Evolution, 59(10), 2005, pp. 2185–2199

BREEDING SYSTEM, COLONY STRUCTURE, AND GENETIC DIFFERENTIATION IN THE *CAMPONOTUS FESTINATUS* SPECIES COMPLEX OF CARPENTER ANTS

MICHAEL A. D. GOODISMAN[1,2,3,4] AND DANIEL A. HAHN[1,5,6]
[1]*Center for Insect Science, University of Arizona, Tucson, Arizona 85721*
[2]*Department of Biochemistry and Molecular Biophysics, University of Arizona, Tucson, Arizona 85721*
[3]*School of Biology, The Georgia Institute of Technology, Atlanta, Georgia 30332*
[4]*E-mail: michael.goodisman@biology.gatech.edu*
[5]*Interdisciplinary Graduate Program in Insect Science, University of Arizona, Tucson, Arizona 85721*
[6]*Department of Entomology and Nematology, University of Florida, Gainesville, Florida 32608*

Abstract.—All social insects live in highly organized societies. However, different social insect species display striking variation in social structure. This variation can significantly affect the genetic structure within populations and, consequently, the divergence between species. The purpose of this study was to determine if variation in social structure was associated with species diversification in the *Camponotus festinatus* desert carpenter ant species complex. We used polymorphic DNA microsatellite markers to dissect the breeding system of these ants and to determine if distinct *C. festinatus* forms hybridized in their natural range. Our analysis of single-queen colonies established in the laboratory revealed that queens typically mated with only a single male. The genotypes of workers sampled from a field population suggested that multiple, related queens occasionally reproduced within colonies and that colonies inhabited multiple nests. *Camponotus festinatus* workers derived from colonies of the same form originating at different locales were strongly differentiated, suggesting that gene flow was geographically restricted. Overall, our data indicate that *C. festinatus* populations are highly structured. Distinct *C. festinatus* forms possess similar social systems but are genetically isolated. Consequently, our data suggest that diversification in the *C. festinatus* species complex is not necessarily associated with a shift in social structure.

Key words.—Formicidae, genetic structure, microsatellites, polyandry, polygyny, relatedness, social insects, speciation.

Received November 3, 2004. Accepted July 12, 2005.

Social insects are remarkable because they form highly cooperative societies (Wilson 1971; Oster and Wilson 1978). However, social insect species differ in many important aspects of their social biology. For example, species-level variation occurs in the number of reproductives within colonies, the number of nests that single colonies inhabit, and the relationships of colonymates (Hölldobler and Wilson 1990; Bourke and Franks 1995; Crozier and Pamilo 1996; Pamilo et al. 1997). Although such variation in social structure is well documented, surprisingly little is known about how variation in social system influences speciation. This is unfortunate because changes in social structure alter the genetic structure of populations, which may affect species diversification (Endler 1977; Barton and Clark 1990; Frank 1998; Avise 2004). In addition, variation in colony- and population-genetic structure may alter the strength of kin selection and kin conflict, potentially leading to changes in the helping behavior of workers, the skew in progeny production among reproductives, and the sex ratio produced by colonies (Bourke and Franks 1995; Crozier and Pamilo 1996; Frank 1998; Keller and Reeve 1999).

One of the key determinants of genetic structure within social insect populations is the breeding system of social groups (Ross 2001). The breeding system incorporates several aspects of the social system, including the number of breeders within groups, their relatedness, and how they partition reproduction. Colonies of many hymenopteran social insects are headed by a single queen (monogyne colonies) that has mated only once. However, in more complex systems, the number of female reproductives in the colony and the number of males with which queens mate may vary. The presence of multiple queens (polygyny) decreases the relatedness of individuals within colonies and creates within-colony genetic substructure. In addition, variation in queen number is associated with a number of important life-history traits that likely influence speciation, including aggression toward nonnestmates and queen dispersal ability (Keller 1993; Bourke and Franks 1995; Ross and Keller 1995; Crozier and Pamilo 1996).

The number of nests that a single colony inhabits is frequently associated with colony queen number and may have additional consequences for the evolution of life-history traits related to speciation (Bourke and Franks 1995; Crozier and Pamilo 1996). Members of single colonies in monogyne species frequently reside within a single nest (monodomy), and the spatial distribution of these nests is thought to affect behaviors such as territoriality, brood raiding, and intraspecific parasitism (Bourke and Franks 1995; Gadau et al. 2003). In contrast, the functional colony units of many polygyne species are separated into multiple nests (polydomy; Bourke and Franks 1995; Crozier and Pamilo 1996). Spreading the colony among multiple nests allows polydomous species to control larger areas (Pamilo and Rosengren 1984; Herbers 1990, 1993). In addition, polydomy can affect genetic structure and alter kin conflict within colonies (Herbers 1990; Pamilo 1990), which may, consequently, affect speciation.

The number of males with which a queen mates may also influence evolutionary diversification. Like colony queen number, multiple mating by queens (polyandry) generally leads to a decrease in nestmate relatedness relative to single mating (monandry) and potentially lowers inclusive fitness benefits received by workers who help raise colonymates.

However, polyandry can be advantageous if it increases the variability of the workforce or reduces conflict within the colony (Boomsma and Ratnieks 1996; Crozier and Fjerdingstad 2001; Strassmann 2001; Brown and Schmid-Hempel 2003).

Between-species hybridization can also substantially affect population genetic structure among social groups. Hybridization yields a mosaic of novel genotypes, which can produce phenotypes that may differ in fitness from either parental species (Barton and Hewitt 1985, 1989; Barton and Gale 1993; Harrison 1993; Arnold 1997). In social insects, hybridization is often associated with remarkable changes in social system. For example, inter- or intraspecific hybridization has been implicated as an important factor in ant caste determination (Julian et al. 2002; Volney and Gordon 2002; Cahan and Keller 2003; Cahan and Vinson 2003), as a causative agent of changes in bee behavior (Clarke et al. 2002; Schneider et al. 2003, 2004), and as a contributor to unusual speciation or gene flow dynamics within populations (Goodisman and Asmussen 1997; Goodisman et al. 1998, 2000; Hochberg et al. 2003). These studies highlight the importance of understanding multiple levels of genetic structure between closely related species, particularly in areas where these species occur in sympatry.

To understand how social structure influences speciation, it is first necessary to have a good working knowledge of the evolutionary relationships among the social taxa of interest. However, such information in social insects is often severely limited by our current ability to positively identify closely related species and infer their phylogenetic relationships (Jenkins et al. 2001; Arevalo et al. 2004; Hunt and Carpenter 2004; Ye et al. 2004). This problem is particularly apparent in the ants (Hymenoptera: Formicidae). Ecologically important in almost all terrestrial ecosystems, ants possess an astounding range of life-history diversity, including differences in queen number, queen mating status, colony founding mechanisms, mating strategies, reproductive skew, and caste ratios (Hölldobler and Wilson 1990; Bourke and Franks 1995; Crozier and Pamilo 1996). In fact, there are numerous examples of species that differ significantly in their ecology or life histories but that cannot be clearly differentiated by morphology alone (Creighton 1950; Umphrey 1996; MacKay and MacKay 1997; Feldharr et al. 2003; Goropashnaya et al. 2004; Kronauer et al. 2004b).

In this study, we attempted to understand the factors contributing to the population-genetic structure of a single species complex within the ant genus *Camponotus* and determine if diversification in the complex is associated with changes in social structure. *Camponotus* is the second most diverse genus within the ants and has been shown to be ecologically important in numerous locales worldwide (Hölldobler and Wilson 1990; Bolton 1995; Brady et al. 2000). However, the systematics of several groups within *Camponotus* is unclear (Creighton 1950; Snelling 1968, 1970, 2000; MacKay and MacKay 1997, 2000). Indeed, significant genetic structuring has been found within some groups that are morphologically indistinguishable, suggesting that molecular genetic data are a necessary complement to morphological data for understanding the evolutionary relationships within *Camponotus* and other ant genera (Bolton 1995; Umphrey 1996; Brady et al. 2000; Feldharr et al. 2003; Goropashnaya et al. 2004).

Our study focuses on the *Camponotus festinatus* (Buckley) species complex, whose members are distributed throughout the southwestern United States and northern Mexico (Creighton 1950; Snelling 1968). The morphological variation present in this group has long been recognized and has led several authors to partition this variance into consistent varieties, subspecies, or forms (Emory 1893; Wheeler 1902, 1910). This paper focuses on the colony and population-genetic structure of three of the *C. festinatus* forms that live in southern Arizona.

The two desert forms, *C.* nr. *festinatus* desert dark (hereafter desert dark) and *C.* nr. *festinatus* desert light (hereafter desert light), occur in the deserts of southern Arizona ranging from approximately 700 to 1500 m in elevation. The two forms differ morphologically in that the cuticle of the desert dark form is mostly brown, while that of the desert light form tends to yellow. These colors do not reflect the maturation process of the ants, but rather represent relatively discrete differences in adult pigmentation. Indeed, the desert dark and light forms can also be reliably distinguished by other morphological features, such as the length of their antennal scapes, and have been shown to differ in their life histories with respect to allocation to fat storage (A. N. Lazarus, D. A. Hahn, S. P. Cover, and J. J. Wernegreen, unpubl. data; D. A. Hahn, unpubl. data). Moreover, the distinct forms have never been found occupying the same nest.

The third form included in this study, *C.* nr *festinatus* midelevation, is typically found in the oak-grassland transition zone in southern Arizona. The midelevation form generally occurs at slightly higher elevations (1200 to 2500 m) than the desert forms, although there is a zone of overlap where the desert and midelevation forms occur in sympatry. Midelevation individuals are distinguished from the two desert forms by the head shape of their minor workers, which tapers more drastically behind the eyes, and the presence of fully erect hairs on their antennal scapes, rather than the decumbent hairs characteristic of the desert forms. The occurrence of the morphological and life-history differences among forms suggests that they could be separate species.

We investigated the breeding and social structure of the desert dark, desert light, and midelevation forms of *C. festinatus*. Specifically, we analyzed the genotypes of workers reared in laboratory colonies to assess whether queens mated with multiple males. We then examined worker genotypes in field-collected colonies to determine if multiple queens inhabited single colonies. We were particularly interested in determining if the breeding system of the *C. festinatus* forms differed, a result that would indicate that selection might have operated differently in the distinct forms. Next, we developed a new method of spatial analysis that facilitated the identification of colony boundaries and genetic structure among colonies within a field site. We also investigated whether the forms of *C. festinatus* were genetically distinct and whether they formed hybrids in a natural population. Finally, we provide general suggestions for untangling the relationships of species complexes in other social insect groups.

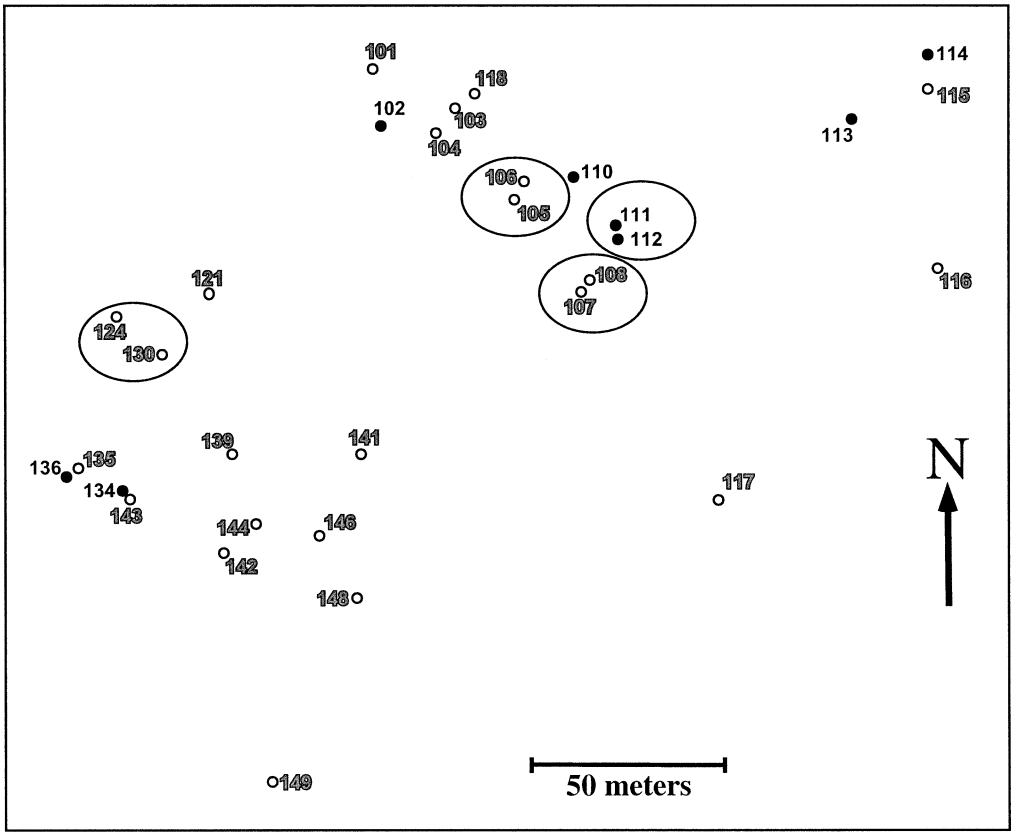

FIG. 1. Locations of 31 field-sampled *Camponotus festinatus* nests. Nests containing desert dark and midelevation form ants are denoted by filled circles with black lettering and empty circles with gray lettering, respectively. Circled pairs of nests contain workers belong to single colonies.

MATERIALS AND METHODS

Sample Collection

We analyzed worker genotypes in both laboratory and field colonies to examine the breeding system of *C. festinatus*. Single-queen colonies were established in the laboratory by introducing newly mated queens, captured in the vicinity of Tucson, Arizona, into trays. The desert dark and desert light forms of *C. festinatus* were chosen for laboratory analysis because queens of these forms are readily captured after their mating flights and reproduce under laboratory conditions. Colonies were allowed to develop for 3–5 years in the lab at 28–30°C and ambient humidity. Ants were fed a diet of frozen *Nauphoeta cinerea* or *Manduca sexta* larvae three times a week, and 50% honey solution with 50 mg each of Vanderzants vitamins (Sigma, St. Louis, MO) and Wesson's Salt Mixture per 100 ml (Sigma). Approximately 15 adult workers were sampled from each colony. In addition, the reproductive queens from three colonies were sacrificed for analysis.

Camponotus festinatus workers of the desert dark and midelevation forms were obtained from field colonies sampled from a single site. We selected these forms for study, because they are known to occur sympatrically in relatively high densities (Fig. 1). The collection site was located opposite Sycamore Canyon in the Atascosa Mountains in Santa Cruz County near Nogales, Arizona (31°26.29′N, 111°11.00′W), at an elevation of 1255 m above sea level. The field site was 60–80 km from the location where the laboratory-reared queens were captured. Between two and 27 adult worker ants were collected from nests located under small rocks throughout an area of 7500 m^2. Worker larvae were also obtained from two nests. All samples were placed in 95% ethanol for preservation and subsequent genetic analysis.

Laboratory Procedures

Genomic DNA was extracted from ants using a modification of the Chelex protocol (Walsh et al. 1991) as described by Crozier et al. (1999). Polymerase chain reaction (PCR) amplification was used to determine the genotypes of individual ants at the four polymorphic microsatellite loci, Ccon12, Ccon20, Ccon42, and Ccon70, which were originally cloned from the congener *Camponotus consubrinus* (Crozier et al. 1999). In addition, genotypes were assayed at the polymorphic microsatellite locus Cfes1, which was detected in an EST screen of *C. festinatus* (Genbank accession CK656570; forward amplification primer 5′-GAGAAGTCTAGAGAAAAGT-3′; reverse amplification primer 5′ TTTGTCAATGTTTAATAAGTG-3′). The PCR products were visualized by end-labeling the forward primers of Cfes1, Ccon12, Ccon20, Ccon42, and Ccon70 with the fluorescent dyes 6-FAM, HEX, HEX, 6-FAM, and NED, respectively. PCRs were conducted in a final volume of 10 μl containing

1 μl genomic DNA and 0.5 U Taq DNA polymerase (New England Biolabs, Ipswich, MA), and a final concentration of 200 μM dNTPs, 0.5 μM of each of the forward and reverse PCR primers, and 1X New England Bioloabs PCR buffer.

The PCR cycling profiles for the five markers began with an initial denaturation at 94°C for 2 min, and then proceeded with 40 cycles of 94°C for 30 sec, 45°C for 45 sec, and 72°C for 45 sec, followed by a final extension of 72°C for 10 min. PCR products from all loci were combined in a 10 μl cocktail containing 1.0-μl, 2.0 μl, 2.0 μl, 2.0 μl, and 3.0 μl of Cfes1, Ccon12, Ccon20, Ccon42, and Ccon70 PCR product, respectively. Two microliters of this cocktail were combined with a labeled size standard, electrophoresed, and scored on an ABI PRISM 3100 Genetic Analyzer (Applied Biosystems, Foster City, CA).

Statistical Analyses

We calculated the genetic diversity at each microsatellite locus for the laboratory and field colonies independently. Allele frequencies were estimated using the program GENEPOP 3.2 (Raymond and Rousset 1995), weighting estimates from each colony equally. The variability at each locus was quantified by Nei's (1987) estimate of gene diversity.

Laboratory samples

We directly examined the genotypes of workers sampled from the monogyne laboratory colonies to determine the minimum number of males with which each queen mated. In general, male mate number is readily determined in Hymenoptera, because males are haploid and females are diploid. However, multiply mated queens can be overlooked if insufficient numbers of worker offspring are sampled and if male mates contribute unequally to offspring production. We estimated the probability of missing male mates to determine if such errors were likely under our sampling scheme. In addition, we calculated the probability that we failed to detect male mates of the queen, because the queen's putative mates possessed identical genotypes. This likelihood is quantified by the nondetection error, which is the probability that two males will share the same multilocus genotype. In haplodiploid taxa such as the Hymenoptera, this probability is the product of the sum of the squared allele frequencies at each microsatellite locus (Boomsma and Ratnieks 1996).

Workers within social insect colonies are typically related and, therefore, should not be considered independently of each other in population analyses. To avoid the potential problems caused by this genetic nonindependence, we used a resampling technique that yielded unbiased measures of population genetic structure. Specifically, we created 10 new datasets, each of which contained genetic information from a single worker randomly selected from each colony. We used these reduced datasets for most analyses, and took the arithmetic mean from the analyses of the 10 datasets as our best estimates for population parameters and associated probabilities.

We examined the laboratory-reared workers for evidence of genetic differentiation between the desert dark and desert light forms. We used probability or exact tests, as implemented by the program GENEPOP 3.2, to determine if there was significant allelic or genotypic differentiation between the color forms (Raymond and Rousset 1995). We also used GENEPOP 3.2 to calculate the magnitude of differentiation between the color forms through the estimation of the statistics F_{ST} and ρ_{ST}, the latter being an estimator of genetic differentiation that considers variation in allele size as an informative measure of divergence.

We examined the genotypic structure of workers from each form to determine if the samples were derived from a single, randomly mating population. If samples were derived from distinct populations, then an increase in homozygosity relative to Hardy-Weinberg expectations might arise through the Wahlund effect (Hartl and Clark 1989). We used both probability tests, where the probability of the observed sample of genotypes defines the rejection region, and score tests, where the alternative hypotheses are defined as either excess or deficiency of heterozygotes, to estimate the significance of deviations from Hardy-Weinberg expectations (Rousset and Raymond 1995). The magnitude of disequilibrium was quantified by the statistic F_{IS}.

The relatedness of laboratory-reared workers was estimated using the program Relatedness 5.0 (Queller and Goodnight 1989). We expected the relatedness of workers to equal 0.75 if workers were full sisters produced by a single, oncemated queen. Lower estimates would be obtained if queens were frequently polyandrous. Standard errors for estimates were obtained by jackknifing over colonies, and *t*-tests were used to determine if calculated relatedness estimates differed from specified values (i.e., 0.75) or from each other. We also used the estimate of worker relatedness from laboratory colonies to calculate the effective mating frequency of queens, which incorporates information on both the number of times a queen mates and the unequal contribution of her male mates to progeny production (Starr 1984).

Field samples

Camponotus festinatus workers collected from distinct field-sampled nests may belong to single colonies (polydomy). To determine if polydomy was common, we tested for genetic structure among nests in our field site in two ways. First, we examined the relationship between pairwise geographic distance between nests and genetic distance, *F*, as estimated by Weir and Cockerham's (1984) method using GENEPOP 3.2. The magnitude of the correlation was measured by Spearman's correlation coefficient, r_S, and the significance of the correlation was determined by a Mantel test. The Mantel test, which was carried out by GENEPOP, compares the matrices of genetic and geographic distances to determine if the observed correlation differs from correlations obtained by permuting the observed data. If polydomy was common and the effects of polydomy ranged over relatively large distances, we expected a significant, positive relationship between pairwise genetic distances and geographic distances.

Second, we examined the data for evidence of genetic structure over varying geographic scales. The motivation for this technique is derived from the possibility that genetic structure may be limited to certain spatial scales within sites. For example, if new colonies within sites are founded by

queens partaking in extensive nuptial flights, and colonies inhabit multiple nests, then genetic similarity of individuals from nests may only be detectable over the distance that a single colony occupies (i.e., a few meters). That is, the relationship between genetic similarity and geographic distance may be limited, because workers from distinct colonies will not be related regardless of their proximity. In this case, extensive queen dispersal coupled with limited colony range would lead to genetic structure on a microgeographic scale only, and a widespread relationship between geographic and genetic distance, as predicted under standard models of isolation by distance, would not be detectable (Rousset 1997).

To test for this possibility, we compared estimates of genetic differentiation (F) for pairs of nests that were separated by a specific range of distances (D_1 to D_2 meters, with $D_2 > D_1$) to pairs of nests that were separated by distances greater than that range (D_2 to ∞ meters; Goodisman and Hahn 2004). We first calculated estimates of F for all pairs of nests in the field site. We then calculated (1) the mean F-value for all pairs of nests that were separated by at least D_1 meters but no more than D_2 meters and (2) the mean F-value for all pairs of nests separated by more than D_2 meters. The difference between (1) and (2), defined as *dif-D*, is expected to be negative if individuals from nest pairs separated by D_1 to D_2 meters are more similar to each other than individuals from nests separated by distances greater than D_2 meters. For example, if nests occupied by a single colony generally lie within 5 m of one another, then nests separated by $D_1 = 0$ to $D_2 = 5$ m should show lower values of genetic differentiation (F) than nests separated by more than $D_2 = 5$ m.

The significance of the test statistic, *dif-D*, was calculated using a resampling protocol whereby nest pairs were randomly assigned values of F from the original dataset with replacement. We then calculated the new test statistic *rdif-D*, which represented the difference in the mean values of F for the randomly constructed dataset. This procedure was repeated 10,000 times. We considered there to be evidence of genetic structure if the observed value of *dif-D* was less than 5% of the calculated *rdif-D* values.

If evidence of polydomy was apparent from either of the statistical tests above, we attempted to group nests into true colony units. To determine which nests belonged to distinct colonies, we compared the genotypic frequencies of workers from all pairs of nests by means of an exact test using the program GENEPOP 3.2 (Goodisman and Crozier 2002). As was the case with the laboratory workers, the mean estimate obtained from 10 datasets containing a single worker per colony was used for calculations. Significant differentiation, after correcting for the multiple tests performed (Rice 1989), suggested that workers sampled from distinct nests did not belong to the same colony. Nonsignificant differentiation was generally taken as evidence that workers were derived from the same colony. However, because few workers were sampled from some nests (e.g., nests 102, 104, 106, 108, 111, 117, and 139), leading to a substantial loss in power, we used partially subjective means to determine colony boundaries. Specifically, nests that contained relatively few workers and were separated by considerable distances were not necessarily viewed as belonging to the same colony unit, even if the workers from the nest pair were not significantly differentiated. Any error of misidentifying colony boundaries (i.e., considering individuals from distinct nests as belonging to distinct colonies, when, in fact, they belonged to the same colony) would be conservative in that it would decrease our estimates of nestmate relatedness relative to their true values.

After grouping workers from distinct nests into colony units, we attempted to determine the social structure of *C. festinatus* field colonies. We examined the genotypes of workers to determine if one or many queens contributed to worker production in *C. festinatus* colonies. Single queens were considered to head colonies if the workers therein possessed a maximum of three alleles at any locus and their genotypes conformed to those expected under Mendelian segregation of alleles from a singly mated diploid female and haploid male. This method could result in missing some instances of multiple matrilines within nests if few workers were sampled from nests or if reproducing queens were closely related.

As was the case with the laboratory colonies, we use 10 reduced datasets, each of which consisted of a single randomly selected worker from each field colony, for population analyses. We took the mean of the 10 datasets as our best estimates for population parameters. We tested for Hardy-Weinberg disequilibrium within forms using GENEPOP 3.2 to determine if workers from each form were part of the same population. We expected to find disequlibrium if colonies of different forms were not part of the same randomly mating population.

We next examined the field samples to determine if the desert dark and midelevation forms were genetically differentiated. In addition, allele and genotype frequencies were compared between laboratory and field-sampled ants to detect differentiation within and between *C. festinatus* forms. The significance of allelic and genotypic differentiation between the forms was estimated using exact tests, and the magnitude of differentiation, as measured by the statistics F_{ST} and ρ_{ST}, was also determined.

The program RELATEDNESS 5.0 was used to estimate the relatedness of workers from field-sampled *C. festinatus* colonies. Standard errors for estimates were obtained by jackknifing over colonies. We used *t*-tests to determine if relatedness estimates differed from specified values or if the relatedness of desert dark and midelevation form workers differed significantly.

The method of Pritchard et al. (2000) was used to establish if field-sampled desert dark and midelevation form workers originated from more than a single interbreeding group. This algorithm uses a Bayesian clustering method to assign individuals to K distinct populations while simultaneously estimating allele frequencies in those populations. The method assumes that alleles from distinct populations will be in Hardy-Weinberg and linkage equilibrium; deviations from equilibrium within a sample signal the presence of population structure. We were specifically interested in determining if sampled ants could be differentiated according to form and potentially distinguishing the presence of hybrids.

Two distinct models of population structure were considered within the clustering framework. In the first model, we considered the probability of observing our genotypic data given $K = 1$–10 populations and no admixture between populations. The second model allowed for the possibility that

TABLE 1. Nei's gene diversity (and number of alleles) at five microsatellite loci in four samples of *Camponotus festinatus* ants.

	Sample			
Locus	Laboratory desert dark	Laboratory desert light	Field desert dark	Field midelevation
Cfes1	0.86 (8)	0.93 (14)	0.58 (3)	0.83 (10)
Ccon12	0.90 (11)	0.94 (14)	0.22 (2)	0.87 (10)
Ccon20	0.53 (4)	0.48 (4)	0.37 (4)	0.14 (5)
Ccon42	0.38 (8)	0.86 (9)	0 (1)	0.62 (6)
Ccon70	0.97 (23)	0.92 (17)	0.62 (5)	0.35 (5)
Mean	0.73 (10.8)	0.83 (11.6)	0.36 (3.0)	0.56 (7.2)

individuals resulted from admixture from the $K = 1$–10 theoretical populations. In both cases, the program Structure 2.1 (available via http://pritch.bsd.uchicago.edu/structure.html) was used to derive the most likely value of *K*. In addition, estimates of the probability of population membership, for the no-admixture model, or fractional membership of each individual into each of the *K* theoretical populations, for the admixture model, were also obtained. Simulations assumed correlated allele frequencies within populations, and burnin and simulation length were set to 10,000.

Microsatellite Locus Quality

A concern when applying microsatellite markers to population genetic data is the occurrence of false signals of evolutionary processes that may originate from errors in the generation of the data themselves. For example, a deficit of heterozygotes, which typically signals the presence of selection, population structure, or inbreeding, can also arise through errors in the PCR amplification of alleles. Two such errors include null alleles and allele dropout. A null allele at microsatellite locus arises when an allele fails to PCR-amplify because it carries a mutation in the region of primer binding. In contrast, allele dropout takes place when particular pairs of alleles occur in a single heterozygous individual. If the alleles differ substantially in length, then the shorter allele may outreplicate the longer allele during PCR, ultimately leading to the longer allele remaining undetected. In either case, individuals that are heterozygous for distinct alleles would incorrectly be scored as homozygous, thereby inflating estimates of homozygosity (Jones and Ardren 2003).

There is no evidence that null alleles or allele dropout influenced our study of genetic variation in *C. festinatus*. High-frequency null alleles would have been evident in our analysis of parentage in laboratory colonies. If null alleles were present at high frequency, then some proportion of matings would occur between queens heterozygous for normal alleles and males carrying the null allele (e.g., AB queen × 0 male). Such matings would result in progeny with a 1:1 ratio of one of the two maternal alleles and the null paternal allele (worker genotypes A0 and B0). The genotypes of these individuals would be naively interpreted as homozygous for the maternal alleles (AA and BB). However, a 1:1 ratio of homozygous genotypes among progeny is not possible in a haplodiploid genetic system. A singly mated queen cannot produce this genotypic ratio, and a multiply mated queen would be expected to produce some heterozygous genotypes. Consequently, the presence of two, distinct homozygote genotypes at a given locus within a family would immediately be suspect. However, our analysis of family groups revealed no instances of such anomalous genotypic ratios. Therefore, we have no evidence that null alleles are present in our dataset.

Our data also did not show evidence of allele dropout. If allele dropout occurred frequently at our loci, then we would expect many putative homozygotes to consist of short alleles. Such a pattern would result from amplification bias in heterozygotes where the allele of shorter size would outreplicate the longer allele during PCR amplification. We tested whether the smallest allele present in putative homozygotes was significantly smaller than the smallest allele present in heterozygotes, as expected if homozygotes frequently resulted from allele dropout of larger alleles. We considered all five loci for all four samples (laboratory/field desert/midelevation), resulting in a total of 18 statistical tests (some loci in some populations were monomorphic). We found that the smallest allele in homozygous genotypes was significantly greater than that smallest allele in heterozygous genotypes in 14 cases, in direct contrast to the effect produced by allele dropout. In three cases there was no significant difference between homozygous and heterozygous allele size, and in one case the smallest allele for homozygotes was significantly smaller than that in heterozygotes. Consequently, these calculations indicate that allele dropout did not occur in our dataset. Overall, our analyses suggest that our data are robust and not affected by errors arising from PCR amplification of microsatellite markers.

RESULTS

The multilocus genotypes of 510 workers were assayed from 37 laboratory colonies (13.78 ± 1.53 workers per colony, $\bar{x} \pm$ SD) established by single queens. The genotypes of the three desert dark form queens were also determined. Workers from 26 and 11 of these laboratory colonies were designated as desert dark and desert light, respectively, based on morphological analysis. In addition, 327 workers from 31 nests were sampled from our field site (13.55 ± 7.51 workers per nest). Workers from eight of these nests were categorized as desert dark, while those from the remaining 23 nests were categorized as midelevation (Fig. 1). Identification was based on phenotypic (i.e., color, morphology) information. No nest contained workers of more than one form.

The five microsatellite markers used in this study proved to be highly variable, although there were substantial differences in variability among samples (Table 1). In total, the

TABLE 2. Genetic differentiation between *Camponotus festinatus* forms. Values above and below the diagonal are estimates of F_{ST} and ρ_{ST}, respectively. All forms differed significantly from each other ($P < 0.0001$), as determined by allelic and genotypic probability tests.

	Laboratory desert dark	Laboratory desert light	Field desert dark	Field midelevation
Laboratory desert dark	—	0.093	0.380	0.219
Laboratory desert light	0.388	—	0.351	0.206
Field desert dark	0.865	0.682	—	0.444
Field midelevation	0.854	0.648	0.862	—

loci Cfes1, Ccon12, Ccon20, Ccon42, and Ccon70 possessed 22, 22, 9, 16, and 36 alleles, respectively. The field samples displayed lower diversity than the laboratory samples, with the field desert dark forms having the lowest variation as a group. The distribution of allele frequencies among the four samples also varied substantially (see Appendix available online only at http://dx.doi.org/10.1554/04-672.1.sl).

Levels of Multiple Mating by Queens

Direct analysis of the genotypes of workers from the 37 laboratory colonies allowed us to estimate the number of males with which queens mated. We first considered the probability that we would underestimate male mate number because of the finite variation of our markers. Considering the desert dark and desert light laboratory samples independently, the probability that two males would have the same haploid genotype given the variability of our markers was 0.00047 and 0.00013, respectively. Consequently, it is unlikely that we failed to detect male mates because of the insufficient variability of our markers. Another potential source of error could arise due to the process of sampling. That is, we may have failed to detect male mates by chance because our worker sample size was finite. However, we found that this probability was also relatively low. For example, if both males contributed equally to progeny production, then the probability of sampling 14 workers (the mean number sampled per colony) from the same patriline is $2 \times 0.5^{14} = 0.00012$. Skew in male reproductive success would need to be substantial, with one male contributing approximately 75% of offspring, for the probability of missing a male mate through sampling effects to exceed 0.05. Therefore, we do not expect sampling biases to contribute major error to our estimate of male mate number.

We found that most queens from our laboratory colonies mated with a single male. In total, the genotypes of workers from 32 of the 37 nests were consistent with having been produced by a singly mated queen. Four of the 37 nests contained workers that would have required double mating by the queen, and one nest contained workers that would have required triple mating by the queen. Therefore, the mean number of mates per queen from these data comes to 1.16. However, upon closer inspection, we found that the worker genotypes from the colonies headed by three of the putatively doubly mated queens and the triply mated queen could more parsimoniously be explained by mutation events or by a single contaminating worker sample. Under these assumptions, only one of the 37 queens was multiply (doubly) mated, and our best estimate of mean number of mates per queen falls to 1.03.

Genetic Structure of Laboratory Samples

We examined the laboratory samples for evidence of genetic differentiation between the two desert forms. We found evidence for highly significant allelic and genotypic differentiation between desert dark and desert light *C. festinatus* ($P < 0.0001$). The magnitude of dissimilarity was also fairly substantial, with our estimates of differentiation equaling $F_{ST} = 0.09$ and $\rho_{ST} = 0.39$ (Table 2). The relatively large estimate of ρ_{ST} was caused, in part, by the locus-specific estimates at Cfes1 and Ccon70, where the two color forms differed not only in the actual alleles present but also in the size ranges of alleles (online Appendix).

We next tested for evidence of deviations from Hardy-Weinberg equilibrium within laboratory desert color forms. The applied probability test revealed no significant disequilibrium in the desert dark form ($F_{IS} = -0.01$, $P > 0.40$) and weak but not statistically significant evidence for disequilibrium in the desert light form ($F_{IS} = 0.16$, $P < 0.10$). Because of the marginally significant result, we conducted further analysis using the statistically more powerful, one-sided tests with predefined rejection regions of heterozygous excess or deficit. We found that the desert light form possessed a modestly significant deficit of heterozygotes (heterozygote excess: desert light, $P > 0.9$; desert dark, $P > 0.1$; heterozygote deficit: desert light, $P < 0.05$; desert dark, $P > 0.8$).

The average relatedness of workers sampled from the laboratory colonies was 0.74 ± 0.01 ($\bar{r} \pm$ SEM) when differences in allele frequencies between the color forms were considered (Table 3). This estimate of nestmate relatedness did not differ significantly from the value of 0.75 expected if all colonies were headed by a single, once-mated queen ($t_{36} = 0.86$, $P > 0.35$), and yielded an estimate of effective mating frequency of 1.02. In addition, worker relatedness of desert dark

TABLE 3. Relatedness of *Camponotus festinatus* workers from laboratory and field colonies.

Locus	Laboratory desert dark	Laboratory desert light	All laboratory samples	Field desert dark	Field midelevation	All field samples
Cfes1	0.72	0.81	0.76	0.79	0.70	0.72
Ccon12	0.71	0.79	0.73	0.83	0.62	0.63
Ccon20	0.78	0.54	0.72	0.75	0.89	0.83
Ccon42	0.73	0.82	0.78	1.00	0.64	0.64
Ccon70	0.73	0.66	0.71	0.75	0.69	0.71
Mean ± SEM	0.73 ± 0.03	0.75 ± 0.02	0.74 ± 0.01	0.77 ± 0.06	0.68 ± 0.11	0.69 ± 0.09

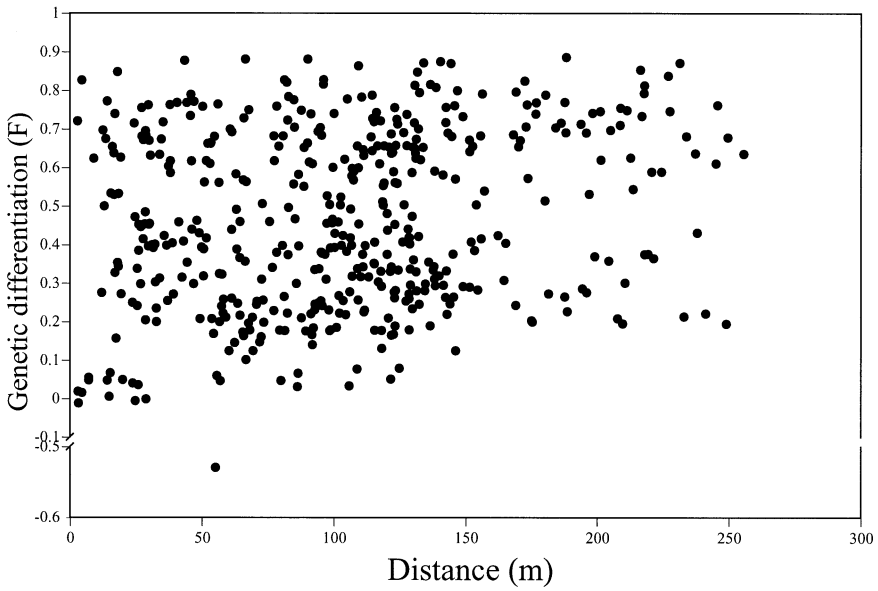

FIG. 2. Relationship between pairwise estimates of genetic distance and geographic distance for *Camponotus festinatus* nests.

and desert light *C. festinatus* did not differ significantly ($t_{34} = 0.60$, $P > 0.5$). As expected, the relatedness estimates for the one colony that was clearly headed by a doubly mated queen ($r = 0.49$) was lower than the value of 0.75 and quite close to the estimate of 0.5 expected if the doubly mated queen used the sperm of each male equally. Nevertheless, consideration of the entire dataset supports the hypothesis that *C. festinatus* queens almost always mate once.

Social Structure of Field Samples

We examined the relationship between pairwise estimates of genetic differentiation for ants collected from distinct nests and the distance between nests to determine if polydomy was common in field populations of *C. festinatus*. The correlation between nest distance and genetic similarity of workers within nests ($r_S = 0.179$) differed significantly from zero as judged by a Mantel test ($P = 0.014$; Fig 2).

To better understand the nature of the significant correlation, we compared the pairwise estimates of differentiation for nests separated by D_1 to D_2 meters to the differentiation of nests separated by distances greater than D_2 meters. As expected under a simple model of polydomy, significant genetic structure was observed over relatively short distances (< 10 m) in both the desert dark and midelevation forms as well as both forms combined (Table 4). The signal grew weaker at moderate distances (25–50 m) but then reemerged as the distances between nests increased. This unusual pattern was observed in both the desert dark and midelevation forms, suggesting that it may reflect some real aspect of colony movement or colonization.

Because our analysis of genetic structure suggested that *C. festinatus* sometimes formed polydomous colonies, we attempted to group individuals sampled from distinct nests into true colonies of related individuals. We used the significance of genotypic differentiation between nests in conjunction with the proximity of nests to determine if individuals belonged to the same colony. Our analyses revealed four instances of polydomy. Specifically, we determined that workers from the following pairs of nests belonged to the same colonies: 105 and 106, 107 and 108, 111 and 112, 124 and 130 (Fig. 1).

We examined the genotypes of field-sampled workers to determine the breeding system of natural *C. festinatus* colonies. We assumed that field-sampled desert dark and midelevation *C. festinatus* queens mated with only a single male, as indicated by our study of laboratory colonies. This assumption is justified, even in the midelevation form, as queen mate number (i.e., whether queens mate multiply vs. singly) is not an evolutionarily labile trait. Typically, all species within a hymenopteran social insect genus are either monandrous or polyandrous (although the extent of multiple mating does vary among species within polyandrous genera; see Strassmann 2001).

We found that the genotypes of workers from 20 of the 27 colonies conformed to the simplest type of social system found in hymenopteran social insects; these nests appeared to be headed by a single, once-mated queen. However, the genetic structure in the remaining seven colonies (101, 103, 118, 121, 124/130, 141, and 142, where ''/'' indicates that the two members of the two nests belong to the same colony) was more complex. The genotype of workers from all these colonies required either reproduction by multiple queens or the mating of a single queen with at least three males. Because only one of the 37 laboratory-reared queens was found to mate multiply (see above), the latter possibility is unlikely. Consequently, it appears that *C. festinatus* colonies sometimes contain multiple matrilines.

Another notable outcome of our analysis was that all seven of the colonies that contained offspring from multiple matrilines were midelevation form. This result initially suggested that the desert dark and midelevation forms differed in social structure. However, because the majority (21 of 27) of the colonies sampled were midelevation in our field population, the probability that all seven multiple-matriline col-

TABLE 4. Genetic structure of *Camponotus festinatus* nests over varying spatial scales. In this analysis, the mean genetic differentiation of pairs of nests separated by distances of D_1 to D_2 meters was compared against the mean genetic differentiation of pairs of nests separated by distances greater than D_2 meters. The resulting difference in means (*dif-D*) was compared against the distribution of differences in means for random nest pairs at various significance limits (*rdif-D*). The number of nest pairs separated by distances of D_1 to D_2 meters and distances greater than D_2 meters are given. Significant differences are given in bold.

	D_1	D_2	Number of nest pairs separated by D_1 to D_2 meters	Number of nest pairs separated by more than D_2 meters	*dif-D*	*rdif-D* 0.05	*rdif-D* 0.01	*rdif-D* 0.001
Desert dark	0	10	1	27	**−0.317**	−0.221	−0.317	−0.357
	10	25	3	24	0.047	−0.128	−0.171	−0.204
	25	50	1	23	0.161	−0.179	−0.231	−0.262
	50	75	6	17	**−0.148**	−0.096	−0.131	−0.166
	75	100	3	14	−0.033	−0.114	−0.149	−0.184
	100	125	3	11	0.022	−0.114	−0.152	−0.185
	125	150	4	7	**−0.181**	−0.109	−0.151	−0.194
	150	240	6	1	**−0.290**	−0.173	−0.22	−0.266
Midelevation	0	10	4	249	**−0.309**	−0.148	−0.234	−0.356
	10	25	11	238	**−0.200**	−0.094	−0.129	−0.180
	25	50	38	200	0.015	−0.055	−0.076	−0.101
	50	75	34	166	**−0.122**	−0.061	−0.087	−0.116
	75	100	40	126	**−0.060**	−0.056	−0.078	−0.104
	100	125	56	70	**−0.086**	−0.058	−0.081	−0.106
	125	150	34	36	**−0.109**	−0.083	−0.116	−0.157
	150	175	11	25	0.035	−0.145	−0.205	−0.275
	175	200	12	13	−0.048	−0.169	−0.233	−0.314
	200	225	9	4	0.164	−0.259	−0.364	−0.475
	225	240	2	2	0.318	−0.316	−0.624	−0.624
All ants	0	10	8	456	**−0.182**	−0.133	−0.18	−0.244
	10	25	27	429	−0.061	−0.071	−0.103	−0.136
	25	50	60	369	0.007	−0.05	−0.071	−0.091
	50	75	58	311	**−0.107**	−0.053	−0.076	−0.107
	75	100	70	241	**−0.048**	−0.048	−0.067	−0.087
	100	125	94	147	**−0.08**	−0.044	−0.064	−0.087
	125	150	68	79	−0.061	−0.058	−0.084	−0.109
	150	175	24	55	−0.006	−0.084	−0.118	−0.162
	175	200	21	34	−0.033	−0.101	−0.146	−0.191
	200	225	21	13	−0.007	−0.124	−0.171	−0.224
	225	250	12	1	−0.062	−0.279	−0.342	−0.427

onies would be midelevation by chance is not particularly small (calculated as the probability that all polygyne nests would be midelevation given that seven nests were polygyne; $P = 21/27 \times 20/26 \ldots \times 15/21 = 0.13$). In addition, the variability of the desert dark field samples was substantially lower than that of the midelevation samples (Table 1), a factor that would diminish our ability to detect multiple matrilines in desert dark ants. Most importantly, we note that the relatedness of desert dark and midelevation form ants did not differ significantly (Table 3). Therefore, there is no convincing evidence that the breeding system of *C. festinatus* desert dark and midelevation form ants differs.

Genetic Structure of Field Samples

We analyzed the field-sampled workers' genotypes for evidence of genetic structure within and between the desert dark and midelevation forms. The genotype frequencies were inconsistent with Hardy-Weinberg equilibrium in the midelevation form ($P < 0.005$, midelevation; $P > 0.7$, desert dark). Subsequent analysis revealed that this departure from equilibrium was due to a heterozygote deficit ($P < 0.0001$, midelevation; $P > 0.7$, desert dark) and not a heterozygote excess ($P > 0.9$, midelevation; $P > 0.3$, desert dark). These significant deviations were accompanied by a relatively poor match between the expected and observed frequency of heterozygotes under Hardy-Weinberg equilibrium in the two forms ($F_{IS} = 0.16$, midelevation; $F_{IS} = -0.17$, desert dark).

Tests for genetic differentiation between the forms were unambiguous (Table 2). The allele and genotype frequencies differed significantly between all forms sampled (allelic and genotypic tests for all pairwise comparisons; $P < 0.0001$). This significant differentiation was consistent with the large magnitude of differentiation estimated by ρ_{ST} and F_{ST} between samples (Table 2). Of interest, we discovered that the desert dark field samples were strongly differentiated from the desert dark laboratory samples. We also note that the estimates of ρ_{ST} were substantially greater than the estimates of F_{ST}, a result that reflects that the sizes of the alleles, in addition to the frequencies of the alleles, differed between the samples (online Appendix).

The relatedness of *C. festinatus* field sampled ants was relatively high (Table 3). The overall estimate of relatedness of workers (0.69 ± 0.03) was not significantly different from the value of 0.75 expected if all colonies were headed by a single, once-mated queen ($t_{26} = 0.66$, $P > 0.5$). However, the average relatedness estimate for colonies deemed to contain multiple matrilines (0.44) was substantially lower than the overall mean estimate. We found that the mean relatedness of desert dark form ants (0.77) and midelevation form ants (0.68) did not differ significantly ($t_4 = 0.70$, $P > 0.5$).

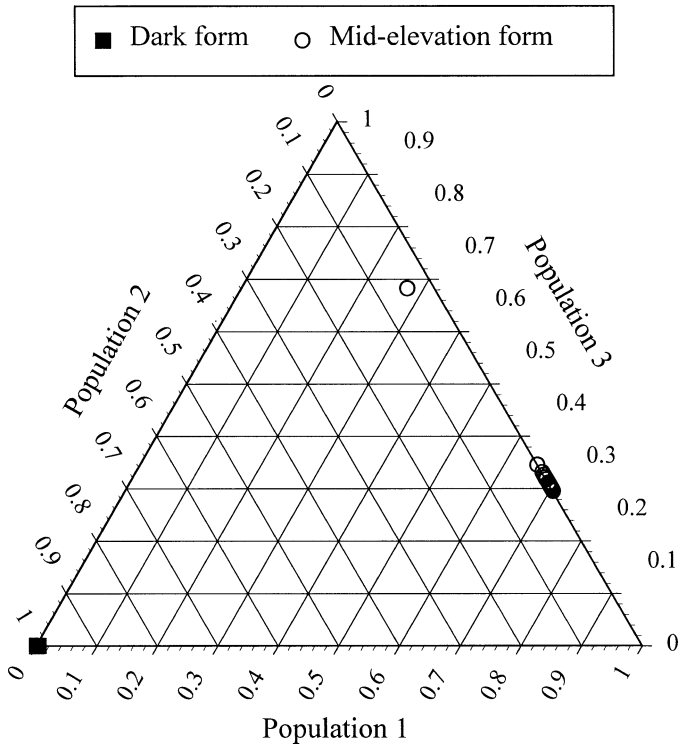

FIG. 3. Probability of membership of desert dark and midelevation *Camponotus festinatus* ants into populations 1, 2, and 3 of $K = 3$ theoretical populations under a model of no-admixture.

This further substantiated the hypothesis that social structure of desert dark and midelevation *C. festinatus* did not differ.

Analyses of the field data using a model-based clustering algorithm yielded additional insights into the nature of differentiation of the forms. Using the no-admixture model, the field-sampled *C. festinatus* ants were clustered into $K = 3$ populations with high probability ($P > 0.999$; Fig. 3). The probability of the number of populations equaling some other value of K was very low ($P < 0.001$ for all other probabilities combined). The no-admixture clustering-algorithm succeeded in sorting the ants into their color forms based on genotype (Fig. 3). The desert dark form ants were strongly differentiated from the midelevation form ants. In addition, all the desert dark form ants were determined to be part of a single theoretical population (population 2), as they all lie in one of three corners of the ternary diagram in Figure 3. In contrast, the midelevation form ants showed more complex characteristics. Although midelevation form ants were distinct from desert dark form ants, midelevation form ants were apparently comprised of mixtures of genes from two populations, theoretical population 1 and population 3 (Fig. 3).

The results of the clustering algorithm with admixture lent some support to two different estimates of K. The probability that the field samples were derived from $K = 2$ populations under the no-admixture model was fairly high ($P \approx 0.84$). In addition, the $K = 2$ population model successfully classified desert dark and midelevation form *C. festinatus* into distinct groups, with the exception of colony 116, which was misclassified (Fig. 4). However, the admixture model also provided some support to a model of $K = 3$ populations ($P \approx 0.18$; the probability of $K = 1$ or $4 \leq K \leq 10$ was $P < 0.0001$).

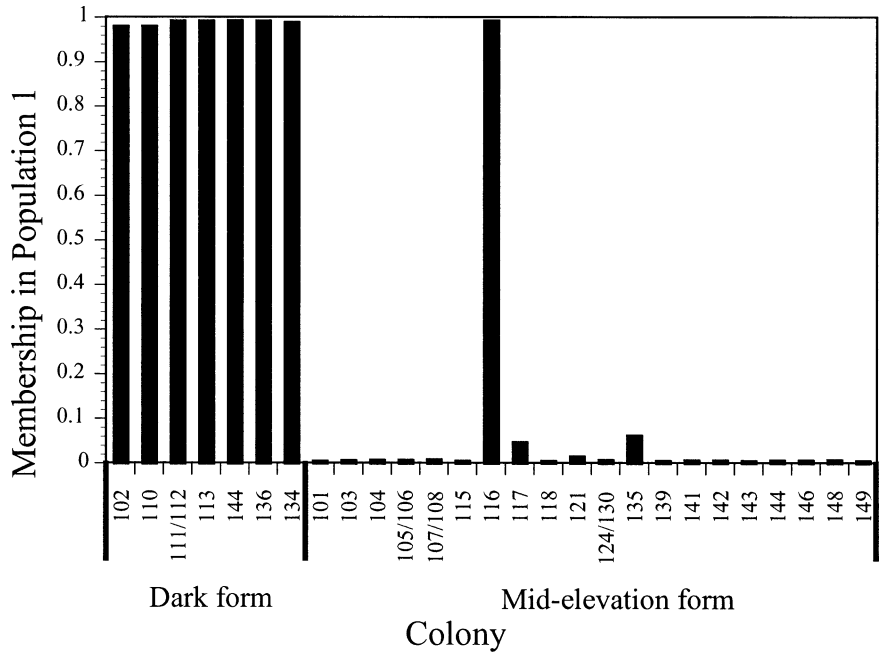

FIG. 4. Proportional membership of desert dark and midelevation form *Camponotus festinatus* ants into population 1 of $K = 2$ theoretical populations under a model of admixture. The proportional membership of each individual into population 2 equals one minus the proportional membership in population 1 (1 − P1). Based on morphological analysis, ants from the first seven and last 20 colonies listed were originally classified as desert dark and midelevation *C. festinatus*, respectively.

Discussion

Population genetic structure affects the evolution of life-history traits, which may subsequently affect species diversification in social organisms (Endler 1977; Barton and Clark 1990; Bourke and Franks 1995; Crozier and Pamillo 1996; Frank 1998; Avise 2004). We used a combination of laboratory and field studies to explore the genetic structure of a social insect taxon at a variety of levels including within colonies, among colonies in a single field site, between spatially separated populations, and across morphologically distinguishable forms. Using highly polymorphic DNA microsatellite markers, we successfully resolved questions concerning the breeding structure, nesting biology, spatial structure, and species status within this complex group. We also gained a greater understanding of whether changes in social system were associated with diversification.

Breeding System

Our analysis of queen mate number in both desert dark and desert light *C. festinatus* strongly indicated that queens typically mated only once. The genotypes of workers sampled from 36 of the 37 monogyne laboratory colonies were parsimoniously explained by queen monandry. Under such a scenario, worker relatedness would be expected to be close to 0.75. Indeed, our estimates of worker relatedness (0.73 and 0.75 for desert dark and desert light, respectively) were very close or equal to this value.

Single mating by desert dark and desert light *C. festinatus* queens is consistent with results from other studies in *Camponotus* (Gadau et al. 1996, 1998; Seppä and Gertsch 1996; Satoh et al. 1997; Crozier et al. 1999; Goodisman and Hahn 2004) and most other hymenopteran social insects (Bourke and Franks 1995; Boomsma and Ratnieks 1996; Crozier and Pamilo 1996; Strassman 2001). Single-mating appears to be an ancestral trait in the social Hymenoptera, and substantial polyandry has only evolved in the ant genera *Atta*, *Acromyrmex*, *Dorylus*, and *Pogonomyrmex*, the bee genus *Apis*, and wasp genus *Vespula* (Schmid-Hempel 1995; Boomsma and Ratnieks 1996; Crozier and Fjerdingstad 2001; Strassmann 2001; Brown and Schmid-Hempel 2003; Kronauer et al. 2004a). Presumably, multiple mating incurs considerable costs in time and energy and increases the risk of predation or contraction of diseases or parasites. Consequently, selection for multiple mating likely occurs only when ecological conditions dictate that it is important to acquire additional sperm for reproduction, produce a genetically variable worker force, or achieve a reduction in intracolony conflict (Boomsma and Ratnieks 1996; Strassmann 2001; Brown and Schmid-Hempel 2003).

Another important feature of the breeding structure of insect societies is the number of queens cohabiting within colonies (Keller 1993). If we assume that desert dark and midelevation form queens from our field populations were singly mated, as was the case in the desert dark and desert light laboratory colonies, then analysis of worker genotypes indicates that workers from approximately 25% of *C. festinatus* field-sampled colonies were derived from multiple matrilines. This suggests that multiple queens inhabited colonies, the primary reproductive queen within colonies changed over time, or brood raiding occurred in this species.

Ants in the genus *Camponotus* generally display life-history characteristics associated with monogyny, such as strong aggression toward nonnestmates, widespread dispersal through extensive nuptial flights, worker caste polymorphism, and independent colony foundation. In contrast, species whose colonies are normally headed by multiple queens often exhibit low aggression toward nonnestmates, relatively weak dispersal, an absence of worker caste polymorphism, and reproduction through colony budding (Hölldobler and Wilson 1990; Bourke and Franks 1995; Crozier and Pamilo 1996). However, recent studies, primarily conducted with molecular markers, have uncovered several cases of polygyny or queen replacement in *Camponotus* (Satoh 1989; Carlin et al. 1993; Akre et al. 1994; Gertsch et al. 1995; Gadau et al. 1998, 1999; Fraser et al. 2000). These studies show that social structure may be more complex than previously appreciated in some *Camponotus* species, and the association between colony queen number and other life-history traits likely occurs as a continuum rather than the dichotomous suites of traits drawn above.

Population averages for nestmate relatedness in both desert dark and midelevation field-sampled *C. festinatus* colonies were high (Table 3), despite the evidence for multiple matrilines within colonies. Moreover, relatedness within colonies containing workers from multiple matrilines was also fairly high ($r = 0.44$), suggesting that queens within nests were related. Indeed, queens cohabiting within nests are related in most social insects (Crozier and Pamilo 1996). The exceptions tend to occur in nonequilibrium situations, such as when a social insect has recently invaded a new environment or occupies a disturbed habitat (Hölldobler and Wilson 1990; Bourke and Franks 1995; Crozier and Pamilo 1996).

Spatial Patterns of Population Genetic Structure in the Field

Single social insect colonies sometimes inhabit multiple nests, a syndrome known as polydomy. Polydomy is common in ants and may occur if nest space is limiting, nests are frequently disrupted, moving into multiple nesting sites increases foraging efficiency, or if intracolony conflict can be reduced by expanding into multiple nests (Hölldobler and Wilson 1977, 1990; Pamilo and Rosengren 1984; Banschbach and Herbers 1996a,b; Cerda et al. 2002). In natural populations, a signature of polydomous colonies is the discovery of genetic structuring over short geographic distances.

We detected genetic structure within our field site and were able to group workers from distinct nests into single colonies based on their genotypes (Fig. 1). Our analysis of genetic patterns within our field site showed that pairs of nests that were separated by less than 10 m were significantly more similar than pairs separated by more than 10 m (Table 4). This pattern of structure fell off as the distances between nest pairs increased (10–50 m). This drop-off in signal is expected in polydomous ants if colonies inhabit multiple nests that remain in proximity (Goodisman and Crozier 2002). Polydomy may be facilitated by the nesting biology of *C. festinatus*, which resides in loose soil under rocks or fallen trees,

rather than forming conspicuous mounds (Cokendolpher 1990). In addition, this result is consistent with the observation that polydomy is frequently associated with polygyny in ants (Bourke and Franks 1995; Crozier and Pamilo 1996).

Our analysis of genetic structure also uncovered additional patterns within the field site. Unexpectedly, workers from pairs of nests separated by relatively large distances (e.g., 50–150 m in the midelevation form) were significantly more similar than expected by chance alone. This pattern was evident in both desert dark and midelevation forms, suggesting that it is not simply an artifact of one of the datasets. It is unlikely that these patterns are signatures of extensive polydomy or colony budding in this species for two reasons. First, *C. festinatus* colonies are relatively small, making polydomy or budding an unlikely source for spatial structure at this scale. Second, polydomy or budding would be expected to leave patterns of genetic structure over intermediate distances (i.e., 10–50 m) if it left such patterns over greater distances.

It is difficult to reconcile the patterns of genetic structuring with the known biology of this species. A possible explanation for these unusual patterns is that they reflect the way this site was colonized over a period of years. This site was located on a slope in the foothills of the Atascosa Mountains in the high desert grassland-to-oak transition zone near a dirt road and a cattle loading pen. This area could have been affected by both natural events such as fire or flooding, and anthropogenic factors such as grazing or pesticide application. Colonizing queens from any one year may have been derived from relatively few parent colonies and, therefore, would have formed a related group of individuals when compared to colonizing queens from other years. In addition, this site may have been colonized in patches of approximately 100 m^2, such that one patch within the site was colonized per year. Under such a scenario, workers sampled from colonies within patches would be more similar than workers sampled from colonies between patches, and genetic similarity of nests over fairly large distances could result. Testing this complex scenario, however, would require monitoring the spatial distribution and demography of colonies in both intentionally disturbed and undisturbed sites over a period of many years.

Genetic Differentiation within Forms

We discovered strong genetic differences between the laboratory- and field-sampled desert dark form. Indeed, the magnitude of differentiation between the field and laboratory desert dark form exceeded some estimates of differentiation between forms (Table 2). This suggests that spatial or temporal variation in allele frequencies exists within forms of the *C. festinatus* species complex, as the samples were collected at distinct sites in different years. Additional evidence of genetic differentiation within forms arises from the fact that the genetic diversity of laboratory desert dark samples was higher than that of the field desert dark samples (Table 1). Moreover, laboratory desert light ants displayed nonrandom association of alleles at the microsatellite loci, suggesting a deficit of heterozygotes relative to Hardy-Weinberg equilibrium. Such patterns could result from the Wahlund effect (Hartl and Clark 1989), whereby ants were sampled from multiple distinct populations resulting in a heterozygote deficit. Overall, these data suggest that forms within this species group may still be poorly defined, and more data from other populations may be required to gain a full understanding of the magnitude of variability in this species.

Genetic Differentiation between Forms

The three forms of *C. festinatus* ants possessed distinct complements of alleles (Table 2). Moreover, the field- and laboratory-sampled desert dark ants, which were collected from distinct sites, also differed significantly in allele frequencies. Measures of differentiation between the forms that incorporated information on allele size (ρ_{ST}) were substantially larger than those that considered only allele identity (F_{ST}). This result indicates that gene flow between the forms has been restricted for a substantial period of time (Hardy et al. 2003). In addition, we were able to group the field-sampled ants into discrete theoretical populations that largely coincided with form, and our analyses did not uncover evidence of hybridization between the desert dark and midelevation forms. Therefore, based on the combined lines of evidence, we consider the desert dark, desert light, and midelevation forms of *C. festinatus* to be reproductively isolated. Significant genetic structure has similarly been shown among populations separated by comparable distances in another taxonomically controversial group, the Florida carpenter ant, *C. floridanus* (Deyrup et al. 1988; Gadau et al. 1996).

Our analyses suggested that the field-collected desert dark form ants belonged to a single interbreeding group (Figs. 3, 4). The midelevation form ants, however, displayed somewhat more variation. There was some evidence from the genetic data that individuals classified as midelevation form originated from multiple populations. This is distinctly possible, given the current difficulty in morphologically distinguishing the different forms within this species complex and the observed differentiation between field- and laboratory-sampled desert dark form ants. Workers from one midelevation form, field-sampled colony (116, Fig. 4) were deemed to be genetically distinct from other midelevation form ants. Further inspection of this colony indicated a very high estimate of colony-specific relatedness relative to other midelevation form colonies ($r = 0.97$). However, ants from this nest were also distinct from desert dark form ants; the smallest estimate of differentiation between workers from the focal nest and workers from any other field-sampled nests was $F = 0.68$. These data indicate that individuals from this colony were strongly differentiated from the other *C. festinatus* samples. Consequently, it appears that workers from this nest may originate from a breeding group separate from desert dark, desert light, and midelevation *C. festinatus*.

In summary, our results indicate that substantial restrictions in gene flow occur within and among *C. festinatus* forms. These findings suggest that population subdivision may be more common in ants than previously recognized. In addition, such patterns of genetic structure may have important impacts on speciation, life-history evolution, and the distribution morphological variation within ants.

Conclusions

One of the aims of this study was to determine if species diversification in social insect taxa was associated with changes in social structure. We found that distinct forms within *C. festinatus* were genetically differentiated, suggesting incipient or complete speciation. However, we found no evidence that the forms differed in their breeding or social structure. Consequently, our data indicate that speciation in ants can occur without significant changes in social structure.

In addition, by documenting the breeding structure, nest structure, and spatial genetic structure within *C. festinatus*, we have taken the first steps toward understanding the evolution of this species complex. Current research on *C. festinatus* is focused on understanding the phylogeography of the group by using a combination of morphological and molecular genetic analyses of the ants and their intracellular endosymbiotic bacteria (A. N. Lazarus, D. A. Hahn, S. P. Cover, and J. J. Wernegreen, unpubl. data) and characterizing the life-history differences displayed by the different forms (D. A. Hahn, unpubl. data). These studies will allow for the reconstruction of the evolutionary history of the *C. festinatus* species complex, which is critical for understanding the observed variation within this group. The large geographic range covered by this species complex combined with the apparent morphological and molecular divergences make *C. festinatus* a promising model for studying speciation and selective forces associated with speciation events in social insects.

Using our experiences with the *C. festinatus* species complex as a guide, we make the following recommendations for others who want to understand evolutionary relationships within complex social insect taxa. A thorough morphological analysis should be the first step in characterizing any group suspected to contain multiple distinct lineages. This will require careful examination of multiple castes from many distinct nests and geographic locations. Statistical approaches to morphological analyses have been used successfully to separate taxa within ants in the past, and new analytical tools derived from studies of functional morphology and developmental allometry should facilitate further progress in using continuous characters in systematic studies (Snelling 1968; Umphrey 1996; Zelditch et al. 2004).

In addition to morphological analysis, molecular genetic tools should be used to understand patterns of gene flow within taxa. Numerous markers, including variable DNA microsatellites, AFLPs, and single nucleotide polymorphisms, can be useful in this arena. The type of marker, in addition to the number of markers and samples necessary, will vary depending on the group of interest and the level of analysis. However, we make the following conservative recommendations. Microsatellite markers are particularly useful because they are codominant and tend to possess many alleles, properties that lead to substantial power in determining colony boundaries and population differentiation. A thorough molecular genetic study will include analysis of the breeding system, spatial analyses of colony and population structure, and phylogenetic analyses and biogeography. To thoroughly analyze breeding system and distinguish colony boundaries, we recommend sampling 30 individuals per nest unit and 30 nest units per site. When considering multiple taxa, samples should be compared from multiple areas of both allopatry and sympatry. In addition, molecular data derived from symbiotic organisms should also be included for comparison when possible (Clark et al. 2000; Abbot and Moran 2002). We acknowledge that gathering evidence from all of the above sources is a daunting task, but such complex studies may be required to understand the factors contributing to evolutionary diversification of social taxa.

Acknowledgments

We are grateful to M. A. Wells and D. E. Wheeler for providing the laboratory facilities and resources necessary for this study. We thank A. Cutter, B. J. Haeck, J. K. Hatt, J. Krenz, and C. Schmidt for help collecting ants; N. Buck for caring of ants; A. Lazarus and S. Cover for identification of the three forms; M. Deyrup, B. Johnson, C. Johnson, and J. Hunt for useful discussion; and A. Lazarus, U. Mueller, J. Wernegreen, and four anonymous reviewers for helpful comments on the manuscript. This research was supported in part by a National Institutes of Health Postdoctoral Excellence in Research and Teaching fellowship administered through the University of Arizona Center for Insect Science (GM00708-02).

Literature Cited

Abbot, P., and N. A. Moran. 2002. Extremely low levels of genetic polymorphism in endosymbionts (*Buchnera*) of aphids (*Pemphigus*). Mol. Ecol. 11:2649–2660.

Akre, R. D., L. D. Hansen, and E. A. Myhre. 1994. Colony size and polygyny in carpenter ants (Hymenoptera: Formicidae). J. Kans. Entomol. Soc. Am. 67:1–9.

Arevalo, E., Y. Zhu, J. M. Carpenter, and J. E. Strassmann. 2004. The phylogeny of the social wasp subfamily Polistinae: evidence from microsatelite flanking sequences, mitochondrial COI sequence, and morphological characters. BMC Evol. Biol. 4:8.

Arnold, M. L. 1997. Natural hybridization and evolution. Oxford Univ. Press, Oxford, U.K.

Avise, J. C. 2004. Molecular markers, natural history, and evolution. Sinauer, Sunderland, MA.

Banschbach, V. S., and J. M. Herbers. 1996a. Complex colony structure in social insects. I. Ecological determinants and genetic consequences. Evolution 50:285–297.

———. 1996b. Complex colony structure in social insects. II. Reproduction, queen-worker conflict, and levels of selection. Evolution 50:298–307.

Barton, N. H., and A. G. Clark. 1990. Population structure and processes in evolution. Pp. 115–173 *in* K. Wöhrmann and S. K. Jain, eds. Population biology: ecological and evolutionary viewpoints. Springer, Berlin.

Barton, N. H., and K. S. Gale. 1993. Genetic analysis of hybrid zones. Pp. 13–45 *in* R. G. Harrison, ed. Hybrid zones and the evolutionary process. Oxford Univ. Press, New York.

Barton, N. H., and G. M. Hewitt. 1985. Analysis of hybrid zones. Annu. Rev. Ecol. Syst. 16:113–148.

———. 1989. Adaptation, speciation and hybrid zones. Nature 341: 497–503.

Bolton, B. 1995. A new general catalogue of the ants of the world. Belknap Press, Cambridge, MA.

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

Bourke, A. F. G., and N. R. Franks. 1995. Social evolution in ants. Princeton Univ. Press, Princeton, NJ.

Brady, S. G., J. Gadau, and P. S. Ward. 2000. Systematics of the ant genus *Camponotus*: a preliminary analysis using data from the mitochondrial gene cytochrome oxidase I. Pp. 131–139 *in*

A. D. Austin and M. Downton, eds. Hymenoptera: evolution, biodiversity, and biological control. CSIRO Press, Collingswood, Australia.
Brown, M. J. F., and P. Schmid-Hempel. 2003. The evolution of female multiple mating in social Hymenoptera. Evolution 57: 2067–2081.
Cahan, S. H., and L. Keller. 2003. Complex hybrid origin of genetic caste determination in harvester ants. Nature 424:306–309.
Cahan, S. H., and S. B. Vinson. 2003. Reproductive division of labor between hybrid and nonhybrid offspring in a fire ant hybrid zone. Evolution 57:1562–1570.
Carlin, N. F., H. K. Reeve, and S. P. Cover. 1993. Kin discrimination and division of labour among matrilines in the polygynous carpenter ant, *Camponotus planatus*. Pp. 362–401 *in* L. Keller, ed. Queen number and sociality in insects. Oxford Univ. Press, Oxford, U.K.
Cerda, X., A. Dahbi, and J. Retana. 2002. Spatial patterns, temporal variability, and the role of multi-nest colonies in a monogynous Spanish desert ant. Ecol. Entomol. 27:7–15.
Clark, M. A., N. A. Moran, P. Baumann, and J. J. Wernegreen. 2000. Cospeciation between bacterial endosymbionts (*Buchnera*) and a recent radiation of aphids (*Uroleucon*) and the pitfalls of testing for phylogenetic congruence. Evolution 54:517–525.
Clarke, K. E., T. E. Rinderer, P. Franck, J. G. Quezada-Euan, and B. P. Oldroyd. 2002. The Africanization of honeybees (*Apis mellifera* L.) of the Yucatan: a study of a massive hybridization event across time. Evolution 56:1462–1474.
Cokendolpher, J. C. 1990. The ants (Hymenoptera, Formicidae) of western Texas. II. Subfamilies Ecitoninae, Ponerinae, Pseudomyrmecinae, Dolichoderinae, and Formicinae. Texas Tech Univ. Press, Lubbock, TX.
Creighton, W. S. 1950. Ants of North America. Harvard Univ. Press, Cambridge, MA.
Crozier, R. H., and E. J. Fjerdingstad. 2001. Polyandry in social Hymenoptera: Disunity in diversity? Ann. Zool. Fenn. 38: 267–285.
Crozier, R. H., and P. Pamilo. 1996. Evolution of social insect colonies: sex allocation and kin selection. Oxford Univ. Press, Oxford, U.K.
Crozier, R. H., B. Kaufmann, M. E. Carew, and Y. C. Crozier. 1999. Mutability of microsatellites developed for the ant *Camponotus consobrinus*. Mol. Ecol. 8:271–276.
Deyrup, M. A., N. Carlin, J. Trager, and G. Umphrey. 1988. A review of the ants of the Florida Keys. Fla. Entomol. 71: 163–176.
Emory, C. 1893. Beiträge zur Kentniss der nordamsrikanischen Ameisen-fauna. Zool. Jahrb. Syst. 7:663–682.
Endler, J. A. 1977. Geographic variation, speciation, and clines. Princeton Univ. Press, Princeton, NJ.
Feldhaar, H., B. Fiala, J. Gadau, M. Mohamed, and U. Maschwitz. 2003. Molecular phylogeny of *Crematogaster* subgenus *Decacrema* ants and the colonization of *Macarange* trees. Mol. Phylogenet. Evol. 27:441–452.
Frank, S. A. 1998. Foundations of social evolution. Princeton Univ. Press, Princeton, NJ.
Fraser, V. S., B. Kaufmann, B. P. Oldroyd, and R. H. Crozier. 2000. Genetic influence on caste in the ant *Camponotus consobrinus*. Behav. Ecol. Sociobiol. 47:188–194.
Gadau, J., J. Heinze, B. Hölldobler, and M. Schmid. 1996. Population and colony structure of the carpenter ant *Camponotus floridanus*. Mol. Ecol. 5:785–792.
Gadau, J., P. J. Gertsch, J. Heinze, P. Pamilo, and B. Hölldobler. 1998. Oligogyny by unrelated queens in the carpenter ant, *Camponotus ligniperdus*. Behav. Ecol. Sociobiol. 44:23–33.
Gadau, J., S. Brady, and P. S. Ward. 1999. Systematics, distribution, and ecology of an endemic California *Camponotus quercicola* (Hymenoptera: Formicidae). Ann. Entomol. Soc. Am. 92: 514–522.
Gadau, J. C.-P. Strehl, J. Oettler, and B. Hölldobler. 2003. Determinants of intracolonial relatedness in *Pogonomyrmex rugosus*: mating frequency and brood raids. Mol. Ecol. 12:1931–1938.
Gertsch, P., P. Pamilo, and S.-L. Varvio. 1995. Microsatellites reveal high genetic diversity within colonies of *Camponotus* ants. Mol. Ecol. 4:257–260.
Goodisman, M. A. D., and M. A. Asmussen. 1997. Cytonuclear theory for haplodiploid species and X-linked genes. I. Hardy-Weinberg dynamics and continent-island model. Genetics 147: 321–338.
Goodisman, M. A. D., and R. H. Crozier. 2002. Population and colony genetic structure of the primitive termite *Mastotermes darwiniensis*. Evolution 56:70–83.
Goodisman, M. A. D., and D. A. Hahn. 2004. Colony genetic structure of the ant *Camponotus ocreatus* (Hymenoptera: Formicidae). Sociobiology 44:21–33.
Goodisman, M. A. D., D. D. Shoemaker, and M. A. Asmussen. 1998. Cytonuclear theory for haplodiploid species and X-linked genes. II. Stepping-stone models of gene flow and application to a fire ant hybrid zone. Evolution 52:1423–1440.
Goodisman, M. A. D., K. G. Ross, and M. A. Asmussen. 2000. A formal assessment of the effects of gene flow and selection in the fire ant *Solenopsis invicta*. Evolution 54:606–616.
Goropashnaya, A. V., V. B. Federov, and P. Pamillo. 2004. Recent speciation in the *Formica rufa* group ants: inference from mitochondrial DNA phylogeny. Mol. Phylogenet. Evol. 32: 198–206.
Hardy, O. J., N. Carbonnel, H. Freville, and M. Heuertz. 2003. Microsatellite allele sizes: a simple test to assess their significance on genetic differentiation. Genetics 163:1467–1482.
Harrison, R. G. 1993. Hybrid zones and the evolutionary process. Cornell Univ. Press, Ithaca, NY.
Hartl, D. H., and A. G. Clark. 1989. Principles of population genetics. Sinauer, Sunderland, MA.
Herbers, J. M. 1990. Reproductive investment and allocation ratios for the ant *Leptothorax longispinosus*: sorting out the variation. Am. Nat. 136:178–208.
———. 1993. Ecological determinants of queen number in ants. Pp. 262–293 *in* L. Keller, ed. Queen number and sociality in insects. Oxford Univ. Press, Oxford, U.K.
Hochberg, M. E., B. Sinervo, and S. P. Brown. 2003. Socially mediated speciation. Evolution 57:154–158.
Hölldobler, B., and E. O. Wilson. 1977. The number of queens: an important trait in ant evolution. Naturwissenschaften 64:8–15.
———. 1990. The ants. The Belknap Press of Harvard Univ. Press, Cambridge, MA.
Hunt, J. H., and J. M. Carpenter. 2004. Intra-specific nest form variation in some Neotropical swarm-founding wasps of the genus *Parachartergus*. J. Kans. Entomol. Soc. 77:448–456.
Jenkins, T. M., R. E. Dean, R. Verkerk, and B. T. Forschler. 2001. Phylogenetic analyses of two mitochondrial genes and one nuclear intron region illuminate European subterranean gene flow, taxonomy, and introduction dynamics. Mol. Phylogenet. Evol. 20:286–293.
Jones, A. G., and W. R. Ardren. 2003. Methods of parentage analysis in natural populations. Mol. Ecol. 12:2511–2523.
Julian, G. E., J. H. Fewell, J. Gadau, R. A. Johnson, and D. Larrabee. 2002. Genetic determination of the queen caste in an ant hybrid zone. Proc. Natl. Acad. Sci. USA 99:8157–8160.
Keller, L. 1993. Queen number and sociality in insects. Oxford Univ. Press, Oxford, U.K.
Keller, L., and H. K. Reeve. 1999. Dynamics of conflicts within insect societies. Pp. 153–175 *in* L. Keller, ed. Levels of selection in evolution. Princeton Univ. Press, Princeton, NJ.
Kronauer, D. J. C., C. Schoning, J. S. Pedersen, J. J. Boomsma, and J. Gadau. 2004a. Extreme queen-mating frequency and colony fission in African army ants. Mol. Ecol. 13:2381–2388.
Kronauer, D. J. C., B. Hölldobler, and J. Gadau. 2004b. Phylogenetics of the new world honey ants (genus *Myrmecocystus*) estimated from mitochondrial DNA sequences. Mol. Phylogenet. Evol. 32:416–421.
MacKay, W. P., and E. E. MacKay. 1997. A revision of the Neotropical ants of the *montivagus* species complex, genus *Camponotus*, subgenus *Myrmentoma*. Sociobiology 30:319–334.
———. 2000. A note in response to Mr. Roy Snelling's review of the *Camponotus montivagus* complex. Sociobiology 36:613–615.

Nei, M. 1987. Molecular evolutionary genetics. Columbia Univ. Press, New York.
Oster, G. F., and E. O. Wilson. 1978. Caste and ecology in the social insects. Princeton Univ. Press, Princeton, NJ.
Pamilo, P. 1990. Sex allocation and queen-worker conflict in polygynous ants. Behav. Ecol. Sociobiol. 27:31–36.
Pamilo, P., and R. Rosengren. 1984. Evolution of nesting strategies of ants: genetic evidence from different population types of *Formica* ants. Biol. J. Linn. Soc. 21:331–348.
Pamilo, P., P. Gertsch, P. Thorén, and P. Seppä. 1997. Molecular population genetics of social insects. Annu. Rev. Ecol. Syst. 28: 1–25.
Pritchard, J. K., M. Stephens, and P. Donnelly. 2000. Inference of population structure using multilocus genotype data. Genetics 155:945–959.
Queller, D. C., and K. F. Goodnight. 1989. Estimating relatedness using genetic markers. Evolution 43:258–275.
Raymond, M., and F. Rousset. 1995. GENEPOP (version 1.2): population genetics software for exact tests and ecumenicism. J. Hered. 86:248–249.
Rice, W. R. 1989. Analyzing tables of statistical tests. Evolution 43:223–225.
Ross, K. G. 2001. Molecular ecology of social behaviour: analyses of breeding systems and genetic structure. Mol. Ecol. 10: 265–284.
Ross, K. G., and L. Keller. 1995. Ecology and evolution of social organization: insights from fire ants and other highly eusocial insects. Annu. Rev. Ecol. Syst. 26:631–656.
Rousset, F. 1997. Genetic differentiation and estimation of gene flow from *F*-statistics under isolation by distance. Genetics 145: 1219–1228.
Rousset, F., and M. Raymond. 1995. Testing heterozygote excess and deficiency. Genetics 140:1413–1419.
Satoh, T. 1989. Comparisons between two apparently distinct forms of *Camponotus nawai* Ito (Hymenoptera: Formicidae). Insectes Soc. 36:277–292.
Satoh, T., K. Masuko, and T. Matsumoto. 1997. Colony genetic structure in the mono- and polygynous sibling species of the ants *Camponotus nawai* and *Camponotus yamaokai*: DNA fingerprinting analysis. Ecol. Res. 12:71–76.
Schmid-Hempel, P. 1995. Parasites and social insects. Apidologie 26:255–271.
Schneider, S. S., L. J. Leamy, L. A. Lewis, and G. DeGrandi-Hoffman. 2003. The influence of hybridization between African and European honeybees, *Apis mellifera*, on asymmetries in wing size and shape. Evolution 57:2350–2364.
Schneider, S. S., G. DeGrandi-Hoffman, and D. R. Smith. 2004. The African honey bee: factors contributing to a successful biological invasion. Annu. Rev. Entomol. 49:351–376.
Seppä, P., and P. Gertsch. 1996. Genetic relatedness in the ant *Camponotus herculeansus*: a comparison of estimates from allozyme and DNA microsatellite markers. Insectes Soc. 43: 235–246.
Snelling, R. R. 1968. Studies on California ants. 4. Two species of *Camponotus*. Proc. Entomol. Soc. Wash. 70:350–358.
———. 1970. Studies on California ants. 5. Revisionary notes on some species of *Camponotus* subgenus *Tanemyrmex*. Proc. Entomol. Soc. Wash. 72:390–397.
———. 2000. A review of the *Camponotus montivagus* complex. Sociobiology 36:599–611.
Starr, C. K. 1984. Sperm competition, kinship, and sociality in the aculeate Hymenoptera. Pp. 427–464 *in* R. L. Smith, ed. Sperm competition and the evolution of animal mating systems. Academic Press, New York.
Strassmann, J. 2001. The rarity of multiple mating by females in the social Hymenoptera. Insectes Soc. 48:1–13.
Umphrey, G. J. 1996. Morphometric discrimination among sibling species in the *fulva-rudis-texana* complex of the ant genus *Aphaenogaster*. Can. J. Zool. 74:528–559.
Volny, V. P., and D. M. Gordon. 2002. Genetic basis for queen-worker dimorphism in a social insect. Proc. Natl. Acad. Sci. USA 99:6108–6111.
Walsh, P. S., D. A. Metzger, and R. Higuchi. 1991. Chelex 100 as a medium for simple extraction of DNA for PCR-based typing from forensic material. Biotechniques 10:506–513.
Weir, B. S., and C. C. Cockerham. 1984. Estimating *F*-statistics for the analysis of population structure. Evolution 38:1358–1370.
Wheeler, W. M. 1902. A new agricultural ant from Texas with remarks on the known North American species. Am. Nat. 36: 85–100.
———. 1910. The North American ants of the genus *Camponotus*. Ann. NY Acad. Sci. 20:295–354.
Wilson, E. O. 1971. The insect societies. Harvard Univ. Press, Cambridge, MA.
Ye, W., C.-Y. Lee, R. H. Scheffrahn, J. M. Aleong, N.-Y. Su, G. W. Bennett, and M. E. Scharf. 2004. Phylogenetic relationships of nearctic *Reticulitermes* species with particular reference to *Reticulitermes arenincola* Goellner. Mol. Phylogenet. Evol. 30: 815–822.
Zelditch, M. L., D. L. Swiderski, H. D. Sheets, and W. L. Fink. 2004. Geometric morphometrics for biologists: a primer. Elsevier, London.

Corresponding Editor: U. Mueller