

T. Giraud · R. Blatrix · C. Poteaux · M. Solignac  
P. Jaisson

## High genetic relatedness among nestmate queens in the polygynous ponerine ant *Gnamptogenys striatula* in Brazil

Received: 26 November 1999 / Revised: 7 September 2000 / Accepted: 7 September 2000

**Abstract** *Gnamptogenys striatula* is a polygynous ant species, in which all workers are potentially able to mate. The reproductive status, relatedness and pedigree relationships among nestmate queens and winged females in a Brazilian population were investigated. We collected all the sexual females of 12 colonies (2–44 queens per colony, plus 2–18 winged females in 3 colonies). Dissections revealed that 98% of the queens were inseminated and that the queens in the most polygynous colonies did not lay equal numbers of eggs. The sexual females and a sample of the population were genotyped using eight microsatellite markers. Relatedness among nestmate queens was among the highest recorded to date ( $0.65 \pm 0.25$ ), and tests of pedigree relationship showed that they were likely to be full-sisters, and sometimes cousins. Mated winged females were always full-sisters, the estimated genetically effective queen numbers were low and tests of pedigree relationship showed that only a few queens in the colony could be the mothers. These results suggest that the high queen-queen relatedness in polygynous colonies of *G. striatula* is maintained by an unusual mechanism: winged females are mostly produced by only one or a few queens, and these groups of full-sisters are recruited back into their original nest after mating.

**Keywords** *Gnamptogenys striatula* · Microsatellites · Ants · Polygyny · Relatedness

### Introduction

Genetic relatedness has formed the core of studies on the evolution of social insects ever since Hamilton (1964a, 1964b) presented his concept of inclusive fitness. In eusocial insects, workers forgo their own reproduction and help to rear the progeny of their colony's queen. Genes promoting such worker behaviour should be eliminated, but the lack of direct reproduction by workers can be compensated if copies of the genes are present in the related reproducers being aided. In social Hymenoptera, colonies are indeed often composed of closely related individuals (Crozier and Pamilo 1996).

Secondary polygyny (many reproductive queens in colonies) is common in ants (Hölldobler and Wilson 1990; Keller 1993, 1995), but raises two sets of evolutionary questions (reviewed in Keller 1995). The first concerns the maintenance of the sterile caste of workers. Nonacs (1988) showed that relatedness among colony members decreases rapidly as the number of queens increases, even if joining queens originate from the nest, due to recruitment over several generations and outbreeding. As polygyny can greatly reduce relatedness between brood and workers, why do workers still raise the queens' progeny, instead of reproducing themselves? Reproductive altruism may be maintained in species with morphologically specialised castes, even when there is very low relatedness among colony members, because workers are trapped in their role (Queller and Strassmann 1998). The most problematic case is certainly polygynous species with weak morphological caste specialisation. If workers have the option of becoming egg-layers, but choose not to do so, they must be acquiring some indirect genetic returns. Several studies have shown that relatedness between workers is sometimes surprisingly high in polygynous species (e.g. Queller et al. 1988; Strassmann et al. 1991; Herbers 1993;

Communicated by: F. Ratnicks

T. Giraud · R. Blatrix · C. Poteaux · P. Jaisson (✉)  
Laboratoire d'Ethologie Expérimentale et Comparée,  
Université Paris 13, avenue J.B. Clément,  
93430 Villetaneuse, France  
e-mail: pierre.jaisson@leec.univ-paris13.fr  
Tel.: +33-1-49403218, Fax: +33-1-49403975

T. Giraud  
IE-ZEA, Bâtiment de Biologie, Université de Lausanne,  
1015 Lausanne, Switzerland

M. Solignac  
Laboratoire Populations, Génétique et Evolution, CNRS,  
91198 Gif-sur-Yvette Cedex, France

Rosengren et al. 1993; Crozier and Pamilo 1996; Bourke et al. 1997), compared to that predicted by models (Nonacs 1988; Hughes et al. 1993). Closely related colony members in polygynous nests may arise from inbreeding, reproductive dominance of one queen and/or high relatedness among queens. Nestmate queens in polygynous species are generally related, but to a degree that varies greatly between species, some species having nestmate queens that are almost unrelated (reviewed in Keller 1995). But the maintenance of closely related queens is also a problem in polygynous colonies. The split sex ratio theory (Boomsma and Grafen 1991; Boomsma 1993) suggests that when colonies have different numbers of queens, those with the fewest queens will have a higher relatedness asymmetry (i.e. sisters will be relatively more related to each other than to their brothers), and will predominantly rear females, whereas those with the most queens will invest more in male production. If queens are produced by a few mothers and are accepted back into their nest, they can indeed remain closely related.

The second set of questions raised by secondary polygyny concerns the evolution of colonies with multiple queens. As the reproductive output per single queen often decreases as the number of queens increases (Michener 1974; Wilson 1974; Keller and Vargo 1993), why do young queens join established nests? It has been suggested that strong constraints on colony founding favours young queens seeking adoption by an established nest (Keller 1995), and that relatedness among nestmate queens may lower the cost of sharing reproduction.

*Gnamptogenys striatula* (Hymenoptera: Ponerinae) is distributed throughout Central and South America where it can be found in open habitats (J. Delabie, personal communication) and in humid forests (Lattke 1995). All workers are potentially able to mate in this species, and some colonies contain gamergates (mated, fertile workers producing diploid offspring) instead of queens. Both queenright (with morphologically differentiated queens) and queenless (with gamergates) colonies are polygynous: under natural conditions several differentiated queens or several gamergates live together in the same nest, without conflict (up to 60 nestmate queens). Removing the queens allows workers to mate and reproduce in laboratory colonies (Blatrix and Jaisson 2000). Colonies contain up to 1,200 workers (mean±SD=372±298,  $n=51$ ; B. Rumsa, unpublished data). In this study, we assessed the relatedness among queens to determine whether the maintenance of the worker caste and the joining of queens in established nests could be mediated by kin selection in *G. striatula*. We then attempted to identify the mechanisms by which the level of relatedness is maintained. This was done by investigating the reproductive abilities of queens and the genetic relationships among nestmate queens and winged females in queenright colonies.

## Methods

### Sample collection and reproductive status

Thirty-two complete field colonies were collected along a 50-km transect in north-east Brazil near Itabuna (Fig. 1) in January and February 1999. The names of the colonies reflect their locations: the first letter indicates the collecting site, the number indicates the circle 100 m in diameter, and the last letter, the circle 15 m in diameter from which the colony was collected.

Eight workers from each sampled colony were screened for eight variable microsatellite markers to estimate the allele frequencies in the population and to assess population structure. We then genotyped all the surviving queens from the colonies C2j, C3e, C4c, C4d, C4f, C6a, I2a, I2b, I2f, I2h, B1b and B1c; these colonies had 3, 10, 3, 13, 2, 44, 3, 2, 12, 2, 12 and 43 queens, respectively. Winged females were found in 3 colonies (17 in colony C6a, 2 in colony I2a and 18 in colony B1c), and these were also genotyped.

Queens and winged females were dissected to determine whether they were mated and were egg-laying. Egg-laying activity was estimated by the presence and the number of mature (with chorion) oocytes and yellow bodies. Some females were not suitable for dissection, as they had been preserved in alcohol.

### DNA extraction and microsatellite amplification

The gasters (terminal parts of the abdomen) of queens were used for ovarian dissections and their heads and alitrunks (thorax and frontal part of the abdomen) were frozen for DNA extraction. Genomic DNA was isolated using the QIAamp DNA Mini Kit (Qiagen).

Primer sequences and amplification conditions for the eight microsatellite loci (L2, L4, L6, L8, L12, L16, L19 and L20) were as described in Giraud et al. (1999). Polymerase chain reaction products were separated in 6% polyacrylamide gels and visualised by autoradiography. Alleles were scored by length in base pairs.

### Data analysis

Fst values (Weir and Cockerham 1984), and the corresponding probabilities were calculated on the sample of eight workers collected from the different nests, using GENEPop software (Raymond 1995).

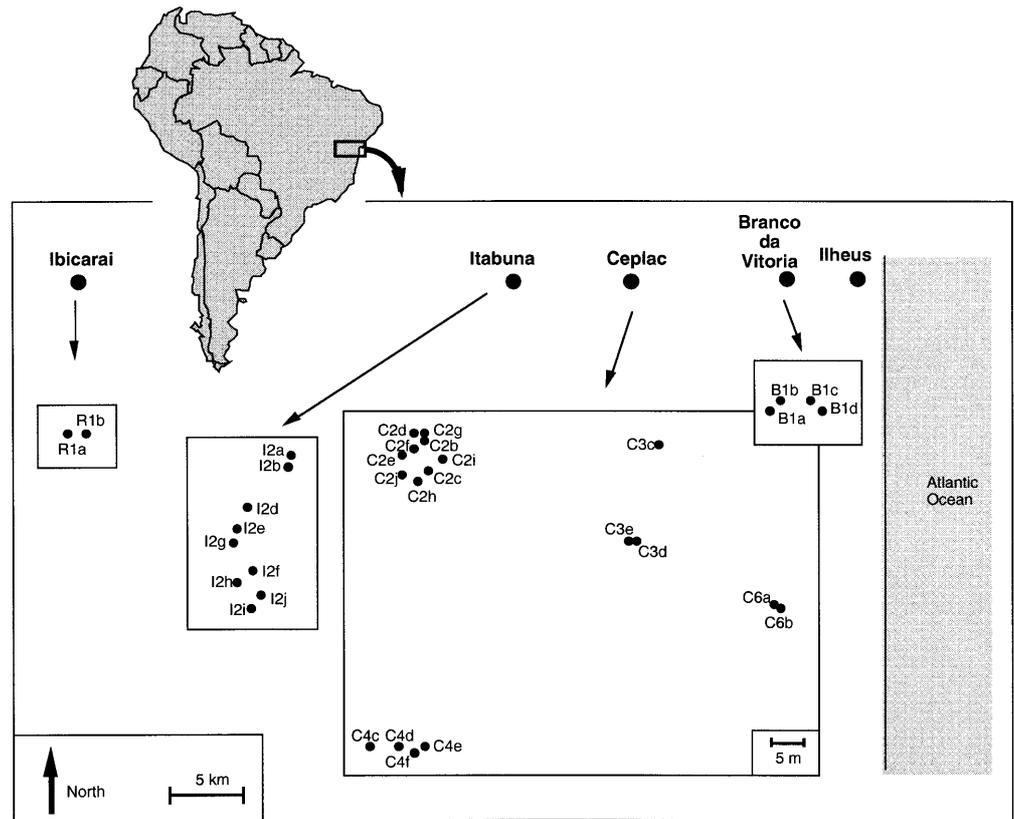
Regression estimates based on shared alleles (Queller and Goodnight 1989) were used to characterise the relatedness among colony members. Relatedness ( $R$ ) is an estimate of the probability of gene sharing among individuals beyond the baseline probability set by the frequency of the gene in the population (e.g. Bourke and Franks 1995, pp. 14–38; Crozier and Pamilo 1996), which is based on Grafen's (1985) relatedness coefficient. Standard errors of  $R$  were obtained by jack-knifing over loci. Calculations were performed using the computer program RELATEDNESS 5.0 by Goodnight and Queller, obtained from the Web site: <http://www-bioc.rice.edu/~kfg/GSoft.html>. Individuals were weighted equally. As there was a significant population structure at the level of the locality, the relatedness estimates among queens were calculated using the allelic frequencies (all nests being weighted equally) of the locality from which the colony had been collected.

Genetically effective queen number, i.e. the number of single-mated, equally reproducing queens that will lead to the observed genetic diversity among the female offspring in a randomly mating population, was calculated for colonies with winged females by the formula from Pedersen and Boomsma (1999):

$$n_e = \frac{3 - r_Q}{4r_F - r_Q}$$

where  $r_Q$  is the average relatedness among queens reproducing in the same colony and  $r_F$  is the estimated relatedness among female

**Fig. 1** Map of the locations in Brazil where the colonies of *Gnamptogenys striatula* were collected in January and February 1999. *Insets* details within each collection area, scales as in Ceplac



offspring. The assumptions of monandry and outbreeding are met in *G. striatula* (Giraud et al., 2000).

The expected relatedness of nestmate queens under the assumption of adoption of daughter queens over several generations was calculated by the formula from Pamilo (1991):

$$r = \frac{3}{3n + 1}$$

where  $n$  is the number of queens in the nest.

Hypotheses of pedigree relationships between pairs of females were tested using Kinship 1.2 software (Goodnight and Queller 1999), obtained from the Web site: <http://www-bioc.rice.edu/~kfg/GSoft.html>. This program calculates the ratio of likelihood between a primary and a null hypothesis. To determine the pedigree relationships among nestmate queens, the primary hypothesis was “full-sisters” and the null hypothesis was “unrelated”. When this test was significant, the primary hypothesis “full-sisters” was also tested against the null hypothesis “cousins by their mothers”. When the first test was not significant, the primary hypothesis “cousins by their mothers” was tested against the null hypothesis “unrelated”. To determine the pedigree relationships among nestmate winged females, the primary hypothesis was “full-sisters” and the null hypothesis was “unrelated”. When this test was significant, the primary hypothesis “full-sisters” was also tested against the null hypothesis “having mothers related at the level estimated among queens in their colony”. To determine the pedigree relationships of each queen towards nestmate winged females, the primary hypothesis was “mother” and the null hypothesis was “unrelated”. When this test was significant, the primary hypothesis “mother” was also tested against the null hypothesis “queen related to the mother at the level estimated in the colony”. To enter the hypotheses settings in Kinship, we considered that queens were all single-inseminated, as we have shown that this species is monandrous (Giraud et al., 2000)

## Results

### Number of reproductive females

Twenty-seven of the 32 collected colonies of *G. striatula* were polygynous. The number of queens in the 12 colonies analysed with microsatellite markers varied from 2 to 44, with a mean ( $\pm$ SE) of  $13.6 \pm 14.7$  (Table 1). Winged females were found in 3 nests, and their number ranged from 2 to 18, with a mean of  $12.3 \pm 9.0$  (Table 1).

Dissections showed that 10 of the 17 winged females from colony C6a, and all winged females from colonies I2b and B1c had mated. All the dissected queens had mated, except one each from colony I2b and B1b, and two from colony B1c. Almost all queens had developed oocytes, except in the colonies with the greatest numbers of queens, where several mated queens had no oocytes. In these colonies, the queens with mature oocytes included a few queens with many oocytes (typically five), whereas the others had only one. These queens were considered to be the main egg-laying queens (Table 1).

### Population structure

The  $F_{st}$  calculated between Itabuna, Ceplac and Branco da Vitoria was highly significant ( $F_{st} = 0.096$ ,  $P < 0.00001$ ), indicating geographical differentiation between these

**Table 1** Colonies of *Gnamptogenys striatula* for which females were genotyped. The number of queens and winged females genotyped is indicated; the number of queens originally present in the colony are in brackets. The numbers of females actually mated and with oocytes are also indicated, when the information was available

Colony	Number of queens	Number of queens with oocytes	Number of winged females
C2j	3	3	0
C3e	10	10	0
C4c	3 [4]	3	0
C4d	13 [18]	nd <sup>a</sup>	0
C4f	2	2	0
C6a	44	5 (2 laying most of the eggs <sup>b</sup> )	17 (10 mated)
I2a	3 [5]	nd <sup>a</sup>	0
I2b	3 (1 unmated) [7]	2	2 (mated and egg-laying)
I2f	12	8	0
I2h	2 [3]	2	0
B1b	12 (1 unmated)	11	0
B1c	43 (2 unmated) [45]	37 (5 laying most of the eggs <sup>b</sup> )	18 (mated, 12 laying few eggs)

<sup>a</sup> Queens were not dissected

<sup>b</sup> Queens with more oocytes than their nestmates

locations. Background allele frequencies used to calculate relatedness were therefore estimated separately for each location.

#### Relatedness among sexual females and pedigree relationships

Nestmate queens were closely related (Table 2), with a mean ( $\pm$ SE) of  $0.65 \pm 0.25$ . The relatedness values among queens were significantly higher than those expected under the assumption of adoption of daughter queens over several generations (Table 2). There was no significant correlation between the number of queens in a colony and their relatedness (Pearson coefficient:  $r = -0.24$ ,  $P = 0.45$ ). Tests of pedigree relationships showed that almost all the queens in 8 colonies were likely to be full-sisters, the other queens probably being cousins. There were two groups of full-sisters in colony I2f (Table 2), which were cousins. The pattern obtained with the Kinship program for nest C6a was too complex to assign the queens to clear groups of full-sisters, but the queens were closely related, all the queens being at least cousins. Only in nest C4c were the queens not significantly related. Non egg-laying queens (without oocytes) and weakly egg-laying queens (one oocyte) were closely related to their nestmate queens with many oocytes (Table 2).

The relatedness estimates among winged females (young sexual females, since they had not yet shed their wings) were also high. Tests of pedigree relationships indicated that nestmate winged females were full-sisters (Table 2). Winged females were also related to queens of the same nest, although much less so than to their nestmate winged females (Table 2). Genetically effective queen numbers were estimated between 1.3 and 1.5 (Table 2), indicating that winged females were produced by only one or two queens. Tests of pedigree relationships showed that some of their nestmate queens, but only a few, could have been their mother (Table 2). Only in nest C6a was the pattern too complex to assign mothers to the winged queens, but there were only two main egg-laying queens in this nest, and they were possible mothers of the winged females.

## Discussion

This study shows that queenright colonies of *G. striatula* contain varying numbers of queens, 98% of them being mated. The relatedness values among nestmate queens are among the highest recorded for polygynous social insects (Herbers 1993; Rosengren et al. 1993; Keller 1995; Crozier and Pamilo 1996; Evans 1996). This raises the question of how relatedness can be so high, when models show that it declines rapidly with an increase in the number queens, even if joining queens are close kin (Nonacs 1988; Hughes et al. 1993). The queen-queen relatedness values observed in *G. striatula* are much higher than those expected if daughter queens are recruited over several generations. Inbreeding can lead to higher values of relatedness, but inbreeding does not seem to occur in *G. striatula* (Giraud et al., 2000).

The split sex ratio theory provides another hypothesis for the maintenance of high queen-queen relatedness. It predicts that colonies having lowest relatedness asymmetry will produce mostly males, and the others mostly females (Boomsma and Grafen 1991; Queller 1993; Queller et al. 1993; Sundström 1994; Chapuisat and Keller 1999). Thus, colonies with the most queens and the lowest relatedness asymmetry will rear mostly males, whereas colonies with few queens will invest more in females. Young queens will then be produced by very few mothers and will therefore be closely related. But this does not fit our data, since daughter winged females were found in the colonies with the most queens.

Our results suggest that the high queen-queen relatedness in polygynous colonies of *G. striatula* is due to reproductive skew for the production of sexuals. Dissection data, relatedness regression analysis, estimations of genetically effective queen numbers and pedigree relationship tests show that large groups of winged females are produced by only one or a few queens, and that these full-sisters are adopted after mating into their nest of origin. This process of recruitment can explain the maintenance of very high relatedness among nestmate queens. It can also account for the lack of correlation between the number of nestmate queens and their relatedness. Such a correlation is expected, even if joining females

**Table 2** Relatedness values ( $R$ ) among queens and winged females from 12 polygynous colonies of *G. striatula*, expected relatedness of nestmate queens under the assumption of adoption of daughter queens over several generations ( $r_{\text{exp}}$ ), genetically effective queen number ( $n_e$ ), and pedigree relationships. The SEs on  $R$  were calculated by jack-knifing over loci

Colony	Focal individuals	Number of females	$R \pm \text{SE}$	$r_{\text{exp}}$	$n_e$	Pedigree relationships
B1b	Among queens	12	0.85±0.12	0.08		11 full-sisters ( $P < 0.001$ ) and a cousin
B1c	Among queens and winged females	58	0.36±0.12			
	Among queens	43	0.93±0.06	0.02		30 full-sisters ( $P < 0.01$ ) and 10 cousins
	Among winged females	18	0.62±0.16		1.3	18 full-sisters ( $P < 0.01$ )
	Between queens and winged females	58	0.18±0.14			3 possible mothers of the winged females ( $P < 0.001$ ), belonging to the group of main egg-laying queens
	Between main egg-laying queens and other queens	43	0.87±0.11			
C2j	Among queens	3	0.14±0.42	0.30		2 full-sisters ( $P < 0.05$ )
C3e	Among queens	10	0.94±0.05	0.10		9 full-sisters ( $P < 0.01$ ) and a cousin ( $P < 0.01$ )
C4c	Among queens	3	0.29±0.48	0.30		$P > 0.05$
C4d	Among queens	13	0.70±0.14	0.07		One group of 8 full-sisters ( $P < 0.001$ ) and 3 cousins ( $P < 0.01$ )
C4f	Among queens	2	0.71±0.20	0.42		2 full-sisters ( $p < 0.001$ )
C6a	Among queens and mated winged females	54	0.56±0.18			
	Among queens	44	0.55±0.28	0.02		Complex pattern, at least cousins ( $P < 0.05$ )
	Among mated winged females	10	0.59±0.31		1.3	10 full-sisters ( $P < 0.001$ )
	Between queens and mated winged females	54	0.56±0.28			Complex pattern, but main egg-laying queens are possible mothers of the winged females
	Between main egg-laying queens and other queens	44	0.58±0.14			
I2a	Among queens	3	0.78±0.21	0.30		3 full-sisters ( $P < 0.01$ )
I2b	Among queens and winged females	5	0.48±0.07			
	Among queens	3	0.42±0.21	0.30		2 full-sisters ( $P < 0.001$ )
	Among winged females	2	0.55±0.30		1.5	2 full-sisters ( $P < 0.05$ )
	Between queens and winged females	5	0.50±0.30			1 possible mother of the winged females ( $P < 0.001$ )
I2f	Among queens	12	0.56±0.16	0.08		One group of 7 full-sisters ( $P < 0.001$ ) and one group of 4 full-sisters ( $P < 0.01$ )
	Between main egg-laying queens and other queens	8	0.57±0.14			
I2h	Among queens	2	0.90±0.12	0.42		2 full-sisters ( $P < 0.001$ )

originate from the nest, when queens are recruited over several generations, and if there is outbreeding (Nonacs 1988; Hughes et al. 1993). Keller (1995) found such a correlation across several ant species. But if joining females belong to large groups of full-sisters produced by one or a few queens, there should no longer be a correlation between the number of nestmate queens and their relatedness.

There are other examples of polygynous species in which queens are not equivalent in their reproductive success (e.g. Ross 1988; Heinze and Smith 1990; Heinze

1993; Keller and Reeve 1994; Bourke and Franks 1995, pp. 258–298). Some polygynous ant species have even been found to be functionally monogynous, with only one major egg-layer per colony (e.g. Buschinger 1968; Ito 1990; Heinze 1993). But their system appears different from that of *G. striatula* in two aspects. First, functional monogyny is established and maintained by aggressive interactions among potential queens (Heinze 1993), whereas no aggression between females has been observed in *G. striatula* (R. Blatrix, personal observation). Second, the relatedness among members of these func-

tionally monogynous colonies has been measured in only a few studies, but it does not appear to be extremely high, because there is a high turnover of the functional queens (Heinze 1995).

High queen-queen relatedness has been found in a few other polygynous social insects, but the mechanisms responsible for its maintenance were often not investigated (Keller 1995), or it was due to split sex ratios (Hastings et al. 1998), cyclical oligogyny (Hughes et al. 1993) or serial polygyny (Gadagkar et al. 1993; Seppä 1994).

This paper provides evidence for an original mechanism of queen recruitment in polygynous social insects, and offers clues to the answers to the evolutionary questions posed by polygyny in *G. striatula*. The high relatedness among nestmate queens should ensure that the brood and workers are closely related, and kin selection may therefore play a role in keeping the *G. striatula* workers to their role, despite their ability to become reproductives.

High queen-queen relatedness has also been suggested to lower the costs of sharing reproduction in polygynous colonies. Our results demonstrate that only one or a few queens per nest produce winged females, but almost all the queens are mated, and many have developed ovaries. This indicates that the other queens may lay workers and/or males, and would indeed mean that queens are sharing reproduction. Further studies are needed to test this hypothesis, but kin selection may then also play a role in the recruitment of queens into established colonies in *G. striatula*.

**Acknowledgements** We thank Yves Brygoo and the Pathologie Végétale group (INRA, France) for allowing part of the work to be done in their laboratory, Dominique Vautrin for technical assistance, Jacques Delabie for field and taxonomy expertise, Emmanuelle Baudry for many invaluable contributions, Laurent Keller, Jes S. Pedersen, Jacqui Shykoff and two anonymous reviewers for comments on earlier versions of the manuscript, and Owen Parkes for correcting the English text. This research was partly funded by the Cellule des relations internationales de l'Université Paris 13. The experiments conducted for this study comply with the current laws of France.

## References

- Blatrix R, Jaisson P (2000) Optional gamergates in the queenright ponerine ant *Gnamptogenys striatula* Mayr. *Insectes Soc* 47: 193–197
- Boomsma JJ (1993) Sex ratio variation in polygynous ants. In: Keller L (ed) *Queen number and sociality in insects*. Oxford University Press, Oxford, pp 86–109
- Boomsma JJ, Grafen A (1991) Intraspecific variation in ant sex ratios and the Trivers-Hare hypothesis. *Evolution* 44:1026–1034
- Bourke AFG, Franks NR (1995) *Social evolution in ants*. Princeton University Press, Princeton, NJ
- Bourke AFG, Green HAA, Bruford MW (1997) Parentage, reproductive skew and queen turnover in a multiple-queen ant analysed with microsatellites. *Proc R Soc Lond B* 264:277–283
- Buschinger A (1968) Mono- und Polygynie bei Arten der Gattung *Leptothorax* Mayr (Hymenoptera: Formicidae). *Insectes Soc* 15:217–226
- Chapuisat M, Keller L (1999) Testing kin selection with sex allocation data in eusocial Hymenoptera. *Heredity* 82:473–478
- Crozier RH, Pamilo P (1996) *Evolution of social insect colonies*. Oxford University Press, Oxford, pp 111–119
- Evans JD (1996) Queen longevity, queen adoption, and post-humous indirect fitness in the facultatively polygynous ant *Myrmica tahohensis*. *Behav Ecol Sociobiol* 39:275–284
- Gadagkar R, Chandrashekara K, Chandran S, Bhagavan S (1993) Serial polygyny in the primitively eusocial wasp *Ropalidia marginata*: implications for the evolution of eusociality. In: Keller L (ed) *Queen number and sociality in insects*. Oxford University Press, Oxford, pp 189–214
- Giraud T, Blatrix R, Solignac M, Jaisson P (1999) Polymorphic microsatellite DNA markers in the ant *Gnamptogenys striatula*. *Mol Ecol* 8:2143–2145
- Giraud T, Blatrix R, Poteaux C, Solignac M, Jaisson P (2000) Population structure and mating biology of the polygynous ponerine ant *Gnamptogenys striatula* in Brazil. *Mol Ecol* 9: 1835–1841
- Goodnight KF, Queller DC (1999) Computer software for performing likelihood tests of pedigree relationship using genetic markers. *Mol Ecol* 8:1231–1234
- Grafen A (1985) A geometric view of relatedness. In: Dawkins R, Ridley M (eds) *Oxford surveys in evolutionary biology*. Oxford University Press, Oxford, pp 28–89
- Hamilton WD (1964a) The genetic evolution of social behaviour I. *J Theor Biol* 7:1–16
- Hamilton WD (1964b) The genetic evolution of social behaviour II. *J Theor Biol* 7:17–52
- Hastings MD, Queller DC, Eischen F, Strassmann JE (1998) Kin selection, relatedness, and worker control of reproduction in a large-colony epiponine wasp, *Brachygastra mellifica*. *Behav Ecol* 6:573–581
- Heinze J (1993) Queen-queen interactions in polygynous ants. In: Keller L (ed) *Queen number and sociality in insects*. Oxford University Press, Oxford, pp 334–361
- Heinze J (1995) Reproductive skew and genetic relatedness in *Leptothorax* ants. *Proc R Soc Lond B* 261:375–379
- Heinze J, Smith TA (1990) Dominance and fertility in a functionally monogynous ant. *Behav Ecol Sociobiol* 27:1–10
- Herbers JM (1993) Ecological determinants of queen number in ants. In: Keller L (ed) *Queen number and sociality in insects*. Oxford University Press, Oxford, pp 262–293
- Hölldobler B, Wilson EO (1990) *The ants*. Harvard University Press, Cambridge, Mass
- Hughes CR, Queller DC, Strassmann JE, Solis CR, Negron-Sotomayor JA, Gastreich KR (1993) The maintenance of high genetic relatedness in multiple queen colonies of social wasps. In: Keller L (ed) *Queen number and sociality in insects*. Oxford University Press, Oxford, pp 153–170
- Ito F (1990) Functional monogyny in *Leptothorax acervorum* in Japan. In: C Veeresh, B Mallik, CA Viraktamath (eds) *Social insects and the environment*. Proc 11th Int Congr IUSSI. Oxford and IBH Publishing, New Delhi, p 251
- Keller L (ed) (1993) *Queen number and sociality in insects*. Oxford University Press, Oxford
- Keller L (1995) Social life: the paradox of multiple queen colonies. *Trends Ecol Evol* 10:355–360
- Keller L, Reeve HK (1994) Partitioning of reproduction in animal societies. *Trends Ecol Evol* 9:98–102
- Keller L, Vargo EL (1993) Reproductive structure and reproductive roles in colonies of eusocial insects. In: Keller L (ed) *Queen number and sociality in insects*. Oxford University Press, Oxford, pp 16–44
- Lattke JE (1995) Revision of the ant genus *Gnamptogenys* in the new world (Hymenoptera: Formicidae). *J Hymenoptera Res* 4:137–193
- Michener CD (1974) *The social behavior of the bees*. Harvard University Press, Cambridge, Mass
- Nonacs P (1988) Queen number in colonies of social Hymenoptera as a kin-selected adaptation. *Evolution* 42:566–580
- Pamilo P (1991) Evolution of colony characteristics in social insects. 2. Number of reproductive individuals. *Am Nat* 138:412–433
- Pedersen JS, Boomsma JJ (1999) Effect of habitat saturation on the number and turnover of queens in the polygynous ant, *Myrmica sulcidonis*. *J Evol Biol* 12:903–917

- Queller DC (1993) Genetic relatedness and its components in polygynous colonies of social insects. In: Keller L (ed) Queen number and sociality in insects. Oxford University Press, Oxford, pp 132–152
- Queller DC, Goodnight KF (1989) Estimating relatedness using genetic markers. *Evolution* 43:258–275
- Queller DC, Strassmann JE (1998) Kin selection and social insects. *Bioscience* 48:165–175
- Queller DC, Strassmann JE, Hughes CR (1988) Genetic relatedness in colonies of tropical wasps with multiple queens. *Science* 242:1155–1157
- Queller DC, Strassmann JE, Solis CR, Hughes CR, DeLoach DM (1993) A selfish strategy of social insect workers that promotes social cohesion. *Nature* 365:639–641
- Raymond M (1995) A population genetics software for exact tests and eucumenicism. *J Hered* 86:248–249
- Rosengren R, Sundström L, Fortelius W (1993) Monogyny and polygyny in *Formica* ants: the result of alternative dispersal tactics. In: Keller L (ed) Queen number and sociality in insects. Oxford University Press, Oxford, pp 308–333
- Ross KG (1988) Differential reproduction in multiple-queen colonies of the fire ant *Solenopsis invicta* (Hymenoptera: Formicidae). *Behav Ecol Sociobiol* 23:341–355
- Seppä P (1994) Sociogenetic organization of the ants *Myrmica ruginodis* and *Myrmica lobicornis*: number, relatedness and longevity of reproducing individuals. *J Evol Biol* 7:71–95
- Strassmann JE, Queller DC, Solfs CR, Hughes CR (1991) Relatedness and queen number in the neotropical wasp, *Parachartergus colobopterus*. *Anim Behav* 42:461–470
- Sundström L (1994) Sex ratio bias, relatedness asymmetry and queen mating frequency in ants. *Nature* 367:266–268
- Weir BS, Cockerham CC (1984) Estimating F-statistics for the analysis of population structure. *Evolution* 38:1358–1370
- Wilson EO (1974) The population consequences of polygyny in the ant *Leptothorax curvispinosus*. *Ann Entomol Soc Am* 67:781–786