

Nest site limitation and colony takeover in the ant *Leptothorax nylanderi*

Susanne Foitzik^{a,b} and Jürgen Heinze^a

^aZoologisches Institut I, Universität Erlangen-Nürnberg, Staudtstraße 5, D-91058 Erlangen, Germany and ^bLS Verhaltensphysiologie und Soziobiologie, Theodor-Boveri-Institut (Biozentrum der Universität), Am Hubland, D-97074 Würzburg, Germany

Ecological constraints on the success of independent colonies are thought to strongly shape the organization of ant societies. One of the most important factors is probably the availability of suitable empty nest sites. By population censuses, laboratory experiments, and microsatellite analyses, we investigated the colony and population structure of the small, myrmicine ant *Leptothorax (Myrafant) nylanderi* in a deciduous forest near Würzburg, Germany, where nest sites appear to be strongly limited, especially in late summer. Colonies of *L. nylanderi* inhabit cavities in rotting branches, hollow acorns, grass stems, etc. After hibernation, a temporary overabundance of empty nest sites facilitates the fragmentation of larger colonies into smaller buds, which, because the species is monogynous, are in part queenless. Nest sites become scarce in summer due to rapid decay, and both established colonies and young founding queens face a severe shortage of suitable nest sites. This leads to the fusion of established, unrelated colonies, which after initial fighting permanently merge and live together. Typically only one queen survives after fusion. Similarly, young mated queens may seek adoption in alien nests instead of founding their own colonies solitarily, and here again only a single queen survives. This temporary intraspecific parasitism may be an important first step in the evolution of obligatory permanent parasitism, which is widespread in the genus *Leptothorax*. **Key words:** colony takeover, competition, *Leptothorax*, nest site limitation, population structure, social parasitism. [*Behav Ecol* 9:367–375 (1998)]

The social and genetic structure of ant colonies is strongly shaped by ecological factors such as habitat structure, climate, or the occurrence of competitors (Herbers, 1993; Hölldobler and Wilson, 1977, 1990; Passera et al., 1996; Rosengren and Pamilo, 1983). In cavity-dwelling ants in temperate and boreal forests, such as the small myrmicine *Leptothorax*, the availability of nest sites for founding new colonies is thought to be especially important (Bourke and Heinze, 1994; Herbers, 1986). *Leptothorax* colonies typically consist only of a few dozen workers, and, because they are incapable of constructing their own nests, they more or less completely depend on abandoned pupal cavities of longhorn beetles in rotting sticks or on hollow acorns, hazelnuts, or grass stems. In species where several queens may co-occur within a single colony (facultative polygyny), the limitation of nest sites appears to lead to an increase in the number of queens and workers per nest. Consequently, experimental seeding of a 100-m² plot with additional nest sites caused a decrease in the number of workers and queens per nest and an increase in the number of nest sites used by individual colonies of North American *Leptothorax (Myrafant) longispinosus* relative to control areas. Colonies responded to the presence of nest sites by fractionating into subunits (Herbers, 1986). Together with overwintering mortality, the availability of nest sites seems to also affect queen number and the degree of reproductive skew among nest-mate queens in boreal *Leptothorax* (s. str.). Where habitats are saturated, young queens return to their maternal nests after mating nearby and form a social hierarchy, in which only the highest-ranking individual lays eggs (functional monogyny, Bourke and Heinze, 1994).

In contrast to *L. longispinosus* and boreal *Leptothorax* (s. str.), most *L. (Myrafant)* living in the temperate deciduous

woods of Central Europe are obligatorily monogynous, i.e., their colonies contain only a single queen (Buschinger, 1968), and new colonies are established by solitary foundresses. Sexu-als mate during nuptial flights (Chauvin, 1947, Plateaux, 1978) and young mated queens therefore cannot easily return to their maternal nests when they fail to find an uninhabited nest site suitable for founding. How do these queens react to habitat saturation, and how does nest site limitation affect the structure of mature colonies of monogynous species?

Previous studies in a dense population of *Leptothorax (Myrafant) nylanderi* near Würzburg, Germany, suggested intense competition for nest sites (Heinze et al., 1996). In this study we present results from population genetic studies and field and laboratory experiments that clearly document that nest site fragility and habitat saturation lead to takeover of nests, the fusion of mature colonies, and temporary intraspecific parasitism, all resulting in a much higher genetic heterogeneity within societies than presumed from the life history of a monogynous and monandrous ant.

METHODS

Field site and laboratory culture

Our study site is an open pine–oak forest with sandy soil on shell-limestone in Sommerhausen, 15 km south of Würzburg, Germany (10°02′–10°03′ E; 49°42′–49°43′ N). The interior part of the forest consists mainly of pine (*Pinus sylvestris*), while at the forest edges oaks (*Quercus petraea*) are dominant. In light areas, shrubs of elder (*Sambucus nigra*) and thickets of rose (*Rosa canina*) and blackthorn (*Prunus spinosus*) occur. Colonies of *L. nylanderi* inhabit small cavities in acorns, rotting sticks, grass stems, hazelnuts, and pine cones.

Colonies were discovered either by following foragers back to their nests or by opening potential nest sites. For further investigation, all ants in the nest were collected with an aspirator and transferred to the laboratory, where they were counted and then either frozen for genetic analyses (see below) or cultured in 10 × 10 × 2.5 cm³ plexiglass boxes con-

Address correspondence to S. Foitzik. E-mail: sfoitzik@biologie.uni-erlangen.de.

Received 18 August 1997; revised 1 December 1997; accepted 26 January 1998.

© 1998 International Society for Behavioral Ecology

sisting of three chambers connected by small passages with a moistened plaster floor (Buschinger, 1974; Heinze and Ortius, 1991). A cavity between a piece of cardboard and a microscope slide, kept apart by a plexiglass frame, served as a nest. Dissections were carried out as described by Buschinger and Alloway (1978).

Field and laboratory experiments

We investigated seasonal fluctuations of colony composition in the Sommerhausen population by collecting and counting the individuals of all colonies found on different 32-m² plots in summer (June–July) and fall (September–October) in 2 consecutive years (1995, 1996). Queens of multiqueen colonies were dissected under a binocular microscope to examine ovarian development. We especially noted the presence of sperm in the spermatheca and also the occurrence of yellow bodies, which indicate that queens have previously produced eggs.

The durability of nest sites in the field was studied in three 2 m × 1 m observation plots, where all colonies had been located in June 1996 by observing at least five foragers leaving or entering a nest. The condition and type of the nest material was noted, and its location was marked with a small flag. After 47, 62, or 72 days, respectively, all nests were opened, their inhabitants were counted, and the condition of the nest material was examined again. As a measure of size, we crudely estimated the approximate volume, V , of the complete nest site from its diameter, τ , and its length, l , assuming that nest sites are cylindrical in shape ($V = \pi \tau^2 l$). We did not measure the volume of the nest cavity itself because it cannot easily be determined with a large sample size. Furthermore, we observed colonies enlarging the nest cavities (Foitzik S, unpublished data), and nest volume is therefore a constantly changing parameter.

To investigate the immigration rate of colonies into empty areas in the field, we removed all colonies and potential nest sites from 4-m² plots in June and July 1995 (eight plots) and 1996 (eight plots). The colonies were censused to obtain the natural population density of the study sites. The experiment consisted of two treatments in a full-factorial design—leaf litter/no litter and nest site replacement/no replacement. In the first treatment, in half of the plots all leaf litter was removed, and the other plots remained untouched. In the second treatment, all removed inhabited nests were replaced by empty, artificial wood nests (2–3 cm thick and 6-cm long pine wood sticks with a 5-cm long and 5-mm wide hole, partially closed by a wooden splinter) in half of the plots, while in the other plots no artificial nests were added. Two months later, we thoroughly searched the plots and collected and censused all colonies found. The colony composition of the colonies that had immigrated into the plots was analyzed.

The behavior of founding queens was studied with 42 queens, which were collected in late September 1995, when they had at most laid their first few eggs but had not yet produced larvae or adult workers. On the same day, we collected queenless colonies. The minimum distance between the nests of founding queens and queenless colonies was 5 m. The queenless colonies were transferred into the laboratory and allowed to move into artificial nests as described above. Three hours after the colony had completely moved into the laboratory nest in the left chamber of the plexiglass box, a founding queen was added into the middle chamber. In 15 experiments, only the nest site with the queenless colony was provided; in 27 experiments, the box contained an additional empty nest site in the right chamber. We carefully observed the behavior and location of queens for 30 min per day for 4

days, and after 2 months the final location of the queen was noted.

Colony takeover was investigated in 38 arena experiments. In each test a freshly collected colony was allowed to move into a laboratory nest located in a 50-cm × 30-cm arena. The queen was marked by tying a knot of 30- μ m thin copper or tungsten wire between petiole and postpetiole. A second newly collected colony with a differently marked queen was added into the arena 5 days later without providing a nest. We carefully monitored the arena during the next 2 h and for 30 min over the following 5 days. Aggression in the arena or the nest and the position of the queens was noted. After 7 days we transferred the nests into a three-chambered plexiglass box.

Colony takeover in the field was investigated in the following way. Colonies were collected in mid-August and at least 15 workers from each colony were frozen (–70°C) for genetic analyses. The colonies were standardized by “pruning” in 4 different size classes consisting of 38 colonies each: queenless colonies with 15 (A), 30 (B), and 60 (C) workers, and colonies with a queen and 30 workers (D). We chose colonies in such a way that for standardization at most 25 workers had to be removed. For groups A, B, and C, colonies were used that had already been queenless when collected in the field. All pupae were removed from the colonies to exclude the adoption of a colony's own young queens. The ants were then allowed to move into artificial wood nests and after 10 days in the laboratory these nests were distributed on the forest floor along a 38-m long straight line in 1-m intervals. We marked artificial nests with a flag bearing the colony number. Exactly 2 months later, on 22 October 1996, we collected all artificial nests and counted the inhabiting colonies. All colonies containing a queen were subjected to a genetic analysis (see below): the genotypes of at least 10 workers and the queen from each colony were compared with the genotypes of 2 workers from the colony that originally had moved into the artificial nest site in the laboratory. Where the queen and the 10 workers differed in their genotypes from the two workers from the original colony, additional individuals (up to 25% of all workers) were tested. In some colonies of group D, the genotypes of 10 workers from the original colony were determined.

Genetic analyses and statistics

DNA was isolated by Chelex extraction (Walsh et al., 1991, as modified by Thorén et al., 1995). Frozen ants were homogenized in 100 μ l distilled water (dd H₂O) and 7 μ l dithiothreitol (DTT) buffer (0.93 g DTT and 20 μ l sodium acetate, pH 5.2, diluted in 6 ml distilled water). Thereafter, 10 μ l of proteinase K (10 mg/ml) and 100 μ l 10% Chelex solution were added, and proteins were digested for at least 1 h at 56°C. After vortexing for 10 s, the homogenate was boiled for 8 min, mixed again thoroughly, and centrifuged at 12000 rpm for 3 min.

The 25- μ l reaction mixture for one polymerase chain reaction contained 2.5 μ l of the ant DNA extract (approximately 10 ng DNA), 10x Taq polymerase buffer (Goldstar: 20 mM Tris HCl, pH 8.3, 50 mM KCl), 2 mM MgCl₂, 1.25 mM of each dNTP, 0.65 μ M of each primer, and 1 unit of Taq polymerase (Goldstar). Polymerase chain reaction was performed with a Perkin Elmer GeneAmp 2400 thermal cycler. Locus L-18 was amplified over 32 cycles with 1 min 93°C, 1 min 54°C, and 1 min 72°C. L-18 is a moderately variable microsatellite marker with at least 11 alleles in the investigated population. Allele frequencies range from 0.022 to 0.269, the observed heterozygosity is 0.746 (Foitzik et al., 1997). Details on the electrophoretic separation and visualization of DNA fragments with Sybr-green have been described by Foitzik et al. (1997).

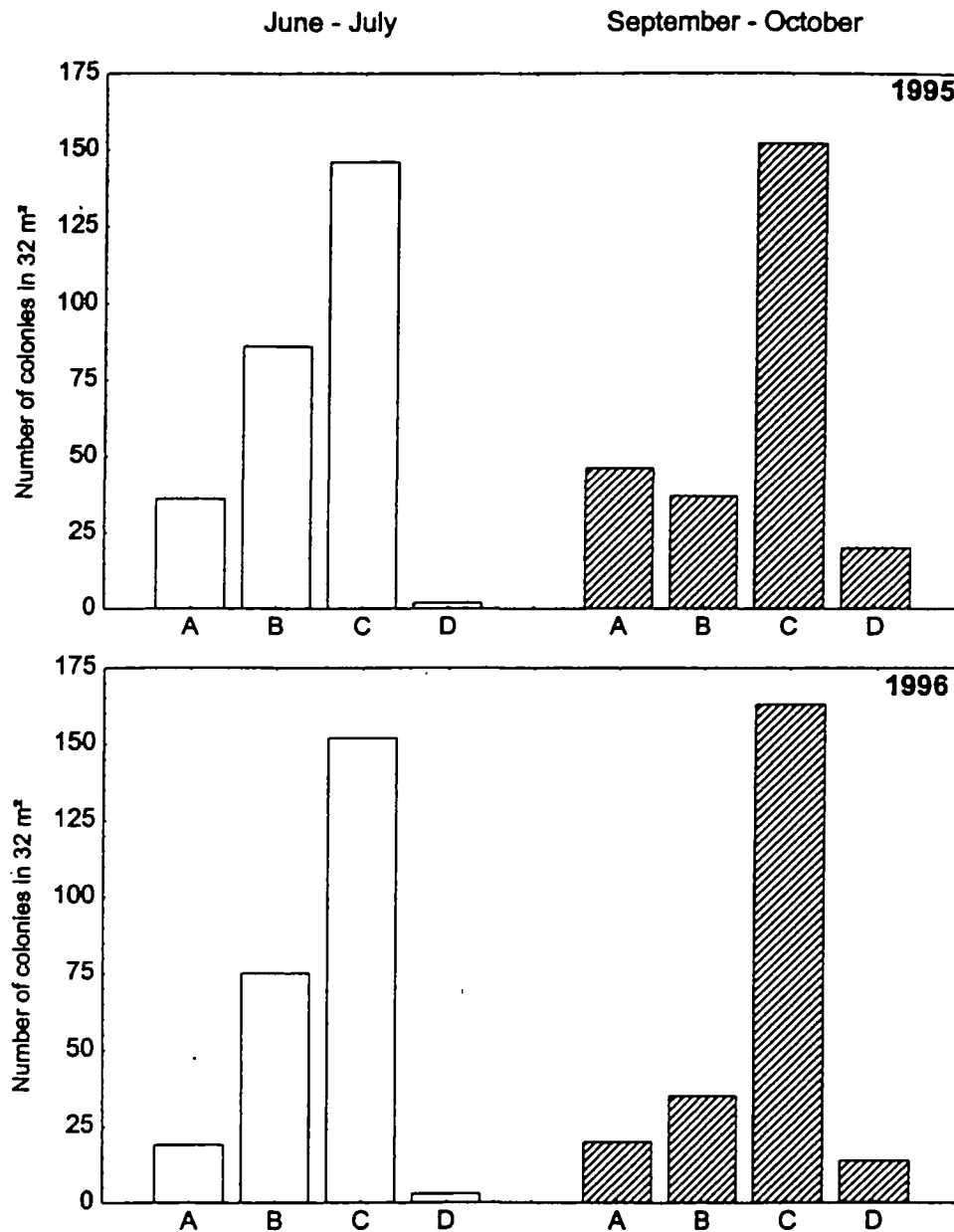


Figure 1
Composition of the Sommerhausen population of the ant *Leptothorax nylanderi* in different seasons and years. The proportions of founding queens (A), queenless (B), monogynous (C), and multiqueen (D) colonies are shown. The results of a log-linear analysis of the data (see Table 1) suggest a strong influence of season on the frequency distribution.

All statistical analyses were performed with Statistica 4.5 (StatSoft, 1993).

RESULTS

Population structure

The influence of the season and year on the frequency of colonies with different social organization in the Sommerhausen population was investigated with log-linear analysis. The frequency distribution of founding queens (A), queenless (B), monogynous (C), and multiqueen (D) colonies was strongly affected by the season ($\chi^2 = 57.70, p < .0001$). The distribution differed also, though less strongly, between the years ($\chi^2 = 15.23, p < .01$). In both years, the frequency of queenless colonies was increased in summer (Figure 1; Table 1). Furthermore, though *L. nylanderi* is considered to be monogynous (Buschinger, 1968; Plateaux, 1978), 20 of 207 colonies collected in fall 1995 (9.0%) and 14 of 212 colonies collected in fall 1996 (6.6%) contained more than a single queen (total

Table 1

Influence of season and year on the frequency of colonies of the ant *L. nylanderi* with different social organization

Factor	df	χ^2	<i>p</i>
Social organization	3	727.19	.0000001
Season	1	1.01	.315
Year	1	1.91	.167
Social organization + season	3	57.70	.0000001
Social organization + year	3	15.23	.01
Season + year	1	-0.00	1.000

Log-linear analysis of the frequency table; χ^2 tests of partial association for different factors.

Table 2

Number of workers, reproductive status of queens, and genotype of workers and queens at microsatellite locus L-18 in 12 multiqueen colonies of *L. nylanderi* collected in fall 1996

Colony	Queens present	Yellow bodies in queen(s)	No. of workers	Genotypes of queens			Genotypes of workers (n)
				a	b	c	
IX 2	a, b	a	40	HH	EE		HH (12)
IX 11	a, b	b	55	GG	EE		DE (12)
X 27	a, b	a	30	HH	DD		EH (12)
XIV 21	a, b	a	69	EF	BB		BD (12)
XVI 20	a, b	a	36	EF	HH		EF (7), EE (5)
IX 23	a, b, c	c	15	DF	DE	II	II (8), GG (3)
IX 20	a, b, c	b	16	EG	GG	HK	EG (4), GG (2), EE (5)
X 28	a, b	b	14	FJ	EE		FJ (3), FF (2), EG (7)
XII 8	a, b	a, b	52	II	GG		II (10), GG (2)
XIII 11	a, b	a, b	58	DL	FF		DD (4), DL (3), FF (5)
XV 18	a, b	a, b	9	DK	JJ		DK (2), DD (3), JJ (7)
XVI 5	a, b	a	98	HH	LF		HH (5), LF (4), FF (3)

All queens and approximately 11 to 12 workers per colony were analyzed.

numbers of colonies excluding founding queens). Multiqueen colonies were considerably more frequent in fall than in summer. Two or more queens were found to be inseminated in 33 of a sample of 34 multiqueen colonies, where all queens were dissected. In some cases, the ovaries of both queens contained yellow bodies, suggesting that both queens had previously been laying eggs (Table 2). According to microsatellite analyses in 12 multiqueen colonies, nest-mate queens typically did not share alleles at locus L18 and therefore were considered not to be first-degree relatives (they could, however, be cousins, aunts, and nieces, etc.). Only in one colony were two of three queens possibly first-degree relatives. Six of the analyzed 12 colonies contained workers that appeared to be offspring of both queens; in one colony, in addition to progeny of 1 of the 3 present queens, workers were found whose genotypes did not match those of any queen (Table 2).

Nest site fragility and colony movements

Of 18, 15, and 14 nest sites found to be inhabited by *L. nylanderi* in June 1996, only 10, 9, and 6 (60, 55.5, and 42.9%)

still contained ants when opened after 47, 62, and 72 days, respectively. The calculated half-life of nest sites therefore does not exceed 10 weeks in summer. Larger nest sites (overall volume, not the volume of the nest cavity) were more durable and less frequently abandoned than small nest sites (Mann-Whitney *U*-test, $U = 81.0$, $p < .0001$, $n_1 = 25$, $n_2 = 22$; Figure 2). In a larger sample of colonies, the size of nest sites was correlated with colony size (Spearman rank test, 300 colonies, $r_s = .63$, $p < .001$, Figure 3).

Colony movement was directly observed in the field during four occasions. The rapid immigration of colonies into areas from which we removed all colonies also suggests frequent colony migrations. Colonies were collected from sixteen 4-m² plots, which each had initially contained an average of 32.4 ± 5.1 (SD) nests (i.e., more than 8 nests/m²). Resettlement of these areas was generally high: in 1 plot, 30 new colonies were found after 2 months. The immigration rate depended strongly on the availability of nest sites (two-way ANOVA: influence of nesting sites: $df = 1$, $F = 42.52$, $p < .0001$; influence of leaf litter: $df = 1$, $F = 6.78$, $p < .05$; Figure 4). In plots where no artificial nest sites were provided, on aver-

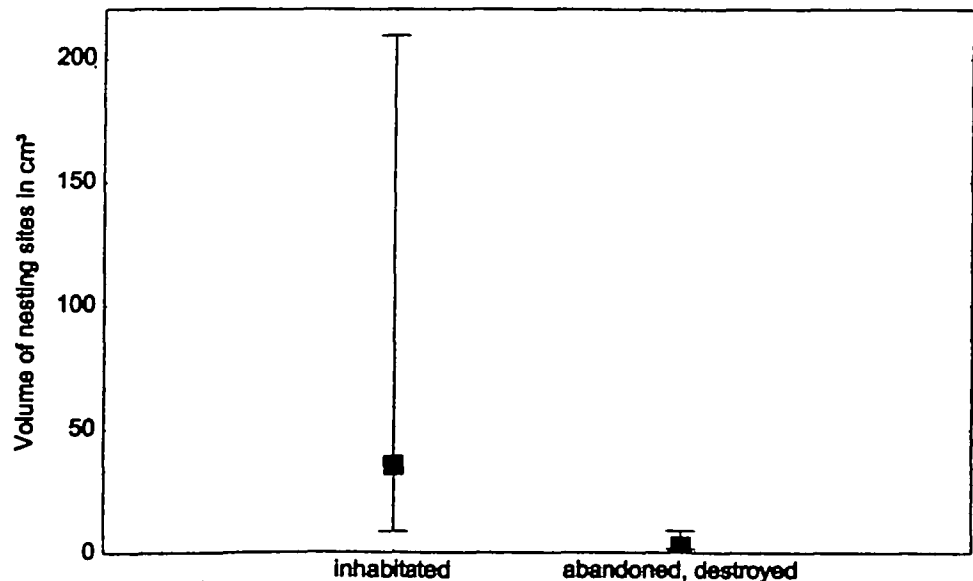


Figure 2

Influence of the nest site volume (not the volume of the nest cavity itself) on its fragility and suitability for habitation. The median and 25–75% quartiles are shown. Inhabited nest sites were located in the field in June 1996 and inspected again 47–72 days later.

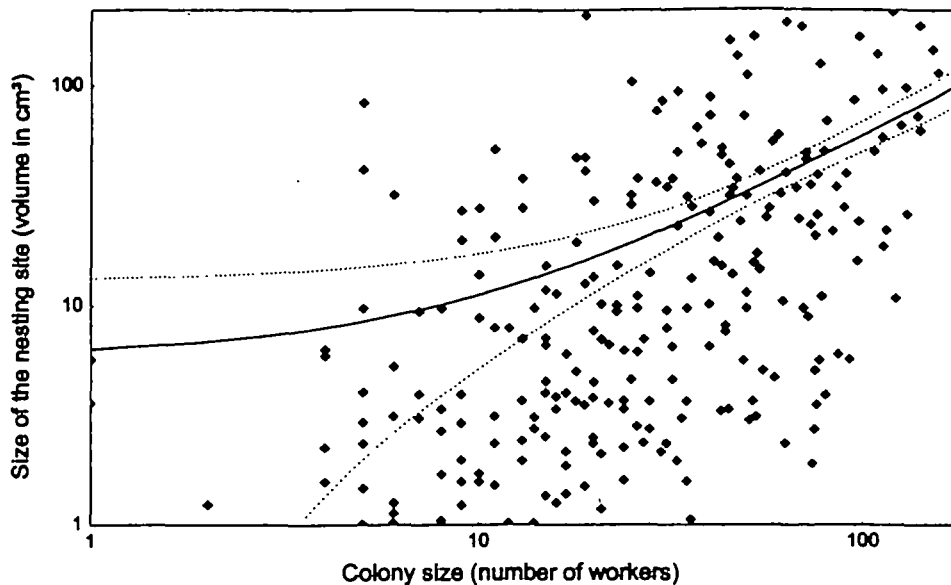


Figure 3

Correlation between colony size and volume of the nest site (not the volume of the nest cavity itself). The linear regression line and 95% CI (dotted lines) are given. For a better presentation of the numerous small colonies, a logarithmic scale was used for both colony size and nest volume.

age, 4.1 ± 3.0 ($n = 8$) colonies were found, in contrast to 19.6 ± 8.5 ($n = 8$), where a nest site had been added for each removed colony. Colony composition differed strongly between treatments (log-linear analysis: $\chi^2 = 83.82$, $p < .0001$). In plots with nest site replacement, fewer founding colonies (7% versus 33%) and more queenless colonies (42% versus 24%) were found compared to the plots where no nest sites had been added.

Behavior of founding queens

Two months after founding queens had been given the choice to either join a queenless colony or found solitarily without a nest site in the laboratory, 13 of 15 queens were present in the originally queenless colonies. Similarly, when queens were

given the choice between a nest site inhabited by a queenless colony and an empty nest, 19 of 27 queens had entered the queenless colony. Aggression between workers and the founding queens were observed only during the first 4 days after the experiment began. Queens reacted to worker antagonism by retreating or crouching. Five queens were observed to leave the nest after being attacked by workers but reentered later and were eventually accepted by the workers. In four cases the queens were violently attacked and killed by the workers. Thirty of those colonies that had adopted founding queens were kept in the laboratory for more than a year, and all queens were still alive after this period and laid eggs, which were tended by the unrelated workers.

Competition for nest sites and colony fusion

In all experiments, where a colony without a nest was placed into an arena with a single, already inhabited nest, workers of the newly added colony discovered the nest within 15 min and entered through the nest entrance. Nest defense by resident workers was generally inefficient: the nest entrance was typically left unguarded, and fighting between alien and resident workers started only after direct physical contact. Fighting between workers did not elicit aggressive reactions by workers close by, suggesting that the resident workers do not use an alarm pheromone to recruit nest mates for the defense of their nest. Resident workers unsuccessfully tried to remove the foreign workers by carrying them away for distances of 15–30 cm. More and more alien workers entered the nest, and if the resident colony was considerably smaller, they started to carry brood into the nest within 10 min. In 8 of 38 experiments, the workers of the intruding colony could not successfully enter the nest. In 7 of these 8 cases, the resident colony was notably larger than the intruding colony; in the remaining case the resident colony contained 120 and the intruding colony 140 workers (binomial test, $p = .03$).

In the 30 cases where alien workers could enter the nest, they sooner or later began to attack the resident queen. In some experiments, the queen of the intruding colony was carried into the nest within the first 30 min of the experiment. In most cases, both queens were attacked by the workers of the alien colony. The queens tried to escape by crouching and generally did not leave the nest. In seven cases, queens carried

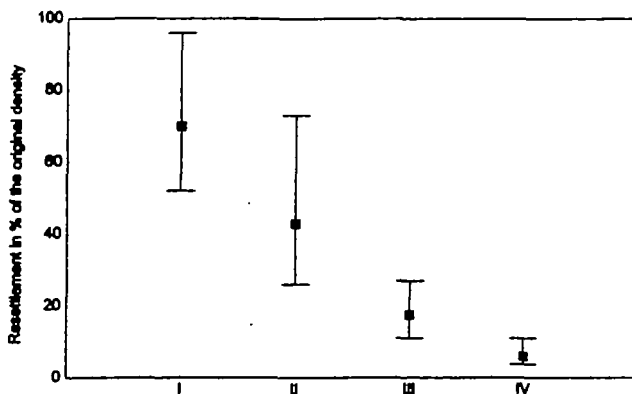


Figure 4

Resettlement rate of experimental plots from which all inhabited nests of *L. nylanderi* were removed. Plots were checked for newly immigrated colonies 2 months later. The experiment consisted of two treatments in a full-factorial design, each repeated in four plots: In group I, an artificial nest site was added for each removed, inhabited nest and leaf litter was left in place. In group II, artificial nest sites were added as before, but all leaf litter was removed. In group III, no nest sites were added and leaf litter was left untouched, and in group IV, no nest sites were added and all leaf litter was removed. Both the presence of nest sites and of leaf litter have an influence on resettlement rate. The original density was 32.4 ± 5.1 SD colonies per plot. Median and 25–75% quartiles of the resettlement rate are given.

Table 3
Usurpation rate of artificial nest sites containing colonies of *L. nylanderi* during 2 months in the field

Experiment	A	B	C	D
Original number of queens	0	0	0	1
Original number of workers	15	30	60	30
Number of nest sites inhabited after 2 months	25	20	24	18
Nests containing alien queens after 2 months (%)	14 (56)	9 (45)	6 (25)	7 (39)

Details on genetic composition of colonies are given in Table 4. Each experiment (A–D) repeated 38 times.

out of the nest by workers tried to reenter, even if still attacked by workers.

Aggression between workers from the resident and the intruder colony became less intense during the first 3 days of the experiment, and no fighting could be observed after 5 days. In 30 of 38 cases, the two colonies finally merged and coexisted in the same nest. In no case did the resident colony emigrate from the nest or carry out brood. Workers from both original colonies were still interacting peacefully in six additional mixed colonies after approximately 180 days.

After fusion, most colonies quickly became monogynous. In 14 of the 30 fusions, 1 of the 2 queens was killed during the process of colony merging or she escaped and remained alone or with 2 or 3 workers in the arena, where she eventually died. In 16 cases, 2 queens and workers from both colonies lived alongside in the same nest for 1 to more than 64 days. In 5 multiqueen nests, we observed aggression among the queens leading to the death of 1 queen. Here, 1 queen was already injured, but it is unknown whether injuries were inflicted by workers or by the attacking queen.

In 6 of the 30 merged nests, both queens died, and in 6 both were still alive at the end of the experiment. In 11 of the remaining 18 single-queen colonies, the queen of the larger colony survived, whereas the queen of the smaller colony survived in 7 colonies ($\chi^2 = 0.45$, ns). In five of the merged colonies, the resident queen survived ($\chi^2 = 1.87$, ns).

The occurrence of fusion of colonies and the adoption of founding queens in the field was investigated by placing 152 artificial nests inhabited by *L. nylanderi* colonies in the field. Two months later, 87 nests (57.2%) were still inhabited, though most of the nest sites had decayed to a certain degree. The presence of queens in nests that had been queenless at the beginning of the experiment and a strong increase in the number of workers in some nests suggested that alien queens and/or workers had invaded some of the nests and either replaced or merged with their original inhabitants. Especially those nests that had initially contained only a small group of workers and brood now were inhabited by often large, monogynous colonies (Table 3). In four colonies, belonging to groups C and D, two inseminated queens were present 2 months later. To identify whether nest sites had been completely taken over by alien colonies with a queen or whether queenless worker groups had adopted founding queens, we compared the genotypes at microsatellite locus L18 of workers taken from the colonies before the experiment and workers and queens found in the nests 2 months later. Nests either contained (1) a mixture of original and alien workers and an alien queen, (2) the original queen and workers and alien workers, (3) only an alien queen and alien workers, or (4) only individuals from the original colony (Table 4). The frequency of nest takeover did not differ significantly between colonies of similar size with and without queens (groups B and D, $\chi^2 = 0.06$, $df = 1$, $p = .81$). Microsatellite analysis also showed that at least

5 of the 11 examined colonies from group D had contained 2 or more matriline before the experiment started, suggesting that colony takeover or fusion indeed occur in the field without any experimental manipulation. Note that because of the possibility that alien workers by chance had the same genotypes at L18 as the workers originally inhabiting the colony, our results give only a lower bound on colony fusion and takeover.

DISCUSSION

Nest site limitation is a common trait in populations of cavity-dwelling ants in boreal and temperate forests and strongly affects the structure of their populations. In nearctic *Leptothorax longispinosus*, for example, the availability of empty nest sites influences the number of queens per nest and the number of nests inhabited by individual colonies (Herbers, 1986). Our study suggests that in its monogynous, monandrous congener *Leptothorax nylanderi*, nest site limitation leads to nest usurpation and temporary intraspecific parasitism.

Our study population in a pine–oak forest near Würzburg is extraordinarily dense—nest density is the highest ever recorded for *Leptothorax*. The abundance of nest sites apparently changes tremendously during the year. Nest sites, such as grass stems, acorns and dead twigs, are not very durable and become rare in spring and summer due to decay: half of the nest sites had become uninhabitable within 10 weeks of summer. On the other hand, few new natural nest sites had become available during this period, whereas in fall and winter new nest sites presumably are added through storms and seed production by oaks, pines, and hazelnuts.

The high percentage of queenless colonies found in early summer suggests that large colonies split into several subunits early in spring when nest sites are available. Later in the year, nest fragility and high colony density lead to strong competition for empty nests. Small colonies and founding queens appear to be affected most because, according to the positive correlation between the volume of the nest site and colony size (number of workers), they are restricted to the most ephemeral, smallest nest sites. Because of the rapid decay of their nests, small colonies probably have to move to new nests several times per year. Frequent colony movement was evident in the field from the fast resettlement of areas experimentally freed of all colonies. In experimental plots where we added an artificial nest site for each removed colony, between 52% and 96% of the original colony density of more than 8 colonies/m² was reached within 2 months in summer by immigration of colonies from neighboring areas. In these plots many colonies were queenless, once more indicating that colonies split when nest sites are available. Areas where no artificial nest sites had been provided after colony removal were repopulated to a much lower degree and especially by founding queens. Removal of leaf litter had a weaker effect on resettlement rate.

Table 4

Composition of colonies of *L. nylanderi* inhabiting artificial nest sites before these were placed in the field ("original composition") and 2 months later

	Original composition		Composition after 2 months		Origin of workers		Origin of queens	
	Queens	Workers	Queens	Workers	Original	Alien	Original	Alien
A4	0	15	1	116	5	11	0	1
A6	0	15	1	26	1	12	0	1
A17	0	15	1	24	3	10	0	1
A19	0	15	1	56	0	17	0	1
A21	0	15	1	154	0	44	0	1
A25	0	15	1	31	1	12	0	1
A27	0	15	1	109	5	9	0	1
A28	0	15	1	91	8	11	0	1
A30	0	15	1	36	2	8	0	1
A31	0	15	1	121	1	18	0	1
A33	0	15	1	0	0	0	0	1
A36	0	15	1	21	7	5	0	1
A37	0	15	1	67	0	19	0	1
B4	0	30	1	87	3	7	0	1
B16	0	30	1	163	8	11	0	1
B18	0	30	1	70	3	7	0	1
B22	0	30	1	86	4	6	0	1
B27	0	30	1	149	1	33	0	1
B29	0	30	1	36	4	6	0	1
B30	0	30	1	142	5	5	0	1
B36	0	30	1	114	6	5	0	1
B38	0	30	1	31	5	5	0	1
C3	0	60	1	154	7	4	0	1
C7	0	60	1	39	0	14	0	1
C8	0	60	2	17	0	14	0	2
C21	0	60	1	96	0	24	0	1
C32	0	60	2	92	3	9	0	2
C36	0	60	1	123	1	22	0	1
D2	1	30	1	28	8	3	0	1
D4	1	30	1	104	4	9	1	0
D8	1	30	1	13	5	0	1	0
D11	1	30	1	65	13	0	1	0
D15	1	30	1	31	5	0	1	0
D17	1	30	1	86	3	10	0	1
D18	1	30	1	86	13	0	1	0
D19	1	30	1	111	17	0	1	0
D23	1	30	2	49	13	0	1	1
D24	1	30	2	93	25	0	1	1
D26	1	30	1	42	4	9	0	1
D27	1	30	1	70	1	11	0	1
D28	1	30	1	60	5	0	1	0
D30	1	30	1	26	14	0	1	0
D33	1	30	1	68	2	8	0	1
D35	1	30	1	38	5	0	1	0
D36	1	30	1	85	5	0	1	0
D38	1	30	1	87	13	0	1	0

The origin of workers and queens was determined by analyzing the microsatellite locus L18. Workers denoted "original" or "alien" had genotypes compatible or not compatible, respectively, with the assumption that they were full sisters of workers belonging to the colony originally inhabiting the nest site when it was placed in the field. Not all workers of a colony were genotyped.

Colonies collected in fall that contained two or more queens most likely originated from colony fusion or from the adoption of young founding queens and thus also reflect nest site limitation. In arena experiments, homeless colonies began to move into occupied nest sites if no other nest sites were available. Contrary to our expectations, both colonies stayed together after initial fighting, though after a few weeks typically only one of the two queens was still alive. According to the presence of unrelated workers in some colonies in the field, nest takeover and colony fusion also occur under natural conditions. Furthermore, 2 months after artificial nest sites with colonies of *L. nylanderi* were placed in

the field, more than one-third of these nest sites contained an alien queen, and some were evidently invaded by a complete alien colony. Larger colonies were usurped to a lower degree than smaller colonies, but the presence of a resident queen apparently did not influence takeover rate.

Founding queens readily invaded laboratory colonies, and it appears that they do the same in the field. Queens co-occurring in field colonies were not closely related, suggesting that supernumerary queens were not daughters or sisters of the resident queens.

Both nest takeover and colony fusion are easily understood as adaptations to habitat saturation and nest site lim-

itation in monogynous ants and might considerably increase the fitness of homeless colonies or founding queens. It is surprising, however, that resident workers do not defend their nests more vigorously against intruders and that workers from two different monogynous colonies eventually merge to form a single, stable society.

In the case of queenless colonies, one could argue that workers might benefit from their nest being usurped by a founding queen or from fusion with a colony containing an unrelated queen. Both circumstances could ensure that sexual brood still present in the queenless colony will be raised to adulthood long after all related workers have died of old age. However, it first appears that most queenless nests of *L. nylander* in the Sommerhausen population are fragments of polydomous colonies, which will move together in fall (Heinze et al., 1996). Second, workers of *L. nylander* are quite long-lived (Plateaux, 1978) and will typically be able to raise all female sexual offspring still present in the colony once the queen has died. In addition, workers in queenless colonies produce their own sons from haploid eggs (Heinze et al., 1997), but can no longer do so after adopting an alien queen. Hence, we doubt that the adoption of an unrelated queen is adaptive for workers in queenless colonies.

Similarly, because in the fusion of two colonies with queens only one queen will survive for more than a few weeks and the queens are not closely related, the workers from one colony will serve an alien queen and tend brood to which they are not closely related without further increasing their inclusive fitness. They thus suffer the fate of "intraspecific slavery" similar to those workers that are pillaged as pupae from colonies defeated in territorial contests in honeypot ants (Hölldobler, 1976, 1981) and other species, including North American *Leptothorax* (Alloway, 1980). In the interspecific raids of slave-making ants (Buschinger, 1970, 1986), typically only brood is pillaged, whereas adult workers flee or are killed, probably because larvae or pupae more easily imprint to an alien colony odor (Carlin, 1988; Hölldobler and Wilson, 1990). We have previously shown that nest material has a strong influence on colony odor in *L. nylander* (Heinze et al., 1996), which probably proximately explains the easy fusion of genetically distinct colonies in this species.

Founding queens may similarly invade the nests of established, alien colonies when facing a severe shortage of nest sites. In several polygynous *Leptothorax*, queens mate in the vicinity of their maternal nests and thus can easily seek adoption in their original colonies (e.g., Heinze et al., 1995; Herbers and Grieco, 1994; Stille et al., 1991; Stuart et al., 1993). Sexu- als of *L. nylander* mate during a nuptial flight (Chauvin, 1947; Plateaux, 1978) and their return into the maternal nests is therefore unlikely. On average about 58 young queens are produced per square meter, and most of these probably attempt to found their own colonies solitary. About 21% of the queens found cooperatively with other foundresses in a pleometrotic association (Foitzik S, unpublished data). Our data show that some queens seek adoption into alien queenless colonies, where once they are adopted they exploit the present workers to rear their own young and thus strongly increase the growth of their own colonies. Others invade colonies containing a resident queen, and as polygyny in *L. nylander* is a temporary phenomenon, only one queen will survive. Whereas resident queens of *L. nylander* typically stop to produce eggs in the fall, young adopted queens develop their ovaries and attempt to lay eggs (Foitzik S, unpublished data). As fertility presumably is important if workers take part in the decision about which queen survives (e.g., Fletcher and Blum, 1983), egg-laying in fall may be a tactic of founding queens to increase their chances of replacing resident queens.

The invasion of established colonies by founding queens

can be seen as temporary intraspecific social parasitism. The use of the term "parasitism" in this context is somewhat controversial. In a strict sense, "parasitism" means a "symbiosis in which members of one species exist at the expense of members of another species" (Wilson, 1971). Here we follow a less restricted usage that includes cases where one of several reproductive tactics in a species is to reproduce or rear young at the expense of conspecific individuals. The term "intraspecific parasitism" has widely been used to describe the phenomenon of birds laying their eggs in nests of other members of the same species (e.g., Andersson, 1984; Birkhead et al., 1990; Møller, 1987), of wasps and bees usurping nests built by conspecifics (Cervo and Dani, 1996; Field, 1992; MacDonald and Matthews, 1975; Makino, 1989; Taylor, 1939), and to refer to the reproduction of ant queens reducing the inclusive fitness of all other resident colony members (Elmes, 1973; Rosengren et al., 1993). "Facultative intraspecific parasitism" has been seen as a first step toward obligatory interspecific parasitism in wasps (Taylor, 1939). In ants, interspecific parasitism is especially common in the genus *Leptothorax* and related taxa. Intraspecific parasitism, where foundress queens invade unrelated orphaned colonies, has hitherto only been reported from the red imported fire ant, *Solenopsis invicta*, where a small queen morph is thought to invade alien colonies and has apparently lost the capability of founding solitarily (Tschinkel, 1996). In *L. nylander*, all foundresses, even those provided with an empty nest site, chose to invade queenless colony fragments over nesting solitarily, suggesting that intraspecific parasitism in this ant is an option open to all young queens.

We thank two anonymous reviewers for helpful comments on the manuscript. The studies were supported by the Deutsche Forschungsgemeinschaft (Heisenberg-grant He 1623/6-1 to J.H., He 1623/7-1, Graduiertenkolleg "Grundlagen des Arthropodenverhaltens," University of Würzburg).

REFERENCES

- Alloway TM, 1980. The origin of slavery in Leptothoracine ants (Hymenoptera: Formicidae). *Am Nat* 115:247-261.
- Andersson M, 1984. Brood parasitism within species. In: *Producers and scroungers: strategies of exploitation and parasitism* (Barnard CJ, ed). London: Croom Helm; 195-227.
- Birkhead TR, Burke T, Zann R, Hunter FM, Krupa AP, 1990. Extra-pair paternity and intra-specific brood parasitism in wild zebra finches *Taeniopygia guttata* revealed by DNA fingerprinting. *Behav Ecol Sociobiol* 27:315-324.
- Bourke AFG, Heinze J, 1994. The ecology of communal breeding: the case of multiple-queen leptothoracine ants. *Phil Trans R Soc Lond B* 345:359-372.
- Buschinger A, 1968. Mono- und Polygynie bei Arten der Gattung *Leptothorax* Mayr (Hymenoptera: Formicidae). *Insect Soc* 15:217-226.
- Buschinger A, 1970. Neue Vorstellungen zur Evolution des Sozialparasitismus und der Dulosis bei Ameisen (Hym., Formicidae). *Biol Zbl* 89:275-299.
- 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, 1986. Evolution of social parasitism in ants. *Trends Ecol Evol* 1:155-160.
- Buschinger A, Alloway TM, 1978. Caste polymorphism in *Harpagoxenus canadensis* M. R. Smith (Hymenoptera: Formicidae). *Insect Soc* 25:332-350.
- Carlin NF, 1988. Species, kin and other forms of recognition in the brood discrimination behavior of ants. In: *Advances in myrmecology* (Trager JC, ed). Leiden: E.J. Brill; 267-295.
- Cervo R, Dani FR, 1996. Social parasitism and its evolution in *Polistes*. In: *Natural history and evolution of paper wasps* (Turillazzi S, West-Eberhard MJ, eds). Oxford: Oxford University Press; 98-112.
- Chauvin R, 1947. Sur l'élevage du *Leptothorax nylander* (Hyméno-

- tère Formicidae) et sur l'essaimage *in vitro*. Bull Soc Zool France 72:151–157.
- Elmes GW, 1973. Observations on the density of queens in natural colonies of *Myrmica rubra* L. (Hymenoptera: Formicidae). J Anim Ecol 42:761–771.
- Field J, 1992. Intraspecific parasitism as an alternative reproductive tactic in nest-building wasps and bees. Biol Rev 67:79–126.
- Fletcher DJC, Blum MS, 1983. Regulation of queen number by workers in colonies of social insects. Science 219:312–314.
- Foitzik S, Haberl M, Gadau J, Heinze J, 1997. Mating frequency of *Leptothorax nylanderi* ant queens determined by microsatellite analysis. Insect Soc 44:219–227.
- Heinze J, Foitzik S, Hippert A, Hölldobler B, 1996. Apparent dearenemy phenomenon and environmental-based recognition cues in the ant *Leptothorax nylanderi*. Ethology 102:510–522.
- Heinze J, Lipski N, Schlehmayr K, Hölldobler B, 1995. Colony structure and reproduction in the ant, *Leptothorax acervorum*. Behav Ecol 6:359–367.
- Heinze J, Ortius D, 1991. Social organization of *Leptothorax acervorum* from Alaska (Hymenoptera: Formicidae). Psyche 98:227–240.
- Heinze J, Puchinger W, Hölldobler B, 1997. Worker reproduction and social hierarchies in *Leptothorax* ants. Anim Behav 54:849–864.
- Herbers JM, 1986. Nest site limitation and facultative polygyny in the ant *Leptothorax longispinosus*. Behav Ecol Sociobiol 19:115–122.
- Herbers JM, 1993. Ecological determinants of queen number in ants. In: Queen number and sociality in insects (Keller L, ed). Oxford: Oxford University Press; 262–293.
- Herbers JM, Grieco S, 1994. Population structure of *Leptothorax ambiguus*, a facultatively polygynous and polydomous ant species. J Evol Biol 7:581–598.
- Hölldobler B, 1976. Tournaments and slavery in a desert ant. Science 192:912–914.
- Hölldobler B, 1981. Foraging and spatiotemporal territories in the honey ant *Myrmecocystus mimicus* Wheeler (Hymenoptera: Formicidae). Behav Ecol Sociobiol 9:301–314.
- Hölldobler B, Wilson EO, 1977. The number of queens: an important trait in ant evolution. Naturwissenschaften 64:8–15.
- Hölldobler B, Wilson EO, 1990. The ants. Cambridge, Massachusetts: Harvard University Press.
- MacDonald JF, Matthews RW, 1975. *Vespula squamosa*: a yellowjacket wasp evolving towards parasitism. Science 190:1003–1004.
- Makino S, 1989. Usurpation and nest rebuilding in *Polistes riparius*: two ways to reproduce after the loss of the original nest (Hymenoptera: Vespidae). Insect Soc 36:116–128.
- Møller AP, 1987. Intraspecific nest parasitism and anti-parasite behaviour in swallows, *Hirundo rustica*. Anim Behav 35:247–254.
- Passera L, Roncin E, Kaufmann B, Keller L, 1996. Increased soldier production in ant colonies exposed to intraspecific competition. Nature 379:630–631.
- Plateaux L, 1978. L'essaimage de quelque Fourmis *Leptothorax* Roles de l'éclaircissement et de divers autres facteurs. Effet sur l'isolement reproductif et la répartition géographique I, II. Ann Sci Nat Zool Biol Anim 12(20):129–192.
- Rosengren R, Pamilo P, 1983. The evolution of polygyny and polydomy in mound-building *Formica* ants. Act Entomol Fenn 42:65–77.
- Rosengren R, Sundström L, Fortelius W, 1993. Monogyny and polygyny in *Formica* ants: the result of alternative dispersal tactics. In: Queen number and sociality in insects (Keller L, ed). Oxford: Oxford University Press; 308–333.
- StatSoft, 1993. Statistica for Windows 4.5. Tulsa, Oklahoma: StatSoft, Inc.
- Stille M, Stille B, Douwes P, 1991. Polygyny, relatedness and nest founding in the polygynous myrmicine ant *Leptothorax acervorum* (Hymenoptera: Formicidae). Behav Ecol Sociobiol 28:91–96.
- Stuart RJ, Gresham-Bissett L, Alloway TM, 1993. Queen adoption in the polygynous and polydomous ant *Leptothorax curvispinosus*. Behav Ecol 4:278–281.
- Taylor LH, 1939. Observations on social parasitism in the genus *Vespula* Thomson. Ann Entomol Soc Am 32:304–315.
- Thorén P, Paxton R, Estoup A, 1995. Unusually high frequency of (CT)_n and (GT)_n microsatellite loci in a yellowjacket wasp, *Vespula rufa* (L.) (Hymenoptera: Vespidae). Insect Mol Biol 4:141–148.
- Tschinkel WR, 1996. A newly-discovered mode of colony founding among fire ants. Insect Soc 43:267–276.
- Walsh PS, Metzger DA, Higushi R, 1991. Chelex 100 as a medium for simple extraction of DNA for PCR-based typing from forensic material. Biotechnology 10:506–513.
- Wilson EO, 1971. The insect societies. Cambridge, Massachusetts: Harvard University Press.