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Research article

Mating frequency in *Apis florea* revisited (Hymenoptera, Apidae)

K. A. Palmer and B. P. Oldroyd

School of Biological Sciences, A12, University of Sydney, NSW 2006, Australia, e-mail: kpalmer@bio.usyd.edu.au; boldroyd@bio.usyd.edu.au

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Summary. Effective and actual mating frequency of *Apis florea* queens was estimated using 92–159 worker pupae from each of five colonies. Mating frequency ranged from 13–19. The mean effective paternity frequency was 10.1 ± 1.2 (± SE). These estimates are significantly greater than those previously reported (5–14 matings). Our new estimates show that the effective mating frequency of *Apis florea* queens is similar to that found in the rest of the genus.

Key words: Polyandry, microsatellites, multiple mating, relatedness, *Apis.*

Introduction

High intracolonial genetic relatedness is thought to have facilitated the evolution of eusociality in Hymenoptera, but some of the most advanced social insect societies are actually characterised by very low relatedness as a consequence of extreme polyandry or polygyny (Crozier and Pamilo, 1996). The most extreme cases of multiple mating by queens are found in the genus *Apis.* Some *Apis* queens are estimated to mate with 44 males or more (Moritz et al., 1996).

Moderate levels of polyandry (6–10 matings) lead to decreased variance in average brood viability (Page, 1980; Page and Metcalf, 1982) and decreased worker-queen conflict over sex ratios (Moritz, 1985; Queller, 1993), and it seems likely that these two factors provided the key selective forces for the transition from presumed ancestral monandry to polyandry (Palmer and Oldroyd, 2000). However, additional matings beyond 6–10 have a minimal affect on these two parameters (Fuchs and Moritz, 1998), and it is difficult to see how these factors alone could have driven the evolution of the extreme levels of polyandry observed in all extant species of *Apis* (Boomsma and Ratnieks, 1996). This is especially so as the costs associated with additional matings are suspected to be high for this genus (Moritz, 1985). Several explanations for the evolution of extreme multiple mating have been offered, most suggesting that increased genetic diversity of workers increases the fitness of the colony (Keller and Reeve, 1994) either by increasing task specialization (Fuchs and Moritz, 1998) or reducing susceptibility to disease (Sherman et al., 1988; Shykoff and Schmid-Hempel, 1991).

A. florea occupies a basal position within the genus *Apis* (Alexander, 1991). Furthermore, based on published estimates (Oldroyd et al., 1995), it appears to have an exceptionally low mating frequency of 5–14. This could be construed as indicating that *A. florea* is intermediate between the ancestral condition of moderate polyandry (6–10 matings) and the derived condition of extreme polyandry (> 10 matings). The aim of this note is to correct that perception. Oldroyd et al. (1995) examined only 24–71 bees per colony based on the expectation from sperm count studies (Koeniger et al., 1989; Woyke 1993) that *A. florea* mates less than four times. Recent technological advances, including the use of Chelex for DNA extraction (Walsh et al., 1991) and automated DNA analysers for electrophoresis and data capture, have greatly increased the number of bees that can be feasibly examined. Because of these advances we are now able to present a more comprehensive analysis of mating frequency in *A. florea*. Understanding whether extreme polyandry is a universal trait in the genus *Apis* is required to properly interpret the evolutionary origin and significance of this trait.

Materials and methods

Worker pupae, pre-pupae and larvae were obtained from combs collected near Lampang, Northern Thailand in 1993, some of which were originally examined by Oldroyd et al. (1995). Ninety-four workers were taken from colony 1, which had not been previously examined. Colonies 2, 3, 4 and 5 were analysed using 92, 93, 93 and 159 workers respectively.

DNA was extracted from pre-pupae and larvae using the Chelex® method of extraction (Walsh et al., 1991). DNA extractions were amplified by polymerase chain reaction (PCR) with primers specific to the same 5 microsatellite loci (A8, A76, A88, A107, B124) as used by Oldroyd et al. (1995), according to the methods of Oldroyd et al. (2000). Genotypes of queens and drones were inferred from the observed worker genotypes according to the methods of Oldroyd et al. (1997). Allele frequencies were estimated from drone alleles observed across all colonies. The expected frequency of each drone genotype in the population was estimated by multiplying the allele frequencies at each

locus. The expected frequency of non-detected patrilines (d_p) was calculated according to Foster et al. (1999) and Boomsma and Ratnieks (1996). We calculated the effective number of matings and the average coefficient of relatedness according to Pamilo (1993).

Results and discussion

We observed 13–19 patrilines (Table 1) in the five colonies studied. Mean observed paternity frequency was 16.0 ± 2.5, mean effective paternity frequency 10.1 ± 1.2 and mean relatedness 0.302 ± 0.005. In every case, the estimates of mating frequency are much greater than the earlier ones (Oldroyd et al., 1995).

Within each colony studied, all patrilines did not contribute equally to the worker population (χ^2 test performed on each colony, in all cases $p < 0.001$). We found a significant positive correlation between the number of workers from each patriline and the expected frequency of the drone genotype (Spearman $\rho = 0.529$, $df = 78$, $p < 0.01$). This could partially explain the unequal distribution of workers in each patriline.

Table 1. Genotypes[a] of queens and drones for five microsatellite loci, number of workers sired by each drone and expected frequency of drone genotype for the five colonies studied

	Microsatellite loci					Number of workers	Expected frequency of drone genotype
	A8	A76	A88	A107	B124		
Number of alleles	5	2	4	4	3		
Colony 1							
Queen allele 1	*166*	*197*	*143*	*105*	*192*		
Queen allele 2	*168*	*197*	*145*	*108*	*194*		
Drone 1	166	197	143	105	192	21	0.040
Drone 2	166	197	143	110	192	14	0.0378
Drone 3	166	197	150	110	194	6	0.0014
Drone 4	166	197	139	105	192/194	2	0.0219
Drone 5	168	197	139	110	192/194	1	0.0198
Drone 6	168	197	139	105	192/194	3	0.0210
Drone 7	168	197	143	110	192	20	0.0363
Drone 8	168	197	150	110	192/194	5	0.0030
Drone 9	166/168	197	143/145	110	194	2	0.0901
Drone 10	166	199	143	105	192	8	0.0116
Drone 11	166	199	143	118	192	4	0.0010
Drone 12	166	199	145	118	192	5	0.0051
Drone 13	168	199	145	118	192	3	0.0005
					Total	94	
Colony 2							
Queen allele 1	*166*	*197*	*143*	*105*	*194*		
Queen allele 2	*168*	*197*	*143*	*110*	*194*		
Drone 1	166	197	139	105	190	2	0.0030
Drone 2	166	197	139	110	190	3	0.0028
Drone 3	166	197	143	110	190	2	0.0092
Drone 4	166	197	143	105	192	9	0.0402
Drone 5	166	197	143	110	192	4	0.0379
Drone 6	166	197	139	110	192	3	0.0114
Drone 7	166	197	143	110	194	3	0.0348
Drone 8	168	197	139	105/110	192	2	0.0226
Drone 9	168	197	143	105	192	6	0.0385
Drone 10	168	197	143	108	192	7	0.0099
Drone 11	168	197	143	110	192	23	0.0363
Drone 12	168	197	143	105/110	194	3	0.0601
Drone 13	168	199	143	110	194	10	0.0085
Drone 14	170	197	143	105/110	190	4	0.0044
Drone 15	170	197	143	105	192	3	0.0093
Drone 16	170	199	139	108	192	2	0.0002
Drone 17	178	199	143	105/110	192	2	0.0033
Drone 18	178	199	143	110	194	4	0.0013
					Total	92	

[a] Due to differences in methodology allele sizes are not always identical to those reported in Oldroyd et al. (1995), but differences between alleles remain consistent between the two studies.

Table 1 (continued)

	Microsatellite loci					Number of workers	Expected frequency of drone genotype
	A8	A76	A88	A107	B124		
Number of alleles	5	2	4	4	3		
Colony 3							
Queen allele 1	*166*	*197*	*143*	*105*	*194*		
Queen allele 2	*172*	*199*	*145*	*118*	*192/194*		
Drone 1	166	197	143/145	105	192/194	10	0.1096
Drone 2	166	199	143/145	105	192	6	0.0176
Drone 3	166	199	143/145	105	194	9	0.0141
Drone 4	166	199	143/145	110	194	2	0.0134
Drone 5	168	197	143/145	105	192/194	20	0.1049
Drone 6	168	197	139	105	192/194	1	0.0209
Drone 7	168	197	143	110	194	3	0.0291
Drone 8	168	197	143/145	105	190	3	0.0142
Drone 9	168	199	139	110	192/194	1	0.0057
Drone 10	168	199	143/145	105	192/194	17	0.0305
Drone 11	168	199	143/145	110	192/194	11	0.0287
Drone 12	170	199	145	105	192/194	2	0.0025
Drone 13	178	199	139	105	192/194	2	0.0009
Drone 14	178	199	139	110	192/194	4	0.0009
Drone 15	178	199	143	105	192/194	2	0.0031
					Total	93	
Colony 4							
Queen allele 1	*168*	*197*	*143*	*105*	*192*		
Queen allele 2	*168*	*197*	*143*	*110*	*194*		
Drone 1	166	197	143	105	194	2	0.0323
Drone 2	166	197	143	110	192	4	0.0379
Drone 3	166	197	143	110	194	5	0.0305
Drone 4	166	197	145	105	192	4	0.0205
Drone 5	166	197	145	105	194	5	0.0165
Drone 6	166	197	145	108	192	1	0.0005
Drone 7	166	197	145	110	192	3	0.0194
Drone 8	166	197	145	110	194	4	0.0156
Drone 9	168	197	143	105	192	10	0.0385
Drone 10	168	197	143	105	194	12	0.0310
Drone 11	168	197	143	108	194	1	0.0080
Drone 12	168	197	143	110	192	8	0.0363
Drone 13	168	197	143	110	194	11	0.0292
Drone 14	168	197	145	105	192	10	0.0197
Drone 15	168	197	145	105	194	5	0.0158
Drone 16	170	197	145	105	192	1	0.0048
Drone 17	170	197	145	105	194	1	0.0038
Drone 18	170	197	145	110	192	3	0.0045
Drone 19	170	197	145	110	194	3	0.0036
					Total	93	
Colony 5							
Queen allele 1	*168*	*197*	*143*	*105*	*192*		
Queen allele 2	*166/168*	*197*	*145*	*105*	*192*		
Drone 1	166/168	197	143	105	190	8	0.0192
Drone 2	166/168	197	143	108	190	1	0.0049
Drone 3	166/168	197	143	110	190	2	0.0181
Drone 4	166/168	197	145	108	190	1	0.0025
Drone 5	166/168	197	145	110	190	17	0.0092
Drone 6	166/168	197	139	105	192	4	0.0238
Drone 7	166/168	197	143	105	192	33	0.0786
Drone 8	166/168	197	145	105	192	23	0.0402
Drone 9	166/168	197	145	108	192	8	0.0103
Drone 10	166/168	197	143	110	192	10	0.0741
Drone 11	166/168	197	143	105	194	15	0.0633
Drone 12	166/168	197	143	110	194	10	0.0597
Drone 13	166/168	197	143	108	194	9	0.0163
Drone 14	166/168	197	143	108	192	13	0.0202
Drone 15	166/168	197	145	105	194	5	0.0324
					Total	159	

It also indicates that our paternity frequencies may be underestimates due to some patrilines being undetected due to males of identical genotype siring two different patrilines. However, the non-detection error within this study was low (d_p = 0.016). A possible reason for this is that the non-detection error is underestimated as it is based on allele frequencies of putative drones. Therefore the estimated distribution of allele frequencies is less skewed than the true distribution.

These results demonstrate that *A. florea* queens mate many more times than previously estimated and a similar number of times as the rest of the genus *Apis*. Thus high mating frequency appears to be ubiquitous to the genus. Why this should be so remains an important unanswered question in social insect biology.

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