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Research article

Kin recognition and the paradoxical patterns of aggression between colonies of a Mojave desert *Pheidole* ant

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Abstract. Populations of the desert seed-harvesting ant *Pheidole xerophylla* are often characterized by high nest density leading to competitive interactions between foragers from different nests. We investigated the inter-nest aggression, spatial distribution and genetic structure of a *P. xerophylla* population of the Mojave Desert in Southern California. Inter-nest aggression was quantified by standardized staged encounters in a neutral arena. Genetic relatedness within nests and relatedness between nests were calculated using allelic frequencies at four microsatellite-DNA loci. We found a bimodal distribution of inter-colony aggression levels with a first mode at low aggression levels and another mode at much higher aggression levels. Inter-colony aggression levels were largely non-transitive. No effect of geographical distance on inter-nest aggression levels was detected. Despite high amounts of variation in inter-colony relatedness (–0.24 to 0.37) this variable did not correlate with the level of aggression between nests. Intra-nest relatedness ranged from 0.40 to 0.75 and close inspection of worker genotypes within colonies revealed a high proportion of polygynous colonies or a mixture of polygyny and polyandry. Aggression levels among nests was found to decrease with increasing intra-nest relatedness. These results do not support the idea that aggression is modulated by a nestmate recognition mechanism based on overall genetic similarity. Instead, the absence of transitivity found in inter-colony aggression and bimodal distribution of aggression levels are compatible with a common label acceptance model of nestmate recognition and suggest that label diversity may be encoded by a limited number of loci.

Keywords: Nestmate recognition, intraspecific competition, endogenous cues, exogenous cues, common label acceptance model.

Introduction

Kin recognition, the capacity to discriminate related individuals from unrelated ones, is the prerequisite for the evolution of complex social interactions between organisms and as such has received considerable attention (Fletcher and Michener, 1987; Hepper, 1991). From a functional point of view, kin recognition can simply be understood as a mechanism allowing animals to maximize their lifetime reproductive success. This can be achieved by ensuring that their reproductive effort is directed towards their own offspring or through actions that promote the reproduction of related individuals (Hamilton, 1964a, b; Fletcher, 1987). Not surprisingly a large proportion of studies of kin recognition have focused on organisms that exhibit elaborate social systems and among those many have investigated kin recognition in social insects (Michener and Smith, 1987; Breed and Bennett, 1987; Jaisson, 1991). Many insect societies and ant societies provide an obvious context for the evolution of kin recognition because they feature non-reproducing workers that pass on their genes through relatives, such as their reproducing sisters and brothers of the reproductive cast. Kin recognition mechanisms are necessary for workers to recognize nestmates from non-nestmates thus enabling them to combine their efforts towards colony growth and reproduction (Breed and Bennett, 1987; Jaisson, 1991).

Important efforts have been made in order to understand the perceptual mechanisms involved in kin-recognition among colony members of ant societies. It is evident from multiple examples of social parasitism that many ants are not innately capable of recognizing kin and some learning process occurs. Slave making ants steal the worker brood of other ants so that once hatched their imagos help them maintaining their colony (Breed and Bennett, 1987; Jaisson, 1991). This phenomenon has been reproduced in numerous experiments and across many species thereby indicating that newly hatched workers associate their nestmates with kin. Once primed, or familiarized, those workers will subsequently discriminate non-nestmates even if they happen to be related to them (Breed and Bennett, 1987; Jaisson, 1991). Familiarization in ants involves odors and it has been shown that the queen plays an important role in producing colony odor with workers playing a lesser role in most species (Breed and Bennett, 1987). Colony odor may have an endogenous component that is dependent on the queen and worker's genotypes (e.g. Stuart, 1988; Crosland, 1990; Stuart and Herbers, 2000). Colony odors may also be influenced by the worker's diet or other exogenous odor components determined by characteristics of the habitat that may contribute to its distinct familiar signature (Breed and Bennett, 1987; Chen and Nonacs, 2000; Silverman and Liang, 2001). Evidence from a few species suggest that individuals would not only use familiarization and habituation to distinguish colony odor but could also discriminate kin from non-kin by comparing their own phenotype or that of related individuals with that of other individuals, a process referred to as 'phenotype matching' (Lucy and Sherman, 1983; Wilson, 1987; Fletcher, 1987; Stuart, 1987; Provost 1991). In such instances, newly hatched ants would prime themselves using their own characteristic odor or that of related individuals and use this as a referential to differentiate individuals that resemble them and those that do not (Crozier, 1987; Fletcher, 1987). In this case again, local environmental conditions could be of importance because of their effect on phenotypic characteristics such as the cuticular hydrocarbons involved in odor cues (Vander Meer and Morel, 1998).

One of the main problems faced by experimenters when attempting to reveal perceptual mechanisms of kin recognition and differentiate between recognition mechanisms is to identify appropriate situations where the organism under study will be likely to use such perceptive mechanisms (Waldman et al., 1988). This requires a good understanding of the species life histories and behavior so that an adequate 'functional context' is identified before testing for the occurrence of kin recognition or for one or the other kin recognition perceptual mechanisms. We studied the patterns of aggression between *Pheidole xerophylla* colonies in relation to their spatial distribution and genetic structure in the Mojave Desert in Southern California. This seed-harvesting ant defends foraging areas and their nests are often over-dispersed suggesting that colonies may compete for resources (Bernstein, 1979; Bernstein and Gobbel, 1979). In a previous study (Langen et al., 2000) of the same *P. xerophylla* population we found remarkable variation in aggression levels between colonies. We hypothesized that aggression patterns between *P. xerophylla* colonies might be modulated by kin recognition mechanisms. If this species exhibits limited post-nuptial dispersal, foragers from different colonies may be related and could gain substantial inclusive fitness gains by being less aggressive to nearby related colonies than to unrelated ones. The recognition systems of *Pheidole* have not been extensively investigated but it is known that ants from that genus can differentiate other ant species and change their defensive behavior accordingly (Carlin and Johnston, 1984; Feener, 1986, 1987). In one species, they were also found to adjust the production of soldiers in response to intraspecific competition (Passera et al., 1996). *P. xerophylla* exhibits considerable variation in aggression levels between colonies and our previous study showed that part of the variation in aggression level was explained by workers from neighboring colonies being less aggressive towards each other than workers from colonies located farther away (Langen et al., 2000). This 'dear enemy phenomenon' was only detectable at short distances (<3m) and probably occurred as a result of habituation from repeated encounters with foragers from neighbor colonies (Langen et al., 2000). An alternative explanation for the observed aggression patterns at short distances would be that *P. xerophylla* only tolerates related colonies in the vicinity of their own nests. The latter would again argue for the presence of a kin-recognition mechanism in *P. xerophylla* that would allow ants to reliably assess the relatedness of other colonies and subsequently affect their behavior towards them. The existence of such system could explain the high variance in aggression levels that were observed across all distances in our previous study (Langen et al., 2000).

In order to determine whether patterns of aggression and relatedness in *P. xerophylla* support a phenotype-matching model of kin-recognition, we studied 15 *P. xerophylla* colonies from a single site in the Mojave Desert. We combined data of aggression levels between colonies with estimates of relatedness within colonies and relatedness between colonies calculated from allelic frequencies at polymorphic microsatellite DNA loci. Comparisons were not biased towards the short distances at which the dear-enemy phenomenon was previously detected, but rather we focused on a broad range of distances within the study site. These data allowed us to investigate the relationships between aggression between colonies, their spatial distribution and their genetic structure. The results are discussed in the light of our current understanding of the evolution of kin-recognition mechanisms and their importance for shaping the behavior and population structure of ants.

Methods

Study site and species

The study was conducted in April 1999 at the Sweeney Granite Mountains Desert Research Reserve in the Eastern Mojave Desert, San Bernardino County, California (34° 48´ N, 115° 39´ W). The population of *P.*

xerophylla under study is located next to the Yucca Bajada Campground (elevation = 1,200 m), an area of typical Mojave Bajada habitat dominated by *Hymenoclea salsola*, *Salazaria mexicana*, *Yucca shidigera* and *Larrea tridentata*. For further information on the Granite Mountains Reserve, see Luke and André (1999).

P. xerophylla was referred to as *P. tucsonica* in our previous work (Langen et al., 2000) and other studies but has recently been synonymized under *P. xerophylla* (Wilson, 2003). *P. xerophylla* is a small (minors 3–4 mm) blackish seed-harvesting ant that forages along trunk trails and nests in the soil (Wheeler and Wheeler, 1973, 1986). They have dimorphic castes (major and minors) but more than 95% of the workers on the soil surface were minors during our study. In our study area *P. xerophylla* exhibits a colony density of 3 ± 0.0 per 100 m^2 and shares the habitat with *P. gilvescens*, a species that has a similar ecology (Langen et al., 2000). 15 colonies were marked with small flags and inter-nest distances were measured with a measuring tape (Fig. 1).

Measure of aggression

Stage encounters were conducted as described in Langen et al. (2000) but new data was collected for the study described here. We collected workers by aspiration from the soil surface near nests. Multiple openings of the same species less than 20.0 cm apart were presumed to be the same colony. Ants were held in 25-ml collection vials until an encounter was staged. This method has proved to yield consistent fight-scores between observers and to be highly repeatable (r = 0.77, one-tailed p = 0.0001, n = 16) (Langen et al., 2000). Briefly, ten minors of each of the two test colonies were placed in a neutral arena for 3 min thereafter we estimated every 30 sec and for 4 min the number of dyadic fights between ants in an instantaneous visual scan. This method yields estimates of aggression that range from 0 to 10 for each scan (8 scans per fights) (see Langen et al., 2000 for details). These estimates were averaged to yield a mean aggression level per fight. Despite the non-independence of the 8 estimates, for the sake of describing the variation in aggression levels within encounter, we present confidence intervals around the mean aggression levels for the 60 pair-wise comparisons that could be made. These were the comparisons involving colonies 1 to 5 versus all other colonies (1 to 15). In addition to staging between-colony encounters, 5 within-colony fights were staged as control using 20 workers from colony 1 to 5. In all cases the estimated aggregation levels were null.

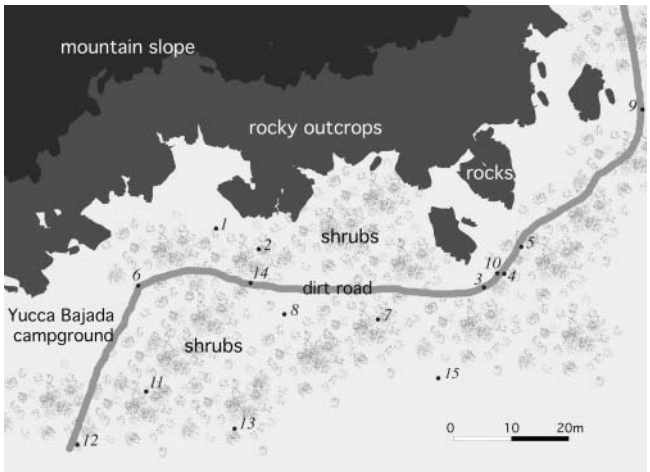

Fig. 1. Schematic representation of the field site at Yucca Bachada or Yucca 'flats' in the Sweeney Mountain reserve. The 15 *P. xerophylla* colonies involved in the study are numbered from 1 to 15.

Genetic analyses

Ant DNA for PCR reactions was extracted using a phenol-chloroform extraction protocol and purified with ethanol precipitation protocol. Individual ants were ground in digestion solution (100 mM NaCl, 50 mM Tris, 1 mM EDTA, 0.5% SDS, and 200 µg/ml proteinase K) and incubated two hours at 55°C. Microsatellites were analyzed using primers and methods developed for the ant *Pheidole pallidula* (Fournier et al., 2002). Amplification reactions were carried out in a 10 µl volume containing 10 mM Tris-HCl, 50 mM KCl, 2.5 mM $MgCl_2$, 0.25 mM of each dNTP, 600 µM of each primer, 1.5 U Taq polymerase and 1 µl of genomic DNA (approximately 10 ng ant DNA). Amplified fragments were visualized on 5% polyacrylamide/6 M urea sequencing gels using an automated 377 ABI sequencer.

Calculation of relatedness

Workers' genotypes were determined at 4 microsatellite loci: *Ppal-03*, *Ppal-12*, *Ppal-33* and *Ppal-77* (Fournier et al., 2002; Fournier, 2004). Allelic frequencies were estimated from workers genotype frequency using the program Genepop 3.2a (Raymond and Rousset, 1995) (available at http://wbiomed.curtin.edu.au/genepop/). The number of alleles ranged from 9 to 14 and the expected heterozygosity ranged from 0.80 to 0.91, under the assumption of Hardy-Weinberg equilibrium (Fournier, 2004). Inbreeding and the average within-colony genetic relatedness among nestmate workers were assessed using relatedness 4.2c and 5.0 (Queller and Goodnight, 1989) (available at http://gsoft.smu.edu/GSoft.html). Colonies were weighted equally. Standard errors were obtained by jackknifing over colonies. Estimates of symmetrical relatedness between pairs of colonies were calculated using the same software. For those analyses individuals were weighted equally and standard errors were obtained by jackknifing over loci.

Test of association between aggression, relatedness and geographical distances

Partial correlations between the measures of aggression levels, relatedness, mean within-colony relatedness and geographical distances between ants colonies were performed using a Mantel test based on a permutation procedure (Smouse et al., 1986) and written by P. Nonacs (available upon request). Linear relationships presented in the result section are based on linear regression procedure. 60 pair-wise estimates of level of aggressions could be made and those were correlated with the corresponding 60 measures of relatedness, intra-nest relatedness and geographical distances.

Investigation of colony social structure

For each colony and each locus we examined the genotypes of 10 of their workers for evidence of polyandry or polygyny. Queens were assumed to contribute two alleles to the allelic pool and males, one allele. Because monandry is predominant in ants (Boomsma and Ratnieks, 1996; Strassmann, 2001) the data were first analyzed assuming polygyny as the most parsimonious explanation for the patterns of genotypes. In this case, each singly-mated queens present in the colony are expected to contribute 3 alleles to the colony and the minimum number of queens present can be simply inferred from the number of alleles detected (less than 4 alleles = 1 queen, 4–6 alleles = 2 queens, etc.). Next, and since we could not strictly rule out polyandry, we analyzed the data assuming polyandry as the most parsimonious scenario. As an example, when 3 alleles were observed the single male allele was expected to occur in all workers in combination with either of the two alleles from the queen. When this did not occur, the most parsimonious explanation was that the queen was mated multiply. We used this technique to record the minimum number of males required to explain the 10 worker genotypes. In some cases, the patterns of alleles still could only be explained if two

queens were hypothesized. In the simplest cases, the presence of homozygote workers allowed simple identification of 1 or 2 queen alleles but additional alleles were involved in the genotypes thereby revealing an additional queen. In more complex cases, and when enough alleles were present, polygyny could be inferred in the absence of homozygotes because the genotypic patterns could not be explained assuming only two maternal alleles.

In addition, we calculated the effective number of breeders, i.e. the number of breeders weighted by their relative contribution to the offspring. The effective mean number of reproductive queens N_e per colony was inferred from relatedness among workers:

$$N_e = \frac{4r_{fs} - r_q - 2r_m}{4r_w - r_q - 2r_m}$$

where r_{fs} is the average relatedness among workers offspring from the same queen (relatedness of full sisters $r_{fs} = 0.75$), r_q is the average relatedness among nestmate queens, r_m is the average relatedness between the males inseminating a same reproductive queen ($r_m = 0$ because we assumed large mating flights in *P. xerophylla*) and r_w is the average relatedness among worker nestmates in the study population (Ross, 1993). The lowest and highest values of N_e were estimated assuming that queens were unrelated ($r_q = 0$) or that the average relatedness among nestmates queens was equal to the mean worker relatedness ($r_q = r_w$).

The effective number of mates (M_e) of a single queen was estimated from the mean relatedness within female brood and is:

$$M_e = \frac{1}{2r_w - 0.5}$$ (Pamilo, 1993).

Results

Patterns of aggression between colonies

Staged encounters in a neutral arena between workers from different nests yielded levels of aggression ranging from 0 (no fights) to 10, the maximum level of aggression (all ants engaged in fights) (Fig. 2a, Table 1). The mean level of aggression was 3.54 ± 3.29 SD and the modal aggression value was equal to 0. Cluster analyses showed that the distribution could be interpreted as a significantly bimodal distribution with a first mean aggression level at 1.02 ± 1.25 SD and a second at 7.07 ± 1.41 SD ($F_{1,58} = 297.7$, $p < 0.0001$). Close examination of the aggression levels between nests revealed patterns of transitivity in some cases and no patterns in others. Consider colonies 2, 4 and 6, for example. Workers from colony 2 do not fight with those of colony 4 and similarly tolerate workers from colony 6 (Table 1). When those same workers from colony 4 face workers from colony 6, this results in 14 out of the 20 workers biting each other (Table 1).

Investigation of social structure

The four loci tested *Ppal-03*, *Ppal-12*, *Ppal-33* and *Ppal-77* exhibited between 8 to 14 alleles with allelic frequencies ranging from 0.01 to 0.19 (Table 2). Average within-nest relatedness per loci at the population level was 0.65 ± 0.06 for locus *Ppal-03*, 0.50 ± 0.05 for *Ppal-12*, 0.53 ± 0.05 for *Ppal-33* and 0.65 ± 0.05 for locus *Ppal-77*. The average relatedness for all loci among all colonies was equal to 0.57 ± 0.03. The population's inbreeding coefficient *Fis* for all loci was equal to 0.019 ± 0.016. Neither that value nor the individual *Fis* values per loci significantly differed from zero (All $p > 0.05$). Relatedness within colonies across all loci ranged from 0.42 to 0.75 (Table 3). Close examination of genotypes from the 10 analyzed workers per colony revealed more than 3 alleles in at least one locus in 14 out of 15 colonies (93.3 %) (Table 3). The maximum amount of alleles found in one colony was 7 for *Ppal-12* in colony 11. Considering that males and females contribute respectively one and two alleles to the patterns of genotypes of the 10 analyzed workers, we first examined the minimum number of females required to produce the observed patterns of the 10 worker genotypes assuming monandry. Under such assumptions, polygyny involving at least 2 females was found in all nests (structure A, Table 3). When polyandry was considered the most parsimonious explanations for the observed genotypes, it was found in all nests, involved 2 to 4 mating per queen, and was combined with polygyny in 5 of the 15 colonies (33.3 %) (structure B, Table 3). These 5 colonies exhibited significantly lower values (0.43 ± 0.03 SD) of relatedness

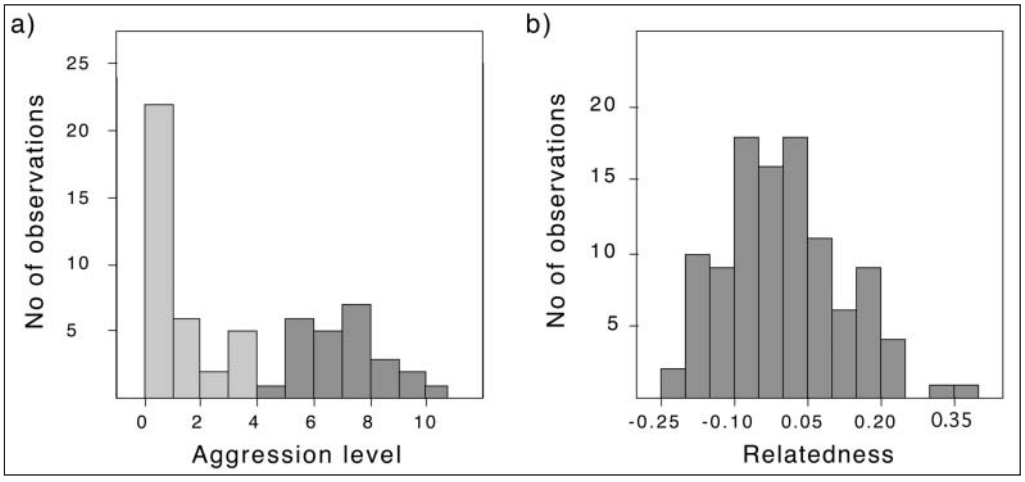

Fig. 2. (a) Bimodal distribution of aggression levels (n = 60) and (b) distribution of relatedness (n = 105) between *P. xerophylla* colonies.

Table 1. Matrices of pair-wise estimates of symmetrical relatedness (below diagonal) and aggression level (above diagonal) between pairs of *Pheidole* colonies. Aggression levels were measured for 60 pair-wise comparisons and relatedness for all 105 combinations between 15 nests. All estimates are presented ± 95 % C.I. An example of non-transitivity in aggression between the colonies 2, 4 and 6 is highlighted together with matching relatedness values among those colonies (see text for details).

	1	***2***	*3*	***4***	*5*	***6***	*7*	*8*	*9*	*10*	*11*	*12*	*13*	*14*	*15*
1		6.1 ± 1.4	0.0	7.6 ± 1.3	0.0	0.7 ± 0.6	7.4 ± 0.4	0.0	0.0	8.1 ± 1.5	2.4 ± 0.4	0.0	1.0 ± 0.4	5.1 ± 1.4	0.6 ± 0.9
2	0.23 ± 0.20		5.5 ± 1.0	0.0	5.1 ± 1.2	0.0	1.0 ± 0.4	0.0	0.6 ± 0.6	0.6 ± 0.4	1.5 ± 0.6	3.4 ± 1.3	3.9 ± 1.1	0.0	0.0
3	–0.01 ± 0.30	0.20 ± 0.22		7.1 ± 1.3	0.4 ± 0.4	5.4 ± 0.4	1.5 ± 0.6	0.4 ± 0.4	3.6 ± 0.4	1.6 ± 0.4	7.7 ± 0.6	3.1 ± 0.7	0.0	7.1 ± 1.4	3.9 ± 0.9
4	–0.01 ± 0.59	0.00 ± 0.17	–0.01 ± 0.31		8.9 ± 0.7	7.0 ± 1.0	0.0	9.4 ± 0.4	9.1 ± 0.3	0.0	5.6 ± 0.4	10.0 ± 0.0	6.9 ± 1.7	5.5 ± 2.0	7.6 ± 0.4
5	–0.07 ± 0.24	–0.14 ± 0.32	0.17 ± 0.14	–0.02 ± 0.07		2.6 ± 0.6	0.1 ± 0.3	1.4 ± 0.6	0.7 ± 0.4	6.7 ± 1.4	6.7 ± 1.5	0.4 ± 0.4	8.4 ± 0.6	6.7 ± 1.2	4.7 ± 0.6
6	0.17 ± 0.21	0.01 ± 0.27	–0.11 ± 0.29	0.17 ± 0.38	0.03 ± 0.56										
7	0.14 ± 0.37	–0.10 ± 0.56	–0.16 ± 0.30	–0.06 ± 0.24	–0.05 ± 0.27	0.11 ± 0.37									
8	0.11 ± 0.36	–0.15 ± 0.24	–0.16 ± 0.23	–0.02 ± 0.46	0.13 ± 0.55	0.16 ± 0.35	0.08 ± 0.58								
9	–0.16 ± 0.30	0.09 ± 0.39	–0.10 ± 0.33	0.04 ± 0.14	–0.17 ± 0.17	0.08 ± 0.33	0.06 ± 0.40	-0.01 ± 0.30							
10	0.02 ± 0.44	–0.08 ± 0.30	0.00 ± 0.27	0.16 ± 0.24	0.01 ± 0.51	0.10 ± 0.13	0.10 ± 0.24	0.00 ± 0.38	0.03 ± 0.23						
11	–0.08 ± 0.31	–0.10 ± 0.29	–0.02 ± 0.35	0.02 ± 0.25	–0.01 ± 0.24	–0.14 ± 0.21	0.11 ± 0.35	–0.17 ± 0.26	0.00 ± 0.38	0.13 ± 0.54					
12	–0.20 ± 0.15	–0.19 ± 0.18	–0.05 ± 0.37	0.08 ± 0.47	0.04 ± 0.52	–0.18 ± 0.11	0.19 ± 0.59	–0.09 ± 0.27	0.19 ± 0.46	0.03 ± 0.58	0.37 ± 0.44				
13	0.02 ± 0.27	0.05 ± 0.35	0.02 ± 0.32	–0.09 ± 0.10	–0.02 ± 0.31	–0.10 ± 0.06	–0.09 ± 0.27	–0.12 ± 0.05	–0.05 ± 0.12	–0.12 ± 0.17	–0.01 ± 0.11	0.01 ± 0.20			
14	–0.24 ± 0.19	–0.09 ± 0.36	0.07 ± 0.29	–0.13 ± 0.20	–0.07 ± 0.17	–0.10 ± 0.29	–0.07 ± 0.17	0.03 ± 0.34	0.19 ± 0.52	0.07 ± 0.31	–0.08 ± 0.12	0.04 ± 0.38	0.23 ± 0.57		
15	0.19 ± 0.14	0.22 ± 0.21	–0.01 ± 0.53	–0.06 ± 0.43	–0.08 ± 0.56	–0.14 ± 0.37	–0.02 ± 0.19	–0.17 ± 0.05	–0.03 ± 0.37	–0.19 ± 0.33	0.05 ± 0.57	0.07 ± 0.47	0.35 ± 0.42	0.06 ± 0.71	

than the 10 remaining ones (0.64 ± 0.08) (*t*-test unequal variances: $t = 6.75$, df = 13, $p < 0.0001$) (Table 2).

The weighted estimators of the mean number of breeders per colony show a lower level of polygyny. Indeed, the effective mean number of reproductive queens per colony was N_e = 1.31 or 1.41, depending on whether queens were assumed to be unrelated ($r_q = 0$) or related ($r_q = r_w$). The effective mean number of matings by queens was 1.55. The effective number of breeders for each colony is given in Table 3.

Patterns of relatedness between colonies

Pair-wise values or relatedness *R* (Queller and Goodnight, 1989) between all colonies were calculated based on all four loci (Table 1). Between-colony relatedness ranged from –0.24 (colonies 1 and 14 which shared no alleles in none of the loci) to 0.37 (colonies 11 and 12) (Fig. 2b, Table 1). Here again, close examination of the estimates of relatedness between nests did not reveal clear patterns of transitivity.

Relationship between aggression, relatedness and geographical distance

A Mantel test revealed no significant association between aggression levels, relatedness and geographical distance between pairs of colonies (*p*-values based on 1000 simulations) (Fig. 3 and 4a). Partial correlations were respectively, 0.139 between aggression and geographical distance ($p = 0.320$), –0.027 between relatedness and geographical distance ($p = 0.862$), and 0.08 between aggression and relatedness ($p = 0.598$). In order to test if genetic variability of the ant colonies involved in the staged encounters influenced aggression levels, we calculated the variable 'max intra-nest relatedness', equivalent to the highest value of intra-nest relatedness for each pairs of colonies. There was a significant negative association between aggression level and max within-colony relatedness of colony pairs (Fig. 4b).

We also tested the relationship between geographical distance, relatedness between colonies and aggression category, equivalent to 1 or 2 depending on which of the two peaks

Table 2. Allelic frequencies for microsatellite DNA loci *Ppal-03*, *Ppal-12*, *Ppal-33*, *Ppal-77* in the *P. xerophylla* population of Yucca Bachada in the Mojave desert.

Locus	*a*	*b*	*c*	*d*	*e*	*f*	*g*	*h*	*i*	*j*	*k*	*l*	*m*	*n*
Ppal03	0.086	0.095	0.118	0.032	0.027	0.395	0.114	0.068	0.064	0	0	0	0	0
Ppal12	0.039	0.113	0.019	0.027	0.08	0.145	0.082	0.038	0.097	0.134	0.057	0.033	0.103	0.033
Ppal33	0.07	0.157	0.043	0.047	0.043	0.139	0.15	0.079	0.047	0.107	0.074	0.01	0.033	0
Ppal77	0.193	0.168	0.182	0.136	0.101	0.126	0.07	0.023	0	0	0	0	0	0

Table 3. Estimates of within-colony relatedness (±SD), maximum number of alleles per locus observed, minimum number of queens (f) and males (m) required for explaining the genotypic patterns observed in offspring workers in 15 colonies of *P. xerophylla* from the Mojave desert (Structure A assumes polygyny as the most parsimonious explanation for the patterns observed whilst Structure B assumes polyandry as the most likely explanation). The effective number of queens (N_e) and the effective number of matings by queens (M_e) are also indicated.

Colony	**Relatedness**	**Alleles**	**Structure A**	**Structure B**	N_e		M_e
					$r_{qq} = 0$	$r_{qq} = r_w$	
B1	0.71 ± 0.09	3	2f, 2 m	1f, 2 m	1.05	1.07	1.08
B2	0.56 ± 0.06	5	2f, 2 m	1f, 3 m	1.33	1.44	1.60
B3	0.57 ± 0.15	4	2f, 2 m	1f, 3 m	1.31	1.42	1.55
B4	0.48 ± 0.08	4	2f, 2 m	2f, 4 m	1.55	1.74	2.14
B5	0.52 ± 0.08	5	2f, 2 m	1f, 4 m	1.45	1.60	1.86
B6	0.45 ± 0.12	5	2f, 2 m	2f, 3 m	1.66	1.88	2.48
B7	0.63 ± 0.05	4	2f, 2 m	1f, 3 m	1.19	1.25	1.31
B8	0.75 ± 0.06	5	2f, 2 m	1f, 3 m	1.00	1.00	1.00
B9	0.55 ± 0.11	4	2f, 2 m	1f, 3 m	1.36	1.48	1.66
B10	0.42 ± 0.11	5	2f, 2 m	2f, 4 m	1.78	2.05	2.93
B11	0.44 ± 0.11	7	3f, 3 m	2f, 3 m	1.70	1.93	2.60
B12	0.73 ± 0.11	5	2f, 2 m	1f, 3 m	1.02	1.03	1.04
B13	0.40 ± 0.10	5	2f, 2 m	2f, 3 m	1.87	2.16	3.31
B14	0.69 ± 0.07	4	2f, 2 m	1f, 3 m	1.08	1.11	1.13
B15	0.69 ± 0.03	4	2f, 2 m	1f, 2 m	1.08	1.11	1.13

of the bimodal distribution of aggression the pair-wise comparison clustered with. No significant association was found. Partial correlations were respectively, 0.106 between category of aggression and geographical distance ($p = 0.446$), –0.023 between among-nest relatedness and geographical distance ($p = 0.906$), and 0.068 between aggression category and relatedness ($p = 0.576$).

Discussion

The variation in relatedness and aggression among colonies reported here suggests that the population structure of these desert seed-harvesting ants could indeed provide a functional context in which kin selection may act to shape the behavior of ants and the spatial distribution of their nests. Aggression levels in staged encounters ranged from no aggressive interactions, where ants workers merely touched each other with their antennae and resumed their search for an escape from the neutral arena, to all-out fights. In such instances every single individual present in the neutral arena was involved in fights with non-nestmates resulting in clumps of fighting ants. These results are consistent with previous findings by Langen et al. (2000). Ants that are involved in such highly aggressive combinations of colonies in a neutral arena will fight for hours on end until exhaustion or death. Several colonies lived at such close proximity that it could reasonably be assumed that they often interact while harvesting seeds. Colonies 10 and 4, for example, were found to be relatively similar genetically and were also located approximately 2 m from each other. These colonies would clearly benefit by tolerating each other's workers while fighting off or displacing those from less related colonies - in this case colonies 3 and 5 located nearby (see Fig. 1). A nestmate recognition system based on kin recognition would therefore not only prevent costly competitive interactions between related nests but

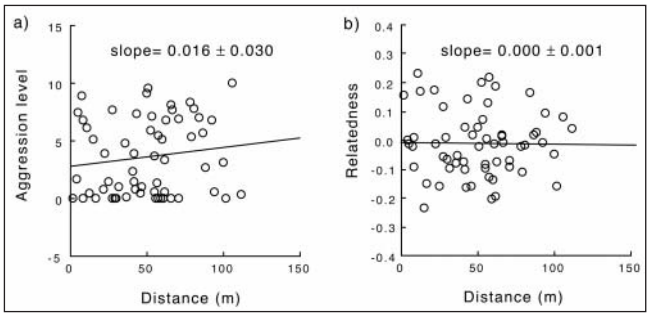

Fig. 3. Relationship between: a) pair-wise aggression levels and geographical distances; and b) relatedness between pairs of *P. xerophylla* colonies and the geographical distance between them. The slope and confidence intervals are indicated. Significance levels of the regressions were tested using permutation tests and were respectively $p = 0.172$ in a) and $p = 0.765$ for b).

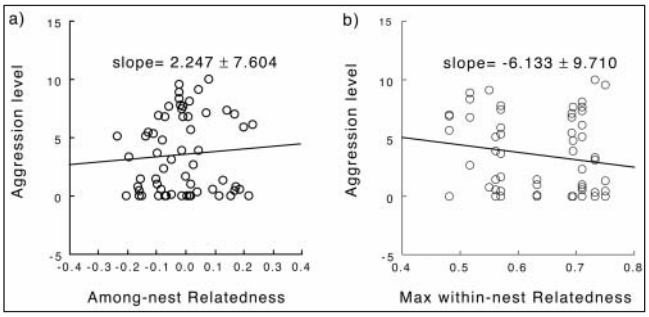

Fig. 4. Relationship between: a) pair-wise aggression levels and the relatedness between pairs of nests; and b) pair-wise aggression levels and maximum within-colony relatedness of the two colonies considered, in *P. xerophylla* colonies. The slope and confidence intervals are indicated. Significance levels of the regressions were tested using a multivariate permutation test and were respectively $p = 0.791$ in a) and $p = 0.028$ for b).

also help them out-compete less-related ones. However, we found no relationship between levels of aggression between ants from different nests and the relatedness between them. Colony 10, for example, exhibited relatively low levels of aggressions towards colony 3 (aggression level = 1.6). We also found no relationship between geographical distance and aggression levels, a result that is consistent with our previous study in which we found evidence of the dear enemy phenomenon only at very short distances (≤3 m) (Langen et al., 2000).

The most important findings of this study are the absence of transitivity between pair-wise aggression levels and a distribution of aggression levels that strongly deviated from uni-modality. The bimodal distribution of aggression levels suggest that colonies either tolerated each other or did not based on a non-transitive recognition mechanism. A simple model of recognition that would tolerate the extreme non-transitive patterns observed in our study is the 'common label acceptance model' (Crozier and Dix, 1979). In this model, individuals from one colony tolerate non-nestmates if at least one component ant of the non-nestmates' odor matches an odor component that was previously learned by familiarization (Crozier and Pamilo, 1996), or known innately as originally proposed by Crozier and Dix (1979). Different pairs of colonies would each differ in the odor labels they would share, resulting in either no aggression or high aggression levels and a bimodal distribution of aggression level. Thus the model would explain the patterns of non-transitivity as well as the bimodal distribution of aggression levels observed here. Intermediate levels of aggression were also observed in our study and could be interpreted either as measurement errors, recognition errors, lower action threshold of some individuals due to polyethism, or evidence that foreign labels are not homogenously distributed among nestmates. In the latter case, the proportion of fighting ants could depend on the proportion of ants carrying the foreign label. Mintzer (1982) reported a lack of transitivity in aggression levels among workers from different colonies of *Pseudomyrmex ferruginea* that would be compatible with this explanation.

It should be noted that the common label acceptance model, when based on phenotype matching rather than familiarization, cannot work in polygynous species because it predicts aggression within colonies between unrelated nestmates. Polygyny and/or polyandry such as that found in our study population would therefore be incompatible with a common label acceptance model of recognition based on genetic similarity and this could explain the absence of correlation between aggression levels and relatedness among colonies. Generally speaking, a mechanism of discrimination between colonies based on genetic cues such as phenotype-matching in its strict sense (priming of juvenile ants with their own odors or that of related individuals) would be more likely to be found in species that are monogynous and monandrous. In such species, the limited genetic background of the colony would guarantee the low amounts of variation in cues necessary for accurate kin recognition (Vander Meer and Morel, 1988). Polygyny and/or polyandry are expected to decrease the efficiency of recognition based on genetic cues because of the increased variance in cues within colonies and lower overall similarity between related colonies (Vander Meer and Morel, 1998). This lower efficiency would increase recognition errors and consequently decrease the benefits of discrimination (Keller, 1997).

The absence of correlation between aggression level and among-nest relatedness found here despite a negative correlation between within-nest relatedness and aggression levels, further supports the idea that the cues used for kin recognition in *P. xerophylla* are probably not based simply on overall genetic similarity. Colonies that were more polygynous and/or polyandrous – as evidenced by lower within-nest relatedness – elicited stronger aggression suggesting that label diversity was higher and could be controlled by a limited number of highly polymorphic loci. Non-additive effects among genes coding for odor labels, templates or olfaction could generate the extremes in aggression behavior observed between

nests provided that few genes are involved. In support to this hypothesis there is strong evidence that the invasive species, *Linepithema humile* owes its expansive unicolonial structure to the loss of genetic diversity at loci coding for recognition cues associated with its introduction in North America and Europe (Tsuitui et al., 2000; Suarez et al., 2002; Giraud et al., 2002). This is only understandable if few genes are involved since recombination between a few loci and alleles with additive effects would already destroy these patterns. The low number of genes involved in producing recognition cues or perceiving them has also been underlined in studies of the social structure of *Solenopsis invicta* in Northern America. In introduced bottlenecked populations, a simplified di-allelic system at a single locus has been shown to dramatically affect recognition and social behavior (monogyny versus polygyny) (Keller and Ross, 1998). It was later found that the same di-allelic gene, now known to code for an odor-binding protein, is present in 4 other related *Solenopsis* species (Krieger and Ross, 2002; Keller and Parker, 2002). Thus the lack of transitivity and extreme level of variation in aggression levels between colonies observed in *P. xerophylla* could be linked to the interaction between non-additive genetic effects affecting the endogenous cues themselves and the effects of variation in the number of queens producing those cues. In addition we cannot dismiss the potential effects of exogenous cues that could also interact with genetic components.

In our study, we were limited in the number of markers available because only 4 markers designed for *P. pallidula* could be successfully amplified and were polymorphic in *P. xerophylla*. As a result, confidence intervals on our *R* estimates were generally high and we lacked sufficient power to detect small effects between aggression and relatedness. Nevertheless, correlations between aggression and relatedness have been found in other species using essentially similar designs but in a different functional context. For example, Beye et al. (1998) found a significant correlation between aggression and relatedness in the polydomous and highly polygynous ant *Formica pratensis* using comparable methods (4 microsatellite loci and an average of 11 workers per colony). Significant correlations between genetic and geographical distance and aggression have also been found in *Leptothorax longispinosus* (Stuart and Herbers, 2000). In these species, a significant number of colonies are polydomous, showing high genetic similarity, short physical distances between nests, and little or no aggression (Beye et al., 1998; Pirk et al., 2001; Stuart and Herbers, 2000). Correlations between aggression and genetic distances have been found in this species in polydomous but also in predominously monodomous colonies suggesting a role for endogenous cues (Stuart and Herbers, 2000). Interestingly, Pirk et al. (2001) found a positive relationship between the intranest relatedness of recipient colonies and their level of aggression to experimentally introduced non-nestmates. The results suggested that ants from different mounts in this polydomous species more closely fitted a foreign label rejection model i.e. individuals accept others as kin if they do not possess any foreign labels. As underlined by the authors, however, the possibility remains that a combination of mechanisms characteristic of two or more models may be present in *F. pratensis* (Pirk et al., 2001).

Facultative polygyny, as reported in this study for *P. xerophylla* has been documented in three other species of the genus *Pheidole*: *P. morrisi* (Wilson, 2003), *P. desertorum* (Helms, 1999) and *P. pallidula* (Fournier et al., 2002, 2003). Polyandry, on the other hand, has been properly evaluated in the genus *Pheidole* only for *P. pallidula* (Boomsma and Ratnieks, 1996; Strassmann, 2001; Fournier et al., 2002). Thus the possibility that *P. xerophylla* is polyandrous remains to be explored. Further study would also be required to assess if the level of aggression observed in neutral arenas truly reflects the level of fighting between non-nestmates in foraging areas or if the workers from one colony simply give way to those from another resulting in competitive displacement from resources. The role and aggressive behavior of majors that are known be recruited in case of conflicts on trunk-trails also remains to be investigated (Wheeler and Wheeler, 1973).

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