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Ecology of *Leptothorax* ants: impact of food, nest sites, and social parasites

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Abstract In a long-term field manipulation, we demonstrate strong reactions of *Leptothorax longispinosus* ant colonies to food- and nest-site supplementation. Demographic and genetic responses varied over small geographic scales, and the two ecological factors interacted with the presence of the social parasite *Protomognathus americanus*. We conducted a 2×2 experiment in three blocks and found that the blocks, which were less than 100 m apart, reacted very differently to the treatments. Blocks differed in degree of polygyny, intranest relatedness, colony size, productivity, and sexual investment. Furthermore, these differences were associated with the presence of slave-making ants and the local availability of nest sites. Nest-site supplementation had a strong effect only in the site with the highest prevalence of social parasites, influencing there the density and investment patterns of colonies. *L. longispinosus* ants in the least parasitized area were strongly affected by both food- and nest-site supplementation. There, food supplementation led to a decrease in the number of queens per colony and consequently to an increase in intranest relatedness, while colonies in nest-site-supplemented areas invested fewer resources in males and produced a female-biased allocation ratio. By contrast, in a third block with a very low intracolony relatedness, food supplementation induced

an absolute and relative higher investment in males. We conclude that ecological factors influencing social organization in insect societies cannot be studied in isolation, because the interactions among factors produce far richer responses than any one variable.

Keywords Social organization · Ecological parameters · Genetic structure · Sex allocation · Ants · Social parasites

Introduction

For nearly half a century studies on social insects have been strongly influenced by theories of inclusive fitness (Hamilton 1964), sex ratio (Trivers and Hare 1976; Boomsma and Grafen 1990, 1991), and reproductive skew (Vehrencamp 1983). Tests of these models have been facilitated by the development of powerful genetic markers such as microsatellites, allozymes, and RFLPs. As a result, a large literature evaluating intracolony relatedness in social insects, especially in ants and wasps, is now available (see reviews by Bourke and Franks 1995; Crozier and Pamilo 1996). By contrast, evaluation of model predictions about ecological parameters has been slower because they are difficult to measure and can interact with the social structure of colonies. Field studies have analyzed the relative importance of internal genetic and external ecological factors (Deslippe and Savolainen 1994, 1995; Banschbach and Herbers 1996; Eickwort et al. 1996; Foitzik and Heinze 1998; Herbers and Banschbach 1998, 1999; Pedersen and Boomsma 1999a; DeHeer et al. 2001; Ingram 2002a, 2002b), but most use correlation inference rather than direct experimentation. Although controlled field experiments have the greatest power to disentangle multiple causation, they are seldom employed because they are very difficult and time-consuming to carry out. Thus, our understanding of how theories of social evolution apply to natural populations remains poor. In this experimental field study, we examine the relative importance of nest sites and food in the ecological context of variable social parasite

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density for the North American forest ant *Leptothorax longispinosus*.

In cavity-dwelling ants in temperate and boreal forests, such as the small myrmicine *Leptothorax*, the availability of nest sites for the foundation of new colonies is thought to be especially important (Herbers 1986a; Bourke and Heinze 1994; Foitzik and Heinze 1998). *Leptothorax* colonies typically consist of only a few dozen workers, and many species are incapable of constructing their own nests. Instead they rely on cavities produced by other insects. Natural nest sites such as rotting sticks, hollow acorns, hazelnuts, or grass stems are ephemeral, with a half-life of as little as 10 weeks in summer (Foitzik and Heinze 1998). Together with overwintering mortality, the availability of nest sites appears to affect numerous dimensions of social structure. For example, in saturated habitats, newly mated queens of *Leptothorax* (s.str.) return to their maternal nests and form a social hierarchy in which only the highest-ranking individual lays eggs (functional monogyny; Bourke and Heinze 1994). Similarly, in the monogynous *L. nylanderi*, shortage of suitable nest sites in the summer leads to colony usurpation by unrelated founding queens and takeovers by mature colonies. Subsequently, aggression among these unrelated queens produces monogyny so that colony takeovers and usurpations are evident only in the signature of low worker-worker relatedness (Foitzik and Heinze 1998; Foitzik et al. 2003). In other species, several queens may co-occur peacefully, and the degree of polygyny is linked to the availability of nesting opportunities for newly mated queens. Experimental seeding with additional nest sites caused a decrease in the number of workers and queens per nest in our focal species *L. longispinosus*. At the same time the number of nest sites utilized by individual colonies increased. Hence, colonies responded to enhanced nest-site availability by fractionating into subunits (Herbers 1986a). These last experiments, conducted on a population in upstate New York, prompted us to broaden our approach, because *L. longispinosus* colonies occur at high densities there (Herbers 1989). We predicted that adding nest sites would cause shifts in social organization to produce colonies that are small and rarely polygynous and that have high intranest relatedness. Such shifts in relatedness in turn can affect investment strategies of *Leptothorax* ants (Herbers 1990; Backus 1995; Hammond et al. 2002).

The second ecological parameter we studied, food availability, is known to influence investment in hymenopteran colonies (Strohm and Linsenmaier 1997). The quality and quantity of food is thought to be of critical importance in the differentiation process of diploid hymenopteran larvae into queens versus workers and thus is involved in the proximate control of allocation ratios (Hölldobler and Wilson 1990; Wheeler 1986). Theory predicts that colonies enjoying high resource availability should produce female-biased sex ratio by increasing either the propagule size (Backus and Herbers 1992) or the numbers of new queens (Nonacs 1986a, 1986b; Rosenheim et al. 1996), and experiments have

yielded such effects (Backus 1993; Deslippe and Savolainen 1995; Herbers and Banschbach, 1998, 1999; Morales and Heithaus 1998; Ode and Rissing 2002; Bono and Herbers 2003). Even so, ecological background can influence ant colony response to food supplementation (DeHeer et al 2001; Herbers and Banschbach 1999). Comparisons across populations have implied that *L. longispinosus* is not food-limited in New York compared to other places (Backus and Herbers 1992; Backus 1993; Herbers and Banschbach 1998). Therefore, we expect food supplementation alone to have few effects (most probably on sex investment), but it might interact with nest-site supplementation. If colonies subdivide to enhance living space and foraging efficiency, then adding food resources should weaken the effect of adding nest sites. Similarly, supplementation of nest sites and food could provide ideal conditions for the establishment of new colonies, giving rise to a high density of small colonies in doubly supplemented plots.

Long-term data showed that at the New York site an additional ecological parameter, parasite pressure through raids of the slave-making ant *Protomognathus americanus*, had strong effects on the primary host demography, intranest relatedness, and investment strategies (Foitzik and Herbers 2001a; Herbers and Foitzik 2002). *L. longispinosus* host nests in neighborhoods containing slave-making colonies were more likely to be small and monogynous with high intranest relatedness and a high investment in sexuals versus new workers (Herbers and Foitzik 2002). Demographic shifts in the presence of the social parasite were attributed to frequent and destructive raids by the slave-maker, which reduced the long-term survival of host colonies. Therefore, our manipulation of nest-site and food availability should interact with slave-maker pressure to cause demographic changes. We predicted that additional nest sites would provide refugia for hosts under attack by raiding parties, thereby weakening the link between slave-maker pressure and colony size. Furthermore, addition of nest sites would enhance recruitment of new colonies, increasing overall density. Finally, addition of food to host nests in neighborhoods containing slave-makers should compound life-history shifts by enhancing investment in sexuals.

We describe below an experiment designed to explore how three ecological factors, singly and in concert, affect the social biology of *L. longispinosus*. Our experiment, conducted over three summers, encompasses the spatial and temporal variation known to be important for this ant. However, while we directly manipulated food- and nest-site availability, we planned to control for environmental heterogeneity including natural variation in parasite pressure by using a block design. We were especially interested in examining how interactions among the three ecological parameters might produce effects unpredicted by single-parameter experiments.

Methods

The study system

We conducted our experiment at the Edmond Niles Huyck Preserve near Albany, New York. At 600-m elevation, the preserve contains second-growth deciduous forest, with maple and oak dominating the canopy. The forest has a shallow leaf litter layer where *Leptothorax longispinosus* leads an inconspicuous life, although it is the most common species of the local ant community (Herbers 1989). *L. longispinosus* has small colonies, typically containing only a few dozen individuals, but these occur at high density, inhabiting small sticks, acorns, and other preformed plant cavities. The biology of this ant with its variable social structure is well known (Headley 1943; Alloway 1990; Herbers and Foitzik 2002 and references therein).

Ecological manipulations

In the summer of 1996, twelve 49-m² plots were set up in three blocks located within 100 m of each other. Food- and nest-site manipulations were carried out in a 2×2 factorial design. Thus, each block contained four treatments: control (no manipulation), food supplementation, nest-site supplementation, and both food and nest-site supplementation. Ten milliliters of dead, frozen fruit flies were spread evenly over the food-supplemented plots every week during three growing seasons (from mid-June through mid-September in 1996, from mid-May through mid-September in 1997, and from mid-May to mid-July in 1998). The flies were consumed by the ants, and a similar manipulation strongly influenced sex allocation patterns in one of two experiments on a Vermont population of the same species (Backus and Herbers 1992; Herbers and Banschbach 1998). The nest-site manipulation consisted of adding two artificial nest sites on each square meter of the manipulated plot. These nest sites, cylindrical birch dowels (10 cm long, 1.5 cm in diameter, with a longitudinal 4-mm hole), mimicked the natural nest sites and were readily accepted by the ants (Herbers 1986a).

In late July and early August 1998, we completely excavated all plots. We examined all potential nest sites (artificial and natural ones), mapped the position of each colony, and carried them to the laboratory in Fort Collins. There, each colony was censused and frozen for genetic analysis. We collected the ants in the field shortly before the emergence of the new sexuals and callow workers from the pupae. To estimate investment in reproduction, we combined census data with data on the average dry masses of adult *L. longispinosus* males, new queens, and workers from 49 nests. Ants were dried at 80°C for 5 days and then individually weighed to the nearest 0.01 mg. Average dry mass was multiplied by the number of individuals to estimate the biomass invested in each caste.

We characterized queen number in *L. longispinosus* nests as queenless, monogynous, or polygynous. Polygynous nests contained between 2 and 10 queens, but the majority (78%) was headed by only two or three queens. We calculated the sex allocation ratio for each nest as the relative proportion of energy allocated to male versus female propagules (Crozier and Pamilo 1996). We also computed the reproductive allocation ratio, which is the relative proportion of energy invested in sexual reproduction compared to new workers (Backus 1995; Sundström 1995; Herbers et al. 2001; Reuter and Keller 2001). Finally, we multiplied the number of new workers, males, and queens with their respective dry masses and adjusted the investment in female sexuals with the energetic cost ratio (Boomsma 1989). This resulted in the total annual investment of each nest in micrograms, which was then divided by the number of adult workers to compute per capita productivity.

Genetic structure

Because of the large number of colonies on our plots, it was not feasible to investigate the genetic structure of each nest in detail.

Instead, for plots with 12 or more resident nests, we randomly selected for genetic analysis a smaller rectangle of the plot, which included at least 12 nests. From each nest we genotyped all queens and 4–10 workers. DNA was extracted from whole ants according to the protocol in Foitzik and Herbers (2001b). We genotyped a total of 1,469 individuals belonging to 180 *L. longispinosus* nests.

We used three highly polymorphic microsatellite loci, and multilocus genotypes were assigned with the protocols in Foitzik and Herbers (2001a). We also amplified a portion of the mitochondrial genome and cut the PCR products with five restriction enzymes: *HaeIII*, *TaqI*, *RsaI*, *BamHI*, and *HhaI* (Foitzik and Herbers 2001a). These gave between two and three cutting patterns, which we used to assign a composite haplotype to each individual. The haplotype diversity (Avice et al. 1989) was computed as follows:

$$h = 1 - \sum f_i^2 \quad (1)$$

with f being the frequency of each haplotype.

Data analysis

We analyzed our genetic data by inspection and Mendelian inference to reach conclusions about family structure and parentage. We also estimated allele frequencies and relatedness coefficients with the program Relatedness 4.2, weighting colonies equally (Queller and Goodnight 1989). The high allelic diversity allowed us to compute relatedness estimates over the entire population as well as for each colony. We report below the population-level estimates and their standard errors obtained by jackknifing, which were compared to the expected value with a two-tailed t -test. We used the mitochondrial haplotype data to examine population substructuring with AMOVA (Excoffier et al. 1992) as implemented by the program Arlequin 2.0 (Schneider et al. 2000). The AMOVA subroutine in Arlequin uses cutting-pattern information to estimate distances, thereby incorporating the presumed ancestral relationships among haplotypes. It further partitions variance in haplotype frequency among levels in a hierarchy and then tests for significance by bootstrapping after 1,000 permutations.

We also used the microsatellite and haplotype data to identify units of polydomous colonies (Pedersen and Boomsma 1999b; Foitzik and Herbers 2001a). We analyzed the demographic data at the nest level, because we could not always identify all nests in polydomous colonies and because the nest level is the appropriate unit at which to study sex allocation in this species (Herbers 1990; Herbers and Stuart 1996; Herbers and Banschbach 1998). Nest density was analyzed at the plot level, and below we disentangle the direct effects of our experiment on nest demography from the indirect effects of varying nest density (which might affect demography due to variable degree of colony subdivision).

For all statistical tests we used the program Statistica 4.5. We expected the parameters (food- and nest-site supplementation, block) to strongly interact with each other when influencing nest density, and therefore we included these factors when we examined nest frequency data with a log-linear analysis (Sokal and Rohlf 1995). This powerful bottom-up approach allowed us to study the impact of several parameters and their interaction effects at once. It has the liability, however, that significant interaction effects halt analysis for each factor alone. For other data analysis, attempts to normalize our variables by transformation (log, arcsine) failed, except for dry mass. Therefore, we were forced to use nonparametric tests: Mann-Whitney U test (MW), Kruskal-Wallis test (KW), and chi-square test (CS). When multiple tests were performed within a table, we used the sequential Bonferroni method to correct for table-wide testing (Rice 1989).

Results

Dry mass

The mean dry mass for workers was $216.6 \mu\text{g} \pm \text{SE } 6.2 \mu\text{g}$ ($n=90$), for males $231.1 \mu\text{g} \pm \text{SE } 7.0 \mu\text{g}$ ($n=41$), and for virgin queens $873.4 \mu\text{g} \pm \text{SE } 19.2 \mu\text{g}$ ($n=32$). The masses of new workers, males, and queens did not differ among colonies (MANOVAs; new workers: $F_{45}=1.57$, $P=0.07$; males: $F_{20}=0.96$, $P=0.55$; queens: $F_{15}=1.53$, $P=0.21$), plots (MANOVAs; new workers: $F_{55}=0.28$, $P=0.89$; males: $F_{36}=0.27$, $P=0.90$; queens: $F_{30}=1.57$, $P=0.21$), blocks (MANOVAs; new workers: $F_{88}=0.38$, $P=0.54$; males: $F_{39}=0.40$, $P=0.54$; queens: $F_{30}=3.93$, $P=0.06$), or treatments (ANOVAs; new workers, nest sites: $F_{86}=0.09$, $P=0.76$; food: $F_{86}=1.37$, $P=0.25$; males, nest sites: $F_{37}=0.15$, $P=0.70$; food: $F_{37}=0.05$, $P=0.82$; queens, nest sites: $F_{28}=0.58$, $P=0.45$; food: $F_{28}=0.41$, $P=0.53$). We therefore used the mean masses to compute the energetic cost ratio (Boomsma 1989) of 2.54, a value we use below to calculate allocation ratios for individual nests.

Genetic markers and genetic colony structure

Of the three microsatellite markers, the most polyallelic locus, LXAGT 1, exhibited 26 alleles between 199 and 257 bp with a H_{obs} of 0.893, while both L-18 (142–204 bp, $H_{\text{obs}}=0.943$) and L-5 (119–161 bp, $H_{\text{obs}}=0.855$) showed 21 alleles. A low but nonzero level of inbreeding ($F_{\text{is}}=0.067 \pm 0.014$, two-tailed t -test: $t_{154}=-4.70$, $P<0.001$) was substantiated by the presence of two diploid males in our samples. We did not detect substructuring with our nuclear markers among the different blocks ($F_{\text{st}}=0.005 \pm 0.003$, two-tailed t -test: $t_2=-2.05$, $P>0.10$).

We used the genetic data to assign nest fragments to polydomous colonies. If two nests were within 3 m and at least some workers shared microsatellite genotypes at all three loci as well as mitochondrial haplotypes, we considered them to be subunits of a polydomous colony (conservative probability of error $\ll 0.0001$ given the allele frequencies). Our procedure gave identical results to the rare-genotype method of Pedersen and Boomsma (1999b). Of 180 nests, 38 (21%) belonged to 17 polydomous colonies. That is, a minimum of 10.7% of all colonies were polydomous, each consisting of two or three subunits. There was no effect of treatment or block on the degree of polydomy (log-linear analysis: food: $\chi^2_1=0.39$, $P=0.53$; nest sites: $\chi^2_1=0.04$, $P=0.85$; block: $\chi^2_2=3.00$, $P=0.22$). Polydomous nest fragments were separated by an average of 0.78 ± 0.52 m, with a maximum distance of 2.09 m. Our estimates of worker-worker relatedness were identical for nests ($r=0.583 \pm 0.020$, $n=167$) and for polydomous colonies ($r=0.582 \pm 0.021$, $n=148$); below we report relatedness values for colonies.

Our mitochondrial data allowed us to examine maternally transmitted markers from 547 ants in 174 free-living *Leptothorax longispinosus* nests. We found 15 different haplotypes, each differing from another by no more than

Table 1 Number of nests of the focal species *Leptothorax longispinosus*/its social parasite *Protomognathus americanus* per 49-m² plot

Block	Control	Food	Nest sites	Both
1	13/1	15/6	43/3	34/9
2	27/0	9/0	26/1	25/0
3	39/1	5/6	22/0	25/2

Table 2 Influence of block and food- and nest-site supplementation on the number of *L. longispinosus* and *P. americanus* nests per 49-m² plot. Log-linear analyses with χ^2 tests of partial association for different models are shown. In bold are P values of the best models explaining the data; significant interactions in a log-linear analysis render main effects difficult to interpret

Species	Model	df	χ^2	P value
<i>L. longispinosus</i>	Food	1	11.31	0.0008
	Nest sites	1	15.67	0.0008
	Block	2	1.83	0.40
	Food \times Nest sites	1	12.31	0.006
	Food \times Block	2	3.76	0.15
	Nest sites \times Block	2	10.26	0.0005
<i>P. americanus</i>	Food	1	8.62	0.004
	Nest sites	1	0.03	0.87
	Block	2	15.24	0.0005
	Food \times Nest sites	1	0.20	0.50
	Food \times Block	2	2.35	0.27
	Nest sites \times Block	2	3.62	0.14

one cutting pattern at a single restriction enzyme (Fig. 3). In 10 colonies (5.8%) we found individuals with different haplotypes, indicating the presence of multiple unrelated matrilineal.

Nest density

In total, we collected 283 nests of the focal species *L. longispinosus* (Table 1), and densities varied from 0.10 to 0.88 nests per square meter. A log-linear analysis (Table 2) showed that nest density depended on several factors and their interactions. Nest-site supplementation interacted strongly with block ($\chi^2_2=10.26$, $P<0.005$). Only in block 1 did our addition of nest sites lead to an increase in nest density (Table 1; χ^2 tests; block 1: $\chi^2_1=12.0$, $P<0.0005$; block 2: $\chi^2_1=1.3$, $P=0.26$; and block 3: $\chi^2_1=0.05$, $P=0.83$). Food addition interacted globally with nest-site supplementation, with doubly supplemented plots having more nests than food-only but fewer than nest-site-supplemented plots (Table 2).

Nests of the social parasite *Protomognathus americanus* (which contained slaves of the focal species) varied among plots as well; in particular, plots in block 2 were nearly parasite-free (Table 1). Indeed, slave-maker density differed significantly among blocks (Table 2; median test on plot-based relative slave-maker density: $\chi^2_2=8.0$, $P<0.02$) and was also boosted by our food treatment ($\chi^2_1=8.62$, $P<0.004$). Slave-maker density, then, responded differently than its host to the ecological background.

Table 3 Differences in nest demography and productivity among three blocks. The results for KW tests and the mean \pm SD values are given; starred entries retain statistical significance after Bonferroni correction

	<i>P</i> value	Block 1	Block 2	Block 3
Number of queens	0.0001**	0.83 \pm 0.73	1.85 \pm 1.87	1.17 \pm 1.28
Number of workers	0.005*	35.2 \pm 31.9	32.4 \pm 25.2	21.9 \pm 16.2
Number of worker pupae	0.015*	9.4 \pm 12.9	12.2 \pm 14.6	12.9 \pm 14.58
Number of males	0.01*	4.0 \pm 8.52	4.9 \pm 8.5	1.5 \pm 3.0
Number of females	0.25	2.5 \pm 5.93	1.5 \pm 3.0	1.3 \pm 2.4
Male allocation ratio	0.12	0.61 \pm 0.06	0.58 \pm 0.05	0.44 \pm 0.06
Reproductive allocation ratio	0.04	0.38 \pm 0.04	0.36 \pm 0.03	0.24 \pm 0.02
Per-worker productivity in μ g	0.02*	132.7 \pm 14.5	160.7 \pm 18.9	258.1 \pm 50.7

* $P < 0.05$; ** $P < 0.01$

Given the spatial patchiness of slave-makers in our study site, we included slave-maker presence or absence as an additional factor for investigating host density. The four-factor log-linear analysis yielded a significant three-way interaction among parasites, food supplementation, and block ($\chi^2_2=41.8$, $P < 0.0001$); among parasites, nest-site supplementation, and block ($\chi^2_2=60.1$, $P < 0.0001$); and a three-way interaction between food- and nest-site supplementation and block ($\chi^2_2=13.6$, $P < 0.005$), which had been implicated in the first log-linear model (Table 2). Thus, host nest density responded to our two manipulations as a function of ecological background. In general, addition of nest sites led to an increase in nest density, while food caused a decrease in the number of host nests but an increase in the number of social parasite nests.

Differences among blocks

Because blocking quickly emerged as an important parameter for our results, we examined environmental heterogeneity in more detail. The blocks were close together in the forest, yet offered different microhabitats. Blocks 1 and 2 were more similar to each other than either was to block 3, the principal difference for our focal species being the availability of nesting sites. Block 3 had oaks in the canopy overhead, providing abundant acorns, which the cavity nesting *L. longispinosus* preferentially uses (Herbers 1989). As a result, in the third block, 58% of the occupied natural nesting sites were acorns or beechnuts, while in the other two areas these ephemeral nesting sites constituted less than 20% ($\chi^2_2=19.5$, $P < 0.001$), with colonies instead inhabiting cavities in sticks (85%). Furthermore, though each block was seeded with identical numbers of artificial nest sites, the ants used those additional sites at different frequencies. In blocks 1 and 2, 23% of the available dowels were occupied by our focal species, but in block 3 only 11% contained *L. longispinosus* colonies ($\chi^2_2=21.8$, $P < 0.001$). Thus, nest-site use differed strongly among blocks and doubtless contributed to significant block interactions with other variables.

The demography and investment patterns of host nests also differed among blocks (Table 3). Polygynous nests were more common in block 2 than in the others (Fig. 1; G-test, $\chi^2_4=30.8$, $P < 0.0001$; post-hoc comparisons of blocks 1 and 2: $\chi^2_2=29.4$, $P < 0.01$ and of blocks 2 and 3:

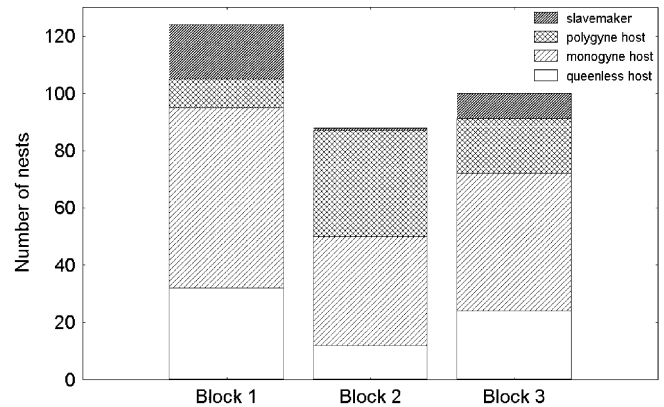


Fig. 1 Number of *Leptothorax longispinosus* nests with different social organization and the number of slave-maker nests found at the three blocks

$\chi^2_2=10.7$, $P < 0.01$; of blocks 1–3, $\chi^2_2=5.0$, $P = 0.05$). Nests in block 3 contained fewer adult workers than did block 1 (MW test: $U=3,677.0$; $n=91$, 105; $P < 0.006$) or block 2 ($U=2,940.5$; $n=91$, 87; $P < 0.003$), possibly owing to the smaller average size of nest sites (i.e., acorns) in block 3. Productivity (dry weight of new workers, queens, and males per adult worker) was highest in block 3 but did not differ between the two other study sites (Table 3). Production of new workers was lowest in block 1, but block 3 had the lowest production of males (Table 3), whereas the production of new queens was roughly equivalent across all blocks. No block effects were found on male allocation ratios or reproductive allocation ratios.

Worker-worker relatedness varied among blocks and plots within blocks (Fig. 2). Overall, intra-colony relatedness was higher in block 1 ($r=0.705 \pm 0.027$) than in block 2 ($r=0.531 \pm 0.039$; t -test; $t_{106}=5.2$; $P < 0.001$) or block 3 ($r=0.472 \pm 0.038$; t -test; $t_{94}=7.3$; $P < 0.001$). No difference in relatedness was found between block 2 and 3 (t -test; $t_{90}=1.5$, $P > 0.10$). Multiple matriline within colonies as indicated by Mendelian inferences based on our microsatellite data were more common in block 3 (75%) than in block 2 (60%), despite the greater frequency of polygyny in the latter (Fig. 1). Overall, queen-queen relatedness in polygynous colonies was 0.471 ± 0.053 , and it did not differ among blocks (t -tests, blocks 1 and 2: $t_{27}=0.95$, $P > 0.20$; blocks 1 and 3: $t_{18}=0.30$, $P > 0.50$; blocks 2 and 3: $t_{33}=1.10$, $P > 0.20$).

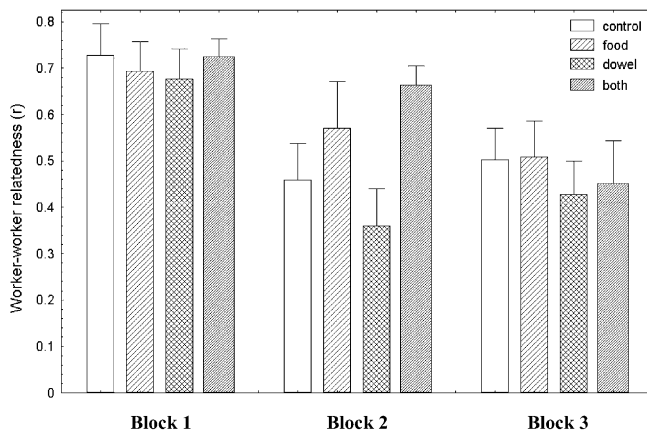


Fig. 2 Intracolony relatedness of workers by block and treatments. Mean \pm SE is given

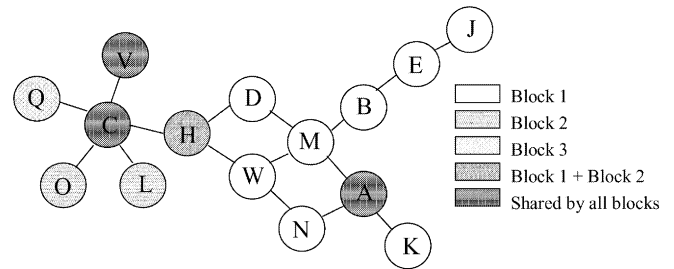


Fig. 3 Relationships among the different haplotypes in the study population. Each haplotype is joined to the next most similar haplotype, which differed at most at one cutting pattern with one restriction enzyme. Haplotype C is the most common haplotype in the entire population, with a frequency of 0.582, followed by haplotype A, with a frequency of 0.207

Table 4 Effect of food- and nest-site supplementation relative to control plots. The results of the KW tests are given, and starred entries retain statistical significance after Bonferroni correction. Means \pm SD are given; bold entries are significantly different from the control

	<i>P</i> value	Control	Food	Nest sites	Both
Block 1					
Number of queens	0.36	0.92 \pm 1.04	0.60 \pm 0.74	0.88 \pm 0.76	0.82 \pm 0.52
Number of workers	0.61	26.9 \pm 15.9	31.7 \pm 39.0	38.2 \pm 35.7	36.2 \pm 28.2
Number of worker pupae	0.11	7.0 \pm 9.5	7.1 \pm 17.3	9.9 \pm 12.1	10.7 \pm 13.2
Number of males	0.61	6.9 \pm 13.1	5.7 \pm 9.1	4.0 \pm 8.9	2.0 \pm 4.8
Number of females	0.004*	3.1 \pm 4.7	5.1\pm7.7	2.0\pm6.0	1.7\pm5.3
Male allocation ratio	0.02*	0.51 \pm 0.48	0.27\pm0.31	0.74\pm0.39	0.69\pm0.43
Reproductive allocation ratio	0.005*	0.62 \pm 0.36	0.76\pm0.38	0.33\pm0.39	0.19\pm0.31
Per worker productivity in μ g	0.23	185.5 \pm 178.3	299.8 \pm 298.5	125.8 \pm 130.8	112.0 \pm 155.5
Block 2					
Number of queens	0.0001**	2.33 \pm 2.27	2.89 \pm 3.14	1.85 \pm 1.26	0.96\pm0.79
Number of workers	0.08	40.4 \pm 28.2	41.7 \pm 37.18	23.3 \pm 18.3	29.7 \pm 20.1
Number of worker pupae	0.04	16.7 \pm 15.5	13.6 \pm 13.0	8.8 \pm 10.8	10.4 \pm 16.8
Number of males	0.0001***	9.2 \pm 10.8	7.8 \pm 12.7	2.8\pm4.2	1.4\pm4.9
Number of females	0.61	2.0 \pm 3.7	1.6 \pm 1.5	1.1 \pm 1.8	1.5 \pm 3.6
Male allocation ratio	0.014*	0.73 \pm 0.33	0.58 \pm 0.32	0.56 \pm 0.44	0.21\pm0.35
Reproductive allocation ratio	0.18	0.40 \pm 0.27	0.36 \pm 0.25	0.37 \pm 0.28	0.23 \pm 0.27
Per worker productivity in μ g	0.06	205.2 \pm 164.6	130.3 \pm 66.7	226.0 \pm 323.8	125.8 \pm 164.1
Block 3					
Number of queens	0.22	0.92 \pm 0.81	0.80 \pm 0.45	0.96 \pm 0.90	1.80 \pm 1.94
Number of workers	0.10	21.3 \pm 15.4	28.2 \pm 18.6	27.3 \pm 18.0	17.0 \pm 14.2
Number of worker pupae	0.09	17.0 \pm 16.7	8.2 \pm 7.8	11.8 \pm 13.9	8.6 \pm 11.2
Number of males	0.014*	0.7 \pm 1.6	4.2\pm4.4	3.0 \pm 4.7	0.9 \pm 1.6
Number of females	0.16	1.7 \pm 2.7	0.0 \pm 0.0	1.4 \pm 3.0	0.8 \pm 1.5
Male allocation ratio	0.006**	0.21 \pm 0.35	1.0\pm0.0	0.62 \pm 0.45	0.44 \pm 0.46
Reproductive allocation ratio	0.12	0.17 \pm 0.21	0.43 \pm 0.42	0.35 \pm 0.35	0.20 \pm 0.25
Per worker productivity in μ g	0.16	294.1 \pm 272.7	102.4 \pm 89.8	188.7 \pm 202.7	394.3 \pm 1010.3

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$

Haplotype diversity was considerably higher in block 1 ($h=0.762$) than in the other two sites (block 2: $h=0.451$; block 3: $h=0.335$). Total haplotype variation resulted from different colonies residing within plots (AMOVA, 72.4% of the variance explained, $P < 0.0001$), but haplotypes also varied among plots within blocks (AMOVA; 13.1% of the variance explained, $P < 0.001$), and the final 14.4% of the variance occurred among blocks (AMOVA, $P=0.05$). Haplotypes unique to a block typically differed by only

one cut (Fig. 3), indicating spatial structure to our mitochondrial markers.

Effects of ecological manipulations

Treatment groups within each block varied demographically (Table 4). In block 1, food-supplemented nests raised more females than did controls, whereas nest-

Table 5 Summary of the differences in slave-maker density, colony demography, genetic composition, and response to treatments among blocks

Parameter type	Parameter	Block 1	Block 2	Block 3
Demography	Slave-maker pressure	High	Low	Moderate/variable
	Nesting sites	Limited, sticks	Abundant, sticks	Abundant, acorns
	Queenless rate	High	Low	Moderate
	Polygynous rate	Low	High	Moderate
Life history	Number of queens	Low	High	Moderate
	Number of workers	High	High	Very low
	Number of worker pupae	Low	High	High
	Number of males	Moderate	High	Low
	Number of females	High	Low	Low
	Investment	Male biased	Male biased	Female biased
	Reproduction/growth	Sexuals	Sexuals	Workers
	Worker productivity	Low	Low	High
	Worker-Worker <i>r</i>	High	Low	Very low
Response to treatment	Haplotype diversity	High	Low	Low
	Effect of food	Increased females, female biased ratio, increase sexuals	Increased relatedness	Increased males, male-biased ratio
	Effect of nest sites	Increased density, decreased females, male-biased ratio, decreased sexuals	Decreased males	
	Effect of both treatments	Decreased females, male-biased ratio, decreased sexuals	Decreased queens, increased relatedness, decreased males, female-biased ratio	

supplemented and doubly supplemented nests raised fewer. Because our treatments did not influence the production of new workers or males at this study site, the observed responses in queen production consequently affected the male allocation and reproductive allocation ratios (Table 4).

By contrast, nests in block 2 responded to our ecological manipulations with changes in the number of resident queens and the production of males. The strongest effects on those plots resulted from an interaction between food- and nest-site supplementation (Table 4). Doubly supplemented plots had a higher fraction of monogynous nests compared to colonies in the control ($\chi^2_2=16.84$, $P<0.001$) or in the food-only plot ($\chi^2_2=14.31$, $P<0.001$). Nests in the plot that was supplemented with both nest sites and food also showed a more female-biased sex investment, because they reared fewer males (Table 4). Finally, worker-worker relatedness increased in response to food supplementation, regardless of nest-site supplementation (Fig. 2). This effect of food on intranest relatedness was not found in other blocks but was presumably caused by the observed decrease in the frequency of polygyny. Thus, manipulating ecological variables affected intranest relatedness only in block 2, where slave-makers were absent.

Nests in block 3 responded weakly to our manipulations. Food-supplemented nests raised more males and thus produced male-biased sex ratios relative to those on other plots. While nests from the three study sites differed in worker number, number of pupae reared, and per-worker productivity (Table 3), none of these variables responded to the experimental manipulation within any given block (Table 4).

Discussion

The social biology of *Leptothorax longispinosus* was known to exhibit strong spatial and temporal variation and therefore we designed our experimental manipulations to control for environmental heterogeneity such as canopy cover, aspect, and soil type. Despite the fact that our study areas were within 100 m of each other, significant differences in social organization, demography, and relatedness showed that biologically relevant heterogeneity occurs on a small scale for these ants (Table 5). Our ecological manipulations affected social and genetic structure of colonies in some sites as expected, while we noted opposing or no responses in other blocks. Food addition increased reproductive output in blocks 1 and 3 but not in block 2. Nest-site manipulation decreased male reproduction in block 1 but not in blocks 2 or 3. Food- and nest-site addition interacted to affect the male allocation ratio in blocks 1 and 2, but in opposite directions. Similarly, worker-worker relatedness was unaffected by our manipulations in blocks 1 and 3, but there were strong effects in block 2. This welter of seemingly confusing results can be resolved when we interpret block differences in the light of their ecological backgrounds.

As expected, slave-maker pressure and the availability and quality of suitable nest sites were found to be the two ecological parameters with the strongest impact on the *L. longispinosus* population at the Huyck Preserve. We suggest that the most important factor differentiating our blocks for *L. longispinosus* was the presence or absence of their social parasite *Protomognathus americanus*. This slave-making ant is known to be patchily distributed in forests (Herbers and Foitzik 2002), and the patchiness evident on our plots (Table 1) correlated strongly with

blocks so that the ratio of slave-makers to hosts was consistently high in all plots of block 1 (Table 1) and was heterogeneous in block 3, while in block 2 only a single slave-maker nest was found in 196 m² of forest floor. When we entered slave-maker presence or absence into our log-linear model of host nest density, this variable significantly interacted with our food manipulations and nest-site addition.

At the Huyck Preserve, the *P. americanus* raids are very destructive and the survival rate of host colonies is negligible (Foitzik et al. 2001). Additionally, the high prevalence of this social parasite means that each nest of *L. longispinosus* suffers from a 50% probability of being raided in any given year (Foitzik and Herbers 2001a). Finally, slave-makers have increased in density markedly over the past 20 years, and of three intensively studied populations, the Huyck Preserve population of *L. longispinosus* shows the strongest evidence of host-parasite coevolution (Herbers and Foitzik 2002). Therefore, we argue that the strong life-history differences between blocks 1 and 2 reflect variation in age structure due to exposure to slave raiding. Generally, larger, polygynous nests of *L. longispinosus* are older than smaller monogynous nests, since in this species polygynous associations arise when monogynous nests adopt newly mated daughters/sisters into the nest. We demonstrated earlier that in areas with large numbers of slave-makers, host colonies tend to be small, queenless, or monogynous, and we attributed these correlates of slave-maker pressure as reflections of frequent openings for newly established colonies in neighborhoods where they rarely mature to the stage of becoming polygynous (Foitzik and Herbers 2001a). Thus, differences between blocks 1 and 2 in the number of resident queens, and consequently in the intranest relatedness, can be explained by the impact of the social parasite on the age structure of host colonies. Furthermore, block 1 contained the greatest number of unique mitochondrial haplotypes and the highest haplotype diversity. That is, where slave-maker pressure was greatest, the between-colony mitochondrial diversity was greatest as well. This enhanced genetic diversity suggests many new colony-founding events in the area, as opposed to fissions of polygynous colonies, and is consistent with our inference that few nests survive slave raiding. Block 2, with virtually no pressure from parasites, displayed host nest demography and social organization predicted by low parasite pressure. The exception to that pattern was that nests in block 2 were not larger than in the highly parasitized block 1 (Table 3), which we attribute to an interaction with nest-site availability. In block 1, nest density increased with nest-site supplementation as new colonies were founded. By contrast, in block 2 colonies grew to be old and polygynous but were highly subdivided due to the high availability of nest sites; in other words, polydomous organization in block 2 yielded nests with worker numbers similar to those in block 1.

Host nest demography in block 3 is best explained with respect to the ecological background of nest-site quantity and quality. A high abundance of small nest sites, such as

acorns and nuts at this site, allowed colonies to split into several polydomous nest units. Consequently, the numbers of workers and queens per nest were especially low. The usage of these ephemeral nest sites might also explain the extremely low intranest relatedness. Nest mate queens were relatives, just as in other blocks, but colonies often contained several worker families, suggesting high queen turnover or mixing of colonies (Evans 1996; Bourke et al. 1997; Foitzik and Heinze 1998). This colony mixing could be caused by the use of ephemeral nest sites, which force colonies to move often.

Ecological differences among sites can explain why our experimental manipulations produced variable results (Table 5). In block 1, nest-site supplementation increased nest density as expected, but colony size increased rather than decreased with the addition of nest sites. This result, in direct contrast to that documented by Herbers (1986a), can be explained by the longer duration of our experiment, which allowed newly established colonies or colony fragments to grow to mature size over the duration of the manipulation. Colonies in these nest-site-supplemented plots still allocated more resources into colony growth versus sexual investment compared to control colonies. In addition, they produced a male-biased sex allocation ratio. Since males are the dispersing sex in this species (Foitzik and Herbers 2001b), this response allowed the colonies to send propagules outside of the locale with high parasite pressure.

Our food manipulations yielded responses in block 1 that were predicted by theory. An increase in food availability led to an increased production of new queens, a more female-biased sex ratio, and greater investment in sexuals relative to new workers. In this species, queens are 2.5 times more costly than males, causing female investment to be limited by food availability (Nonacs 1986a). A food-mediated shift to production of new queens should be especially advantageous when queens are philopatric, as our haplotype data suggest, because local food bonanzas predict good founding opportunities for daughter queens. However, our data contradict the queen-replenishment theory, which predicts that colonies should raise new queens only when the resident queen number is so low that colony production of brood is reduced or colony survival is threatened (Brown et al. 2002).

In block 2, where slave-maker pressure was very slight, addition of nest sites alone affected the biology of *L. longispinosus*, but only slightly. In nest-site-supplemented plots, colonies were, as expected, somewhat smaller and raised fewer new workers and males. However, supplementation with both food and nest sites yielded strong responses. Addition of food and nest sites reduced the number of resident queens, lowered male production, and caused sex allocation ratios to become more female-biased. The decrease in queen number led to higher relatedness in block 2, an effect shared with the larger ant *Myrmica punctiventris* in this same experiment (DeHeer et al. 2001). We suggest that, when released from constraints of nest-site- and food availability,

colonies refrained from readopting daughter queens because independent colony foundation by daughter queens was facilitated. Good ecological conditions can also lead to a higher rate of budding, by which queens spread out over a larger number of nest sites. The effect of double supplementation in block 2 therefore paralleled the differences between this population and another, where nest sites are not limiting and polygyny is uncommon (Herbers 1986b).

Block 3 showed only weak responses to our experimental manipulations, for which we offer two explanations. First, parasite pressure was highly variable at this site: some plots contained more slave-maker colonies than host colonies, while in others the social parasite was totally absent. Thus, variable parasite pressure may have superseded direct effects of food- or nest-site supplementation. Furthermore, the abundant availability of acorns as nest sites led us to expect no response to nest-site addition. Interestingly, the only effect of our manipulation was an increase in the numbers of males and consequently a male-biased allocation ratio in colonies on the food-supplemented plot compared to the control plot. This stands in stark contrast to theory (Nonacs 1986a, 1986b; Rosenheim et al. 1996) and to findings in other blocks but might be caused by the very low relatedness within ant colonies of this block. According to the relatedness asymmetry hypothesis (Boomsma and Grafen 1990, 1991), colonies with a lower relatedness asymmetry than the population average should invest resources mainly in males rather than in virgin queens.

A surprise from our study was that the social parasite itself responded to our manipulations. The number of slave-maker nests increased with the addition of food, opposite to the reaction of their hosts; perhaps raiding activity by the slave-maker was enhanced by food addition, thereby reducing its host density. If so, then the responses of *L. longispinosus* to food supplementation might reflect increased parasite pressure rather than the effects of food supplementation per se.

An earlier study of our focal species showed strong annual variation in response to food supplementation in another population (Backus and Herbers 1992; Herbers and Banschbach 1998). We were unable to incorporate a temporal dimension into our study here, and the spatial heterogeneity we documented may well be matched or exceeded by temporal heterogeneity. Certainly slave-maker pressure has been increasing over the years at our New York site (Herbers and Foitzik 2002), and long-term data show that slave-maker presence or absence has particularly strong effects on *L. longispinosus* demography in this site relative to other locales (Herbers and Foitzik 2002). Our study shows that ecological factors influencing social organization in insect societies cannot be studied in isolation, because the interaction among factors produces far richer responses than any one variable. This complexity, while daunting, nonetheless demonstrates that ecological background provides a fertile arena for exploring eusocial evolution.

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