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# Population structure of *Leptothorax ambiguus*, a facultatively polygynous and polydomous ant species

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**Abstract**

We examined the genetic and spatial structure of *Leptothorax ambiguus* in a Vermont site. Nests of this tiny ant species have variable queen number and comprise larger polydomous colonies, as do their closest relatives in North America. Nests are patchily distributed in the forest, and sometimes occur in local abundance. We collected 121 nests in four years from plots in which all nests were mapped; furthermore, we subjected nests collected in two separate years to starch gel electrophoresis and estimated relatedness according to the Queller–Goodnight (1989) algorithm. Queens that share a nest site also share 33% of their alleles on average, and relatedness among worker nestmates is about 0.5. The existence of diploid males and nonzero $F$-values demonstrate inbreeding in this species, an unusual phenomenon for social insects in general. Mapping data showed that nests with like genotypes tended to cluster in space, forming polydomous colonies. Colonies consisted of 1–6 nest subunits, and about half of all colonies were polygynous. We compare these features of *L. ambiguus* to its close relative *L. longispinosus* and a European congener *L. acervorum*. These comparisons allow us to conclude that an interplay between ecological and genetic factors produces the observed pattern of multiple queening and nest spatial distribution in this species.

The ant tribe Leptothoracini is particularly interesting for students of social evolution since it includes species that vary in the number of queens resident in nests (Buschinger, 1968), various forms of social parasitism have evolved repeatedly

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in this lineage (Buschinger, 1986), and colonies can have complex, polydomous structure (Alloway et al., 1982; Herbers and Tucker, 1986). In North America, three species of the Myrafant subgroup exemplify this complexity: *L. curvispinosus*, *L. longispinosus*, and *L. ambiguus* are facultatively polygynous, with some nests having one queen, others having two, still others having three and so on (Alloway et al., 1982). These three species also are host to social parasites in the genera *Leptothorax* and *Protomognathus* (=*Harpagoxenus*) (Buschinger, 1981). Finally, colonies can consist of two or more physically discrete nesting units, a condition termed polydomy (Alloway et al., 1982). Of these three species, extensive work on *L. curvispinosus* (Headley, 1943; Talbot, 1957; Stuart, 1985; Stuart et al., 1993) and *L. longispinosus* (Headley, 1943; Herbers, 1984; 1986a, 1986b) has been published, but rather less is known about *L. ambiguus* (Herbers, 1983; Alloway and Hodgson, 1990). This paper addresses that gap, and seeks to compare and contrast the population structure of this species with its two closest relatives in North America and a congener, *L. acervorum*, in Europe.

Both *L. longispinosus* and *L. curvispinosus* are widespread and locally abundant. *L. ambiguus*, however, appears to be more patchily distributed both within and between habitats. In our large-scale collection of *L. longispinosus*, we have picked up dozens of *L. ambiguus* nests, and here report on their spatial, demographic, and genetic structure.

## Methods

A population of *L. ambiguus* resident at Mallett's Bay State Park (Chittenden County, Vermont) has been sampled over several years. This site, and the ant community within which *L. ambiguus* is embedded, are described fully in Herbers (1989). Nests of *L. ambiguus* are patchily distributed in these temperate woods and are far outnumbered by their close relative *L. longispinosus* (Herbers, 1989). Our standard collection method is to excavate large plots (typically 36–49 $m^2$) and to map every nest thereby located. This method allows us to collect every nest in a locale without size or nesting type biases. Ant nests are transported to the laboratory where they are censused and frozen for further analysis.

Seven of twelve such plots that were excavated in 1986–87 yielded nests of *L. ambiguus* (Herbers, 1989); two plots in 1990, and two more in 1992 also included nests of our focal species. These plots were all located within a kilometer of each other in the woods.

Nests from the last four plots were examined for genetic variation via horizontal starch gel electrophoresis. Two suitably polymorphic enzymes were found, which represent two presumptive loci. All queens, all sexuals, and up to 20 workers from each nest were scored for PGD and MDH-2. For PGD, we used a discontinuous tris-citrate pH 8.5–LiOOH pH 8.1 buffer system, and for MDH-2 we used a continuous tris-citrate system, pH 6.5; recipes for buffers and stains were adapted from May (1980).

The spatial locations of nests and electrophoretic data were synthesized to identify putative colonies as follows: maps of nest locations in the forest were drawn up, and the genotypes of workers and queens superimposed thereon. Two nests that were within 2 meters of each other and whose workers shared the same set of electrophoretic variants were considered subunits of a larger polydomous colony (cf. Herbers, 1991). This decision rule was applied liberally so as to incorporate nests within larger polydomous colonies as often as possible: for example, if a genotype was found in one nest but not in its neighbor yet its absence in the latter could be a result of chance, the two were considered concolonial.

We analyzed the genetic structure of ant nests and putative colonies via the methods of Queller and Goodnight (1989). The algorithm allowed us to estimate relatedness coefficients among all potential interactants within nests and within colonies.

## Results

### *Demography and genetics of nests*

The database from which we draw inferences is synopsized in Table 1. From 1986–1992 we excavated 18 plots, but only 11 contained nests of *L. ambiguus*. Nest density was highly variable across these plots, as is typical for other leptothoracines in this habitat (Herbers, 1989). The initial set of plots excavated in 1986–87 gave baseline demographic data, while the four plots excavated in 1990 and 1992 gave both demographic and genetic data. The earlier plots were spread over all four seasons, and so we can assess the importance of seasonality in the distribution of queens and workers across nests.

Queen numbers among nests of *L. ambiguus* are shown in Fig. 1. The early samples showed strong seasonal variation in the distribution of queens among nests, such that queenless nests were common in spring and summer, while polygynous nests were more common in fall and winter. This seasonal pattern,

**Table 1.** Demography database for this study.

| Year | Season | No. of plots | No. of nests | Workers per nest | |
|---|---|---|---|---|---|
| | | | | Mean | s.e. |
| 1986 | spring | 2 | 11 | 18.73 | 2.44 |
| 1986 | summer | 3 | 30 | 13.03 | 1.43 |
| 1986 | fall | 1 | 6 | 19.33 | 8.76 |
| 1987 | winter | 1 | 9 | 24.78 | 2.66 |
| 1990 | summer | 2 | 37 | 22.81 | 3.06 |
| 1992 | spring | 2 | 28 | 22.64 | 2.76 |
| Total | | 11 | 121 | 19.95 | 5.06 |

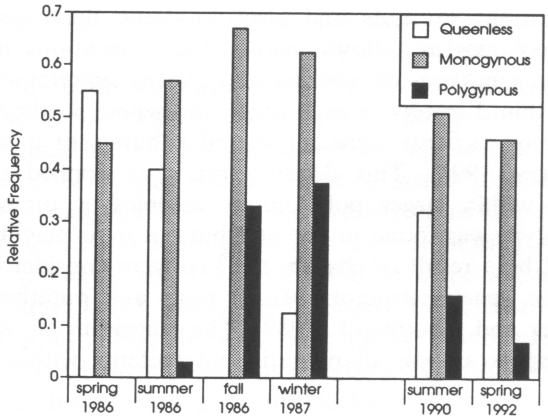


**Fig. 1.** Distribution of queens among nests in our six samples.

which deviates significantly from random ($G$-test, $P < 0.001$) is a reflection of the polydomous colony structure in this and related species: colonies break up in spring to form semi-autonomous nesting units, and re-coalesce in fall for overwintering (Alloway et al., 1982; Herbers, 1986b). The 1990 and 1992 samples, collected in summer and spring respectively, likewise gave numerous queenless nests. Comparisons among years (spring-summer 1986 vs 1990 vs 1992) show considerable annual variation in the frequencies of nests in different queen classes. While those differences were not significant ($G$-test, $P > 0.05$), they echo annual variation in queen numbers for the congener *L. longispinosus* (Herbers, 1990).

Across all samples, we collected nine nests with two queens; 2 nests with three queens; and one each containing five, eight, and ten queens. Overall, therefore, 14 of 121 ($=12\%$) contained multiple queens, 33 ($=27\%$) were monogynous and 74 ($=61\%$) were queenless.

Average numbers of workers per nest are given in Table 1. Nests were quite small, typically containing 20–25 workers and perhaps a queen. The largest nest we collected had 58 workers, and the smallest contained only a queen and a few eggs. Decreasing nest size in summer and increasing nest size in fall and winter, consistent with a polydomous seasonal cycle, are evident from the 1986–87 data. Nest sizes were larger in the 1990–92 samples than in the earlier samples, but no significant differences among samples were found (ANOVA with orthogonal contrasts, $P > 0.05$).

A synopsis of reprodctive data for our samples is given in Table 2. The population sex ratio (total number of males/total number of alates) varied from 0.716 in 1986 to 0.979 in 1990; the population allocation ratio (total dry weight of males/total dry weight of alates) ranged from approximately equal investment in both sexes (1986, 1992) to extremely male-biased (1990). In fact, in 1990 only one female was reared among 37 nests, and there was also a high rate of reproductive failure (Tab. 2). Across all three years, fewer than half of all nests reared sexuals.

**Table 2.** Reproduction by nests of *L. ambiguus*.

| | 1986 | 1990 | 1992 |
|---|---|---|---|
| Population sex ratio (proportion of males) | 0.716 | 0.979 | 0.745 |
| Population dry-weight allocation ratio | 0.457 | 0.940 | 0.493 |
| Proportion of nests rearing alates | 0.481 | 0.263 | 0.567 |
| Median No. of alates reared | 3.5 | 4 | 3 |
| No. of nests rearing males only | 16 | 9 | 7 |
| No. of nests rearing females only | 2 | 0 | 3 |
| No. of nests rearing both males and females | 9 | 1 | 3 |
| Proportion of nests rearing no alates | 0.519 | 0.737 | 0.433 |

Of those, sexual brood size was very small, with medians of 3–4 alates being reared. Finally, a characteristic "split sex ratio" (Nonacs, 1986; Boomsma and Grafen, 1991) was observed such that productive nests tended to rear only males or only females, with few rearing both sexes.

The genetic structure of this population was examined from electrophoretic data. The database upon which those inferences are based is given in Appendix I and synopsized in Table 3. A total of 57 nests was examined for genetic variability; of those, 34 contained queens and 11 reared at least one male. Unfortunately, no nest produced female alates in any sizable quantity (indeed, female alates are in short supply, Tab. 2). Three alleles were segregating at each locus in this population. The most common allele for PGD occurred in high frequency ($>95\%$ in each caste), while the MDH2 locus was more strongly polymorphic. Allele frequency differences among castes were not significantly different from zero ($G$-tests with 4df, $P > 0.05$ for both allozymes). Inspection of the raw data (Appendix I) confirmed that in general these presumptive loci followed Mendelian inheritance as well.

The raw data indicated that single mating is the rule in this population. In 55 of 57 nests, worker genotypes were consistent with the genotypes of the resident queen(s) mated to a single male. In only 2 ($=3.5\%$) was multiple paternity necessary to explain worker genotypes.

We found diploid males in three of the eleven nests ($=27\%$) that reared sexuals. In Nest VT-17 three of the seven males reared were heterozygous and therefore indisputably diploid; in nest VT-22 one of the two males and in VT-44 one of the three was clearly diploid. In those nests additional males that we scored as hemizygous could have been homozygous diploids, and our estimates for the overall frequency of diploid males (42% in diploid-male-producing nests and 10% over all nests) therefore represent lower limits.

We computed $F$-statistics for this population as well. The inbreeding coefficient, based on data from workers, was $F = 0.249 \pm 0.083$ s.e., a value significantly greater than zero (one-tailed $t$-test, $P < 0.01$). Coefficients based on data from queens ($F = 0.114 \pm 0.153$ s.e.) and males ($F = 0.503 \pm 0.213$ s.e.) were not different from zero, but small sample sizes gave those comparisons extremely low power.

Nonzero $F$-values (i.e., an excess of homozygotes) can reflect inbreeding or population subdivision (Wahlund, 1928). To distinguish between these possibilities,

we recomputed $F$-statistics whereby nest were identified by plot of collection origin. The analysis showed no significant differences among plots ($F_{ST} = 0.04$), indicating that the nonzero $F$-values reflect inbreeding rather than the Wahlund effect.

Our genetic database contained 34 nests with queens, but only six of those were polygynous. Similarly, while 11 nests produced males, only 9 reared at least two males. Thus our inferences on relatedness among nestmates, which require at least two individuals in each group, are based on smaller samples than the numbers in Table 3.

Relatedness coefficients are given in Table 4. The expected proportions of alleles shared among nestmates all of whom share the same mother and father (i.e. the

**Table 3.** Genetic profile of nests used to infer relatedness structure.

| | Queens | Workers | Males | Total |
|---|---|---|---|---|
| No. of nests | 34 | 57 | 11 | 57 |
| No. of individuals | 50 | 817 | 44 | 911 |
| Allele frequencies | | | | |
| MDH2 | | | | |
| a | 0.051 | 0.054 | 0.061 | 0.052 |
| b | 0.724 | 0.742 | 0.837 | 0.738 |
| c | 0.225 | 0.204 | 0.102 | 0.210 |
| PGD | | | | |
| a | 0.020 | 0.003 | 0 | 0.006 |
| b | 0.970 | 0.953 | 0.980 | 0.953 |
| c | 0.010 | 0.004 | 0.020 | 0.041 |

**Table 4.** Genetic structure of social insects. (a) relatedness values expected in a colony headed by a single once-mated queen and with no egg-laying by workers (Hamilton 1964); (b) relatedness observed in **nests** and (c) relatedness observed in **colonies** og *Leptothorax ambiguus*. Entries in (b) and (c) are the relatedness estimates ± one standard error (number of nests/colonies). Estimates significantly greater than 0 are **bolded**. Estimates significantly less than their Hamiltonian expectations are *italicized*.

| | | | Relatedness of | | |
|---|---|---|---|---|---|
| | | | Queens | Workers | Males |
| (a) | to | Queens | – | $\frac{1}{2}$ | 1 |
| | | Workers | $\frac{1}{2}$ | $\frac{3}{4}$ | $\frac{1}{2}$ |
| | | Males | $\frac{1}{2}$ | $\frac{1}{4}$ | $\frac{1}{2}$ |
| (b) | to | Queens | 0.328 ± 0.405 (6) | ***0.223 ± 0.097 (35)*** | **0.767 ± 0.194 (6)** |
| | | Workers | ***0.254 ± 0.116 (35)*** | ***0.441 ± 0.075 (57)*** | **0.451 ± 0.107 (11)** |
| | | Males | 0.283 ± 0.319 (6) | 0.014 ± 0.181 (11) | 0.406 ± 0.184 (9) |
| (c) | to | Queens | 0.336 ± 0.243 (11) | **0.324 ± 0.099 (27)** | **0.674 ± 0.181 (8)** |
| | | Workers | **0.358 ± 0.125 (27)** | ***0.496 ± 0.109 (36)*** | **0.457 ± 0.111 (9)** |
| | | Males | 0.308 ± 0.189 (8) | 0.114 ± 0.181 (9) | 0.423 ± 0.196 (7) |

colony is headed by a single, once-mated queen) are given in Table 4a (Hamilton, 1964; Oster and Wilson, 1978). These expectations range from 1/4 for worker-male relatedness to 3/4 for worker-worker relatedness (Hamilton, 1964; Oster and Wilson, 1978). In Table 4b, we present corresponding estimates for nests of *L. ambiguus*, along with standard errors of those estimates and sample sizes.

Elements along the diagonal in Table 4 represent estimates of relatedness among nestmates of the same caste. In nests of *L. ambiguus*, queens in polygynous nests share on average 33% of their alleles; worker nestmates share 44% of their alleles; and males reared in the same nest share 41% of their alleles.

Off-diagonal elements represent relatedness estimates across different castes. Hamilton (1964) first pointed out that the *directionality* of relatedness was important for haplodiploid systems. Inspection of Table 4a shows that for females, relatedness estimates are transitive whereas a male's relatedness to his female relative does not equal that female's relatedness to him. For a colony with one singly-mated queen and no worker egg-laying, males are in fact twice as closely related to their mothers and sisters than the latter are to the males (Table 4a).

Our empirical results on relatedness asymmetry follow the predicted pattern: queens and workers, both females, were related to each other by 22–25%, depending on which caste is the referent. Relatedness estimates between males and females, however, were strongly asymmetric: males shared about 77% of their alleles with queens, while queens shared only 28% of their alleles with those males. Similarly, males had 45% of their alleles in common with worker nestmates, but those workers only shared 1.4% of their alleles with the males. These asymmetries of relatedness, a direct consequence of the haplodiploid mechanism of sex determination (Hamilton, 1964), thus represent a 3-fold imbalance between queens and males, and a 32-fold imbalance between workers and males. The large standard errors on such estimates of relatedness asymmetry preclude detailed analysis of these values, but we note they are in qualitative agreement with predictions of kin selection theory: within a nest, males are more closely related to females than those females are to the males.

Our small sample sizes produced sizable standard errors for some estimates. Of the nine estimates in Table 4b, only five are significantly greater than zero. The large variance in queen-queen relatedness is especially interesting. We found some polygynous nests with sets of queens that appeared to be close relatives, while in other nests queens were cohabiting peacefully even though they could not have been full or even half-sisters. The nest with 10 queens contained three queen genotypes consistent with two **families** of queens. Within this group, some queens were closely related and others were unrelated. Thus the average value for queen-queen relatedness of 0.328 belies considerable variation within and among nests.

We also examined the estimates of Table 4b for deviations from the upper limit expected under perfectly Hamiltonian conditions (Table 4a). Of the estimates in Table 4b, the relatedness of workers to queens, queens to workers, and workers to each other all were significantly below those expectations (2-tailed $t$-tests). Since multiple-mating appears to be relatively unimportant in this population, we attribute reduction of relatedness values below that expected for a monogynous-monoandrous colony to polygyny per se.

The effective queen number (Queller, 1993) is a useful measure of the contribution of polygynous nests to the overall population genetic structure. We were unable to estimate effective queen number from the harmonic mean of queen numbers across nests (Wade, 1985), however, because our samples included queenless nests; harmonic means cannot be computed for data that include zeros, and no transformation exists to allow such a mean to be computed and back-transformed. Rather, we estimated effective queen number from the estimates of worker-worker relatedness according to the formula of Queller (1993). Under an assumption of single-mating, our worker-worker relatedness of 0.441 suggests an effective queen number of 1.93. This value is actually an upper estimate, since Queller's formula (1993) is sensitive to inbreeding.

*Demography and genetics of colonies*

Further inspection of the electrophoretic data suggested additional complexities. Several nests that contained just one queen contained more than one matriline of workers; that is, monogynous nests comprised groups of workers, only some of which were offspring of the queen in residence. Three explanations are possible: 1) monogynous nests are fractions of larger polygymous colonies; 2) the nest had recently lost a second queen, whose worker offspring remain in the nest and 3) there is considerable mixing of workers among different colonies in this species. To examine the first possibility, we identified putative colonies by employing a decision rule that synthesized genetic and spatial data.

Using this decision rule allowed us to identify groups of nests that were members of putative colonies (cf. Fig. 2). From 57 nests, a total of 35 colonies were thus identified, and a summary of colony demographics is given in Table 5. We were unable to identify con-colonial nests for 24 nests, which thereby constituted their own monodomous "colonies", including eight that were queenless. Many of these monodomous nests were on the edges of excavated quadrats, and may indeed have been members of polydomous colonies that were not completely collected. The remaining 33 nests were assigned to polydomous colony groups.

Many polydomous colonies were compact, consisting of only 1 or 2 nest subunits, but we found two five-nest colonies and one six-nest colony (Tab. 5). The distribution of queens among colonies differed from that among nests; in particular, polygyny was more common among colonies ($13/35 = 37\%$) than among nests (12%), cf. Fig. 1). Also, worker number was necessarily higher in colonies ($\bar{x} = 40.08 \pm 6.76$ SE) than in nests (cf. Tab. 1). The largest colony we identified contained six queens and 209 workers, while the smallest contained one queen and 3 workers (Tab. 5).

Of the 35 colonies, eight had no queen. Queenless colonies may have been orphans, but the fact that all of them consisted of a single nest near the plot's edge suggests that a sizable number were in fact fragments of larger queenright colonies. Our data cannot distinguish between orphaning and incomplete collection, but we suspect the latter is a better explanation for queenless colonies.

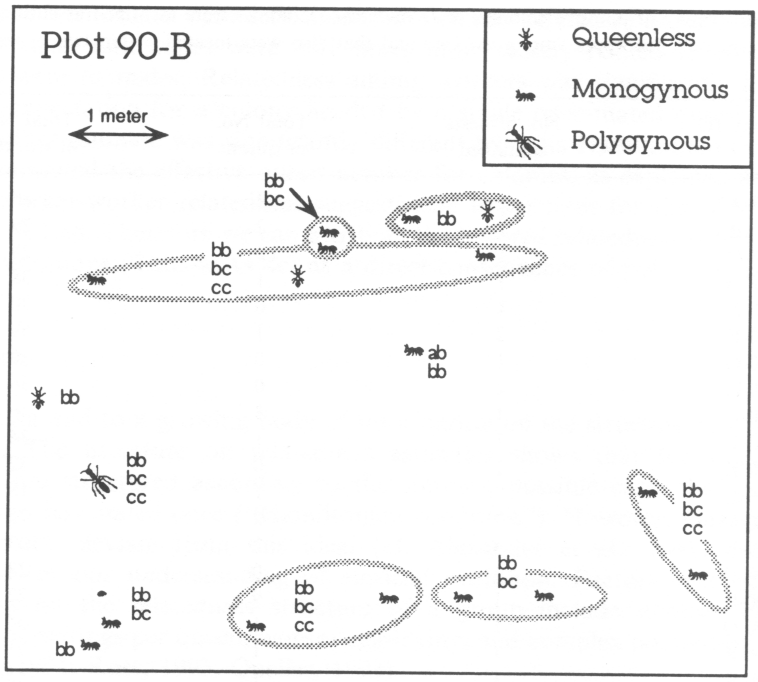


**Fig. 2.** A representative plot showing putative colony boundaries. Worker genotypes at two loci were used to draw these boundaries, but for simplicity we show only genotypes at the MDH2 locus here. The complete data set is given in Appendix I.

For several nests, assignments to polydomous colonies resolved inconsistencies between queen and worker genotypes. For example, Colony N includes workers in nest 101 that could not be offspring of the resident queen but whose mother was probably the queen in nearby nest 94 (cf. Appendix I). Even so, five colonies remained whose worker genotypes were not wholly consistent with the queen(s) genotype(s). These colonies included some workers whose mother could not have been a resident queen (e.g. Nest 41 in Colony C, Appendix I). The most plausible explanation for these is recent queen death, with a transformation from previous polygyny to current monogyny. Our estimate for such events is therefore $5/57 = 8.8\%$ of all nests in the population.

Diploid males were produced in a least two colonies (Tab. 5). Values for inbreeding coefficients derived from worker data ($F = 0.288 \pm 0.112$ s.e.) and from queen data ($F = 0.303 \pm 1.55$ s.e.) were significantly different from zero (one-tailed $t$-tests; $P < 0.01$ and $P < 0.05$, respectively) but the estimate based on male data was not, a test that had extremely low power.

We estimated relatedness coefficients among members of colonies (Tab. 4C). Sample sizes were larger for these than were relatedness estimates within nests, since we had more polygynous colonies and more colonies that reared at least two males.

**Table 5.** Demography of putative colonies of *L. ambiguus*. Colonies were identified as clusters of nests that shared common genotypes among workers and that also were located within two meters of each other.

| Colony ID | No. of nests contained | Total No. of queens | Total No. of workers |
|---|---|---|---|
| 90-A | 1 | 2 | 88 |
| 90-B | 1 | 2 | 29 |
| 90-C | 1 | 2 | 28 |
| 90-D | 1 | 1 | 38 |
| 90-E | 1 | 0 | 20 |
| 90-F | 2 | 1 | 59 |
| 90-G | 1 | 0 | 30 |
| 90-H | 1 | 0 | 36 |
| 90-I | 1 | 3 | 6 |
| 90-J | 1 | 1 | 34 |
| 90-K | 1 | 0 | 32 |
| 90-L | 2 | 1 | 18 |
| 90-M | 2 | 2 | 23 |
| 90-N | 3 | 2 | 103 |
| 90-O | 1 | 0 | 23 |
| 90-P | 1 | 9 | 22 |
| 90-Q | 1 | 1 | 55 |
| 90-R | 1 | 1 | 21 |
| 90-S | 1 | 1 | 15 |
| 90-T | 2 | 2 | 56 |
| 90-U | 2 | 2 | 63 |
| 90-V | 2 | 1 | 60 |
| 92-1 | 1 | 0 | 11 |
| 92-2[1] | 6 | 4 | 209 |
| 92-4 | 5 | 2 | 104 |
| 92-5 | 2 | 1 | 77 |
| 92-6[2] | 5 | 3 | 74 |
| 92-7 | 1 | 0 | 14 |
| 92-8 | 1 | 1 | 14 |
| 92-9 | 1 | 0 | 11 |
| 92-10 | 1 | 1 | 20 |
| 92-11 | 1 | 1 | 22 |
| 92-12 | 1 | 1 | 5 |
| 92-13 | 1 | 1 | 3 |
| 92-14 | 1 | 2 | 20 |

[1] This colony included two nests that produced diploid males.
[2] This colony included one nest that produced diploid males.

The estimates of relatedness among colonies (Tab. 4c) followed the same pattern as estimates of relatedness among nests: there was considerable variation among colonies that, along with small sample sizes, led to sizable standard errors of the relatedness estimates. As a result, only five of the nine estimates in Table 4c are significantly greater than zero. Relatedness asymmetries were evident among

colonies as well: males were 2.19 times more closely related to queens than queens were to males and males were 4.01 times more closely related to workers than workers were to males. Relatedness among workers was significantly lower than 3/4, the expectation for a colony headed by a single once-mated queen (Tab. 4a), but no other estimate was significantly different from its Hamiltonian expectation.

We computed the effective queen number for colonies, as explained above. The colony worker-worker relatedness suggested an upper limit for the effective queen number of 1.68. Therefore we can attribute the lowered relatedness below Hamiltonian expectations (Tab. 4a vs 4c) as a direct consequence of polygyny in colonies.

## Discussion

Our data add to a growing body of information on the structure of social insect colonies. The literature on relatedness estimates shows that for some species, colonies are structured according to the simplest possible case, that of a single queen who has mated once ("Hamiltonian condition"). However, many species of social insects deviate from this ideal (cf. Alexander et al., 1991) and present problems for our understanding of eusocial evolution. Factors that have major influences on the relatedness structure of a group include number of queens, number of matings per queen, worker egg-laying, and complex population structure (Pamilo, 1991; Ross, 1993; Queller, 1993).

Population structure in the North American Myrafant group of the ant tribe Leptothoracini is known to be complex (Alloway et al., 1982; Herbers, 1984). Colonies can be initiated by single queens (haplometrosis) or jointly by several queens sharing a nest site (pleometrosis). Our data support earlier inferences by Alloway et al. (1982) that colonies of *L. ambiguus* can be founded in both fashions. One incipient colony we found had a single queen with a few eggs, while another consisted of two queens and a single worker. Our data also support the conclusions of Alloway et al. (1982) that colonies are polydomous: with our decision rule, well over half of all nests could be identified as subunits of larger colonies (Fig. 2).

There are striking similarities and some important differences between *L. ambiguus* and its close relative *L. longispinosus*. Both species inhabit the same forest types, and nest in cavities like hollow acorns and twigs (Herbers, 1989). Both are facultatively polygynous, but colonies of *L. longispinosus* are founded almost exclusively by single queens, with polygyny developing secondarily when established nests accept daughters back after the mating flight (Herbers and Stuart, 1994). In contrast, colonies of *L. ambiguus* can be initiated cooperatively by several newly-mated queens (pleometrosis) or by single queens (haplometrosis). Thus polygyny in this species can be primary, for colonies founded pleometrotically, or secondary, if haplometrotic colonies accept additional queens later in their life cycle (Hölldobler and Wilson, 1977; Herbers, 1993),

We cannot estimate from our data how often polygyny arises in *L. ambiguus* from pleometrotic colony foundation versus adoption of newly-fertilized queens. Our estimate of queen-queen relatedness ($r = 0.33$) is consistent with a mix of

primary and secondary polygyny. We have no information about the relatedness structure of queens that found colonies pleometrotically in *L. ambiguus*, but in other ant species, such cooperating queens typically are not related (Strassmann, 1989). Understanding the forces that promote strict haplometrosis in *L. longispinosus* but allow for pleometrosus in *L. ambiguus* is an interesting challenge for future work on this group.

Nest demography is similar between the two species as well. The pattern of queen distribution among nests shows considerable annual variation, and nests are small, typically containing 20–25 workers. Reproduction in *L. ambiguus* follows a pattern similar to that documented for *L. longispinosus* (Herbers, 1990): many nests fail to rear sexuals in any given year, and those that do reproduce tend to rear a small number (Tab. 2). There is strong annual variation in the overall population sex ratio and ratio of investment, with a tendency to male-bias in this population. Finally, sex ratios of the nests' reproductive broods exhibit a bimodal distribution. That distribution for *L. ambiguus* results in part from the small number of sexuals reared by any given nest, but it also conforms to a general pattern of "split sex ratios" that characterizes eusocial hymenopterans (Nonacs, 1986).

Relatedness structure is very similar between *L. longispinosus* and *L. ambiguus*: polygyny occurs in both populations, and queens that cohabit tend to share a sizable fraction of their genes. Queens are more closely related in *L. longispinosus* (Herbers and Stuart, 1994) than in *L. ambiguus*, but the differences may not be biologically significant. Relatedness among workers in nests of both species remains high, approximately 0.5 (Herbers and Stuart, 1994 and Tab. 4a herein) showing that the presence of multiple laying queens in a nest can be consistent with a kin selection scenario.

Recent queen death was inferred from our electrophoretic data for fewer than one-tenth of *L. ambiguus* nests. Queen deaths could also be inferred from the proportion of queenless colonies (23%). The former is an underestimate, because of the limited resolution afforded by allozyme data. The latter is an overestimate, since the queenless colonies represented both orphans and incompletely-collected colonies. It seems, then, that queen death occurs at a rate between 10 and 20%, and is not as important in this species as in others like *Myrmica ruginodus* (Seppa, 1994). The orphaning data were not sufficient to estimate mortality rates or life spans for queens (Pamilo, 1993; Seppa, 1994).

We can also compare the genetics of *L. ambiguus* with its European relative *L. acervorum*. The latter species is facultatively polygynous, and its queens are related so that workers share a high proportion of their alleles with nestmates (Douwes et al., 1987; Stille et al., 1991). Even though colonies appear not to be polydomous, Stille and Stille (1993) found strong evidence of population spread by "diffusion" of maternal genotypes across space. This last report did not examine colony boundaries per se, but it seems that clustering of mtDNA haplotypes results not from a polydomous colony structure but from limited dispersal by female sexuals.

Our electrophoretic data had limited resolution for asking detailed questions about genetic structure in these colonies. First, we were unable to ascertain the reproductive status of queens in polygynous nests. Alloway et al. (1982) had found

that about one-half of all queens in polygynous associations were not fertile, but we could only infer fertility from the electrophoretic profile of workers and males. Those data indicate that most queens in polygynous associations were indeed contributing to the pool of offspring in the nest. Our data likewise were unable to give us good information on unequal reproduction among cohabiting queens or worker egg-laying, both factors known to influence genetic structure (Queller, 1993). This poor resolving power was due to two factors: first, electrophoretic data are inherently less powerful for answering such questions than hyper-variable traits such as mtDNA (Stille and Stille, 1993) and microsatellite DNA (Queller et al., 1993). Second, the complexities introduced by polydomous colony structure can overwhelm even highly sensitive data analyses (Herbers and Stuart, 1994). When colonies undergo fission, fusion, worker exchange and the like, genotype mapping may not sort out family structures. Despite the suboptimal resolution of our data, we suspect that reproductive inequity and worker egg-laying are not particularly important. Neither factor has been demonstrated to have a strong effect on any other leptothoracine and the presence of multiple laying queens in colonies appears to explain most of the variation in worker genotypes. However, these must remain open questions for future work.

We were surprised to find diploid males and nonzero inbreeding coefficients in this population. Diploid males, which typically result from inbreeding (Ross, 1993), have not been reported for other leptothoracines (including the closely-related *L. longispinosus*, Herbers and Stuart, 1993) and inbreeding coefficients generally hover around zero in social insects (Crozier and Pamilo, 1994). The $F$ coefficient represented true inbreeding rather than population subdivision, showing there is gene flow across the population we sampled yet a measurable degree of local viscosity. We suspect this inbreeding is a result of the patchy distribution of nests in habitats. Males are not strong flyers (pers. obs.) and their limited dispersal ability may promote mating among relatives when nest density is low and sexuals are reared by separate nests in polydomous colonies. Interpretation of sex ratio information requires larger data sets and more sophisticated analysis than we have provided here, but we point out that the male-biased sex ratios in this population may reflect local mate competition (Sherman and Alexander, 1977; Frank, 1987), a phenomenon linked to inbreeding cycles.

Colony structure has been explored in several other species (Herbers and Stuart, 1993; Herbers, 1990; Snyder and Herbers, 1991; Boomsma et al. 1993). For some, polydomous colonies can be easily identified (*Myrmica punctiventris*, Snyder and Herbers, 1991; *Tapinoma minutum*, Herbers, 1990), whereas for others colony structure is amorphous, with indistinct or absent boundaries (*Rhytidoponera mayri* [=sp. 12], Crozier et al., 1984; *Leptothorax longispinosus*, Herbers and Stuart, 1994). Our data here show that *L. ambiguus* colonies can be readily discerned from genetic data, since clusters of nests often share a worker genotypic profile. Most striking is the conclusion that *L. ambiguus* colonies are structured more strongly than those of its close relative *L. longispinosus*. This result may reflect its relative patchiness in the woods: *L. ambiguus* is found in fewer places and typically in lower abundance than *L. longispinosus* (Herbers, 1989).

Perhaps a competitive regime constrains colony structure in this species. It is likely that *L. ambiguus*, like its congener *L. longispinosus*, is affected by the availability of empty nest sites (Herbers, 1986b, 1989). Further, the virtual absence of *L. ambiguus* female alates in 1990, a year when its congener reared females in the same habitat (Backus, 1992), indicates they may be food-limited as well. If this scenario is correct, then the outcome of interspecific competition is that *L. ambiguus* occupies patches of habitat in relatively low densities. Low nest density would promote local inbreeding, leading to inbreeding depression, as manifested by production of diploid males. Lowered fitness associated with production of diploid males (Ross, 1989), coupled with the absence of similar inbreeding depression in its competitor, could exacerbate the competitive asymmetry between congeners such that *L. ambiguus* would remain constrained to be locally rare and patchily distributed. Thus, we envision ecological forces and genetic systems interacting to produce the observed population structure.

We suggest a direct link between relative rarity and inbreeding in this population. If correct, then the general absence of inbreeding in social insect genetic studies (Crozier and Pamilo, 1994) may be an artefact of how investigators choose study species. Because inbreeding and relatedness estimates typically require large sample sizes (Wilkinson and McCracken, 1985), research doubtless has focused on common, widespread species rather than rare or locally restricted species. Conclusions about the prevalence of inbreeding in social insects therefore must await more studies on less abundant species.

Thus, while the two species share the same macrohabitat and same microhabitat, nuances of their biology differ. Genetic structure within nests is similar but the species differ strongly in colony ontogeny and structuring in space. Their ecological milieus are distinct despite overall similarity in nesting and foraging habits. Such comparisons between *L. ambiguus* and *L. longispinosus* underscore the difficulty of extrapolating an evolutionary explanation for a trait in one species from its history in a relative.

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## Appendix I. Genotypic data inferred from starch gel electrophoresis

| | | | | PGD | | | | | | | MDH-2 | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Plot | COLONY | NEST | caste | b | c | aa | ac | bb | bc | cc | b | c | ab | ac | bb | bc | cc |
| 1990-A | A | 36 | W | | | | | 18 | | | | | | 5 | 8 | | 2 |
| | | | Q | | | | | 2 | | | | | | 1 | 1 | | |
| | | | M | 5 | | | | | | | 5 | | | | | | |
| | B | 38 | W | | | | | 3 | 7 | 5 | | | | | 5 | 2 | 8 |
| | | | Q | | | | | 1 | | | | | | | | | 1 |
| | | | M | 1 | | | | | | | | | | | | | |
| | C | 41 | W | | | | | 30 | | | | | | | 12 | 10 | 7 |
| | | | Q | | | | | 1 | | | | | | | 1 | | |
| | D | 34 | W | | | | | 34 | | | | | | | 22 | 2 | |
| | | | Q | | | | | 1 | | | | | | | 1 | | |
| | E | 33 | W | | | | | 14 | 2 | | | | | | 12 | 2 | |
| | | | M | 2 | 1 | | | | | | 3 | | | | | | |
| | F | 42 | W | | | | | 12 | 2 | | | | | | 14 | | |
| | | 47 | W | | | | | 5 | 2 | | | | | | 15 | | |
| | G | 43 | W | | | 1 | | 8 | 1 | 1 | | | | | 4 | 7 | 1 |
| | H | 56 | W | | | | | | | 12 | | | | | 12 | | |
| | I | 55 | W | | | | | 3 | | | | | | | 7 | | |
| | | | Q | | | | | 3 | | | | | | | 3 | | |
| | J | 48 | W | | | | | 21 | | 1 | | | | | 12 | 3 | 6 |
| | | | Q | | | | | 1 | | | | | | | 1 | | |
| | K | 58 | W | | | | | 10 | | | | | 5 | | | 5 | |
| 1990-B | L | 102 | W | | | | | 10 | | | | | | | 10 | | |
| | | | M | 4 | | | | | | | 4 | | | | | | |
| | | 104 | W | | | | | 15 | | | | | | | 15 | | |
| | | | Q | | | | | 1 | | | | | | | 1 | | |
| | M | 71 | W | | | | | 9 | | | | | | | 4 | 5 | |
| | | | Q | | | | | 1 | | | | | | | | | |
| | | 90 | W | | | | | 10 | | | | | | | 4 | 5 | |
| | N | 70 | W | | | | | 10 | | | | | | | 5 | 2 | 2 |
| | | 94 | W | | | | | 14 | | | | | | | 9 | 1 | 4 |
| | | | Q | | | | | 1 | | | | | | | | 1 | |
| | | 101 | W | | | | | 16 | | | | | | | 1 | 12 | 3 |
| | | | Q | | | | | 1 | | | | | | | 1 | | |
| | O | 72 | W | | | | | 12 | | | | | | | 12 | | |
| | P | 73 | W | | | | | 20 | | | | | | | 5 | 8 | 3 |
| | | | Q | | | | | 10 | | | | | 2 | | 3 | 5 | |
| | | | M | 8 | | | | | | | 7 | 1 | | | | | |
| | Q | 66 | W | | | | | 22 | | | | | | | 16 | | |
| | | | Q | | | | | 1 | | | | | | | 1 | | |
| | R | 69 | W | | | | | 15 | | | | | | | 2 | 14 | |
| | | | Q | | | | | 1 | | | | | | | | 1 | |
| | S | 100 | W | | | | | 21 | 2 | | | | 9 | | 12 | | |
| | | | Q | | | | | 1 | | | | | | | 1 | | |
| | T | 83 | W | | | | | 15 | | | | | | | 12 | 1 | 2 |
| | | | Q | | | | | 1 | | | | | | | 1 | | |
| | | | M | 1 | | | | | | | 1 | | | | | | |
| | | 98 | W | | | | | 13 | | | | | | | 9 | 2 | 2 |
| | | | Q | | | | | 1 | | | | | | | | 1 | |
| | U | 113 | W | | | | | 22 | | | | | | | 11 | 11 | |
| | | | Q | | | | | 1 | | | | | | | | 1 | |
| | | 117 | W | | | | | 14 | | | | | | | 6 | 8 | |
| | | | Q | | | | | 1 | | | | | | | | 1 | |
| | V | 115 | W | | | | | 9 | | | | | | | 6 | 2 | 1 |
| | | 119 | W | | | | | 14 | | | | | | | 9 | 1 | 4 |
| | | | Q | | | | | 1 | | | | | | | 1 | | |
| 1992-A | 1 | 3 | W | | | 1 | | 10 | | | | | | | 10 | | |
| | 2 | 22 | W | | | | | 16 | | | | | | | 3 | 10 | 1 |
| | | | M | 1 | | | | 1 | | | 1 | | | | | 1 | |
| | | | Q | | | | | 1 | | | | | | | | 1 | |
| | | 44 | W | | | | | 14 | | | | | | | 3 | 9 | |
| | | | M | 2 | | | | 1 | | | 2 | | | | | 1 | |
| | | 46 | W | | | | | 15 | | | | | | | 7 | 6 | |
| | | | Q | | | | | 1 | | | | | | | | 1 | |
| | | 47 | W | | | | | 15 | | | | | | | 8 | 5 | |
| | | | Q | | | | | 1 | | | | | | | | 1 | |
| | | 48 | W | | | | | 13 | | | | | | | 4 | 7 | |
| | | | Q | | | | | 1 | | | | | | | | 1 | |
| | | 52 | W | | | | | 10 | | | | | | | 6 | 4 | |
| | 4 | 4 | W | | | | | 18 | | | | | | | 16 | | |
| | | | M | 9 | | | | | | | 9 | | | | | | |
| | | 6 | W | | | | | 9 | | | | | | | 9 | | |
| | | 7 | W | | | | | 14 | | | | | | | 12 | | |
| | | | Q | | | | | 2 | | | | | | | 2 | | |
| | | 8 | W | | | | | 6 | | | | | | | 6 | | |
| | | 13 | W | | | | | 14 | | | | | | | 12 | | |
| | 5 | 2 | W | | | | | 16 | | | | | | | | 8 | 6 |
| | | | Q | | | | | 1 | | | | | | | | 1 | |
| | | 58 | W | | | | | 2 | | | | | | | | 1 | 1 |
| | 6 | 12 | W | | | | | 14 | | | | | 6 | 5 | 1 | | |

| | | | | PGD | | | | | | | MDH-2 | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Plot | COLONY | NEST | caste | b | c | aa | ac | bb | bc | cc | b | c | ab | ac | bb | bc | cc |
| | | | Q | | | | | 1 | | | | | | | 1 | | |
| | | 15 | W | | | | | 9 | | | | | 3 | 4 | 3 | | |
| | | 17 | W | | | | | 10 | | | | | 5 | 4 | 1 | | |
| | | | M | 4 | | | | 3 | | | 4 | | 1 | 2 | | | |
| | | 18 | W | | | | | 12 | | | | | 6 | 3 | 3 | | |
| | | | Q | | | | | 1 | | | | | | | 1 | | |
| | | | M | 2 | | | | | | | 2 | | | | | | |
| | | 49 | W | | | 1 | | 13 | | | | | 5 | 4 | 3 | | |
| | | | Q | | | | | 1 | | | | | | | | 1 | |
| | 7 | 57 | W | | | | | 12 | | | | | | | | 10 | |
| | 8 | 23 | W | | | | | 14 | | | | | | | 11 | 1 | |
| | | | Q | | | | | 1 | | | | | | | 1 | | |
| | 9 | 1 | W | | | 1 | | 10 | | | | | | | 10 | | |
| | 10 | 21 | W | | | | | 9 | 5 | | | | | | 12 | | |
| | | | Q | | | | | | 1 | | | | | | 1 | | |
| | 11 | 54 | W | | | | | 10 | 6 | | | | | | 14 | | |
| | | | Q | | | | | 1 | | | | | | | 1 | | |
| | 12 | 5 | W | | | | | 5 | | | | | | | | 5 | |
| | | | Q | | | 1 | | | | | | | | | 1 | | |
| 1992-B | 13 | 9 | W | | | | | 13 | | | | | 4 | | | 8 | |
| | | | Q | | | | | 1 | | | | | | 1 | | | |
| | 14 | 10 | W | | | | | 14 | | | | | 6 | 4 | | 2 | |
| | | | Q | | | | | 2 | | | | | | 1 | | 1 | |