexuals were repeatedly observed to reject an approaching male and to mate with another male later. Furthermore, males did not invariably attempt to mount the first calling queen. Assuming that mating frequencies reflect attractiveness we examined whether male and female sexuals that had copulated multiply differed in morphology from those that copulated only once. We excluded sexuals that had not mated at all from this study because their motivational status was not known: they might have been without success because they were not attractive or because they were simply not in the mood for sexual engagements. Males that copulated multiply were not significantly larger than males that copulated only once (18 multiple vs. 12 single; head width in mm: $0.49 \pm SD\ 0.03$ vs. 0.51 ± 0.05, $t = 0.964$, $P = 0.343$; thorax length in mm: 1.26 ± 0.06 vs. 1.29 ± 0.12, $t = 1.15$, $P = 0.258$). Similarly, multiply and singly mated queens did not differ in size (multiple vs. single, $N_1 = N_2 = 14$, head width in mm: 0.57 ± 0.05 vs. 0.59 ± 0.02, $t = 1.57$, $P = 0.128$; thorax length in mm: 1.11 ± 0.05 vs. 1.10 ± 0.04, $t = 1.01$, $P = 0.320$). Fluctuating asymmetries of the antennae, tibiae of the forelegs, and wings were neither associated with copulation success in male nor in female sexuals (Table 1).

Offspring genotyping suggested that queens that had mated with multiple males completely or predominantly used sperm of only a single mate for the insemination of their eggs (see below), suggesting that not all matings had been equally successful. "Unsuccessful matings" did not differ from "successful matings" in duration (duration of "unsuccessful matings," n = 14, median 57 s, quartiles 44.5 and 64.5 s; "successful matings," n = 13, median 63 s, quartiles 51.5 and

Table 1. Fluctuating asymmetry of front tibiae, antennae, and wings of multiply and singly mating male (n = 15) and female sexuals (n = 14 female) of the ant, *Leptothorax gredleri*. Wings could not be measured in mated queens

Male sexuals	σ^2 singly mated	σ^2 multiply mated	$F_{14,30}$	P-value
Tibiae of front leg	39×10^{-6}	70×10^{-6}	1.96	> 0.15
Antennae	259×10^{-6}	121×10^{-6}	2.14	> 0.15
Wings	892×10^{-6}	621×10^{-6}	1.43	> 0.25
Female sexuals			$F_{13,28}$	
Tibiae of front leg	7.9×10^{-6}	4.38×10^{-6}	1.86	> 0.15
Antennae	414×10^{-6}	205×10^{-6}	2.01	> 0.15

76.5 s; Mann-Whitney U-test, U = 69.5, p = 0.295). Furthermore, males were not more successful in siring offspring during their first mating than during their second mating (8 first matings vs. 5 s matings, $\chi^2 = 0.692$, p = 0.405).

Genetic analyses

Genetic analysis of workers from 30 natural colonies collected in three adjacent pine stands in Erlangen revealed a considerable population substructure. Summed over all three sites, significantly less heterozygotes were found in workers and queens than expected from panmixia (Table 2). Though the heterozygote deficiency was smaller in individual pine stands than in the whole sample it still was considerably larger than zero in particular in the largest pine stand 1, suggesting that even within individual pine stands mating is not random due to population subdivision (Tables 2, 3). The F_{IS}-value was not significantly larger than zero, indicating that mating does not preferentially occur among relatives.

The relatedness among nest mate workers was $r = 0.68 \pm$ SE 0.04 (95% confidence interval 0.60 – 0.76). The value adjusted for heterozygote deficiency was 0.50 ± 0.06 (95% CI 0.37 – 0.63), i.e., significantly lower than 0.75. A worker relatedness lower than 0.75 despite of functional monogyny (i.e., only one of several mated queens actually lays eggs, Buschinger, 1968b) might reflect either queen replacement (Heinze et al., 1992), the casual adoption of foreign workers in the colony, or multiple mating of the queen. As in our behavioural experiments, almost one third of all mating female sexuals had mated with several males, we investigated whether these queens indeed did use sperm from several mates. The genetic analysis of a total of 111 workers produced by 13 queens that had copulated multiply (11 double mated, one triple mated, one quadruple mated) did not reveal any evidence for multiple parentage, i.e. all examined workers from an individual colony shared the alleles of a single father. Non-sampling probability of a presumed second patriline was calculated for those ten colonies in which we had analysed ten worker offspring each. Assuming equal contribution of both males (i.e., c = 0.5), the probability to have accidentally missed offspring sired by a second male is only 0.002 per colony. With higher paternity skew, i.e. assuming that the minority male contributed only 20% of the sperm (c = 0.2), the probability equals 0.107 for each colony. The probability that we missed the second male though all ten queens had used sperm from a second mate to equally fertilise 20% of their eggs is less than 0.00001. Assuming a maximal undetected paternity skew of 0.2 and the observed frequency of multiple copulation among queens, 0.316, gives a maximum effective male number, m_e (Pedersen and Boomsma, 1999) of 1.11.

Workers from seven queens exclusively showed the microsatellite allele of the first mate of the queen and workers from five queens (including the queen that had mated with four males) had the allele of the second mate. In the case of the triple-mated queen, all workers had the alleles of the third mate.

Table 2. Expected heterozygosity (H_{exp}), observed heterozygosity (H_{obs}), and fixation index (F) at four microsatellite loci in the Erlangen population of the ant, *Leptothorax gredleri*

Subpopulation	Locus	H_{exp}	H_{obs}	F
Pine stand 1	L18	0.682	0.412	0.370
(11 colonies)	LXAGA1	0.455	0.405	0.110
	L5	0.328	0.214	0.350
	LXAGT1	0.507	0.336	0.339
	All	0.493	0.342	0.308
Pine stand 2	L18	0.738	0.743	–0.007
(10 colonies)	LXAGA1	0.625	0.583	0.066
	L5	0.490	0.564	–0.153
	LXAGT1	0.628	0.814	–0.298
	All	0.620	0.676	–0.090
Pine stand 3	L18	0.507	0.292	0.425
(9 colonies)	LXAGA1	0.632	0.629	0.005
	L5	0.497	0.506	–0.019
	LXAGT1	0.531	0.392	0.262
	All	0.542	0.455	0.161
Whole sample	L18	0.800	0.495	0.381
	LXAGA1	0.657	0.534	0.187
	L5	0.491	0.413	0.159
	LXAGT1	0.647	0.516	0.202
	All	0.648	0.489	0.245

Discussion

In an experimental situation, both male and female sexuals of the ant *Leptothorax gredleri* mated with up to four mates. About half of all sexuals did not exhibit any sexual activity, though in our experiments we only used those individuals that had left their nests and therefore were presumably ready to engage in mating. As all individuals were used only in one trial, it is likely that some inactive sexuals might have copu-

Table 3. Estimates of Wright's *F*-statistics from a hierarchical analysis (Lewis and Zaykin, 2001): heterozygote deficiency within colonies is denoted by f, heterozygote deficiency within the whole sample, assuming no subdivision and random mating, by *F*. θ_S and θ_P are the F_{ST} estimates for subpopulations and the whole sample. Confidence intervals were obtained by bootstrapping over loci

	L-18	LXAGA1	L-5	LXAGT1	Mean	95% CI
f (F_{IS})	0.002	–0.396	–0.356	–0.447	–0.271	–0.426 to 0.078
F (F_{IT})	0.436	0.251	0.217	0.258	0.304	0.228 to 0.398
θ_S	0.434	0.464	0.423	0.487	0.452	0.429 to 0.475
θ_P	0.240	0.207	0.179	0.173	0.204	0.175 to 0.230

lated at a later time. Despite of the presence of rivals, several males succeeded in copulating more than once. Scramble competition among males for access to female sexuals, such as observed in harvester ants (Hölldobler, 1976), or other kinds of male-male aggression were never observed. Some males approached and antennated mating pairs but never interfered with copulation. In contrast to harvester ants (Davidson, 1982; Wiernasz et al., 1995; Abell et al., 1999), larger body size apparently does not give an advantage in *L. gredleri*. Both male and female sexuals appeared to be choosy and did not invariably copulate with the first encountered mate, even though these had not mated before. However, we were not able to detect any morphological cue associated with successful mating or mating frequency: female and male sexuals that copulated once differed neither in body size nor fluctuating asymmetry from those that copulated multiply.

Surprisingly, about one third of all queens that copulated at all mated with several males. Subsequent microsatellite analysis of the offspring of multiply mating queens did not reveal any evidence for multiple paternity: all offspring workers produced by an individual queen shared a single paternal allele at all four studied microsatellite loci. Because only ten offspring workers were analysed per multiply mated queen we might accidentally have missed workers belonging to a rare patriline. However, the probability of not sampling a second patriline in the whole sample is extremely low. Furthermore, regular sperm replacement by the last mate appears unlikely as approximately half of the queens exclusively used the sperm of the first and half of the second or third mate.

Sperm clumping might potentially lead to queens nonrandomly using sperm from one male to fertilise eggs during one year and sperm from another male during another year. However, in *Formica truncorum* – at present the only ant species in which genetic data are consistent with such sperm clumping – multiply mated queens were suggested to actively mix sperm and to thus prevent a complete alternation of paternity (Sundström and Boomsma, 2000). Direct analysis of spermathecal content of *L. gredleri* queens might have helped to detect the presence of sperm from different males but would also have failed at higher levels of skew (Gertsch and Fjerdingstad, 1997). Previous genetic analyses in *L. gredleri* and several related taxa did never give evidence for the regular occurrence of multiple mating or sperm clumping. For example, worker relatedness, as estimated from three microsatellite loci, was $0.73 \pm$ SE 0.06 in a neighbouring population of *L. gredleri* with predominantly single-queen colonies (134 workers from 11 colonies, I. Merten and J. Heinze, unpublished), and according to genotyping of spermathecal contents of queens and mother-offspring comparisons, polyandry is rare in *L. acervorum* (Hammond et al., 2001).

The most parsimonious explanation for single paternity in the offspring of multiply mated queens therefore appears to be that queens did not receive sperm from all their mates. This is supported by the observation that the spermathecae of queens of *Leptothorax* and related taxa dissected shortly after an observed copulation occasionally did not contain any sperm (e.g., Buschinger, 1978; Felke and Buschinger, 1999). It has previously been shown that the range of observed copulations overestimates mating frequencies (Boomsma and Ratnieks, 1996). For example, multiple copulations have been observed in *Linepithema humile* (Keller and Passera, 1992), but according to genetic data, queens are singly inseminated (Krieger and Keller, 2000). Unsuccessful copulations in *L. gredleri* were presumably not caused by sperm depletion in males. Males of *Leptothorax* and related taxa have been shown to be capable of successfully inseminating up to ten female sexuals under controlled laboratory conditions (*Harpagoxenus sublaevis*, Winter and Buschinger, 1983).

L. gredleri queens that did not receive sperm during their first copulation obviously mated a second time. It is unclear, however, why some already inseminated queens did not resist mating attempts by a second male though they somehow prevented sperm transfer. Repeated mating by already inseminated queens might be an artefact due to the limited possibilities to escape from approaching males in our flight cage.

The duration of actual copulations varied considerably, but at present it is unclear why. In contrast to *Myrmica* (Woyciechowski, 1990), the duration of a male's first copulation was not significantly different from the duration of its subsequent copulations. Furthermore, the duration of copulations did not differ between those with males whose sperm was later used for the fertilisation of eggs and those during which presumably no or very little sperm was transferred. There was also no difference in the probability of success of a male's first and second mating.

Microsatellite data revealed a large heterozygote deficiency in our study population. In general, this might result from inbreeding or population substructuring (e.g., Pamilo, 1984). Behavioural observations and an F_{IS}-value slightly below zero exclude preferential inbreeding, while the significant F_{ST}-values indicate a considerable population subdivision: neither male nor female sexuals appear to disperse far from their maternal nests. Mating presumably occurs in the vicinity of the nests from which female sexuals originate. In laboratory experiments with larger arenas we observed that *Leptothorax* queens are capable of moving away a few meters from their maternal nests in a series of walks and short flights, though mating directly in the nest was never observed (e.g., Heinze, 1993 and unpublished results). At the same time, behavioural observations and the considerable relatedness of nest mate queens (Heinze, 1995) suggest that they are capable of returning into their maternal nests after mating. *L. gredleri* males have been observed to fly over several meters in the laboratory, but according to our genetic data they rarely bridge the few meters of open ground between individual pine stands.

Acknowledgements

References

Abell, A.J., B.J. Cole, R. Reyes and D.C. Wiernasz, 1999. Sexual selection on body size and shape in the western harvester ant, *Pogonomyrmex occidentalis* Cresson. *Evolution 53*: 535–545.

Alcock, J., E.M. Barrows, G. Gordh, L.J. Hubbard, L. Kirkendall, D.W. Pyle, T.L. Ponder and F.G. Zalom, 1978. The ecology and evolution of male reproductive behaviour in the bees and wasps. *Zool. J. Linn. Soc. 64*: 293–326.

Boomsma, J.J. and F.L.W. Ratnieks, 1996. Paternity in eusocial Hymenoptera. *Phil. Trans. R. Soc. Lond. B 351*: 947–975.

Bourke, A.F.G., H.A.A. Green and M.W. Bruford, 1997. Parentage, reproductive skew and queen turnover in a multiple-queen ant analysed with microsatellites. *Proc. R. Soc. Lond. B 264*: 277–283.

Buschinger, A., 1968a. "Locksterzeln" begattungsbereiter ergatoider Weibchen von *Harpagoxenus sublaevis* NYL. (Hymenoptera, Formicidae). *Experientia 24*: 297.

Buschinger, A., 1968b. Mono- und Polygynie bei Arten der Gattung *Leptothorax* Mayr (Hymenoptera Formicidae). *Insect. Soc. 15*: 217–225.

Buschinger, A., 1974. Experimente und Beobachtungen zur Gründung und Entwicklung neuer Sozietäten der sklavenhaltenden Ameise *Harpagoxenus sublaevis* (Nyl.). *Insect. Soc. 21*: 381–406.

Buschinger, A., 1976. Giftdrüsensekret als Sexualpheromon bei der Gastameise *Formicoxenus nitidulus* (Nyl.) (Hym., Form.). *Insect. Soc. 23*: 215–226.

Buschinger, A., 1978. Genetisch bedingte Entstehung geflügelter Weibchen bei der sklavenhaltenden Ameise *Harpagoxenus sublaevis* (Nyl.) (Hym., Form.). *Insect. Soc. 25*: 163–172.

Buschinger, A. and T.M. Alloway, 1979. Sexual behaviour in the slave-making ant, *Harpagoxenus canadensis* M.R. Smith, and sexual pheromone experiments with *H. canadensis, H. americanus* (Emery), and *H. sublaevis* (Nylander) (Hymenoptera; Formicidae). *Z. Tierpsychol. 49*: 113–119.

Cremer, S. and J. Heinze, 2002. Adaptive production of fighter males: queens of the ant *Cardiocondyla* adjust the sex ratio under local mate competition. *Proc. R. Soc. Lond. B 269*: 417–422.

Davidson, D.W., 1982. Sexual selection in harvester ants (Hymenoptera: Formicidae: *Pogonomyrmex*). *Behav. Ecol. Sociobiol. 10*: 245–250.

Felke, M. and A. Buschinger, 1999. Social organization, reproductive behavior and ecology of *Leptothorax acervorum* (Hymenoptera, Formicidae) from the Sierra de Albarracin in central Spain. *Insect. Soc. 46*: 84–91.

Foitzik, S., M. Haberl, J. Gadau and J. Heinze, 1997. Mating frequency of *Leptothorax nylanderi* ant queens determined by microsatellite analysis. *Insect. Soc. 44*: 219–227.

Gertsch, P.J. and E.J. Fjerdingstad, 1997. Biased amplification and the utility of spermatheca-PCR for mating frequency studies in Hymenoptera. *Hereditas 126*: 183–186.

Hamilton, W.D., 1979. Wingless and fighting males in fig wasps and other insects. In: *Sexual Selection and Reproductive Competition in Insects* (M.S. Blum and N.A. Blum, Eds.), Academic Press, New York, pp. 167–220.

Hammond, R.L., A.F.G. Bourke and M.W. Bruford, 2001. Mating frequency and mating system of the polygynous ant, *Leptothorax acervorum*. *Mol. Ecol. 10*: 2719–2728.

Heinze, J., 1993. Habitat structure, dispersal strategies and queen number in two boreal *Leptothorax* ants. *Oecologia 96*: 32–39.

Heinze, J., 1995. Reproductive skew and genetic relatedness in *Leptothorax* ants. *Proc. R. Soc. Lond. B 261*: 375–379.

Heinze, J. and B. Hölldobler, 1993. Fighting for a harem of queens: physiology of reproduction in *Cardiocondyla* male ants. *Proc. Natl. Acad. Sci. USA 90*: 8412–8414.

Heinze, J. and D. Ortius, 1991. Social organization of *Leptothorax acervorum* from Alaska (Hymenoptera: Formicidae). *Psyche 98*: 227–240.

Heinze, J., N. Lipski and B. Hölldobler, 1992. Reproductive competition in colonies of the ant *Leptothorax gredleri*. *Ethology 90*: 265–278.

Heinze, J., B. Hölldobler and K. Yamauchi, 1998. Male competition in *Cardiocondyla* ants. *Behav. Ecol. Sociobiol. 42*: 239–246.

Hölldobler, B., 1976. The behavioral ecology of mating in harvester ants (Hymenoptera: Formicidae: *Pogonomyrmex*). *Behav. Ecol. Sociobiol. 1*: 405–429.

Hölldobler, B. and S.H. Bartz, 1985. Sociobiology of reproduction in ants. In: *Experimental Behavioral Ecology and Sociobiology* (B. Hölldobler and M. Lindauer, Eds.), G. Fischer, Stuttgart. pp. 237–257.

Hölldobler, B. and E.O. Wilson, 1990. *The Ants*. Harvard University Press, Cambridge, Massachusetts. 732 pp.

Keller, L. and L. Passera, 1992. Mating system, optimal number of matings, and sperm transfer in the Argentine ant *Iridomyrmex humilis*. *Behav. Ecol. Sociobiol. 31*: 359–366.

Kinomura, K. and K. Yamauchi, 1987. Fighting and mating behaviors of dimorphic males in the ant *Cardiocondyla wroughtoni*. *J. Ethol. 5*: 75–81.

Krieger, M.J.B. and L. Keller, 2000. Mating frequency and genetic structure of the Argentine ant *Linepithema humile*. *Mol. Ecol. 9*: 119–126.

Lewis, P.O. and D. Zaykin, 2001. Genetic Data Analysis: Computer program for the analysis of allelic data. Version 1.0 (d16c). Free program distributed by the authors over the internet from http:// lewis.eeb.uconn.edu/lewishome/software.html

Liggett, A.C., I.F. Harvey and J.T. Manning, 1993. Fluctuating asymmetry in *Scatophaga stercoraria* L.: successful males are more symmetrical. *Anim. Behav. 45*: 1041–1043.

Markow, T.A. and J.P. Ricker, 1992. Male size, developmental stability, and mating success in natural populations of three *Drosophila* species. *Heredity 69*: 122–127.

Mühlenberg, M., 1993. *Freilandökologie*, 3rd edn. Quelle und Maier, Heidelberg. 512 pp.

Palmer, A.R., 1994. Fluctuating asymmetry analysis: a primer. In: *Developmental Instability: its Origins and Evolutionary Implications* (T.A. Markow, Ed.), Kluwer, London, pp. 335–364.

Pamilo, P., 1984. Genotypic correlation and regression in social groups: multiple alleles, multiple loci and subdivided populations. *Genetics 107*: 307–320.

Passera, L. and L. Keller, 1994. Mate availability and male dispersal in the Argentine ant *Linepithema humile* (Mayr) (= *Iridomyrmex humilis*). *Anim. Behav. 48*: 361–369.

Pedersen, J.S. and J.J. Boomsma, 1999. Multiple paternity in social Hymenoptera: estimating the effective mate number in single-double mating populations. *Mol. Ecol. 8*: 577–587.

Queller, D.C. and K.F. Goodnight, 1989. Estimating relatedness using genetic markers. *Evolution 43*: 258–275.

Reder, E., H.J. Veith and A. Buschinger, 1995. Neuartige Alkaloide aus dem Giftdrüsensekret sozialparasitischer Ameisen (Myrmicinae: Leptothoracini). *Helv. Chim. Acta 78*: 73–79.

Sundström, L. and J.J. Boomsma, 2000. Reproductive alliances and posthumous fitness enhancement in male ants. *Proc. R. Soc. Lond. B 267*: 1439–1444.

Wiernasz, D.C., J. Yencharis and B.J. Cole, 1995. Size and mating success in males of the western harvester ant, *Pogonomyrmex occidentalis* (Hymenoptera: Formicidae). *J. Insect Behav. 8*: 523–531.

Wilson, E.O., 1971. *The Insect Societies*. Harvard University Press, Cambridge, Massachusetts, 548 pp.

Winter, U. and A. Buschinger, 1983. The reproductive biology of a slavemaker ant, *Epimyrma ravouxi*, and a degenerate slavemaker, *E. kraussei* (Hymenoptera: Formicidae). *Entomol. Gener. 9*: 1–15.

Woyciechowski, M., 1990. Mating behaviour in the ant *Myrmica rubra* (*Hymenoptera, Formicidae*). *Acta Zool. 33*: 565–574.

Zippin, C., 1956. An evaluation of the removal method of estimating animal populations. *Biometrics 12*: 163–189.

Zippin, C., 1958. The removal methods of population estimation. *J. Wildl. Managem. 22*: 82–90.