

Sib-mating in the ant *Plagiolepis pygmaea*: adaptative inbreeding?

N. THURIN & S. ARON

Evolutionary Biology and Ecology, Université Libre de Bruxelles, Brussels, Belgium

Keywords:

ants;
inbreeding;
mate choice;
Plagiolepis pygmaea.

Abstract

Multiple functional queens in a colony (polygyny) and multiple mating by queens (polyandry) in social insects challenge kin selection, because they dilute inclusive fitness benefits from helping. Colonies of the ant *Plagiolepis pygmaea* brash contain several hundreds of multiply mated queens. Yet, within-colony relatedness remains unexpectedly high. This stems from low male dispersal, extensive mating among relatives and adoption of young queens in the natal colony. We investigated whether inbreeding results from workers expelling foreign males, and/or from preferential mating between related partners. Our data show that workers actively repel unrelated males entering their colony, and that queens preferentially mate with related males. These results are consistent with inclusive fitness being a driving force for inbreeding: by preventing outbreeding, workers reduce erosion of relatedness within colonies due to polygyny and polyandry. That virgin queens mate preferentially with related males could result from a long history of inbreeding, which is expected to reduce depression in species with regular sibmating.

Introduction

In a wide range of organisms, inbreeding negatively affects several fitness components by reducing birth weight, survival, fecundity, and/or by increasing susceptibility to pathogens, predation or environmental stress (Keller & Waller, 2002; Acevedo-Whitehouse *et al.*, 2003). However, inbreeding allows to preserve local genetic differences and may be a strategy to enhance social cohesion within groups across generations (Hamilton, 1964, 1972). Inbreeding has been shown to substantially increase relatedness values among individuals and is likely associated with the origin of helping behaviour by kin selection in species as diverse as the gall-inducing thrips (Chapman & Crespi, 1998; Chapman *et al.*, 2000), spiders (Avilés, 1997; Bilde *et al.*, 2005) or the naked mole rats (Reeve *et al.*, 1990). This suggests that, at least in some species, the inclusive fitness benefits from inbreeding may compensate for its negative effects

on various fitness components (Bateson, 1983; Keller & Waller, 2002).

Inbreeding is fairly rare in ants. This probably stems from two mechanisms. In most species, mating takes place during population-wide nuptial flights in which numerous colonies synchronously release male and female sexuals, thus greatly decreasing the probability of close relatives contacting one another (Crozier, 1980; Hölldobler & Wilson, 1990). Such a dispersal strategy is typical of single queen (monogynous) colonies. In multiple queen (polygynous) colonies, young queens usually have reduced dispersal abilities and mate in or close to their natal nest. When males do not disperse before mating, queens can avoid incest by discriminating between nestmates and non-nestmates, and preferentially mating with non-nestmates males (Kaufmann *et al.*, 1992; Keller & Passera, 1993; Passera & Keller, 1994). However, moderate to high levels of inbreeding have been documented in a few species, indicating that inbreeding avoidance may be absent (Cole & Wiernasz, 1997; Sundström *et al.*, 2003; Schrempf *et al.*, 2005; Lenoir *et al.*, 2007; Haag-Liautard *et al.*, 2009).

Colonies of the tiny ant *Plagiolepis pygmaea* contain several hundreds of laying queens. Genetic analyses on

Correspondence: Nicolas Thurin, Evolutionary Biology and Ecology, CP 160/12, Université Libre de Bruxelles, Avenue F.D. Roosevelt 50, B-1050 Brussels, Belgium.
Tel.: +32 2 650 22 67; fax: +32 2 650 24 45; e-mail: nthurin@ulb.ac.be

different populations reveal that within-population inbreeding value F ranges from 0.29–0.39 (Trontti *et al.*, 2005, 2007; Thurin & Aron, 2008), which is consistent with low male dispersal, extensive mating among relatives and adoption of young queens in their natal colony (Trontti *et al.*, 2005). Field observations show that *P. pygmaea* males are capable of flying and do swarm; by contrast, females were hardly ever seen flying. Despite male dispersal, colonies within populations remain highly differentiated ($F_{st} = 0.44$). Overall, this suggests that the males first mate in their natal colony with their sisters, and then leave in search of other mating opportunities with little success. At least two causal explanations, not mutually exclusive, may account for the lack of gene flow between colonies of a same population and the high level of inbreeding found in *P. pygmaea*. First, inbreeding may result from the workers expelling foreign males entering their colonies in search of young virgin queens. If inclusive fitness acts as a driving force for inbreeding, this may be a way for workers to reduce the erosion of relatedness by preventing outbreeding. Second, inbreeding may result from a mate choice of the queens; queens mating preferentially with related males. In this study, we examined both these hypotheses. We first studied the aggressiveness of workers towards related and unrelated males at the period of sexual production. To test for preferential mating, we then performed mate choice experiments where virgin queens were given access to two males, one related and one unrelated. The reciprocal experiment was also set up, by giving males the choice between a related and an unrelated virgin queen.

Methods

Sampling and study species

Twenty five colonies of *P. pygmaea* were collected in southern France at the early beginning of the mating period, during June 2007 and 2008. At this time, some male and female sexuals (virgin queens) already developed into imagos but most were found at the last larval and pupal stages. Samples were gathered from five populations distant for at least 1.5 km and up to 180 km from each other: Aureville, Tarabel, Bordeneuve, Mireval and Cerbère. Data on the genetic structure of the sampled populations are given elsewhere (Trontti *et al.*, 2005; Thurin & Aron, 2008). The ants were housed in standard laboratory nests (Passera, 1969), and fed honey and water *ad libitum*. To avoid mating among sibs in rearing nests, queen pupae were removed from their colonies and reared separately in small units set up with ~1000 workers. After emergence of the pupae, adults were sexually mature within about 1 week (as determined by their dark colour) and ready to mate (personal observations). We therefore had sexuals (virgin males and queens) produced by the same field colony (siblings),

and sexuals produced by different colonies from the same or different populations. In all experiments (see below), the individuals (workers, virgin queens and males) tested were of two types: they either originated from the same colony and were considered as related, or they came from different populations and were considered as unrelated to each other. As expected from a previous study (Thurin & Aron, 2008), preliminary tests performed with individuals from different colonies of the same population gave similar results than those performed with individuals from different populations.

Aggressiveness of workers towards related and unrelated males

Ten workers and two virgin queens from a colony were placed in a fluon-coated Petri dish (35 mm × 10 mm). After 10 min, one male from the same colony (related male) or one male from a different colony (unrelated male) was presented and the behaviour of the workers was observed for 3 min. The level of aggression displayed by the workers towards the introduced male was recorded using a five-point scale (Thurin & Aron, 2008): 1, antennation, tapping the antennae somewhere on the other ant; 2, mandible opening and/or dorsal flexion of the gaster as escalation to chemical defence; 3, biting; 4, gaster application, ventral flexion of the gaster as deposition of formic acid; and 5, fighting, body tangling. Level 1 is referred to as nonaggressive behaviour and levels 2–5 are referred to as aggressive behaviours. The arena was carefully cleaned with ethanol 70% after each encounter to remove potential contamination with formic acid or other chemicals. Five replicates were performed for each pair of nests tested; different workers were used for each trial. The highest level of aggression was recorded for each replicate. The scores of each set of replicates were pooled. For comparison, we also estimated the aggressiveness of workers towards unrelated workers (i.e. workers from different populations) by following the same experimental procedure. Owing to non-normality of the data, the mean aggressiveness of workers towards related males, unrelated males and unrelated workers was compared using the nonparametric Kruskal–Wallis one-way analysis of variance, with pairwise comparisons performed by applying Dunn's multiple comparisons tests.

Mate choice experiments

Each virgin queen was offered a choice of two males, a related male (i.e. originating from the same field colony but reared in a different laboratory nest) and an unrelated male, in a 35 mm × 10 mm Petri dish. Each male was marked with a small spot of colour (white or yellow) on the abdomen (Humbrol-Enamel®). The colour of the spot had no effect on mate choice (Fisher's exact test: $f_1 = 22$, $f_{12} = 17$, $f_{21} = 8$, $f_{22} = 10$, $P = 0.569$). The

reciprocal experiment was also set up, where one male was given the choice between a related and an unrelated virgin queen. We observed the behaviour of the ants for 15 min, and recorded the origin (related or unrelated) of the partners involved in the first mating. Experiments were stopped after the first pairing. Mating behaviour is easily recognizable in *P. pygmaea*, with males relaxing their legs and displaying sharp jolts of the abdomen. To determine the mating status of the queens, they were each placed in a vial with workers for 2 h. Virgin queens can be differentiated easily from successfully mated ones because they shed their wings rapidly after mating (mean time between mating and dealation \pm SD = 23'20" \pm 19'19", $n = 16$). To avoid using the same male twice, males that had mated were kept apart until the end of the experiment. When sexuals did not copulate within 15 min (i.e. in 62% of trials), the experiment was interrupted and the ants were returned to their respective rearing nest. Mating choice experiments data were analysed with a binomial test, under the null hypothesis of no discrimination on the part of the females or the males.

Results

Aggressiveness of workers

There was a great disparity in the level of worker aggressiveness depending on the origin and the gender of the introduced individuals (Kruskal–Wallis test: $H_{0.05, 2, 5, 5} = 58.46$, $P < 0.0001$; Fig. 1). Workers were never aggressive towards related males, and usually ignored them (mean aggression \pm SD = 1.05 \pm 0.22; range of the maximum aggression level: 1–2; $n = 20$). On very rare occasions, they displayed mandible opening or dorsal flexion of the gaster. By contrast, workers were

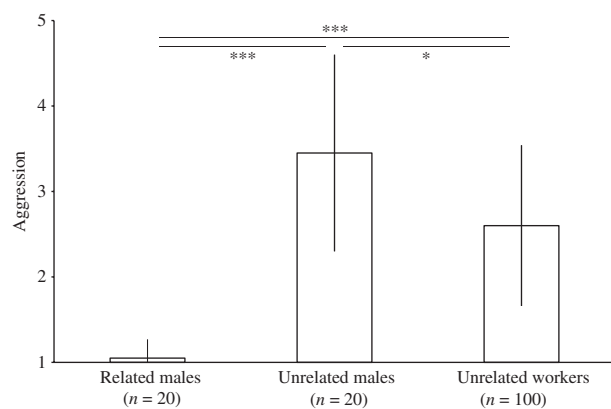


Fig. 1 Mean aggressive behaviour \pm SD of workers towards related males, unrelated males and unrelated workers (Kruskal–Wallis one-way analysis of variance, $H_{0.05, 2, 5, 5} = 58.46$, $***P < 0.001$, $*P < 0.5$).

much more hostile towards unrelated males (mean aggression \pm SD = 3.45 \pm 1.15; range of the maximum aggression level: 1–5; $n = 20$) (Kruskal–Wallis test: $H_{0.05, 2, 5, 5} = 58.46$, $P < 0.001$). They were frequently seen biting and applying the gaster on the males, who ran off. Fighting occurred in 15% of encounters. The aggressive behaviour of workers was even more pronounced towards unrelated males than towards unrelated workers (mean aggression \pm SD = 2.6 \pm 0.94; range of the maximum aggression level: 1–5; $n = 100$) (Kruskal–Wallis test: $H_{0.05, 2, 5, 5} = 58.46$, $P < 0.05$).

Mate choice

When presented simultaneously with a related and an unrelated male, virgin queens mated significantly more often with the related male. Of the 33 queens that mated, 25 (76%) copulated with the related male and eight (24%) with the unrelated male (Binomial test, $n_1 = 25$, $n_2 = 8$, $P < 0.01$; Fig. 2). Evidence that the type of male influenced mating choice was also supported by behavioural observations. When they were presented with two virgin queens, males were forward mating and attempted to copulate with the first female they encountered in 83% of trials. However, in 27% of the experiments where virgin queens were given the choice between two males, they rejected the unrelated male by bending the abdomen away from the male genitalia and/or by biting the male or applying the gaster on the male, driving it away. Such behaviour never occurred during encounters between related individuals. On the contrary, when the male and the virgin queen came from the same colony, the female was observed turning round to direct her abdomen towards the male in 48% of trials, suggesting invitation to copulation.

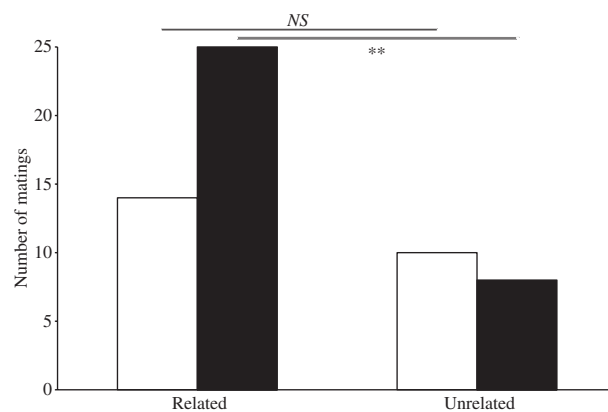


Fig. 2 Number of matings by females (black bars) when presented simultaneously with a related and an unrelated male ($n = 33$), and by males (white bars) when presented simultaneously with a related and an unrelated female ($n = 24$). (Binomial test, NS: $P = 0.27$, $**P < 0.01$).

By contrast, when males were given a choice between a related and an unrelated virgin queen, they mated equally with both types of females (Binomial test, $n_1 = 14$, $n_2 = 10$, $P = 0.27$). It should be noted that when virgin queens had no opportunity to mate with a related male, rejection of unrelated males occurred much less frequently (8% vs. 27% when a related male is present).

Discussion

Two primary mechanisms preventing mating with close relatives and, hence, inbreeding depression, are dispersal of one or both sexes away from their natal nest before mating and recognition of kin (Bateson, 1983; Pusey & Wolf, 1996). Polyandry may also be selected to escape the negative effects of genetic incompatibility due to inbreeding (Tregenza & Wedell, 2002). Intriguingly, our data indicate that at least two mechanisms prevent outbreeding in the ant *Plagiolepis pygmaea*. They show that the behaviour of both the workers and the virgin queens favour mating between relatives: workers actively repel unrelated males, and queens preferentially mate with related males than with unrelated males. These results are consistent with the weak gene flow between colonies and the high level of inbreeding previously reported in this species (Trontti *et al.*, 2005; Thurin & Aron, 2008). Workers are even more aggressive towards unrelated males than towards unrelated workers. Eviction of unrelated males entering colonies in search of mating is consistent with inclusive fitness being a driving force for inbreeding. By doing so, workers prevent outbreeding and, as a consequence, its possible effects on the erosion of relatedness within colonies due to polygyny and polyandry (average queen-mating frequency: 2.89 ± 1.37 ; Trontti *et al.*, 2007). In line with this explanation, within-colony regression relatedness r remains fairly high and ranges from 0.53–0.55 (Trontti *et al.*, 2007). Inbreeding corrected relatedness estimates within-colonies falls to $r = 0.11$. Thus, the breeding system of *P. pygmaea* allows circumventing the loss in inclusive fitness returns caused by dilution of relatedness due to the co-occurrence of multiple queens mating multiply.

A prominent explanation for the evolution and maintenance of polyandry in social hymenoptera stresses the benefits of increased genetic diversity within colonies in terms of improved colony-level resistance to pathogens (Sherman *et al.*, 1998; Baer & Schmid-Hempel, 1999; Tarpay, 2003; Hughes & Boomsma, 2004) or increased productivity through a more efficient division of labour among workers (Crozier & Page, 1985; Robinson & Page, 1988; Mattila & Seeley, 2007). Thus, one should expect polyandry to be selectively favoured if each additional mate increases the chance of obtaining an unrelated partner. This should be even more likely if outbreeding is rare. Despite the possible benefits of polyandry, our data show that virgin queens of

P. pygmaea are capable of differentiating between nest-mate males and non-nestmate (unrelated) males from different populations, and mate significantly more often with related males.

The benefits of inbreeding could greatly differ between females and males in *P. pygmaea*. A potential advantage favouring inbreeding is the increase in relatedness between the queens and their offspring and, hence, their genetic representation in subsequent generations relative to outcrossing females. In hymenoptera, inbreeding may increase relatedness between mother and daughters from 0.5 (under outbreeding) to 0.75 (under inbreeding). Regarding males, mating in the natal nest enhances their chance of encountering females. This strategy may be particularly relevant in *P. pygmaea* for at least two complementary reasons. First, as is usually the case in polygynous ants (Bourke & Franks, 1995), populations of this species show extremely male-biased numerical sex-ratios, the proportion of males varying from 95% to more than 99% (four populations sampled, $n = 67$ colonies, $n = 8239$ male and female sexuals; unpublished data). More than 88% of the colonies produce exclusively a male sexual brood, which reduces dramatically the chance of a dispersing male to find a colony rearing female sexuals. Second, as shown by the present data, the probability that males successfully enter a new colony and mate with unrelated queens seems greatly reduced. In short, inbreeding avoidance by males would lead to considerable loss of breeding opportunities to the individuals. This should result in high 'inbreeding tolerance', as reported in various species of insects, arachnids, birds and mammals (see review of Kokko & Ots, 2006).

Inbreeding increases levels of homozygosity, which usually affects several fitness components leading to inbreeding depression (Keller & Waller, 2002). A major source of inbreeding depression is increased expression of recessive deleterious alleles (Charlesworth & Charlesworth, 1987, 1999). Inbreeding may be particularly damaging in ants because the mating system involves obligate partner-commitment for life (Boomsma *et al.*, 2005; Boomsma, 2007). Mating occurs only during a short period at the beginning of the reproductive lives of sexuals. Males are short-lived. Queens receive a given amount of sperm once and for all. They never re-mate after the breeding period, and rely on the sperm acquired during this unique mating event for all later fertilizations. Inbreeding has been shown to decrease queen life span, male quality and brood survival in the ant *Cardiocondyla obscurior* (Schrempf *et al.*, 2006). In the ant *Formica exsecta*, increased worker homozygosity lessens investment in sexual production, while queen homozygosity results in reduced queen lifespan and colony survival (Haag-Liautard *et al.*, 2009). Another major inbreeding load specific to Hymenoptera is the production of diploid males that arise when there is homozygosity at the sex locus. Diploid males are usually unviable (Petters & Mettus, 1980) or sterile (Cook, 1993); when they are not

sterile, they father a sterile, triploid progeny (Smith & Wallace, 1971; Krieger *et al.*, 1999; de Boer *et al.*, 2007; Cournault & Aron, in press; but see Cowan & Stahlhut, 2004). In social species, diploid males represent an additional high fitness cost because they are produced at the expense of female workers and, since males do not work, they do not contribute to colony productivity (ants, Ross & Fletcher, 1985, 1986; bumblebees, Duchateau & Mariën, 1995; honeybees, Tarry & Page, 2002). Interestingly, despite the high level of inbreeding found in all *P. pygmaea* populations sampled, no diploid males were found in any of them (Trontti *et al.*, 2005; Thurin & Aron, 2008). Two alternative mechanisms may explain this absence. First, diploid males might be eliminated by workers during the larval stage, as documented in *Apis mellifera* and *A. cerana* (Woyke, 1963, 1980). Second, sex could be determined by alternative mechanism such as multi-locus complementary sex determination (*ml*-CSD) (Cook, 1993; Naito *et al.*, 2000) or noncomplementary forms of sex determination like genomic imprinting (Beukeboom, 1995; Dobson & Tanouye, 1998), genic balance (Kerr & Nielsen, 1967) or maternal effect (Crozier, 1971). In the free living ant *Cardiocondyla obscurior*, brother–sister matings in the laboratory over 10 generations resulted in less than 2% diploid males supporting *ml*-CSD with more than 2 loci (Schrempf *et al.*, 2006).

Two complementary mechanisms have been proposed to reduce inbreeding depression in species with regular sibmating (Waller, 1993). First, increased exposure of deleterious mutations in homozygotes should result in to purging of the genetic load. Second, in haplodiploid species, deleterious mutations are regularly exposed to a purifying selection in haploid males. Both predictions have been supported by experimental studies in a variety of organisms e.g. vascular plants (Husband & Schemske, 1996), monkeyflowers (Latta & Ritland, 1994), freshwater snails (Doums *et al.*, 1996), parasitoid wasps (Antolin, 1999). How chronic inbreeding affects queen longevity or colony productivity and survival remains unknown in *P. pygmaea*. A long history of inbreeding might have led to purging of the genetic load due to increased exposure of deleterious mutations in homozygotes, thereby reducing inbreeding depression. Simultaneously, it may have promoted outbreeding depression because of increased genetic variation between parental lineages (Price & Waser, 1979; Waser *et al.*, 2000; Edmands, 2002). Lack of inbreeding depression, but evidence of outbreeding depression have been reported in a number of plants (Edmands, 2002) and, more recently, in the haplodiploid ambrosia beetle *Xylosandrus germanus* (Peer & Taborsky, 2005). In the latter species, offspring from outcrossed matings suffer reduced hatching rates compared with inbred ones. Whether offspring fitness is negatively associated with parental genetic divergence in *P. pygmaea* and other ant species with continuous sibling mating remains to be studied.

Acknowledgments

We thank K. Trontti and L. Sundström for fruitful discussions on inbreeding in *P. pygmaea*, and P. Mardulyn for critical reading of a first draft of the manuscript. Thanks also to J. Evraerts for his help in laboratory. This work was supported by several grants from the Belgian Fond National de la Recherche Scientifique (FRS-FNRS) to NT and SA.

References

- Acevedo-Whitehouse, K., Gulland, F., Greig, D. & Amos, W. 2003. Inbreeding: disease susceptibility in California sea lions. *Nature* **422**: 35.
- Antolin, M.F. 1999. A genetic perspective on mating systems and sex ratios of parasitoid wasps. *Res. Pop. Ecol.* **41**: 29–37.
- Avilés, L. 1997. Causes and consequences of cooperation and permanent-sociality in spiders. In: *The Evolution of Social Behavior in Insects and Arachnids* (B. Crespi & J. Choe, eds), pp. 471–498. Cambridge University Press, Cambridge, UK.
- Baer, B. & Schmid-Hempel, P. 1999. Experimental variation in polyandry affects parasite loads and fitness in a bumble-bee. *Nature* **397**: 151–154.
- Bateson, P. 1983. Optimal outbreeding. In: *Mate Choice* (P. Bateson, ed), pp. 257–277. Cambridge University Press, Cambridge, UK.
- Beukeboom, L.W. 1995. Sex determination in Hymenoptera: a need for genetic and molecular studies. *BioEssays* **17**: 813–817.
- Bilde, T., Lubin, Y., Smith, D., Schneider, J.M. & Maklakov, A.A. 2005. The transition to social inbred mating systems in spiders: role of inbreeding tolerance in a subsocial predecessor. *Evolution* **59**: 160–174.
- de Boer, J.G., Ode, P.J., Vet, L.E.M., Whitfield, J.B. & Heimpel, G.E. 2007. Diploid males sire triploid daughters and sons in the parasitoid wasp *Cotesia vestalis*. *Heredity* **99**: 288–294.
- Boomsma, J.J. 2007. Kin selection versus sexual selection: why the ends do not meet. *Curr. Biol.* **17**: R673–R683.
- Boomsma, J.J., Baer, B. & Heinze, J. 2005. The evolution of male traits in social insects. *Annu. Rev. Entomol.* **50**: 395–420.
- Bourke, A.F.G. & Franks, N.R. 1995. *Social Evolution in Ants*. Princeton University Press, Princeton, NJ.
- Chapman, T.W. & Crespi, B. 1998. High relatedness and inbreeding in two species of haplodiploid eusocial thrips (Insecta: Thysanoptera) revealed by microsatellite analysis. *Behav. Ecol. Sociobiol.* **43**: 301–306.
- Chapman, T.W., Crespi, B.J., Kranz, B.D. & Schwarz, M.P. 2000. High relatedness and inbreeding at the origin of eusociality in gall-inducing thrips. *Proc. Natl Acad. Sci. USA* **97**: 1648–1650.
- Charlesworth, D. & Charlesworth, B. 1987. Inbreeding depression and its evolutionary consequences. *Annu. Rev. Ecol. Syst.* **18**: 237–268.
- Charlesworth, B. & Charlesworth, D. 1999. The genetic basis of inbreeding depression. *Genet. Res.* **74**: 329–340.
- Cole, B.J. & Wiernasz, D.C. 1997. Inbreeding in a lek-mating ant species, *Pogonomyrmex occidentalis*. *Behav. Ecol. Sociobiol.* **40**: 79–86.
- Cook, J.M. 1993. Sex determination in the Hymenoptera: a review of models and evidence. *Heredity* **71**: 421–435.

- Cournault, L. & Aron, S. 2009. Diploid males, diploid sperm production and triploid females in the ant *Tapinoma erraticum*. *Naturwissenschaften* **96**: 1393.
- Cowan, D.P. & Stahlhut, J.K. 2004. Functionally reproductive diploid and haploid males in an inbreeding hymenopteran with complementary sex determination. *Proc. Natl Acad. Sci. USA* **101**: 10374–10379.
- Crozier, R.H. 1971. Heterozygosity and sex determination in haplo-diploidy. *Am. Nat.* **105**: 399–412.
- Crozier, R.H. 1980. Genetical structure of social insect population. in: *Evolution of Social Behavior: Hypotheses and Empirical Tests* (H. Markl, ed.), pp. 129–146. Verlag Chemie, Weinheim.
- Crozier, R.H. & Page, R.E. 1985. On being the right size: male contributions and multiple mating in social Hymenoptera. *Behav. Ecol. Sociobiol.* **18**: 105–115.
- Dobson, S.L. & Tanouye, M.A. 1998. Evidence for a genomic imprinting sex determination mechanism in *Nasonia vitripennis* (Hymenoptera: Chalcidoidea). *Genetics* **149**: 233–242.
- Doums, C., Viard, F., Pernot, A.F., Delay, B. & Jarne, P. 1996. Inbreeding depression, neutral polymorphism, and copulatory behavior in freshwater snails: a self-fertilization syndrome. *Evolution* **50**: 1908–1918.
- Duchateau, M.J. & Mariën, J. 1995. Sexual biology of haploid and diploid males in the bumble bee *Bombus terrestris*. *Insectes Soc.* **42**: 255–266.
- Edmands, S. 2002. Does parental divergence predict reproductive compatibility? *Trends Ecol. Evol.* **17**: 520–527.
- Haag-Liautard, C., Vitikainen, E., Keller, L. & Sundstrom, L. 2009. Fitness and the level of homozygosity in a social insect. *J. Evol. Biol.* **22**: 134–142.
- Hamilton, W.D. 1964. The genetical evolution of social behavior. *J. Theor. Biol.* **7**: 1–52.
- Hamilton, W.D. 1972. Altruism and related phenomena, mainly in social insects. *Annu. Rev. Ecol. Syst.* **3**: 193–232.
- Hölldobler, B. & Wilson, E.O. 1990. *The Ants*. Springer-Verlag, Berlin.
- Hughes, W.O.H. & Boomsma, J.J. 2004. Genetic diversity and disease resistance in leaf-cutting ant societies. *Evolution* **58**: 1251–1260.
- Husband, B.C. & Schemske, D.W. 1996. Evolution of the magnitude and timing of inbreeding depression in plants. *Evolution* **50**: 54–70.
- Kaufmann, B., Boomsma, J.J., Passera, L. & Petersen, K.N. 1992. Relatedness and inbreeding in a French population of the unicolonial ant *Iridomyrmex humilis* Mayr. *Insectes Soc.* **39**: 195–213.
- 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.F. & Waller, D.M. 2002. Inbreeding effects in wild populations. *Trends Ecol. Evol.* **17**: 230–241.
- Kerr, W.E. & Nielsen, R.A. 1967. Sex determination in bees (Apinae). *J. Apic. Res.* **6**: 3–9.
- Kokko, H. & Ots, I. 2006. When not to avoid inbreeding. *Evolution* **60**: 467–475.
- Krieger, M.J.B., Ross, K.G., Chang, C.W.Y. & Keller, L. 1999. Frequency and origin of triploidy in the fire ant *Solenopsis invicta*. *Heredity* **82**: 142–150.
- Latta, R. & Ritland, K. 1994. The relationship between inbreeding depression and prior inbreeding among populations of four *Mimulus* taxa. *Evolution* **48**: 806–817.
- Lenoir, J.C., Schrempf, A., Lenoir, A., Heinze, J. & Mercier, J.L. 2007. Genetic structure and reproductive strategy of the ant *Cardiocondyla elegans*: strictly monogynous nests invaded by unrelated sexuals. *Mol. Ecol.* **16**: 345–354.
- Mattila, H.R. & Seeley, T.D. 2007. Genetic diversity in honey bee colonies enhances productivity and fitness. *Science* **317**: 362–364.
- Naito, T., Ishikawa, M. & Nishimoto, Y. 2000. Two-locus multiple-allele sex determination in the rose sawfly *Arge nigrinodosa*. 3rd International Hymenoptera Congress, Canberra, Australia.
- Passera, L. 1969. Biologie de la reproduction chez *Plagiolepis pygmaea* Latr. et ses deux parasites sociaux *Plagiolepis grassei* Le Mas. et *Plagiolepis xene* St. (Hymenoptera: Formicidae). *Annals Soc. Nat. Zool. Paris* **11**: 327–482.
- Passera, L. & Keller, L. 1994. Mate availability and male dispersal in the Argentine ant *Linepithema humile* (Mayr) (= *Iridomyrmex humilis*). *Anim. Behav.* **48**: 361–369.
- Peer, K. & Taborsky, M. 2005. Outbreeding depression, but no inbreeding depression in haplodiploid ambrosia beetles with regular sibling mating. *Evolution* **59**: 317–323.
- Petters, R.M. & Mettus, R.V. 1980. Decreased diploid male viability in the parasitic wasp, *Bracon hebetor*. *J. Heredity* **71**: 353–356.
- Price, M.V. & Waser, N.M. 1979. Pollen dispersal and optimal outcrossing in *Delphinium nelsonii*. *Nature* **277**: 294–297.
- Pusey, A. & Wolf, M. 1996. Inbreeding avoidance in animals. *Trends Ecol. Evol.* **11**: 201–206.
- Reeve, H.K., Westneat, D.F., Noon, W.A., Sherman, P.W. & Aquadro, C.F. 1990. DNA “fingerprinting” reveals high levels of inbreeding in colonies of the eusocial naked mole-rat. *Proc. Natl Acad. Sci. USA* **87**: 2496–2500.
- Robinson, G.E. & Page, R.E. 1988. Genetic determination of guarding and undertaking in honey bee-colonies. *Nature* **333**: 356–358.
- Ross, K.G. & Fletcher, D.J.C. 1985. Genetic origin of male diploidy in the fire ant, *Solenopsis invicta* (Hymenoptera, Formicidae) and its evolutionary significance. *Evolution* **39**: 888–903.
- Ross, K.G. & Fletcher, D.J.C. 1986. Diploid male production: a significant colony mortality factor in the fire ant, *Solenopsis invicta* (Hymenoptera, Formicidae). *Behav. Ecol. Sociobiol.* **19**: 283–291.
- Schrempf, A., Reber, C., Tinaut, A. & Heinze, J. 2005. Inbreeding and local mate competition in the ant *Cardiocondyla batesii*. *Behav. Ecol. Sociobiol.* **57**: 502–510.
- Schrempf, A., Aron, S. & Heinze, J. 2006. Sex determination and inbreeding depression on an ant with regular sib-mating. *Heredity* **97**: 75–80.
- Sherman, P.W., Seeley, T.D. & Reeve, H.K. 1998. Parasites, pathogens, and polyandry in social Hymenoptera. *Am. Nat.* **131**: 602–610.
- Smith, S.G. & Wallace, D.R. 1971. Allelic sex determination in a lower hymenopteran, *Neodiprion nigroscutum*. *Midd. Canad. J. Genet. Cytol.* **13**: 617–621.
- Sundström, L., Keller, L. & Chapuisat, M. 2003. Inbreeding and sex-biased gene flow in the ant *Formica exsecta*. *Evolution* **57**: 1552–1561.
- Tarpy, D.R. 2003. Genetic diversity within honeybee colonies prevents severe infections and promotes colony growth. *Proc. R. Soc. Lond. B* **270**: 99–103.
- Tarpy, D.R. & Page, R.E. 2002. Sex determination and the evolution of polyandry in honey bees (*Apis mellifera*). *Behav. Ecol. Sociobiol.* **52**: 143–150.

- Thurin, N. & Aron, S. 2008. Seasonal nestmate recognition in the polydomous ant *Plagiolepis pygmaea*. *Anim. Behav.* **75**: 1023–1030.
- Tregenza, T. & Wedell, N. 2002. Polyandrous females avoid costs of inbreeding. *Nature* **415**: 71–73.
- Trontti, K., Aron, S. & Sundstrom, L. 2005. Inbreeding and kinship in the ant *Plagiolepis pygmaea*. *Mol. Ecol.* **14**: 2007–2015.
- Trontti, K., Thurin, N., Sundström, L. & Aron, S. 2007. Mating for convenience or genetic diversity? *Mating patterns in the polygynous ant Plagiolepis pygmaea*. *Behav. Ecol.* **18**: 298–303.
- Waller, N.M. 1993. The statics and dynamics of mating system evolution. In: *The Natural History of Inbreeding and Outbreeding* (N.W. Thornhill, ed), pp. 97–117. University of Chicago Press, Chicago.
- Waser, N.M., Price, M.V. & Shaw, R.G. 2000. Outbreeding depression varies among cohorts of *Ipomopsis aggregata* planted in nature. *Evolution* **54**: 485–491.
- Woyke, J. 1963. What happens to diploid drone larvae in a honeybee colony. *J. Apic. Res.* **2**: 73–75.
- Woyke, J. 1980. Evidence of action of cannibalism substance in *Apis cerana indica*. *J. Apic. Res.* **19**: 51–63.

Received 10 July 2009; revised 22 September 2009; accepted 25 September 2009