Lack of inbreeding avoidance in the Argentine ant *Linepithema humile*

Laurent Keller and Denis Fournier
Institute of Ecology, University of Lausanne, Bâtiment de Biologie, 1015 Lausanne, Switzerland

Although workers might increase their inclusive fitness by favoring closer over more distant kin, evidence suggest that nepotism generally does not occur within colonies of social insects. It has been suggested that this may be due to the cost of recognition errors. We tested whether recognition occurs in a system where a better than random ability to recognize kin should be selected for. Using DNA microsatellites, we show that sexuals of the Argentine ant *Linepithema humile* fail to use genetic cues to avoid sib-mating. When offspring of two queens were allowed to mate, the percentage of matings among siblings was not significantly lower than expected under the hypothesis of random mating. The finding that sexuals fail to use genetic cues to avoid sib-matings cannot be attributed to the cost of recognition errors because any recognition system that would lead to a better than random ability to avoid sib-mating should be selected for when there are costs to inbreeding. These data are thus consistent with the view that kin recognition mediated solely by genetic cues might be intrinsically error prone within colonies of social insects. *Key words:* Argentine ants, inbreeding, kin recognition, *Linepithema humile*, recognition errors, sib-mating. *[Behav Ecol 13:28–31 (2002)]*

Individuals of many species can distinguish kin from non-kin, usually on the basis of proximate cues based on familiarity or association within nests (Fletcher and Michener, 1987). Considerable attention has recently focused on whether individuals can distinguish kin from non-kin within colonies of social insects because such an ability would set the stage for numerous kin conflicts among colony members (Bourke, 1997; Bourke and Franks, 1995; Heinze et al., 1994; Keller and Chapuisat, 1999; Keller and Reeve, 1999; Ratnieks and Reeve, 1992). For example, colonies of social insects frequently contain multiple reproductive queens, or queens that have mated with more than one male (Bourke and Franks, 1995; Chapuisat et al., 1997; Crozier and Pamilo, 1996; Keller, 1993). In such colonies, workers belong to several genetically distinct lineages, which are groups of more related individuals, such as full sisters. Hence, workers might benefit from behaving nepotistically—that is, favoring the individuals most related to them. Earlier studies on honeybees suggested that workers favored full sisters over half sisters (e.g., Getz and Smith, 1983; Page et al., 1989). However, these studies have been justifiably criticized on a number of grounds (Breed et al., 1994; Frumhoff, 1991; Hogendoorn and Velthuis, 1988; Oldroyd et al., 1990), and new empirical studies using molecular markers failed to demonstrate differential family composition during swarming (Kryger and Moritz, 1997). Moreover, studies in a number of ant and wasp species also failed to detect nepotism within colonies (DeHeer and Ross, 1997; Keller, 1997; Strassmann et al., 1997).

Two recent studies showed that subfamilies (offspring from different fathers) tend to have different cuticular hydrocarbon profiles in the honeybee (Arnold et al., 1996, 2000). As cuticular hydrocarbons are probably the chemical labels used by workers to discriminate nest mates from non-nest mates

(Arnold et al., 1996; Bonavita-Cougourdan et al., 1987; Lahav et al., 1999; Lenoir et al., 1999), this raises the possibility that such labels can be used for within-colony discrimination. However, the study by Arnold et al. (1996) also showed considerable overlap in the chemical profile of workers from different patrilines, suggesting that these chemical labels would at best provide a moderately efficient system of recognition for within-colony discrimination.

Two general explanations may account for the lack of nepotism within insect societies (Reeve, 1998). One is that selection has favored uniform treatment of colony members because differential treatment of kin classes incurs costs in colonies where all individuals are related (albeit to a variable degree). For example, the preferential treatment of full sisters over half sisters in a colony where the queen is mated with several males likely will affect colony performance and lead to decreased fitness of half sisters. Hence, nepotism will be selected against if the cost incurred by less related individuals outweighs the benefits provided to more related individuals.

Alternatively, kin-biased behaviors may be disfavored because of the cost of recognition errors. Because no recognition system is perfect, the decision of an individual to behave nepotistically depends on the probability of correctly identifying desirable and undesirable recipients. Recognition mediated by genetic cues might be unstable and error prone. Theoretical studies indicate that allelic diversity of recognition systems may vary over time, and in some ecological circumstances more frequent alleles may be continually favored until fixation, resulting in a loss of diversity at the recognition system (Crozier, 1988, Ratnieks, 1991). Finally, it has been suggested that colony members may also benefit from reducing or eliminating information about kinship within the group when nepotism entails a cost for colony productivity (Reeve, 1998). This phenomenon illustrates the fact that mechanisms may evolve at the colony level to prevent the outbreak of conflicts (Keller and Chapuisat, 1999; Keller and Reeve, 1999; Ratnieks and Visscher, 1989; Reeve, 1998).

The aim of this study was to determine whether genetic cues can be used to discriminate kin from non-kin within colonies as a means to avoid inbreeding. As in most other animals, inbreeding is probably detrimental and rare in ants and other eusocial Hymenoptera (Crozier, 1980; Crozier et al., 1984; Pamilo, 1983; Ross and Carpenter, 1991; Ross and

Fletcher, 1986). Dispersal is probably the primary mechanism preventing inbreeding. In most ants, winged males and females emerge from many nests simultaneously and undergo large mating flights before mating, thus greatly decreasing the probability of close relatives contacting one another (Baudry et al., 1998; Crozier, 1980). However, inbreeding seems to occur in a few monogynous (single queen colonies) ants (Cole and Wiernasz, 1997; but see Cole and Wiernasz, 1999; Pamilo, 1991). In polygynous (multiple queen colonies) ants, females (and sometimes males) have reduced dispersal abilities, and mating occurs in the nest (e.g., Hölldobler and Wilson, 1990; Passera, 1994). In species where mating occurs exclusively between nest mates, preferential mating between related or nonrelated individuals would have to be based on genetically determined cues.

A previous study suggested that male and female sexuals of the Argentine ant *Linepithema humile* (previously *Iridomyrmex humilis*) may use genetic cues to avoid inbreeding. Colonies of this species typically contain multiple queens, and most reproductive individuals mate within their parental nest without dispersing (Keller and Passera, 1993). When females were presented simultaneously with a brother that was reared in the same colony until the pupal stage and an unrelated male produced in another colony, they mated preferentially with the unrelated male (Keller and Passera, 1993). These experiments raised the intriguing possibility that sexuals use genetically derived cues to avoid sib-mating, although it could not be ruled out that colony odor may have been learned during the larval stage. Males of the social bee *Lasioglossum zephyrum* are able to determine the relatedness between females to which they have been exposed and preferentially mate with females that are unrelated to each other (Smith, 1983; Smith and Ayasse, 1987). However, in that case, discrimination is based on learning kin identity of females. To test whether genetic cues are used to avoid sib-mating in the Argentine ant, we used microsatellites (Krieger and Keller, 1999) to determine whether the proportion of sib-matings was lower than expected by chance in unmanipulated colonies containing reproductive individuals produced by two unrelated queens.

MATERIALS AND METHODS

We collected *L. humile* in Port-Leucate, near Perpignan, southern France and set up 120 nests, each with 2 queens and 300 workers but no eggs or brood. Queens in the nest were most likely unrelated, as they were collected from several nests and randomly paired (moreover, the relatedness between nest-mate queens is very low in this population, $r = 0.004$; M. Reuter, F. Balloux, L. Lehman, and L. Keller, unpublished data). These colonies were maintained in the laboratory under standard laboratory conditions (Keller and Passera, 1992, 1993). To ensure that colonies contained only brood from the two queens, colonies were regularly checked for the first 2 weeks and any brood removed. After another 2 weeks we removed the two queens and froze them at $-20°C$ for genetic analyses. The workers then reared the brood that had been produced in the previous 2 weeks. Queenless colonies generally rear male and female sexuals which typically mate in the nest (Keller and Passera, 1992, 1993). We collected all female reproductives that shed their wings, a behavior generally associated with mating, and stored them at $-20°C$ for genetic analyses.

The two mother queens of each colony and the sperm stored in their spermathecae were genotyped at eight microsatellite loci (*Lhum-11, Lhum-13, Lhum-19, Lhum-28, Lhum-35, Lhum-39, Lhum-52, Lhum-62*) (Krieger and Keller, 1999). To isolate sperm DNA, the queen's abdomen was dissected in distilled water and her intact spermatheca removed and placed in extraction buffer. The spermatheca was then ruptured with forceps and the sperm collected with a micropipette for genetic analysis. Queens of this species mate only once (Krieger and Keller, 2000), and in 30 of the 120 colonies the 2 mother queens and their mate (i.e., sperm in their spermatheca) had genotypes that allowed unambiguous maternity assignment of all their offspring. These colonies produced 324 female sexuals that mated and dealated. These females and the sperm in their spermathecae were genotyped at the informative loci (inferred from the genotype of their mothers and fathers) to determine which females mated with a brother versus an unrelated male. Genetic analyses showed that more than five males and five females from each queen were present in 26 out of the 30 colonies. We used only these 26 colonies in further analyses (which represent a total of 273 matings).

Deviation from the null hypothesis of random mating between siblings and unrelated individuals was tested in two ways. First, we estimated for each of the *i* colonies the probability of sib-mating (Rel_i) which depends on the proportion of male and female offspring (that successfully mated) produced by each of the two queens. The probability of sib-mating (Rel_i) is

$$Rel_i = pm_{ai}pf_{ai} + pm_{bi}pf_{bi}$$

where pm_{ai}, pf_{ai}, pm_{bi}, and pf_{bi} are the proportion of male and female offspring (that successfully mated) produced by queens *a* and *b* in colony *i* (inferred from the genotypes of the mated females and the sperm in their spermatheca). We used a paired *t* test (Sokal and Rohlf, 1995) to compare these values with the observed proportion of sib-mating in each of the 26 colonies where both queens contributed to the production of reproductive individuals (all values were arcsine transformed before analysis). We also conducted an analysis on the whole data set by comparing with a binomial test the observed and expected number of matings between siblings under the assumption of random mating, the latter value being:

$$\sum_{i=1}^{26} Rel_i(n_i)$$

where n_i is the number of matings that occurred in colony *i*.

RESULTS AND DISCUSSION

There was no evidence that sexuals avoided mating with siblings in the 26 colonies in which we could unambiguously assign maternity of both the male and female sexuals (total number of matings = 273; see Table 1). A binomial test showed that the percentage (45.1%) of matings among siblings was not significantly lower ($p > .05$) than expected (48.6%) under the hypothesis of random mating. A power analysis demonstrated that the probability of detecting even a relatively weak avoidance of sib-mating ($g = 0.1$; that is, 40% or fewer of the matings occurring between siblings) with an α value = 0.05 and a sample size of 273 was high ($.93 < p < .97$; the power analysis was done by using a 50% theoretical value of sib-mating, the closest value to the expected value in our experiments; Cohen, 1988). An analysis conducted at the colony level (Table 1) also showed no significant difference between the observed (46.6 ± 14.0, $n = 26$) and expected percentages (49.4 ± 12.6, $n = 26$) of matings between siblings (paired *t* test, df = 25, $p = .344$).

The finding that sexuals fail to use genetic cues to avoid sib-matings cannot be attributed to the cost of recognition errors, in contrast to other studies that failed to demonstrate nepotistic behavior (Keller, 1997; Reeve, 1998). This is be-

Table 1
Observed and expected percentages of matings between siblings (under the hypothesis of random mating between sexuals within colonies)

Colony	No. of matings	Matings between sibs (%) Observed	Expected
22	12	25.0	50.0
23	12	75.0	50.0
25	14	50.0	37.5
30	4	50.0	50.0
31	15	60.0	44.0
32	6	50.0	50.0
33	6	50.0	50.0
37	8	50.0	50.0
38	12	50.0	66.7
40	4	50.0	50.0
41	6	50.0	50.0
44	18	16.7	50.0
48	12	50.0	50.0
57	14	40.0	80.0
60	6	50.0	50.0
66	9	66.7	66.7
79	9	66.7	66.7
86	15	20.0	20.0
98	6	50.0	50.0
100	12	25.0	25.0
104	10	50.0	50.0
107	12	33.3	55.6
108	9	33.3	55.6
112	15	40.0	32.0
113	12	50.0	33.3
115	15	60.0	52.0
Total	273		
Mean	10.5	46.6	49.4

Hence, our results are consistent with the view that within-colony recognition mediated by genetic cues is error prone (Crozier, 1988; Ratnieks, 1991), possibly because of meiotic shuffling and/or loss of diversity at the recognition system over evolutionary time (Crozier, 1988; Ratnieks, 1991). Alternatively, low efficiency of kin recognition systems within insect colonies may also stem from colony members benefiting from and actively reducing or eliminating information about kinship within the group (by scrambling recognition labels; Reeve, 1998), if, for example, nepotism would decrease colony productivity. Whatever the mechanism underlying the lack of inbreeding avoidance in the Argentine ant, the demonstration that sexuals fail to avoid sib-matings suggests that genetically mediated recognition cues may not readily be used for within-colony kin recognition.

REFERENCES

Arnold G, Quenet B, Cornuet JM, Masson C, Deschepper B, Estoup A, Gasqui P, 1996. Kin recognition in honeybees. Nature 379:498.

Arnold G, Quenet B, Masson C, 2000. Influence of the social environment on the genetically-based subfamily signature in the honeybee. J Chem Ecol 26:2321–2333.

Baudry E, Solignac M, Garnery L, Gries M, Cornuet JM, Koeniger N, 1998. Relatedness among honeybees (*Apis mellifera*) of a drone congregation. Proc R Soc Lond B 265:2009–2014.

Bonavita-Cougourdan A, Clément JL, Lange C, 1987. Nestmate recognition: the role of cuticular hydrocarbons in the ant *Camponotus vagus* Scop. J Entomol Sci 22:1–10.

Bourke AFG, 1997. Sociality and kin selection in insects. In: Behavioural ecology. an evolutionary approach, 4th ed. (Krebs JR, Davies NB, eds). Oxford: Blackwell; 203–227.

Bourke AFG, Franks NR, 1995. Social evolution in ants. Princeton, New Jersey: Princeton University Press.

Breed MD, Welch CK, Cruz R, 1994. Kin discrimination within honey bee (*Apis mellifera*) colonies: an analysis of the evidence. Behav Process 33:25–39.

Chapuisat M, Goudet J, Keller L, 1997. Microsatellites reveal high population viscosity and limited dispersal in the ant *Formica paralugubris*. Evolution 51:475–482.

Cohen J, 1988. Statistical power analysis for the behavioral sciences. Hillsdale, New Jersey: Lawrence Erlbaum.

Cole BJ, Wiernasz DC, 1997. Inbreeding in a lek-mating ant species, *Pogonomyrmex occidentalis*. Behav Ecol Sociobiol 40:79–86.

Cole BJ, Wiernasz DC, 1999. The selective advantage of low relatedness. Science 285:891–893.

Cook J, Crozier RH, 1995. Sex determination and population biology in the Hymenoptera. Trends Ecol Evol 10:281–286.

Crozier RH, 1980. Genetical structures of social insects populations. In: Evolution of social behaviour: hypotheses and empirical tests (Markl H, ed). Weinheim: Dahlem Konferenzen 1980, Verlag Chemie GmBH; 129–146.

Crozier RH, 1988. Kin recognition using innate labels: a central role for piggy-backing. In: Invertebrate historecognition (Grosberg RK, Hedgecock D, Nelson K, eds). New York: Plenum Press; 143–156.

Crozier RH, Pamilo P, 1996. Evolution of social insect colonies. Sex allocation and kin-selection. Oxford: Oxford Universty Press.

Crozier RH, Pamilo P, Crozier YC, 1984. Relatedness and microgeographic genetic variation in *Rhytidoponera mayri,* an Australian arid-zone ant. Behav Ecol Sociobiol 15:143–150.

DeHeer CJ, Ross KG, 1997. Lack of detectable nepotism in multiple-queen colonies of the fire ant *Solenopsis invicta* (Hymenoptera, Formicidae). Behav Ecol Sociobiol 40:27–33.

Fletcher DJC, Michener CD, 1987. Kin recognition in animals. Chichester, UK: John Wiley.

Frumhoff PC, 1991. The effect of the cordovan marker on apparent

cause any recognition system that would lead to a better than random ability to avoid sib-mating should be selected for when there are costs to inbreeding (e.g., the costs of diploid male production; Cook and Crozier, 1995; Ross et al., 1993; Ross and Fletcher, 1985). In the Argentine ant, the operational sex ratio is highly male biased because there are many more males than females per colony and because both sexes mate only once (Keller and Passera, 1992). Hence, females can easily have access to mates, and they should avoid mating with sibs. Thus, a recognition system that would allow females to mate less frequently with sibs than expected by mating randomly with the males in their colony should be selected for. It is important to note, however, that the selective pressure to avoid sib-matings might not be very strong in our study population because colonies contain a high number of queens (Keller et al., 1989), leading to a relatively low probability of sib-mating even if matings would occur randomly between sexuals. Selection for a mechanism preventing sib-mating is presumably higher in native populations (Argentina and Brazil) where queen number per colony is frequently much lower than in introduced French populations (Pedersen J, Giraud T, and Keller L, unpublished data).

The lack of kin recognition also cannot be attributed to a reduction of recognition alleles following a bottleneck because microsatellite analyses revealed a relatively high genetic diversity in the population under study (Krieger and Keller, 1999). That introduced populations have retained genetic variability is also supported by the finding that some populations from the Mediterranean coast are highly aggressive with other populations and that the level of aggression is strongly associated with the genetic differentiation between populations (Giraud T, Pedersen J, and Keller L, unpublished data).

kin discrimination among nestmate honey bees. Anim Behav 42: 854–856.

Getz WM, Smith KB, 1983. Genetic kin recognition: honey bees discriminate between full and half sisters. Nature 302:147–148.

Heinze J, Hölldobler B, Peeters C, 1994. Conflict and cooperation in ant societies. Naturwissenschaften 81:489–497.

Hogendoorn K, Velthuis HHW, 1988. Influence of multiple mating on kin recognition by worker honeybees. Naturwissenschaften 75: 412–413.

Hölldobler B, Wilson EO, 1990. The ants. Berlin: Springer-Verlag.

Keller L, 1993. Queen number and sociality in insects. Oxford: Oxford University Press.

Keller L, 1997. Indiscriminate altruism: Unduly nice parents and siblings. Trends Ecol Evol 12:99–103.

Keller L, Chapuisat M, 1999. Cooperation among selfish individuals in insect societies. Bioscience 49:899–909.

Keller L, Passera L, 1992. Mating system, optimal number of matings, and sperm transfer in the Argentine ant *Iridomyrmex humilis*. Behav Ecol Sociobiol 31:359–366.

Keller L, Passera L, 1993. Incest avoidance, fluctuating asymmetry, and the consequences of inbreeding in *Iridomyrmex humilis,* an ant with multiple queen colonies. Behav Ecol Sociobiol 33:191–199.

Keller L, Passera L, Suzzoni JP, 1989. Queen execution in the Argentine ant *Iridomyrmex humilis* (Mayr). Physiol Entomol 14:157–163.

Keller L, Reeve HK, 1999. Dynamics of conflicts within insect societies. In: Levels of selection in evolution (Keller L, ed). Princeton, New Jersey: Princeton University Press; 153–175.

Krieger MJB, Keller L, 1999. Low polymorphism at 19 microsatellite loci in a French population of Argentine ants (*Linepithema humile*). Mol Ecol 8:1078–1080.

Krieger MJB, Keller L, 2000. Mating frequency and genetic structure of the Argentine ant *Linepithema humile*. Mol Ecol 9:119–126.

Kryger P, Moritz RFA, 1997. Lack of kin recognition in swarming honeybees (*Apis mellifera*). Behav Ecol Sociobiol 40:271–276.

Lahav S, Soroker V, Hefetz A, Vander Meer RK, 1999. Direct behavioral evidence for hydrocarbons as ant recognition discriminators. 86:246–249.

Lenoir A, Fresneau D, Errard C, Hefetz A, 1999. Individuality and colonial identity in ants: the emergence of the social representation concept. In: Information processing in social insects (Detrain C, Deneubourg JL, Pasteels JM, eds). Basel: Birkhäuser; 219–237.

Oldroyd BP, Rinderer TE, Buco SM, 1990. Nepotism in honey bees. Nature 346:707–708.

Page REJ, Robinson GE, Fondrk MK, 1989. Genetic specialists, kin recognition and nepotism in honey-bee colonies. Nature 338:576–579.

Pamilo P, 1983. Genetic differentiation within subdivided populations of Formica ants. Evolution 37:1010–1022.

Pamilo P, 1991. Life span of queens in the ant *Formica exsecta*. Insect Soc 38:111–119.

Passera L, 1994. Characteristics of tramp species. In: Exotic ants, biology, impact, and control of introduced species (Williams DF, ed). Boulder, Colorado: Westview Press; 23–43.

Ratnieks FLW, 1991. The evolution of genetic odor-cue diversity in social Hymenoptera. Am Nat 137:202–226.

Ratnieks FLW, Reeve HK, 1992. Conflict in single-queen hymenopteran societies: the structure of conflict and processes that reduce conflict in advanced eusocial species. J Theor Biol 158:33–65.

Ratnieks FLW, Visscher PK, 1989. Worker policing in the honeybee. Nature 342:796–797.

Reeve HK, 1998. Game theory, reproductive skew, and nepotism. In: Game theory and animal behavior (Dugatkin L, Reeve HK, eds). New York: Oxford University Press; 118–145.

Ross K, Vargo E, Keller L, Trager J, 1993. Effect of a founder event on variation in the genetic sex-determining system of the fire ant *Solenopsis invicta*. Genetics 135:843–854.

Ross KG, Carpenter JM, 1991. Population genetic structure, relatedness, and breeding systems. In: The social biology of wasps (Ross KG, Matthews RW, eds). Ithaca, New York: Cornell University Press; 451–479.

Ross KG, Fletcher DJC, 1985. Genetic origin of male diploidy in the fire ant, *Solenopsis invicta* (Hymenoptera: Formicidae), and its evolutionary significance. Evolution 39:888–903.

Ross KG, Fletcher DJC, 1986. Diploid male production—a significant colony mortality factor in the fire ant *Solenopsis invicta* (Hymenoptera: Formicidae). Behav Ecol Sociobiol 19:283–291.

Smith BH, 1983. Recognition of female kin by male bees through olfactory signals. Proc Natl Acad Sci USA 80:4551–4553.

Smith BH, Ayasse M, 1987. Kin-based male mating preferences in two species of halictine bee. Behav Ecol Sociobiol 20:313–318.

Sokal RR, Rohlf FJ, 1995. Biometry. The principles and practice of statistics in biological research, 3rd ed. San Francisco: Freeman.

Strassmann JE, Klingler CJ, Arevalo E, Zacchi F, Husain A, Williams J, Seppa P, Queller DC, 1997. Absence of within-colony kin discrimination in behavioural interactions of swarm-founding-wasps. Proc R Soc Lond B 264:1565–1570.