

Michael A. D. Goodisman · Robert W. Matthews
Ross H. Crozier

Mating and reproduction in the wasp *Vespula germanica*

Received: 23 December 2000 / Revised: 21 January 2002 / Accepted: 4 January 2002 / Published online: 22 March 2002
© Springer-Verlag 2002

Abstract We used polymorphic microsatellite markers to study patterns of queen and worker reproduction in annual nests of the wasp *Vespula germanica* in its introduced range in Australia. We found that queens were typically polyandrous (at least 85.4% mated multiply), with the minimum number of male mates ranging from 1 to 7. Calculations based on nestmate worker relatedness ($r=0.46$) yielded an estimate of effective queen mating frequency of 2.35. Queens were unrelated to their mates ($r=-0.01$), indicating that mating occurred at random within Australian *V. germanica* populations. In addition, the distribution of the minimum number of male mates of queens followed a Poisson distribution. This result suggested that the probability of a queen remating was not affected by previous copulations. We also discovered that mates of polyandrous queens contributed unequally to progeny production leading to significant male reproductive skew within nests. Analyses of nestmate male genotypes revealed that queens usually produced most or all males. However, workers were responsible for the production of many males in a few nests, and, in contrast to theoretical expectations, two of these nests were apparently queenright.

Keywords DNA microsatellites · Relatedness · Polyandry · Social insects · Worker reproduction

Introduction

Considerable interest has centered on the mating frequency of queens in the eusocial Hymenoptera (reviewed by Bourke and Franks 1995; Boomsma and Ratnieks 1996; Crozier and Pamilo 1996; Foster and Ratnieks 2001a), because variation in queen mating frequency can alter colony genetic structure, which plays a key role in the evolution and maintenance of worker sterility (Hamilton 1964), the determination of colony-level sex ratios (Trivers and Hare 1976; Boomsma and Grafen 1991), and the behavior of workers in policing reproduction (Ratnieks 1988). A recent survey of eusocial Hymenoptera found that queens in most species mate with one or a few males, and that high levels of polyandry occur in only a few genera (Boomsma and Ratnieks 1996; Foster and Ratnieks 2001a). Understanding the evolution and effects of polyandry in eusocial insects requires documenting both the occurrence of, and life history patterns associated with, multiple mating in these lineages.

Social wasps (Vespidae) promise considerable insight in this regard. Boomsma and Ratnieks (1996) note that “the Vespidae are particularly interesting because they contain species characterized by both single and multiple paternity.” Foster et al. (1999) further propose that “the vespine wasps are a taxon in which paternity data should be particularly informative”, because the genus contains relatively few species and the phylogenetic relationships within the group have been extensively investigated (Carpenter 1987; Schmitz and Moritz 1998).

Our primary objective in this study was to use genetic techniques to determine mating and reproductive patterns of queens of the wasp *Vespula germanica* (Vespidae, Vespinae) in its introduced range of Australia. Native to the Palearctic region, *V. germanica* has been widely introduced around the world. So far as known,

Communicated by F. Ratnieks

M.A.D. Goodisman (✉) · R.H. Crozier
Department of Genetics, La Trobe University, Bundoora,
VIC 3083, Australia

M.A.D. Goodisman · R.H. Crozier
Department of Zoology and Tropical Ecology,
James Cook University, Townsville, QLD 4811, Australia

R.W. Matthews
Department of Entomology, University of Georgia, Athens,
GA 30602, USA

Present address:

M.A.D. Goodisman, Department of Biochemistry,
513 Biological Sciences West, University of Arizona, Tucson,
AZ 85721-0088, USA, e-mail: goodisma@email.arizona.edu,
Fax: +1-520-6219288

annual colonies are founded and headed by a single reproductive queen that produces all the nest's workers (Greene 1991; Spradbery 1991). An observational study in New Zealand suggests that *V. germanica* queens mate with only a single male (Thomas 1960). However, polyandry appears to be frequent among other *Vespula* species (Foster et al. 1999; Foster and Ratnieks 2001a). In this study, we compared *V. germanica* queen mating patterns in three geographically separated populations. Studies of reproductive behavior across populations can be particularly informative, because differences in queen mating frequencies among populations may signal the existence of variation in the selective pressures affecting polyandry.

As a secondary topic, we investigated the reproductive biology of *V. germanica* workers. *Vespula* workers do not mate and, therefore, cannot produce diploid offspring. However, workers are capable of producing males (Spradbery 1973; Greene 1991; Ross and Carpenter 1991) and are expected to do so under some circumstances. In particular, theoretical predictions suggest that worker reproduction will be favored if the effective paternity of queens is less than two. In this case, workers will be more closely related to nephews, which are produced by other workers, than to brothers, which are produced by the queen (Ratnieks 1988). In contrast, if the effective paternity of queens is greater than two, then workers will be more closely related to brothers than to nephews and should prevent other workers from reproducing. In this study, we used microsatellite markers to determine if *V. germanica* workers contribute to the production of males in natural populations and compared our findings to expectations derived from the effective mating frequency of queens.

Methods

V. germanica workers, males, and reproductive queens were collected from three populations in and around the cities of Adelaide, Canberra, and Melbourne in the austral summer and fall of 1998–1999. Wasps were netted at the nest entrance or collected after nests were killed and excavated. Samples were either frozen or immediately placed in 95% ethanol. Whenever possible the entire nest was collected, but since the queen was often not recovered in the nest debris we used the presence of diploid (queen and/or worker) brood as evidence that colonies recently contained a functional queen.

The genotypes of all individuals were assayed at three polymorphic microsatellite loci, Rufa 5, Rufa 18, and Rufa 19, as described by Goodisman et al. (2001). Allele frequencies and nest-mate worker relatedness (r_{ww}) were estimated with the program Relatedness 4.2 (Queller and Goodnight 1989) using the 'deme' function to account for differences in allele frequencies across populations. Nests were always weighted equally, and standard errors for estimates were obtained by jackknifing over nests. We used *t*-tests to determine whether relatedness estimates differed statistically from hypothesized values (e.g., 0.0 or 0.75) and applied Bonferroni corrections when multiple tests were performed (Rice 1989). We also tested for differences in relatedness among populations via a Kruskal-Wallis test on the individual nest-level relatedness estimates from each population.

Polyandry levels in *V. germanica* nests were investigated by two methods. First, we examined genotypic data directly to calcu-

late minimum male mate numbers (m_m) needed to explain worker genotypes. After establishing the reproductive queen's genotype by direct assay or by inference from the genotypes of nestmate workers and males, we determined the genotype(s) of her putative mate(s) by subtracting her alleles from the diploid worker genotypes.

When few workers are sampled or genetic markers display low levels of variation, m_m may underestimate the actual number of male mates (Pamilo 1993; Boomsma and Ratnieks 1996). To investigate possible error due to sampling few individuals per nest, we calculated Spearman's rank order correlation coefficient between the number of workers genotyped in a nest and minimum number of males detected. A significant correlation would suggest that sampling error contributed substantially to our m_m estimates. To investigate possible error due to limited genetic variation at our markers, we calculated the probability of two males having identical genotypes. This nondetection error was quantified by the formula

$$\left(\sum p_i^2\right) \left(\sum q_j^2\right) \left(\sum r_k^2\right) \quad (1)$$

where p_i , q_j , and r_k are the frequencies of alleles i , j , and k at the loci Rufa 5, 18, and 19, respectively (Boomsma and Ratnieks 1996).

To obtain insight into queen mating behavior, we tested the fit of three mathematical models, derived under alternate biological assumptions, to the distribution of nest-level estimates of m_m using a *G*-test for goodness-of-fit. First, if queens were equally likely to mate to any number of males (with the theoretical maximum number of male mates specified by the maximum number observed) then the frequency of each mating class should be equal and the distribution of m_m should conform to a uniform distribution. Second, if queens mated repeatedly until they mated with an 'optimal' male or a male that prevented the queen from mating further, then the values of m_m should follow a geometric distribution. And finally, if the probability of a queen obtaining a mate was independent of whether she had already mated, then the frequencies of mating classes should follow a Poisson distribution. Because we had no data on the number of queens that did not mate, we used the distribution of values of $m_m - 1$ when fitting the Poisson probability function.

The second metric of polyandry we considered, the effective number of mates (m_e), incorporates information both on the number of times that a queen mates and on the males' potentially unequal contribution to her offspring (Starr 1984). An estimate of m_e in single-queen colonies was obtained from worker relatedness estimates as

$$m_e = 2 / (4r_{ww} - 1). \quad (2)$$

We assessed the relatedness of queens to their mates in the 13 nests where the queen's genotype could be determined unambiguously. The genotypes of putative mates were reconstructed from worker genotypes and the relatedness of queens to their mates was estimated as described above.

Reproductive skew of the queens' mates was calculated in a subset of six nests selected because at least 40 workers were sampled, genotypes of the queen and her mates could be determined unambiguously, and all worker offspring could be assigned to only one male. Reproductive skew for a given nest was quantified by the statistic of Pamilo and Crozier (1996) as

$$S = (N_T - N_E) / (N_T - 1) \quad (3)$$

where N_T is the total number of male mates, and N_E the effective number of male mates, for the given worker array. The effective number of male mates can be estimated as $N_E = (n_w - 1) / (n_w \sum y_i^2 - 1)$ where n_w is the number of workers sampled and y_i is the proportional contribution of male i (Pamilo 1993; Pamilo and Crozier 1996). We determined the statistical significance of the male skew using a *G*-test for goodness of fit under the null hypothesis that all males contributed equally to progeny production.

Observed skew within nests could be caused by nondetection of mates. For example, if a queen were to mate with three males but two of these shared the same multilocus genotype, our methods might reveal significant skew between the two detectable male genotypes, even though none might have actually occurred among the three males. To determine the possible importance of such error, the single genotype nondetection error was obtained from (1) with slight modification as $p_i^2 q_j^2 r_k^2$, where p_i , q_j , and r_k are the frequencies of alleles i , j , and k at the loci Rufa 5, 18, and 19, that make up the multilocus genotype of the putative single male mate. We reasoned that if the probability of two males possessing this genotype were low, then nondetection due to limited power of our markers was unlikely to account for the observed skew.

We sought evidence of male production by workers in two ways. First, we directly examined genotypes of sampled males within nests. If all males were queen-produced, their genotypes would reflect those of the queen, and, as a group, they would display no more than two different alleles at a single microsatellite locus. Thus, the presence of more than two alleles at any locus provided evidence for worker reproduction. Second, in nests where at least 25 males had been sampled, we used a G -test to calculate the probability of the observed distribution of male genotypes, assuming that the queen's alleles segregated according to Mendelian laws. If the distribution differed significantly from expectations, we assumed that the deviation was caused by worker reproduction.

Results

We sampled 1,239 workers, 374 males, and 6 reproductive queens from 58 nests – 11 in Adelaide (ADL), 36 in Canberra (CBR), and 11 in Melbourne (MEL). Workers and males were sampled from 55 and 20 nests, respectively, and the genotypes of 21.14 ± 17.23 workers (mean \pm SD) and 6.34 ± 11.91 males were assayed per nest.

Worker genotypes within all nests were consistent with monogyny, confirming that only a single queen headed annual *V. germanica* colonies. The relatedness of workers ($r_{ww} = 0.52 \pm 0.06$, 0.38 ± 0.03 , 0.56 ± 0.06 , and 0.46 ± 0.03 in ADL, CBR, MEL, and all populations combined, respectively) fell significantly below the value of 0.75 expected if queens mated with only a single male ($t_8 = 3.79$, $P = 0.0053$; $t_{34} = 10.82$, $P < 0.001$; $t_{10} = 3.02$, $P = 0.013$; $t_{54} = 9.62$, $P < 0.001$ for the above estimates of r_{ww}). Therefore, these estimates indicated that *V. germanica* queens were polyandrous. Comparison of individual relatedness estimates across the three populations revealed no significant variation ($H_2 = 4.84$, $P = 0.089$).

Our estimates for the minimum number of male mates (m_m) exceeded one in 47 of the 55 nests from which workers were sampled (Table 1), indicating that *V. germanica* queens almost always mated multiply. However, the grand mean value of $\bar{m}_m = 2.73$ suggested that queens mated with relatively few males. The correlation between number of workers sampled and m_m did not differ significantly from zero ($r_s = 0.25$, $P = 0.068$ in all populations combined). This suggested that small worker samples from some nests did not contribute substantial error to our m_m estimate. Nevertheless, the positive correlations obtained indicated that the best estimates for m_m came from colonies from which more than 40 workers were sampled. The nondetection errors arising from males having identical multilocus genotypes obtained from (1) equaled 0.032, 0.155, 0.034, and 0.056 in ADL, CBR, MEL, and all populations combined. These low probabilities suggested that limited genetic variation at the microsatellite loci also did not lead to substantial underestimation of m_m .

We next determined the fit of uniform, geometric, and Poisson distributions to the values of the minimum number of male mates to better understand the processes governing queen mating frequency. We found that the uniform distribution failed to fit the observed data ($G_6 = 43.32$, $P < 0.001$), indicating that queens were not equally likely to fall into the seven observed mating classes. In addition, queen mating frequencies failed to follow the geometric distribution ($G_2 = 52.07$, $P < 0.001$), suggesting that queens did not continue to mate with an indefinite number of males until they mated with an optimal male or a male that prevented the queen from mating further. In contrast, the Poisson distribution did not differ significantly from the observed data ($G_3 = 6.63$, $P = 0.085$). Consequently, we cannot reject the possibility that matings occurred independently of each other, given that a queen mated at least once.

The estimates of worker relatedness from each population were used in conjunction with (2) to calculate the effective number of male mates (m_e). In ADL, CBR, MEL, and all populations combined, $m_e = 1.84$, 3.73, 1.60, and 2.35, respectively. Estimates of m_e typically were below the corresponding estimates of m_m , as expected when there is variation in the proportional contributions of males to offspring. However, in CBR m_e was

Table 1 Mean (\bar{m}_m) and range for the minimum number of male mates necessary to explain the genotype distributions of *V. germanica* workers in three Australian populations. Values are given

as a function of worker numbers sampled per nest and numbers of nests (N) in each category. – No data available

Population	Number of workers genotyped per nest								
	3–8			40–58			All samples combined		
	N	\bar{m}_m	Range	N	\bar{m}_m	Range	N	\bar{m}_m	Range
Adelaide	9	3.33	2–6	0	–	–	9	3.30	2–6
Canberra	18	2.11	1–5	17	2.65	1–4	35	2.37	1–5
Melbourne	3	2.33	1–4	8	3.75	2–7	11	3.36	1–7
All nests combined	30	2.50	1–6	25	3.00	1–7	55	2.73	1–7

Table 2 Male reproductive skew within six *V. germanica* nests from Australia. The number of workers sampled per nest (n_w), minimum number of male mates (m_m), proportional contribution of male mates (y), estimate of reproductive skew (S), P values under the null hypothesis of equal male contributions (P), and probability of a second male possessing the same genotype of the most productive male mate are given for each nest (see text for details)

Nest no.	n_w	m_m	y	S	P	Probability of another male possessing genotype of most productive male mate
20	58	2	0.672, 0.328	0.187	0.0080	0.033
35	40	3	0.525, 0.375, 0.100	0.285	0.0016	<0.001
36	40	2	0.650, 0.350	0.211	0.056	0.0039
39	40	5	0.300, 0.275, 0.200, 0.200, 0.025	0.234	0.014	<0.001
48	40	2	0.900, 0.100	0.815	<0.001	0.084
53	40	2	0.750, 0.250	0.375	0.0012	0.084

substantially greater than m_m indicating that our m_m estimate fell below the actual number of male mates, in part because the population lacked sufficient variation to detect all the queens' mates.

In the 13 nests where queen genotype could be unambiguously determined, we estimated the relatedness of queens to their mates. The values (-0.04 ± 0.09 , 0.06 ± 0.15 , and -0.01 ± 0.07 for CBR, MEL, and both populations combined; no estimates were obtained for ADL) did not differ significantly from zero ($t_8=0.42$, $P=0.68$; $t_3=0.37$, $P=0.38$; $t_{12}=0.09$, $P=0.93$), nor did estimates differ significantly between populations ($H_1=1.17$, $P=0.28$). Therefore, there was no evidence that *V. germanica* queens and their mates were significantly related.

Within six selected nests, paternity skew ranged from $S=0.187$ to 0.815 , with a mean of 0.351 (from equation (3), Table 2). Under the null hypothesis of equal contribution of all males, individual G -tests for goodness of fit indicated that reproductive skew usually led to statistically significant bias in male contribution. The probability of a second male possessing the genotype of the most productive male contributor was relatively high in three nests (Table 2, nests 20, 48, and 53), leaving open the possibility that more than a single male of this genotype contributed to offspring within those nests. In three others (nests 35, 36, and 39), the probability of two males having the same genotype was low, suggesting that multiple males with a similar genotype probably did not mate with the queen. Thus, we conclude that significant male reproductive skew occurred in at least half of the nests for which appropriate data were available.

To determine whether workers produced males, we analyzed male genotypes within 17 nests that produced more than two males (the minimum number needed to detect worker reproduction in our nests). In four nests (three from ADL and one from CBR), sampled males as a group possessed three or more alleles at a locus. In all cases, multiple males showed anomalous genotypes, strongly suggesting that microsatellite mutation was not responsible for the genotypic patterns observed. In addition, in two of these nests, workers were sampled, and all the alleles present in the males were also present in these workers, bolstering the hypothesis that the resident

workers produced males. In ten nests where both males and workers were genotyped, workers possessed at least three alleles, while males possessed no more than two (i.e., we possessed the power to detect worker reproduction); thus, data from these nests were consistent with production of males by the queen alone.

In the six nests from which more than 25 males had been sampled, we examined the distribution of male genotypes for deviations from expectations based on Mendelian segregation. In a single nest from CBR, male genotypes differed from this expectation after Bonferroni corrections ($G_3=16.92$, $P<0.001$). We concluded that this deviation arose because of worker reproduction; the alternate hypothesis of meiotic drive seemed less likely.

Thirteen of the 19 nests from which males were sampled also contained queen or worker brood. (Data on brood presence were unavailable for the remaining six nests.) The presence of diploid brood suggested that the queen was present when the sampled males were produced. Importantly, two of these 13 nests also contained worker-produced males. These results suggest that *V. germanica* workers reproduced in the presence of the queen.

Discussion

Polyandry in *V. germanica*

Our estimates for the minimum number of male mates (m_m) indicated that *V. germanica* queens in Australia were typically polyandrous. However, rather than pairing with extremely large numbers of males – as occurs, for example, in *Apis* bees (Oldroyd et al. 1998) – queens mated with moderate (1–7) numbers of males. The high frequency of polyandry in *V. germanica* contrasts with a report from an observational study in New Zealand, in which queens were reported to have mated with only a single male (Thomas 1960). Our study thus demonstrates the utility of genetic markers for investigating mating systems.

The distribution of the per-queen m_m values provided some insight into the processes involved in *V. germanica* mating behavior. Our data indicated that the probability

of a queen remating was not influenced by the number of times that she had previously mated. This implies that queens did not obtain some optimal number of mates, as would be the case, for example, if queens necessarily required large numbers of mates for reproduction. In addition, the apparent independence of mating events does not support hypotheses that queen mating behavior terminated after mating with a male that possessed some particular optimal attribute or a male that prevented a queen from mating further (e.g., by inserting a mating plug). Our results were also consistent with mating events being randomly distributed among queens, as would be the case if chance elements influenced queen mating success.

The effective number of male mates of *V. germanica* queens ($m_e=2.35$) was similar to other estimates obtained in the genus. *V. squamosa* and *V. maculifrons* queens displayed somewhat higher effective mating frequencies of $m_e=7.14$ and 3.27 , respectively (Ross 1986). In contrast, *V. vulgaris* queens displayed a lower value, with $m_e=1.90$ (Foster and Ratnieks 2001b). In the confamilial genera *Dolichovespula* and *Vespa*, effective mating frequencies have tended to be lower than in *Vespula* (Foster et al. 1999, 2001), but actual effective mating frequency appears to be a highly labile trait that can change over relatively short periods of evolutionary time.

We tested for differences in m_e among the three *V. germanica* populations sampled. This test failed to reveal significant differences in effective paternity across populations. Consequently, our data provided no evidence that queens mated with different numbers of males in distinct populations, as would occur if the ratio of queens to males varied geographically. In addition, we had no reason to believe that male reproductive skew, which also affects estimates of m_e , differed substantially across our sampling sites.

Our estimates of the relatedness of *V. germanica* queens to their mates did not differ significantly from zero. Thus mating appears to have taken place at random within the studied populations. Our results agree with behavioral observations of *Vespula* queens mating away from the nest in mating swarms (Greene 1991), but fail to support observations from laboratory experiments showing *V. maculifrons* queens preferred siblings as mates (Ross 1983). However, the latter study also noted that mating was inhibited by darkness (i.e., the condition within the nest). This further supports the hypothesis that *Vespula* queens mate outside of the nest with unrelated males.

Our genetic analyses also allowed us to investigate aspects of male reproduction in *V. germanica*. Specifically, patterns of male reproductive skew within three of six nests examined indicated that males did not contribute equally to the production of offspring. This result agrees with previous studies of *V. squamosa* and *V. maculifrons*, which found that male reproductive skew was relatively high among workers residing within nests headed by multiply-mated queens (Ross 1986). In contrast, paternal contributions were distributed more equally in *V. vulgaris* (Foster and Ratnieks 2001b). Nevertheless, our results

support the idea that male reproductive skew is common among eusocial Hymenoptera (Boomsma 1996; Boomsma and Ratnieks 1996; Keller et al. 1997; Boomsma and Sundström 1998).

In a recent survey in *Formica* ants, paternity skew in colonies headed by doubly-mated queens tended to be relatively low in species where queens frequently mated twice, as opposed to once (Boomsma and Sundström 1998). Overall, our results are somewhat at variance with this finding. Skew in nests headed by doubly-mated *V. germanica* queens (\bar{S} , Table 2) rose above that expected for a *Formica* species with comparable rates of polyandry ($S=0.147$, from Fig. 1 of Boomsma and Sundström 1998). We note that *Formica* queens rarely mate with as many males as *V. germanica* queens. Thus the disagreement between the observed data and the model may be associated with differences in overall mating strategies between *Vespula* wasps and *Formica* ants.

Current theory suggests that the evolutionary importance of reproductive skew may be related to sex allocation patterns in the eusocial Hymenoptera (Boomsma 1996; Boomsma and Sundström 1998). If workers produce split sex ratios based on the relatedness asymmetry to the sexuals they rear, then males and females may have evolutionary conflicts of interest and the male mates may favor reproductive skew. However, the sex ratio data currently available for *Vespula* suggest that colonies do not produce split sex ratios (e.g., MacDonald et al. 1974; MacDonald and Matthews 1976). Therefore it is unlikely that the reproductive skew observed in *V. germanica* is derived from long-term evolutionary strategies of males based on relative relatedness asymmetries.

Worker reproduction in *V. germanica*

Our results indicated that workers produced males in some nests. Significantly, the presence of worker brood in two of these nests implied that worker reproduction occurred in the presence of the queen. Worker reproduction of males has been documented in other *Vespula* wasps (Spradbery 1973; Ross 1986; Ross and Carpenter 1991), but available data suggest that workers typically reproduce only if the queen dies (Ross 1986).

Worker policing of worker-laid eggs is expected in species in which queens effectively mate with two or more males (Ratnieks 1988). Our finding of worker reproduction in queenright colonies of *V. germanica* contrasts with this expectation. Moreover, previous studies in other *Vespula* wasps failed to find evidence of worker reproduction in the presence of the queen (Ross 1986; Foster and Ratnieks 2001b). We note that we did not directly sample the queens presumed to be heading the two colonies that contained both worker brood and worker-produced males; rather, their existence was inferred by the presence of worker brood. Therefore, it remains possible that either the queen had died or worker-produced males had eclosed very recently. Alternatively, another queen could have usurped the colony, leading to anoma-

lous genotype distributions. Regardless, the potential importance of this result suggests that further study in *V. germanica* is warranted for a better understanding of worker reproduction in the eusocial Hymenoptera.

Acknowledgments We thank P.A. Thorén for providing unpublished microsatellite primer sequences, A.D. Austin, M.E. Carew, J.G. Ewen, J.K. Hatt, M. Sanetra, J.P. Spradbery, and the Ararat Euro Wasp Research Group for providing samples, and J.G. Ewen, E.J. Fjerdingstad, J. Matthews, K.G. Ross, M. Sanetra, and G.J. Thompson for comments on earlier versions of this manuscript. This research was supported in part by a National Science Foundation Postdoctoral Fellowship in the Biosciences #DBI-9804263 (MADG) and a Commonwealth Scientific and Industrial Research Organization McMaster Fellowship (RWM). Nests from Adelaide were obtained with the help of M. Kasper and G. Wood, supported by a grant from the Office of Local Government in South Australia to the South Australian Research and Development Institute, Waite Campus of the University of Adelaide.

References

- Boomsma JJ (1996) Split sex ratios and queen-male conflict over sperm allocation. *Proc R Soc Lond B* 263:697–704.
- Boomsma JJ, Grafen A (1991) Colony-level sex ratio selection in the eusocial Hymenoptera. *J Evol Biol* 3:383–407
- Boomsma JJ, Ratnieks FLW (1996) Paternity in eusocial Hymenoptera. *Philos Trans R Soc Lond* 351:947–975
- Boomsma JJ, Sundström L (1998) Patterns of paternity skew in *Formica* ants. *Behav Ecol Sociobiol* 42:85–92
- Bourke AFG, Franks NR (1995) *Social evolution in ants*. Princeton University Press, Princeton, N.J.
- Carpenter JM (1987) Phylogenetic relationships and classifications of the Vespinae (Hymenoptera: Vespidae). *Syst Entomol* 12:413–431
- Crozier RH, Pamilo P (1996) *Evolution of social insect colonies: Sex allocation and kin selection*. Oxford University Press, Oxford
- Foster KR, Ratnieks FLW (2001a) Paternity, reproduction and conflict in vespine wasps: a model system for testing kin selection predictions. *Behav Ecol Sociobiol* 50:1–8
- Foster KR, Ratnieks FLW (2001b) Convergent evolution of worker policing by egg eating in the honeybee and common wasp. *Proc R Soc Lond B* 268:169–174
- Foster KR, Seppä P, Ratnieks FLW, Thorén PA (1999) Low paternity in the hornet *Vespa crabro* indicates that multiple mating by queens is derived in vespine wasps. *Behav Ecol Sociobiol* 46:252–257
- Foster KR, Ratnieks FLW, Gyllenstrand N, Thorén PA (2001) Colony kin structure and male production in *Dolichovespula* wasps. *Mol Ecol* 10:1003–1010
- Goodisman MAD, Matthews RW, Crozier RH (2001) Hierarchical genetic structure of the introduced wasp *Vespula germanica* in Australia. *Mol Ecol* 10:1423–1432
- Greene A (1991) *Dolichovespula* and *Vespula*. In: Ross KG, Matthews RW (eds) *The social biology of wasps*. Comstock, Ithaca, N.Y. pp 263–305
- Hamilton WD (1964) The genetical evolution of social behaviour I–II. *J Theor Biol* 7:1–52
- Keller L, Sundström L, Chapuisat M (1997) Male reproductive success: paternity contribution to queens and workers in *Formica* ants. *Behav Ecol Sociobiol* 41:11–15
- MacDonald JF, Matthews RW (1976) Nest structure and colony composition of *Vespula vidua* and *V. consobrina* (Hymenoptera: Vespidae). *Ann Entomol Soc Am* 69:471–475
- MacDonald JF, Akre RD, Hill WB (1974) Comparative biology and behavior of *Vespula atropilosa* and *V. pennsylvanica* (Hymenoptera: Vespidae). *Melanderia* 18:1–93
- Oldroyd BP, Clifton MJ, Parker K, Wongsiri S, Rinderer TE et al. (1998) Evolution of mating behavior in the genus *Apis* and an estimate of mating frequency in *Apis cerana* (Hymenoptera: Apidae). *Ann Entomol Soc Am* 91:700–709
- Pamilo P (1993) Polyandry and allele frequency differences between the sexes in the ant *Formica aquilonia*. *Heredity* 70:472–480
- Pamilo P, Crozier RH (1996) Reproductive skew simplified. *Oikos* 75:533–535
- Queller DC, Goodnight KF (1989) Estimating relatedness using genetic markers. *Evolution* 43:258–275
- Ratnieks FL (1988) Reproductive harmony via mutual policing by workers in eusocial Hymenoptera. *Am Nat* 132:217–236
- Rice WR (1989) Analyzing tables of statistical tests. *Evolution* 43:223–225
- Ross KG (1983) Laboratory studies of the mating biology of the Eastern yellowjacket, *Vespula maulifrons* (Hymenoptera: Vespidae). *J Kans Entomol Soc* 56:523–537
- Ross KG (1986) Kin selection and the problem of sperm utilization in social insects. *Nature* 323:798–800
- Ross KG, Carpenter JM (1991) Population genetic structure, relatedness, and breeding systems. In: Ross KG, Matthews RW (eds) *The social biology of wasps*. Comstock, Ithaca, N.Y. pp 451–479
- Schmitz J, Moritz RFA (1998) Molecular phylogeny of Vespidae (Hymenoptera) and the evolution of sociality in wasps. *Mol Phyl Evol* 9:183–191
- Spradbery PJ (1973) *Wasps: an account of the biology and natural history of solitary and social wasps*. Sidgwick and Jackson, London
- Spradbery JP (1991) Evolution of queen number and queen control. In: Ross KG, Matthews RW (eds) *The social biology of wasps*. Comstock, Ithaca, N.Y. pp 336–388
- Starr CK (1984) Sperm competition, kinship, and sociality in the aculeate Hymenoptera. In: Smith RL (ed) *Sperm competition and the evolution of animal mating systems*. Academic Press, New York, N.Y. pp 427–464
- Thomas CR (1960) The European wasp (*Vespula germanica* Fab.) in New Zealand. *N Z Dep Sci Ind Res Inf Ser* 27:1–74
- Trivers RL, Hare H (1976) Haplodiploidy and the evolution of the social insects. *Science* 191:249–263