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Inbreeding in a lek-mating ant species, *Pogonomyrmex occidentalis*

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Abstract In this paper we have two goals. First, we examine the effects of sample size on the statistical power to detect a given amount of inbreeding in social insect populations. The statistical power to detect a given level of inbreeding is largely a function of the number of colonies sampled. We explore two sampling schemes, one in which a single individual per colony is sampled for different sample sizes and a second sampling scheme in which constant sampling effort is maintained (the product of the number of colonies and the number of workers per colony is constant). We find that adding additional workers to a sample from a colony makes it easier to detect inbreeding in samples from given number of colonies; however, adding more colonies rather than more workers per colony always gives greater power to detect inbreeding. Because even relatively large amounts of sib-mating generate relatively small inbreeding coefficients, detection of even substantial deviations from random mating will require very large samples. Second, we look at the amount of inbreeding in a large population of the western harvest ant, *Pogonomyrmex occidentalis*. We find deviations from Hardy-Weinberg equilibrium equivalent to approximately 27% sib-mating in our population ($f = 0.09$). Review of past studies on the population structure of other *Pogonomyrmex* species suggests that inbreeding may be a regular feature of the mating system of these ants. Although *P. occidentalis*is a swarm-mating species, there are a number of features of its population biology which suggest that the effective population size may be small. These include topographical variation that potentially breaks the population into demes, variation in the reproductive output of colonies, and variation in the size of reproductives produced by colonies.

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Introduction

The inbreeding coefficient of a population measures the correlation between alleles at a locus within an individual. Changes in this genetic correlation are due to nonrandom mating within populations. Higher levels of inbreeding can increase the opportunity for selection on altruistic traits, including eusociality, by increasing the genetic variance among groups relative to the variance within a group (Price 1970; Hamilton 1972; Breden and Wade 1981, 1991; Wade and Breden 1981, 1987; Uyenoyama 1984; Michod 1980, 1993). Inbreeding can, in fact, be the crucial factor that tips the balance in favor of the evolution of an altruistic trait (Michod 1980), although Michod (1980) and Ueynoyama (1984) explore some conditions under which it may have the opposite effect. Although much of the theoretical and empirical work on inbreeding has focused on the social insects, these questions apply to many social species.

The genetic structure of social insect populations has been the subject of numerous recent studies (e.g., Kukuk 1989; Blows and Schwarz 1991; Ross 1993). Kukuk (1989) found significant population substructuring and, in particular, substantial inbreeding in the bee *Dialictus zephyrus*. Her genetic evidence was supported by behavioral observations of males, many of which mate near their natal nest. Blows and Schwarz (1991) examined the hierarchical organization of populations of the primitively eusocial bee *Exoneura bicolor*. They found significant heterogeneity among certain subunits, particularly between aggregations at different localities, and different populations had different patterns of organization as revealed by *F*-statistics. Ross (1993) has examined the effects of the breeding system of the fire ant, *Solenopsis invicta*, including the level of inbreeding, on the colony genetic structure. He concluded that there

was no evidence for inbreeding in any of the age or sex categories examined. In a review of the literature on population structure in the social wasps, Ross and Carpenter (1991) note that social wasps generally show little evidence of inbreeding.

Because inbreeding is potentially critical for the evolution of social behavior, it has been measured in many species of social insects. Usually these data are gathered to estimate relatedness as well as inbreeding in the species of interest. Among ants, inbreeding has been characterized as uncommon (Pamilo 1983; Crozier et al. 1984; Ross 1988,1993; Kaufmann et al. 1992; Pamilo et al. 1992; Seppä 1992; Keller and Passera 1993). Typically, the estimated inbreeding coefficient is quite small, and the authors conclude that inbreeding is being actively avoided (e.g., Crozier 1980; Keller and Passera 1993). Breeding systems with synchronous production of reproductives or the production of large mating swarms are often interpreted as mechanisms to ensure outbreeding or prevent incestuous mating. If this is true, then there must be a penalty for inbreeding, such as expression of deleterious recessives (Werren 1993), the production of diploid males (e.g., Crozier 1977; Ward 1980; Ross and Fletcher 1985; Kukuk and May 1990) or perhaps increased fluctuating asymmetry (Keller and Passera 1993).

An alternative to the view that inbreeding is rare is that inbreeding is difficult to measure and therefore rarely detected. If inbreeding reduces worker viability, colony survival and establishment will be a function of the degree of inbreeding (Werren 1993). Measures of inbreeding in established colonies may thus underestimate its true frequency. However, because of the difficulties associated with sampling colonies very early after founding, most investigators will be confined to measuring inbreeding in established colonies. In these instances the probability of detecting inbreeding will be affected by the number of colonies that can be genotyped. Hedrick (1983, pp 61–64) gives a clear description of the relationship between sample size and the power to detect a given level of inbreeding. The measurement of inbreeding has sample size requirements that may not be widely appreciated – very large sample sizes are required to measure inbreeding coefficients accurately. Wright (1978) discusses the fact that even extreme levels of population subdivision may lead to small *F*-statistics. Inferences about the commonness or rarity of inbreeding should be tempered by the power to detect a given amount of inbreeding with a particular sample size.

In this paper we have two goals. First we will examine the power of tests to detect inbreeding in social insects. The type of colony that we examine has a single foundress, which accounts for a large fraction of the total species. We will show that sample sizes of several hundred colonies are required to detect even very large levels of inbreeding. Second, we will measure the level of inbreeding within a large population of the western harvester ant, *Pogonomyrmex occidentalis*. We find evidence for substantial inbreeding in *P. occidentalis*, and reanalyze past genetic studies in other *Pogonomyrmex* to show that inbreeding may be common in this genus of ants.

Materials and methods

The study organism

The western harvester ant, *P. occidentalis*, is one of the most common ant species in the arid lands of western North America. Studies or anecdotal evidence on this and closely related species elsewhere have reported that colonies apparently have long lifespans (15–30 years: Porter and Jorgenson 1988; Keeler 1982, 1993). Colonies are monogynous and may reach a maximum of about 10,000 workers with the possibility of producing 1000 reproductives in one annual reproductive bout (in the closely related *P. owhyeei,* Lavigne 1969). In our population, the median yearly reproductive output is 180 reproductives with a maximum of over 1300 (authors, unpublished work).

Like other species of *Pogonomyrmex* (Hölldobler 1976; Davidson 1982), this is a swarm mating species (Mull and Crist 1993; Wiernasz et al. 1995). An annual mating flight usually occurs on the afternoon of the first sunny day following a sufficient rain (> 5 mm) in mid-July or later. If a small amount of rainfall (e.g., 1 mm) occurs after a long period without rain a partial reproductive flight may be observed, followed by the remainder of winged reproductives (alates) flying with the next substantial rainfall. Mating swarms (leks) occur at the tops of hills; the number and spacing of leks depends on the topography of the area. Nests are begun only by single queens; nest initiation by groups of foundresses or by nest fission is unknown in this species.

Inbreeding simulations

In this section we used simulations to derive the confidence limits for the measurement of inbreeding as a function of sample size. For social insects, the sample size that is relevant for measuring the inbreeding coefficient (the correlation between alleles at a locus) is a function of the number of independent mating units, or colonies, that are sampled. To measure inbreeding the most efficient sampling strategy would be to take a single worker from the largest possible number of colonies. Since data are often collected for other reasons, such as to examine patterns of relatedness, typically a number of individuals are collected from each colony. Because the total sampling effort is usually constrained, there will be a tradeoff in any study between obtaining data that are useful for measuring inbreeding and data that are useful for other purposes. We used two sets of simulations to examine the ability to detect inbreeding in social insects as a function of the number of colonies and the number of workers within a colony which are sampled.

Our approach was to define a life history for a haplodiploid organism that included a varying frequency of sib-mating and determine the sample size required to detect inbreeding. In species that are not self fertilizing, sib-mating (along with parent-offspring mating) represents the most extreme form of inbreeding. In the simulations described below, we used a population of 1000 colonies of a social haplodiploid. The queen mated a single time, and produced all of the reproductive offspring of the colony. Many species of social insects are known to mate multiply (Cole 1983; Page 1986). In such situations, where the queen may both sibmate and outcross, inbred workers within the colony may be less common than expected if they are less fit (because of genetic load, Werren 1993). Our choice of a singly mated queen is thus conservative, maximizing the probability of detecting inbreeding if it is present. In each simulation the allele frequency was initially set to 0.5 for each of two alleles. The ability to detect inbreeding should be maximized when the expected heterozygosity is the greatest (when initial allele frequencies are equal). We are, therefore, estimating

the maximum power to detect inbreeding. The next generation of female reproductives (the new queens) mated either with a sibling (a fraction D of them) or mated randomly ($1-D$) within the population. Our goal was to relate the amount of mating among close relatives that will generate a given inbreeding coefficient, f. After the population reached equilibrium (which happens very rapidly, see Crow and Kimura 1970), the inbreeding coefficient was calculated using a single individual from 20, 50 and 100 colonies chosen randomly from the population and from all 1000 colonies. This procedure was repeated for 1000 iterates for each level of sib-mating. For each sample size, we tested proportions of sib-mating of 0, 0.2, 0.4, 0.6 and 0.8. The inbreeding coefficient, f, including the correction for sample size, was calculated according to the methods given by Weir and Cockerham (1984).

A second set of simulations addressed the distribution of sampling effort that is required to measure inbreeding. We assumed that the sampling effort was constant at 1000 individuals; if more colonies were sampled, then fewer individuals per colony can be genotyped. We asked whether additional individuals per colony versus additional colonies increased the power to detect a given amount of inbreeding. Our simulations used either 1000, 500, 200, 100, 50 or 20 colonies, and employed the randomization procedure of Ross (1993). Because workers within a colony are genetically correlated, it is only appropriate to use a single worker from each colony to determine the observed and expected genotype distributions of the population. As an example, suppose we sampled 20 workers from each of 50 colonies. We performed 50 randomization samples, selecting a single worker (out of 20) from each of the 50 colonies in each randomized sample. For each random sample we calculated the allele frequencies and recorded the observed genotype distributions. The mean allele frequencies for the randomized samples were used to calculate the expected genotype distribution. For each of the 50 sample genotype distributions we calculated an inbreeding coefficient and also calculated the mean of these 50 inbreeding estimates. The procedure is repeated 1000 times for each of the sampling regimes, for the levels of brother-sister mating described above. We calculated the standard deviation of the inbreeding coefficients calculated from the 1000 replicates and the probability of detecting a true level of inbreeding.

Empirical studies

Our study population currently consists of over 1700 colonies of *P. occidentalis* that have been permanently marked with numbered aluminum tags and mapped (Wiernasz and Cole 1995). At the time data were collected for this study (1992), the population consisted of 1000 marked colonies, including first-year nests. The population is located on Bureau of Land Management land approximately 15 km northwest of Fruita (Mesa Co.), Colorado (39° 16′ N, 108° 45′ W) at 1470 m elevation. The habitat is predominantly arid shrubland (primarily *Atriplex corrugata*, *A. confertifolia*, and *Sarcobatus vermiculatus*, Chenopodiaceae) on adobe soil. The site is topographically complex, consisting of six central sets of hills that run roughly north-south. One deep wash, one broad wash (an old road bed) and several shallow washes dissect the site.

The position of washes and hills potentially divides the site into a set of six mating sites (lek domains); the nests within each domain are closer to one hilltop than they are to any other hilltop. Approximately 120 nests in the northeastern portion of the site and some 85 nests which are equally distant to two hills could not be unambiguously assigned to a lek domain. The hilltops were those which had heights of greater than about 6 m; we have not observed leks on hilltops lower than 6 m. A lek is not produced at each site in each year or mating flight. However, leks have been observed at the hilltops which define each of the regions in at least one mating flight during the past two years. The number of nests which were assayed in each of the lek domains is: LD1, 128; LD2, 180; LD3, 66; LD4, 134; LD5, 130; LD6, 141 (Fig. 1). These lek domains were used to divide the site into regions for analysis of F-statistics.

Two workers were collected from each colony, placed in a 1.5-ml microcentrifuge tube and stored at 5°C. We collected only workers that were emerging directly from the nest entrance to ensure proper colony identification. In addition, we collected ten workers from each of 20 colonies in order to screen for electrophoretic variation. Within 1–2 days of collection, ants were shipped to the University of Houston by overnight air freight and stored at –80°C until electrophoresis. Ants were prepared for electrophoresis by removing the gaster and homogenizing the head and thorax in 80 µl of grinding buffer (Selander et al. 1971). We initially screened for variation at twenty enzyme loci using cellulose acetate electrophoresis (Hebert and Beaton 1989; Richardson et al. 1986). We found two highly polymorphic loci, phosphoglucose isomerase (*Pgi*, 2 alleles) and amylase (*Amy*, 5 alleles) and one locus, phosphoglucose mutase (*Pgm*), with two rare alleles (frequency < 0.01). Two polymorphic esterase loci could not be resolved consistently enough to be used in the study. We successfully genotyped a single worker from each of 966 colonies for amylase and from 961 colonies for phosphoglucose isomerase.

We calculated F-statistics separately for each locus using the method of Weir and Cockerham (1984). We calculated the inbreeding coefficient separately for the population as a whole, including nests not assignable to lek domains, and for the site divided into lek domains. We estimated the confidence limits on the F-statistics by jackknifing (Weir and Cockerham, 1984) across the separate lek domains.

Results

Sample size and the measurement of inbreeding

The probability of detecting a given level of sib-mating as a function of the number of colonies sampled is summarized in Table 1. The expected values are from theoretical expectations (Li 1976; Suzuki and Iwasa 1980) and $f = D/(4-3D)$, where D is the fraction of the population that is sib-mating. The confidence intervals are estimated from the distribution of simulated values. We show the estimated upper and lower critical values for a one-tailed test of differences from random mating. We give the one-tailed critical values since one is typically interested in the one-tailed hypothesis that inbreeding is occurring.

In order for an inbreeding coefficient to be significantly larger than that expected for random mating, the coefficient must be greater than the upper confidence limit for random mating. For example, with a sample size of 1000 the inbreeding coefficient must be larger than about 0.06 to be significantly different from zero. This corresponds to the inbreeding coefficient expected from about 20% sib-mating. If the sample size is only twenty colonies, the inbreeding coefficient must be greater than approximately 0.38, which is expected from about 70% sib-mating, in order to be significantly different from random mating.

Using the results of these simulations, it is possible to estimate the effect of sample size on the power to measure inbreeding as a function of the fraction of sib-mating (Fig. 2). The null hypothesis being tested is the usual one of random mating. Typically one would like a minimum power of 0.7–0.8 (the dotted lines included for reference in Fig. 2 and 3). For a sample size of 1000, one has the power to detect a significant difference between random mating and about 35% sib-mating approxi-

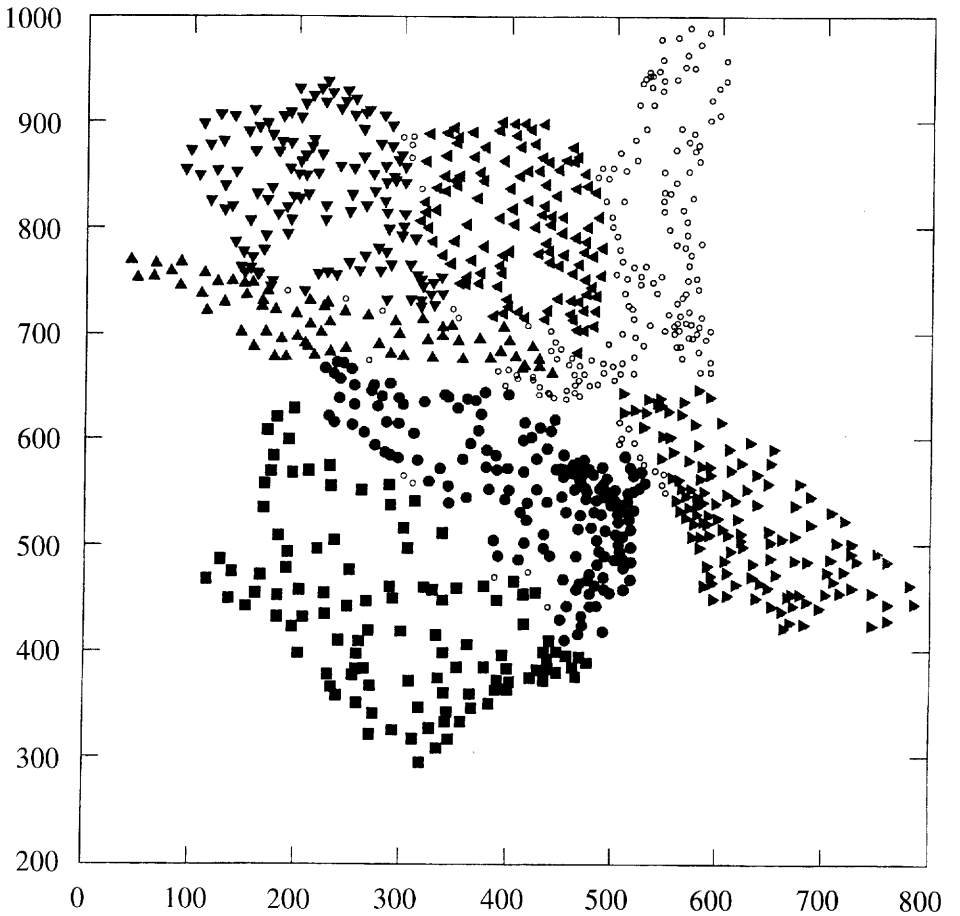

Fig. 1 The study site showing the distribution of colonies used in calculation of the *F*-statistics. The scale is in meters; north is *up*. Each colony is given a *symbol* indicating membership in a particular putative lek domain. *Squares* are in LD 1, *filled circles* are in LD 2, *upward pointing triangles* in LD 3, *downward pointing triangles* in LD 4, *left-pointing triangles* in LD 5, *right-pointing triangles* in LD 6. Colonies which could not be unambiguously assigned to a putative lek domain, but which were used in calculating the overall inbreeding coefficient, are shown as *hollow circles*. This map shows the data for the *Amylase* locus; the colonies for which we have PGI data are virtually identical

mately 70–80% of the time. For samples of 100 colonies, one only has the power to detect about 55–60% sib-mating 70–80% of the time. With a sample of only 20 colonies, one can only detect the difference between pure random mating and pure sib-mating; finer distinctions are not possible. For most social insect species, however, it will be difficult to obtain sample sizes of 1000 colonies or larger. This places inbreeding levels of less than 35% sib-mating beyond our ability to detect them. For samples on the order of 100 colonies, the minimum level of inbreeding we can reliably detect is about 60% sib-mating. These statements only apply to results obtained from a single locus. It seems reasonable to suppose that multiple loci or highly polymorphic loci, such as some microsatellites, would give greater information and allow finer distinctions.

The availability of additional workers from colonies did increase the statistical power of smaller numbers of colonies to detect inbreeding. In Fig. 3 we show the relation between the power to detect a given amount of inbreeding and the number of colonies sampled for a constant sampling effort of 1000 workers. For 20% sib-mating, ($f = 0.06$), none of the samples has acceptable statistical power. For 40% sib-mating ($f = 0.14$), sam-

Table 1 The confidence limits of the inbreeding coefficient as a function of the level of sib-mating

Amount of sib-mating	Expected f	Estimated lower and upper 95% critical values for one-tailed tests for given sample size			
		1000	100	50	20
0	0.0	−0.060, 0.057	−0.142, 0.179	−0.261, 0.231	−0.447, 0.384
0.2	0.059	0.006, 0.112	−0.121, 0.224	−0.189, 0.285	−0.310, 0.421
0.4	0.143	0.091, 0.194	−0.030, 0.302	−0.092, 0.368	−0.226, 0.518
0.5	0.200	0.148, 0.258	0.020, 0.363	−0.073, 0.437	−0.188, 0.578
0.6	0.273	0.226, 0.322	0.110, 0.430	0.023, 0.488	−0.100, 0.616
0.7	0.368	0.318, 0.415	0.204, 0.508	0.130, 0.567	0.016, 0.712
0.8	0.500	0.452, 0.545	0.354, 0.656	0.281, 0.705	0.146, 0.801

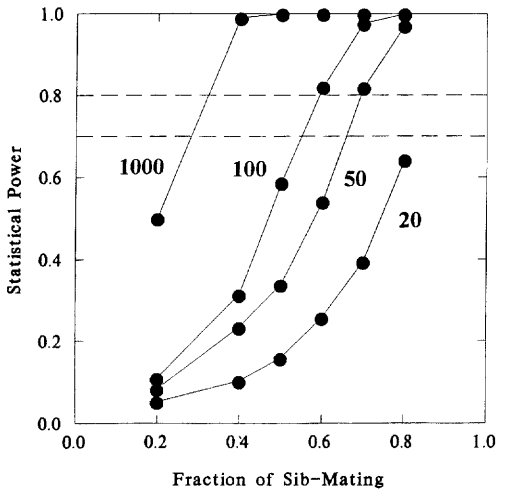

Fig. 2 The relation between statistical power and the fraction of sib-mating for various sample sizes. The *dotted lines* at 0.8 and 0.7 indicate acceptable power levels. Note that sample sizes of fewer than 100 colonies do not provide adequate power to detect inbreeding equivalent to less than 50% sib-mating, while even samples of 1000 colonies do not ensure detection of less than about 30% sib-mating

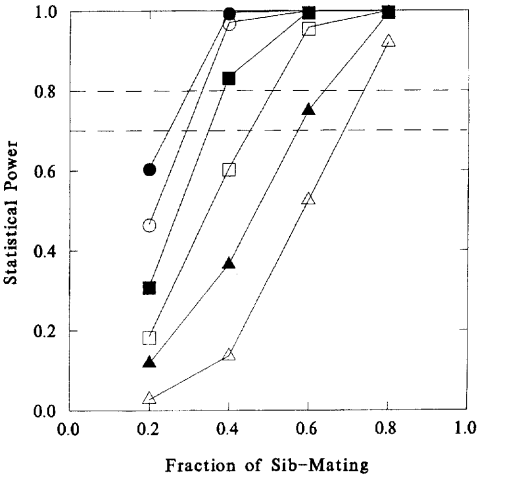

Fig. 3 The statistical power of a constant effort sampling regime. Collection of additional workers per colony allows greater power to detect inbreeding over collecting a single worker per colony, however, the increase in power is less than can be obtained from adding additional colonies. *Lines* show samples of 1000 colonies (1 worker per colony, *solid circles*), 500 colonies (2 workers, *hollow circles*), 200 colonies (5 workers, *solid squares*), 100 colonies (10 workers, *hollow squares*), 50 colonies (20 workers, *solid triangles*) and 20 colonies (50 workers, *hollow triangles*)

ples of 200 colonies or more are required in order to have a 70–80% chance of detecting an inbreeding coefficient that is significantly different from zero. A sample of 50 colonies can be used to detect inbreeding levels of more than about 60% sib-mating ($f = 0.27$). Finally, even a sample of 20 colonies is sufficient to detect 80% sibmating ($f = 0.5$) about 90% of the time.

Inbreeding in *P. occidentalis*

For each locus, the global and the local frequencies for each lek domain are shown in Table 2. Allele frequencies did not differ significantly between regions for either locus. The overall inbreeding coefficients are 0.024 ($P > 0.5$) for *Pgi* and 0.088 ($0.01 < P < 0.025$) for Amylase. The significance of these coefficients is tested by looking at the deviations from Hardy-Weinberg equilibrium with a chi-square. An inbreeding coefficient this large requires approximately 28% full-sib mating (by the relation $f = D/(4-3D)$; Li 1976; Suzuki and Iwasa 1980). The *F*-statistics across the lek domains are given in Table 3. Only the F_{IS} for Amylase is significantly different from zero, but it indicates substantial inbreeding. It would be useful to assign confidence limits for the effective amount of sib-mating in *P. occidentalis*. The simulations suggest two approaches. By interpolation from Table 1, the one-tailed confidence intervals for a population with an inbreeding coefficient of 0.088 and a sample size of 966 would be 0.058–0.162, which indicates effective sib-mating levels of 20–44%. Alternatively, from Table 1, the sibmating rate is significantly greater than 0%, significantly less than 40%, and not significantly different from 20%.

Discussion

Statements about the occurrence of inbreeding are statistical ones which depend on the adequacy of the data. If we have sampled 1000 colonies and find that the inbreeding coefficient is not significantly different from

Table 2 Allele frequencies for the entire population and for each of the putative lek domains which are illustrated in Fig. 1

	PGI^f	Amy^1	Amy^{1A}	Amy^2	Amy^3	Amy^4
Entire Population	0.272	0.062	0.025	0.731	0.047	0.136
Domain 1	0.260	0.064	0.012	0.720	0.052	0.152
Domain 2	0.264	0.048	0.030	0.707	0.072	0.144
Domain 3	0.235	0.068	0.045	0.712	0.038	0.136
Domain 4	0.314	0.065	0.008	0.781	0.023	0.123
Domain 5	0.234	0.065	0.027	0.715	0.062	0.131
Domain 6	0.314	0.072	0.025	0.730	0.028	0.144

Table 3 *F*-statistics for *Pogonomyrmex occidentalis*. F_{rr} is the reduction in heterozygosity across lek domains; F_{ST} is the reduction in heterozygosity due to subdivision; and F_{IS} is the reduction in heterozygosity within lek domains due to inbreeding. The *F*-statistics for the population divided into putative lek domains is presented as the jackknifed estimate of the mean and jackknifed estimate of the standard error across the six lek domains. The significance levels are all given from two-tailed tests. The significance of the *f* for the entire population is based on the deviation from Hardy-Weinberg equilibrium tested with a chi-square

	Amylase	PGI
f – Entire population	0.088**	0.024
F_{IT} – Lek domains	0.088 (0.027)*	0.030 (0.036)
F_{ST}	0.004 (0.002)	0.006 (0.003)
F_{IS}	0.085 (0.027)*	0.024 (0.036)

*$P < 0.05$;** $P < 0.02$

zero, we must keep in mind that with such a sample we have a 98% chance of ruling out that the population is experiencing 40% or higher sib-mating, but only a 60% chance of ruling out the possibility of 20% sib-mating. The value of making a statement about the absence of inbreeding depends on the amount of inbreeding which may influence our system. If we have a sample of ten workers from each of 100 colonies, then we have a high probability of ruling out 50% or more sib-mating. With samples of fewer than 100 colonies we expect to detect only very substantial levels of sib-mating. When interpreting the evidence for random mating based on the lack of significant inbreeding coefficients, it is important to consider the sample sizes that lead to such a conclusion. In many cases the samples from which inbreeding coefficients have been calculated are not large enough to justify any statements about the level of inbreeding in the population. As Michod (1980) demonstrates, as little as 20% sib-mating can markedly influence the rate of spread of altruistic traits.

Estimated inbreeding coefficients need not be concordant within a population (across loci) undergoing inbreeding. The distribution of inbreeding coefficients that is expected under any given level of sib-mating and a given sample size may vary greatly among loci. If two loci are unlinked, then the two estimates of the inbreeding coefficient are equivalent to two random samples from this distribution. Under a given, moderate level of sib-mating, the inbreeding coefficients may even have different signs. For example, in our sample of about 960 colonies we detect significant inbreeding in only one of two loci. If the true inbreeding coefficient is 0.088, equivalent to 28% sib-mating, then with a sample of about 960 colonies we have about a 75% chance of detecting significant inbreeding at any locus.

Two previous studies of population genetic structure in *Pogonomyrmex* provide data to estimate inbreeding. Johnson et al. (1969) presented data from a large number of populations of *P. barbatus* in Texas. They genotyped four workers at one esterase locus from about 25 colonies per population. There are several problems with these data. Since the allele frequencies were calculated using several individuals per colony, they did not reflect the frequencies in the breeding population. Inbreeding coefficients were calculated using all sampled individuals to determine the departures from Hardy-Weinberg equilibrium. This introduces a bias, because the genotypes of workers from the same colony are correlated. Finally, in calculating the inbreeding coefficients, they did not correct for sample size, which biased their estimates of inbreeding downward (Levene 1949).

We simulated their sampling scheme (four workers per colony; 25 colonies) to generate the expected distribution of inbreeding coefficients that should result if queens mate once and randomly. We did not correct these simulation estimates for the deficiency in heterozygotes due to small sample sizes. Because we could not determine the population level allele frequencies, we could not simulate the expected distribution of inbreeding coefficients precisely, but instead consider two sets of frequencies that span the range of likely outcomes. With allele frequencies of 0.5, the inbreeding coefficient based on the worker sample is slightly greater ($f = -0.015$, 13000 replicates) than the mean of the inbreeding coefficient based on the queens ($f = -0.024$, 13000 replicates). When the allele frequencies are 0.8 and 0.2, the worker-based and queen-based inbreeding calculations give essentially identical results (for workers $f = -0.018$, and for queens $f = -0.014$, each 11,000 replicates). Using the worker allele frequencies tends to overestimate *f* by a very small amount (possibly as much as 0.009), so we ignored this effect. The measured inbreeding coefficient is still expected to be negative and must be adjusted upwards (by about 0.02–0.03) to correct for sample size effects. When we correct for sample size (Levene 1949), the data for *P. barbatus* indicate an inbreeding coefficient of about 0.09 (± 0.02 SE) across 31 replicate populations, a value that corresponds to 29% sib-mating. These results suggest that a significant amount of inbreeding is occurring in this species.

We performed similar analyses on a similar data set for *P. badius* (Tomaszewski et al. 1973). They used an average of 'about five workers' per colony, and collected data from fewer colonies (about 15) per site. The extent of the sample size correction necessary (the estimated inbreeding coefficients must be increased by about 0.04) and the uncertainty about the numbers of individuals per colony means that these data give rather poor estimates of the inbreeding coefficient. Nevertheless, the distribution of the inbreeding coefficients estimated from these data suggests that there is significant inbreeding ($f = 0.08 \pm 0.03$ SE), equivalent to 25% sib-mating).

The data for *P. occidentalis*, together with the reanalysis of previous studies of *Pogonomyrmex*, suggest that there may be substantial inbreeding in this genus even though the reproductive system can be characterized as synchronous swarm mating. Perhaps the genus *Pogonomyrmex* is unusual in some fashion that we do not understand. If this apparent inbreeding is real, we expect the production of diploid males to be associated

with early colony death, but this remains to be tested. There are no premating isolating mechanisms that prevent sibmating in *P. occidentalis*; sib-mating can occur in reproductive traps. We have no evidence bearing on the occurrence of mating within the nest, which is known to occur in other species (e.g., Fortelius et al. 1987). It is conceivable that mating occurs within the nest, but that mating at the leks is random.

Two possible factors may account for the high levels of inbreeding in *P. occidentalis*: reduction in effective population size and nonrandom mating. Although our population consists of approximately 1300 colonies, the number of colonies that actually reproduces is considerably smaller. Alate production in colonies is strongly dependent on colony size (B.J. Cole and D.C. Wiernasz, unpublished work). Reproduction is not a simple size threshold, rather the probability of reproducing increases with colony size. Given the size distribution of colonies at the site, we estimate that only about 215 colonies actually reproduced in 1994. Although this is a fairly large number, the local effective size may be considerably smaller if each colony's reproductives attend a single lek. The lek domains potentially fragment the population into a series of small demes. Based on the size-reproduction relationship, the number of reproducing colonies in each domain can be estimated as: LD1, 22 colonies; LD2, 39 colonies; LD3, 13 colonies; LD4, 24 colonies; LD5, 19 colonies; LD6, 42 colonies. While mating swarms do not occur in each lek domain every year, and they may not attract reproductives exclusively from colonies within a lek domain, the possibilities for restricted effective population sizes are clear. The rate of loss of heterozygosity in the site as a whole is about 6 times as fast with a population of 215 colonies rather than 1300 colonies (Crow and Kimura 1970) and the rate of loss of heterozygosity from lek domain 3 (with only 13 colonies of reproductive size) is 100 times faster than from a population of 1300 colonies. If leks only form at given sites in certain years causing the subpopulations to amalgamate in various ways in various years the effective population size is the harmonic mean of the population sizes across lek domains and years (Crow and Kimura 1970). We also know that the colonies which do reproduce vary considerably in the number of alates produced (B.J. Cole and D.C. Wiernasz, unpublished work); this variation will reduce the effective population size still further.

The females of *P. occidentalis* mate nonrandomly (Wiernasz et al. 1995). Males collected *in copula* are significantly larger than males that are not mating (strength of selection = 0.25). This characteristic of *P. occidentalis* is also characteristic of other species of *Pogonomyrmex* (Davidson 1982). This significant sexual selection for increased male body size will affect the genetic structure of the population because it effectively reduces the number of colonies which contribute equally to the pool of reproductives. This will further reduce the effective population size if colonies differ in the average size of the males that they produce.

A possibility that must be considered is that the deviations of the allele frequencies from equilibrium may be due to selection rather than to inbreeding. This is true especially in light of the studies of Ross (1992) and Pamilo (1992) showing that workers and queens can differ substantially in allele frequency. Random mating and selection against heterozygotes at the amylase locus would produce apparent significant inbreeding, as well as the lack of concordance between the amylase and phosphoglucos isomerase loci. Since the selection would have to be on heterozygous individuals rather than against particular alleles, this mechanism seems rather implausible. It is more satisfying to have a general explanation that accounts for deviations from equilibrium in all three species of *Pogonomyrmex*. A selective argument in the other species would require similar sorts of selection at other loci (esterase in *P. barbatus* and amylase in *P. badius*).

The observation of inbreeding in *P. occidentalis* raises several general points about the occurrence of inbreeding in social insects. Paradoxically, an abundant, swarm-mating species, with copious, synchronous reproduction has considerable inbreeding. Furthermore, we find evidence of significant inbreeding in two other species of *Pogonomyrmex* upon reanalysis. Perhaps the lack of widespread evidence for the occurrence of inbreeding in social insects generally is due to the lack of power of statistical tests to detect it. Moderate levels of inbreeding may frequently occur in ants. In light of the evidence presented in this study, one must be cautious about concluding that synchronous swarm-mating species automatically avoid inbreeding

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