Colony Genetic Structure of the Ant *Camponotus ocreatus* (Hymenoptera: Formicidae)

by

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ABSTRACT

An increasing number of studies have uncovered evidence of polygyny or polyandry in the ant genus *Camponotus*. To further increase our understanding of the sociobiology of ants in this genus, we used DNA microsatellite markers to analyze the genetic structure of *C. ocreatus*. We found that the genotypes of workers, gynes, and males in 15 of the 16 nests analyzed were consistent with having been produced by a single, once-mated queen. However, nestmate genotypes in one nest were more complex, signifying the presence of multiple reproductives. In addition, the adjusted estimate of diploid nestmate relatedness, $r = 0.65$, suggested infrequent polygyny or queen polyandry. We also uncovered some evidence of inbreeding between queens and their male mates, and occasional polydomy in this population. Overall our results indicate that the social structure of most *C. ocreatus* colonies is relatively simple, although the genetic structure of a few colonies suggests that more than a single queen and male may contribute to offspring production.

INTRODUCTION

Colony queen number is a key determinant of the genetic structure of social insect populations (Ross 2001). In addition, variation in queen number tends to be associated with particular life history characteristics in ants. For example, species whose colonies are usually headed by a single queen (monogyny) typically display strong aggression towards 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 (polygyny) often exhibit low aggression towards nonnestmates, relatively weak dispersal, an absence of worker caste polymorphism,

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and reproduction through colony budding (Boomsma & Grafen 1990, Hölldobler & Wilson 1990, Crozier & Pamilo 1996).

Ants in the genus *Camponotus* display many life history characteristics associated with monogyny (Fowler 1986, Carlin & Hölldobler 1988, Hölldobler & Wilson 1990). Indeed, *Camponotus* ants have traditionally been characterized as uniformly monogyne (Fowler 1986, Frumhoff & Ward 1992). However, recent studies, primarily conducted with molecular markers, have uncovered several cases of polygyny in this genus (Satoh 1989, Carlin *et al.* 1993, Akre *et al.* 1994, Gertsch *et al.* 1995, Gadau *et al.* 1998, Gadau *et al.* 1999, Fraser *et al.* 2000). These studies suggest that the social structure of *Camponotus* may be more complex than previously appreciated and the association between colony queen number and life history traits as described above may require more careful examination.

In order to increase our understanding of the sociobiology of *Camponotus*, we investigated the sociogenetic structure of the ant *Camponotus ocreatus*. *C. ocreatus* is commonly found in the southwest of the United States (Hunt & Snelling 1975, Blom & Clark 1980, Chew & Chew 1980, Wheeler & Wheeler 1986, Cokendolpher 1990). *C. ocreatus* is particularly remarkable because of its worker polymorphism and aggression displayed by its soldier caste (Lamon & Topoff 1981, Wheeler & Wheeler 1986). Both of these traits are commonly associated with monogyny. Consequently, our principal interest lies in determining if single or multiple queens head colonies in this species.

MATERIALS AND METHODS

We collected *C. ocreatus* workers, prereproductive winged queens (gynes), and winged males from the Sycamore Canyon in the Atascosa Mountains in Santa Cruz County near Nogales, Arizona, USA. The collection site was at 31° 26.29' N, 111° 11.00' W at an elevation of 1255 meters above sea level. Ants were collected from nests located under small rocks throughout an area of 7500 m^2 and placed in 95% ethanol.

Genomic DNA was extracted from ants using a modification of the Chelex® protocol (Walsh *et al.* 1991) as described by Crozier *et al.* (1999). Genotypes of individual ants were determined at the four polymorphic microsatellite loci, Ccon12, Ccon20, Ccon42, and Ccon70, which were amplified using PCR. PCR products were visualized by endlabeling the forward primers of Ccon12, Ccon20, Ccon42, and Ccon70 (Crozier *et al.* 1999) with the fluorescent dyes 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), and a final concentration of 200

mM dNTPs, 0.5 mM of each of the forward and reverse PCR primers, and 1X New England Bioloabs PCR buffer.

The PCR cycling profiles for the four markers began with an initial denaturation at 94 °C for 2 min, and then proceeded with 40 cycles of 94 °C for 30 s, 45 °C for 45 s, and 72 °C for 45 s, followed by a final extension of 72 °C for 10 min. PCR products from all loci were then combined in a 7 µl cocktail containing 1.0 µl, 2.0 µl, 2.0 µl, and 2.0 µl of Ccon12, Ccon20, Ccon42, and Ccon70 PCR product, respectively. Two µl of this cocktail was combined with a labeled size standard and electrophoresed and scored on an ABI PRISM® 3100 Genetic Analyzer.

We used the program RELATEDNESS 4.2 (Queller & Goodnight 1989) to estimate the microsatellite allele frequencies in this population of *C. ocreatus*. The variability at each locus was quantified by Nei's (1987) estimate of gene diversity, $h = 2n(1-\sum_i p_i^2)/(2n-1)$, where n is the number of workers sampled and p_i is the frequency of allele i.

Ants collected from distinct *C. ocreatus* nests may belong to single colonies (polydomy). To determine if polydomy was common, we tested for genetic isolation by distance in our population in two ways. First, we used GENEPOP 3.2 (Raymond & Rousset 1995) to examine the relationship between pairwise geographic distances between nests and genetic distance, *F*, as estimated by Weir and Cockerham's (1984) method. The magnitude of the correlation was measured by Spearman's correlation coefficient, r_S, and the significance of the correlation was determined by Mantel's test as implemented by GENEPOP. If polydomy was common and the effects of polydomy could be seen over relatively large distances, we expected a significant, positive relationship between pairwise genetic distances and geographic distances.

Second, we sought evidence for polydomy over shorter distances by comparing the pairwise values of genetic differentiation, *F*, for nests that were within some predefined distance, *D*, to those that were separated by distances greater than *D*. Specifically, we calculated the mean of pairwise *F* values for all nests within *D* meters of each other and subtracted the mean of pairwise *F* values for nests separated by more than *D* meters. The resulting test statistic, *dif-D*, could not be analyzed using classic parametric statistics because of the nonindependence of pairwise values of genetic differentiation. Consequently, we used a resampling protocol to calculate the significance of *dif-D*. In the algorithm used, we first randomly assign values of pairwise *F* to all nests. The randomly assigned values of *F* were obtained from the observed distribution of pairwise *F* values 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 data set. This procedure was repeated 10,000 times and the resulting *rdif-D* values were ordered from smallest to largest. We considered there to be evidence of genetic isolation by distance if the observed value of *dif-D* was less than 5% of the calculated *rdif-D* values, because our test is one-tailed. That is, we expect mean pairwise values of *F* for nests within distance *D* to be *less* than mean pairwise values of *F* for nests separated by distances greater than *D*.

We directly examined the genotypes of workers, gynes, and males from single nests to determine if the social system of *C. ocreatus* conformed to the simplest type of social insect colony structure: specifically, nests headed by a single, once-mated queen, which produced all workers, gynes, and males. Genetic data were considered to be consistent with this social structure if (1) diploid ants (workers and gynes) possessed a maximum of three alleles at any locus and their genotypes conformed to those expected under Mendelian segregation of alleles from a single diploid female and a single haploid male, and (2) if males possessed a maximum of two alleles at any locus. We also used *G*-tests to determine if diploid female or haploid male single-locus genotypes segregated in the 1:1 ratio expected in colonies headed by a single, once-mated queen. Tests were only conducted in nests where ants displayed genotypic variation.

We next used RELATEDNESS 4.2 to estimate the relatedness of nestmates. We first estimated the relatedness among workers, gynes, and males, which, under a simple social system are expected to equal 0.75, 0.75, 0.5, respectively. Next we estimated the relatedness of workers (y) to gynes (x) and workers (y) to males (x) (x and y directionality *sensu* Crozier & Pamilo 1996). Under a simple social system the relatedness of gynes to workers should equal 0.75, while the relatedness of males to workers should equal 0.25. Standard errors for estimates were obtained by jackknifing over nests, and *t*-tests were used to determine if estimates differed statistically from predicted values.

We then obtained estimates for the level of inbreeding (f) of diploids using the program RELATEDNESS 4.2. The significance of the estimate was determined using a *t*-test, with the standard error determined by jackknifing over nests. Inbreeding can affect measures of genetic relatedness (Pamilo 1985). To adjust the estimates of relatedness obtained in this study for inbreeding, we applied Pamilo's (1985) correction to obtain the adjusted estimate $r^* = [r - 2f / (1 + f)] / [1 - 2f / (1 + f)]$, where r^* is the inbreeding adjusted relatedness estimate, r is

the unadjusted relatedness estimate, and f is the inbreeding coefficient.

Finally, we used the observed genotypes of sampled workers and males to reconstruct the genotypes of queens and their male mates that produced them. We assessed the relatedness of queen's to their male mates using the program RELATEDNESS 4.2 and used t-tests to determine if this estimate differed significantly from zero.

RESULTS

We sampled ants from a total of 16 nests (Fig. 1). An average of 12.06 ± 5.59 ($\bar{x}$ ± s.d.) workers were collected from each nest, and 3.50 ± 3.70

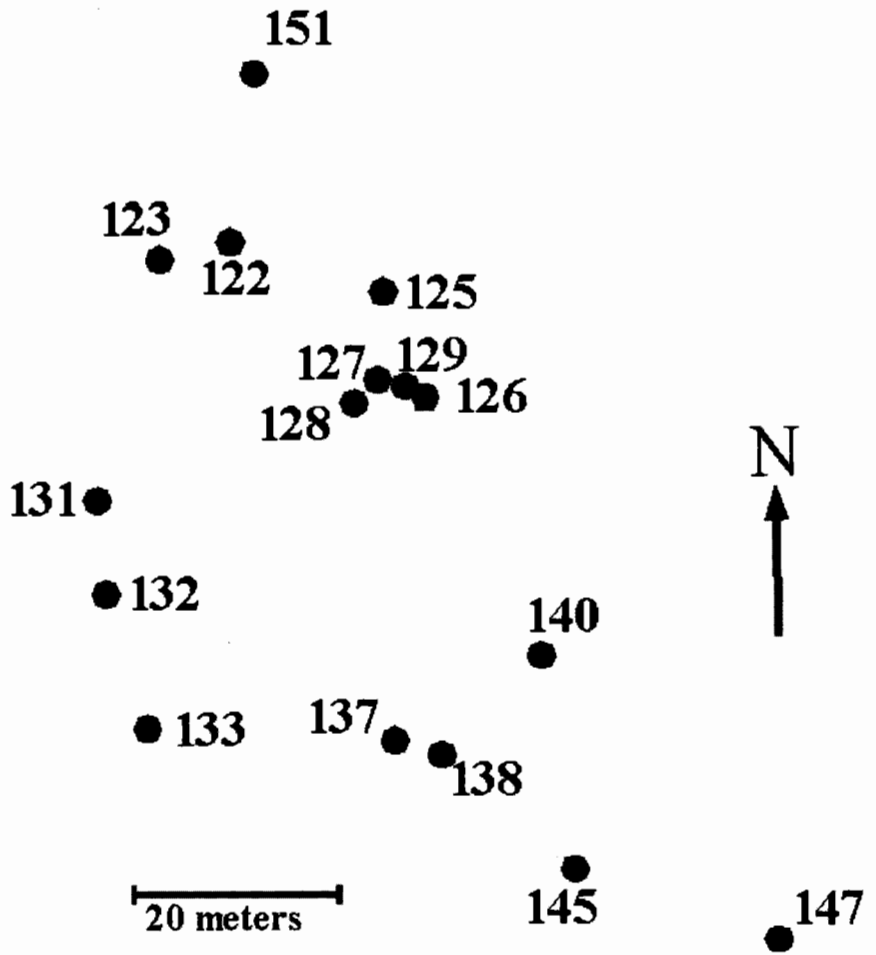

Fig.1. Locations of 16 *C. ocreatus* nests sampled in this study.

Table 1. Numbers of workers, gynes, and males sampled from 16 *C. ocreatus* nests.

Nest	Workers	Gynes	Males	Total
122	6	0	0	6
123	10	0	3	13
125	11	0	0	11
126	3	0	0	3
127	19	0	0	19
128	26	0	0	26
129	18	0	0	18
131	12	0	1	13
132	10	2	0	12
133	14	0	0	14
137	14	0	0	14
138	11	0	0	11
140	13	9	15	37
145	6	1	15	22
147	12	2	10	24
151	8	0	0	8
Total	193	14	44	251

gynes and 8.80 ± 6.57 males were collected from four and five nests, respectively (Table 1). The four microsatellite loci showed modest levels of variation. The loci Ccon12, Ccon20, Ccon42, and Ccon70 displayed three, two, two, and five alleles respectively, with associated gene diversities of 0.43, 0.086, 0.032, and 0.37.

We next examined the relationship between pairwise estimates of genetic differentiation between ants collected from distinct nests and the distance between nests to determine if polydomy was common in *C. ocreatus*. We found that the correlation between these two variables, $r_S = 0.053$, was small (Fig.

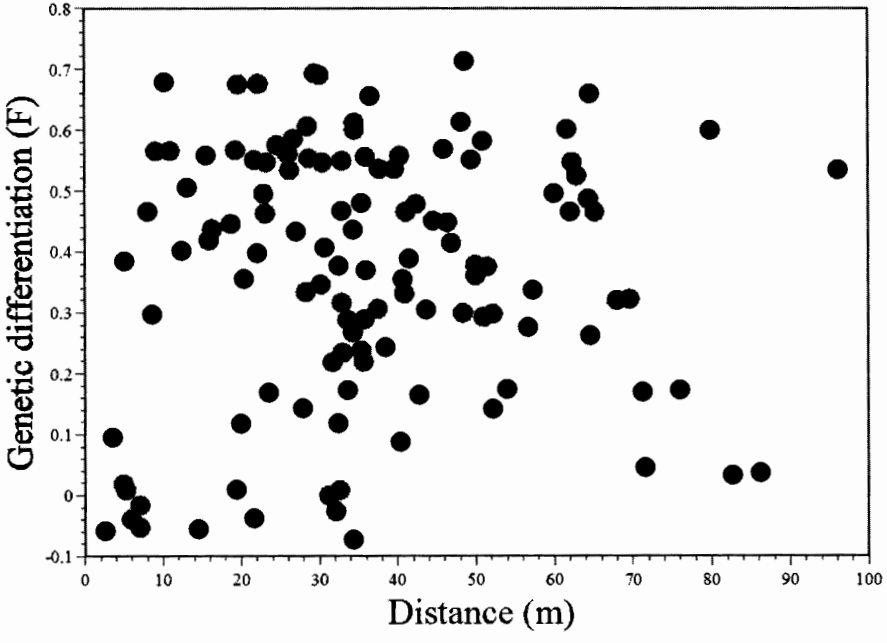

Fig. 2. Patterns of isolation by distance for *C. ocreatus*. Relationship between pairwise estimates of genetic distance, *F*, and geographic distance between nests.

Table 2. Distance, *D*, in meters, numbers of pairs of nests separated by distances less than *D* (*N* < *D*) and greater than *D* (*N* > *D*), difference in mean genetic differentiation of nests separated by distance *D* (*dif-D*), and randomly calculated values of the difference in mean differentiation for nests at various significance limits (*rdif-D*) (see text for details).

				rdif-D		
D	*N* < *D*	*N* > *D*	*dif-D*	0.05	0.01	0.001
10	11	109	-0.23	-0.11	-0.15	-0.20
20	23	97	-0.08	-0.08	-0.11	-0.15
30	43	77	-0.01	-0.06	-0.09	-0.12
40	75	45	-0.03	-0.06	-0.09	-0.12
50	92	28	0.01	-0.07	-0.10	-0.14

2) and not significantly different from zero ($P > 0.2$). We then compared the pairwise estimates of differentiation for nests separated by various distances *D* to those separated by distances greater than *D*. Using this method, we found evidence for genetic isolation by distance over short distances (Table 2). Specifically, nests separated by less than 10 m were significantly more similar to each other ($P < 0.001$) than expected by chance. A marginal effect of genetic isolation by distance was also observed for nests separated by less than 20 m ($P = 0.05$), and no effect was observed for distances greater than 20 m. Consequently, we conclude that polydomy does appear to occur infrequently in *C. ocreatus*. However, the potential statistical problems arising from sampling ants from distinct nests belonging to the same colony should not bias our study because polydomy was rare in our study population.

By direct examination of worker, gyne, and male genotypes, we found that 15 of the 16 nests 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, which produced all males and gynes. However, in nest 147 workers as a group possessed three distinct genotypes at the locus Ccon70. In addition, the males from this nest possessed four haploid genotypes in total. Consequently, more than a single mating pair must have produced the offspring sampled from this nest.

We conducted a total of 16 *G*-tests on the diploid female and haploid male genotypes in 13 nests whose inhabitants were qualitatively consistent with having been produced by a single, once-mated queen and showed genotypic variation (Tests were not conducted on colony 147, which clearly showed complex genetic patterns). Of the 16 tests conducted, only one showed a significant deviation from the 1:1 ratio

Table 3. Estimates and standard errors for the relatedness ($r \pm$ S.E.) of *C. ocreatus* nestmates at four microsatellite loci and all loci combined. Values are given for the relatedness of nestmate workers (r_{ww}), gynes (r_{gg}), males (r_{mm}), workers to gynes (r_{wg}), workers to males (r_{wm}), and queens to their male mates (r_{qm}).

Locus	r_{ww}	r_{gg}	r_{mm}	r_{wg}	r_{wm}	r_{qm}
Ccon12	0.76 ± 0.05	1.00 ± 0.00	1.00 ± 0.00	1.00 ± 0.00	0.81 ± 0.18	0.33 ± 0.11
Ccon20	0.88 ± 0.11	1.00 ± 0.00	1.00 ± 0.00	1.00 ± 0.00	1.00 ± 0.00	0.70 ± 0.28
Ccon42	0.45 ± 0.45	1.00 ± 0.00	1.00 ± 0.00	1.00 ± 0.00	1.00 ± 0.00	-0.50 ± 1.40
Ccon70	0.68 ± 0.09	1.00 ± 0.00	0.53 ± 0.28	0.24 ± 0.34	-0.09 ± 0.16	0.02 ± 0.12
All	0.74 ± 0.03	1.00 ± 0.00	0.70 ± 0.17	0.90 ± 0.04	0.27 ± 0.05	0.23 ± 0.09

expected ($G_1 = 3.96$, $P = 0.047$, other results not shown). The occurrence of this single marginally significant deviation in 16 statistical tests is expected by chance alone (Rice 1989), and we conclude that the statistical tests are consistent with a single once-mated queen having produced the progeny in these 13 nests.

The relatedness of nestmate *C. ocreatus* ants was relatively high (Table 3). The relatedness of workers, 0.74, was very close to, and not significantly different from, the value of 0.75 expected ($t_{15} = 0.25$, $P > 0.8$). The estimate of the relatedness of gynes was at its maximum value of 1.0 with no variation, because all gynes were genotypically identical within each of the nests from which multiple gynes were sampled. In addition, the estimate of workers to gynes, 0.90, was significantly greater than expected under the simplest model ($t_3 = 4.10$, $P < 0.03$). These unusually high estimates of gyne relatedness presumably result from sampling error, as relatively few gynes were obtained from each of only three nests (Table 1).

The overall relatedness of diploid individuals (gynes and workers) within nests, 0.74, was very close to that for workers, given that they made up most of the sample, and didn't differ from the expected value of 0.75 ($t_{15} = 0.15$, $P > 0.8$). The estimate for males, 0.70, did not differ significantly from 0.5 ($t_2 = 1.14$, $P > 0.3$), and the estimate of workers to males, 0.27, was also close to the expected value of 0.25 ($t_4 = 0.32$, $P > 0.7$). The estimate of inbreeding for diploid individuals (workers and gynes), 0.15 ± 0.09, was high, but not significantly different from zero ($t_2 = 1.76$, $P > 0.05$). Nevertheless, we corrected the relatedness of diploids down to 0.65 to account for the potential inbreeding.

We next turned our attention to the relatedness of queens to their male mates. We were able to determine the genotypes of queens and their mates in 14 of the 16 nests. In nest 147, the complex genotypic composition of workers and males (see above) precluded determination

of parental genotypes and, consequently, this nest was omitted from analysis. In addition, in nest 137 all workers were heterozygous at loci Ccon12 and Ccon70, thereby rendering it impossible to determine the queen and male genotypes with certainty. To avoid biases in estimation of relatedness that would arise if this nest were omitted, we constructed four distinct data sets, each containing a different possible combination of potential queen-male genotypes at these loci. The mean of the four relatedness estimates, 0.23, was taken as our best estimate. We found that this value differed marginally from zero ($t_{14} = 2.61$, $P < 0.05$). Therefore queens appear to mate with related males leading to a slightly inbred population.

DISCUSSION

Ants in the genus *Camponotus* have been characterized as primarily monogyne (Fowler 1986, Frumhoff & Ward 1992, Carlin *et al.* 1993). However, recent studies have now uncovered evidence of polygyny in several *Camponotus* species, including *C. planatus* (Carlin *et al.* 1993), *C. ligniperda* (Gertsch *et al.* 1995), *C. quercicola* (Gadau *et al.* 1999), *C. yamaoki* (Satoh 1989, Sanada *et al.* 1997) and *C. consubrinus* (Fraser *et al.* 2000). Our results revealed that the genotypes of workers and males in 15 of the 16 nests examined conformed to those expected if a single, once-mated queen headed *C. ocreatus* colonies. Consequently, *C. ocreatus* appears to be predominantly monogyne.

However, the workers in one nest possessed genotypes incompatible with having been produced by a single, once-mated queen, and the males from this nest possessed four alleles as a group, including a single allele that was not present in the workers. Perhaps the simplest explanation for this result is that two queens that both produced workers and males headed this colony. If so, *C. ocreatus* would represent another instance of polygyny in the genus *Camponotus*. However, another explanation is that this colony was founded by a single, doubly-mated queen, which produced the sampled workers, and then subsequently died. The queen's daughters then could have become reproductively active and produced males. Indeed, such a scenario is possible, as *Camponotus* workers are known to produce male eggs in the absence of the queen (Hölldobler & Wilson 1990). Although, this explanation also requires a mutation at one of the microsatellite markers in males, an event which occurs relatively rarely (Goldstein & Schlotterer 1999).

If this latter scenario is true, we cannot reject the possibility that other queens in our study site mated multiply. In fact, our estimate of the effective number of mates of queens, $m_e = 2 / (4r - 1)$, where r is the

inbreeding-correct relatedness estimate of diploid offspring, equals 1.25 (Starr 1984). The estimate of m_e incorporates information both on the number of times that a queen mates and the potential unequal contribution to progeny production of males mated to polyandrous queen. Consequently, our data suggest that *C. ocreatus* queens mate multiply some proportion of the time (assuming that polygyny is absent or very rare). This type of mating system appears to be common in *Camponotus*, as previous studies have discovered that, although most *Camponotus* queens mate only once (Gadau *et al.* 1996, 1998, Satoh *et al.* 1997, Crozier *et al.* 1999), a low frequency of polyandry does occur (Seppä & Gertsch 1996, Gadau *et al.* 1998).

Perhaps the most surprising result of this study is that *C. ocreatus* appears to undergo some degree of inbreeding. This result is unexpected, given that monogyny is typically associated with outbreeding in ants (Hölldobler & Wilson 1990, Bourke & Franks 1995) (but see Hasegawa & Yamaguchi 1995, Cole & Wiernasz 1997, Sundström *et al.* 2003 for exceptions). Inbreeding is expected to result in substantial costs to queens, because queens that mate to related males increase their probability of undergoing a 'matched' mating: a mating where the queen shares an allele with her mate at her sex determining locus (or loci). If a queen partakes in a matched mating then some fraction of her diploid offspring may be male. The production of diploid males is expected to entail a substantial cost, because diploid males are generally subfertile and do not partake in useful work (Crozier & Pamilo 1996). We genotyped 44 males in this study and none appeared to be diploid. Nevertheless it is possible that diploid males in *C. ocreatus* are either eliminated from colonies before they reach maturity or that the colonies of queens who engage in matched matings fail to become established due to the cost brought on by diploid male production (*e.g.*, Ross & Fletcher 1986). Regardless, the suggestion of inbreeding in this ant is of considerable interest and its causes and consequences require further investigation.

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