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Polyandry in the genus *Apis*, particularly *Apis andreniformis*

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Abstract Using four polymorphic microsatellite loci, we found that four *Apis andreniformis* queens collected in Thailand each mated at least 10–20 times, producing an average relatedness, g_{ww}, of workers of 0.30 ± 0.007, and an average effective number of matings of 9.1 ± 2.2. The degrees of polyandry and intracolonial genetic relatedness in *A. andreniformis* are similar to those in *A. mellifera*, slightly more than in *A. florea*, and up to 6 times less than in *A. dorsata*. We argue that while presently favoured hypotheses for the evolution of polyandry in monogynous social insects may adequately explain the evolution of up to five or six matings, they are inadequate to explain the extreme polyandry (10–60 matings) observed in *Apis*. One alternative possibility is that colony fitness is a non-additive function of the fitness of individual subfamilies. Such behavioral over-dominance may mean that queen fitness is increased by high levels of polyandry, which increase the probability of desirable combinations of worker genotypes occurring in one colony. The special attributes of honey bees which may lead to behavioral over-dominance include colony aggregation (which may increase the incidence of disease), and frequent long-distance migration.

Key words Polyandry · Multiple mating · *Apis* · Behavioral dominance · Microsatellites

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Introduction

Because of the apparent inclusive fitness benefits of monandry (Hamilton 1964; Gadagkar 1990), and the potential costs of polyandry (due, for example, to increased risk of predation and sexually transmitted disease: Moritz 1985), evolutionary explanations for the wide-spread existence of polyandry in social insects (Page and Metcalf 1982) have been frequently sought (Crozier and Page 1985). Recent interest has focused on genetic variance (GV) hypotheses (Pamilo 1993; Keller and Reeve 1994) which suggest that queen and colony fitness is increased by the greater intra-colonial genetic diversity that is the consequence of polyandry.

The GV hypotheses can be divided into two broad categories. The first group relate to postulated fitness increases stemming from genetic diversity within the worker population. These hypotheses suggest that genetic diversity within the worker population leads to greater colony fitness because colonies comprised of particular combinations of worker genotypes are fitter than colonies comprised of just one genotype (Moritz and Hillesheim 1989; Oldroyd et al. 1991a,b, 1992a,b, 1993, 1994; Fuchs and Schade 1994). The second set of hypotheses arise from male haploidy, and relate to the effects of sex determination on brood viability (Page 1980; Page and Metcalf 1982; Ratnieks 1990), and to conflict between workers and queens over optimal sex ratios (Moritz 1985; Ratnieks and Boomsma 1995).

The subset of hypotheses relating to worker diversity are:

1. Genetic variance allows an increased expression of caste (Crozier and Page 1985) or task (Calderone et al. 1989; Calderone and Page 1991; Oldroyd et al. 1991a,b, 1992a, 1993, 1994; Fewell and Page 1993; Dreller et al. 1995) polymorphism.
2. Genetic variance increases the range of environments a colony can tolerate (Crozier and Page 1985; Oldroyd et al. 1992a,b, 1995b, 1996).

3. Genetic variance increases colonial resistance to parasites and pathogens (Sherman et al. 1988; Shykoff and Schmid-Hempel 1991 a,b).
4. Polyandry helps colonies buffer environmental variance (Crozier and Page 1985; Page et al. 1995).

The subset of hypotheses relating to sex determination and sex ratios suggest:

1. In swarming species, because queens with low brood viability due to diploid male production have low fitness, polyandry has evolved because it reduces variance in the production of diploid males among colonies (Shaskolsky 1976; Page 1980; Ratnieks 1990; Crozier and Pamilo 1996).
2. That polyandry reduces conflict between queens and workers over preferred sex ratios (Moritz 1985; Pamilo 1993; Queller 1993; Ratnieks and Boomsma 1995).

There are other, less plausible, hypotheses for the evolution of polyandry. For example, polyandry might foster beneficial intra-spermathecal sperm competition (as has been proposed for some snakes: Madsen et al. 1992). This hypothesis was discarded by Crozier and Page (1985) because there is no reason to expect that traits which help sperm succeed in fertilisation would be well correlated with colony fitness. It has also been proposed that polyandry is necessary to provide enough sperm for a long-lived queen to continue laying (Cole 1983), but this seems implausible in the light of evidence that queens discard most of the spermatozoa they receive during mating (Crozier and Page 1985).

Oldroyd et al. (1996) argued that only the GV hypotheses, coupled with behavioral dominance, could explain the evolution of extreme polyandry (beyond six matings). For example, a particular subfamily might confer resistance to a particular viral infection on the whole colony by rapidly removing infected eggs, but have no resistance to an important parasite. Another subfamily might diligently search out parasites, but have no viral resistance. If this is the case, then selection by pathogen resistance is "unambiguously in favour of polyandry" (Crozier and Pamilo 1996, p. 106). Other hypotheses are inadequate to explain extreme polyandry. Higher levels of polyandry do not further reduce the variance in diploid male production among colonies (Crozier and Page 1985). Selection on individual queens for increased polyandry in response to workers biasing the production of reproductive offspring towards females (Trivers and Hare 1976) can explain the evolution of double mating or treble mating (Ratnieks and Boomsma 1995), but cannot explain extreme polyandry (Queller 1993; Oldroyd et al. 1996).

Understanding the evolution of extreme polyandry in *Apis* may be approached by comparative studies of species which have different life histories and different ecological ranges. If the number of matings is well correlated with a particular life history trait, such as migration, across a variety of species, then the evolutionary antecedents of polyandry may be suggested. Furthermore, the development of a large number of polymerase chain reaction microsatellite primers from *A. mellifera* by Estoup et al. (1994) now permits the precise determination of mating frequency in honey bees. Some *A. mellifera* microsatellite sequences and primers are sufficiently conserved to permit their use across species (Moritz et al. 1995; Oldroyd et al. 1995b, 1996). These studies have demonstrated that the degree of polyandry across the genus is uniformly high (> 5 matings), but extremely variable, both within and among the species so far examined (Table 1).

Table 1 Levels of polyandry, intra-colonial genetic relationships in the genus *Apis* as determined by microsatellite analysis, and levels of colony aggregation

Species	Number of matings			Coefficient of relatedness	N^a	Colony aggregations (colonies/aggregation)
	Observed		Effective			
	Mean ± s.e	Variance				
A. mellifera[b]	13.8 ± 2.5	30.7	12.4 ± 2.2	0.30 ± 0.009	5	1–7[g]
A. florea[c]	8.0 ± 1.6	5.5	5.6 ± 1.0	0.35 ± 0.02	5	Low 1–2[h]
A. dorsata[d]	26.7 ± 6.6	117.6	20.0 ± 6.6	0.29 ± 0.007	4	Up to 120[i]
A. dorsata[e]	18.0 ± 1.6	38.4	25.65 ± 1.05	0.27 ± 0.02	6	Up to 120[i]
A. andreniformis[f]	13.5 ± 2.3	20.3	9.1 ± 0.83	0.30 ± 0.007	4	Unknown

[a] Estimate based on N colonies
[b] Estoup et al. (1994)
[c] Oldroyd et al. (1995b)
[d] Oldroyd et al. (1996)
[e] Recalculated from Moritz et al. (1995). Their original estimates, based on weighted means, are: observed number of patrilines = 16.3 ± 2.4. $E(k)$ = 30.17 ± 6.0, $\bar{m}$ = 25.6 ± 11.6
[f] This study
[g] Oldroyd et al. (1995a)
[h] Personal observations
[i] Personal observations, Koeniger and Koeniger (1980)

On the basis of the morphology of the endophallus and the tibia of the male, *A. andreniformis* (Smith 1858) has recently been reconfirmed as a separate species from its sympatric cogener *A. florea* (Wu and Kuang 1986; Wongsiri et al. 1990). More recently, detailed comparative studies of *A. florea* and *A. andreniformis* have shown substantial differences in the morphology of the workers (Rinderer et al. 1995), the morphology of the nests (Rinderer et al. 1996) and the hour of mating (Rinderer et al. 1993). Wongsiri et al. (1996) review the comparative biology of *A. andreniformis* and *A. florea*, and in addition to the foregoing, note a number of significant behavioral differences between the two species.

The distribution of *A. andreniformis* is unclear, but it has been found in at least seven Thai provinces, in China, India, Burma, Laos, Vietnam, Malaysia and the Philippines (Otis 1991; Wongsiri et al. 1996).

Koeniger et al. (1990) reported that the mean volume of semen contained in the spermathecae of two *A. andreniformis* queens sampled in Johore, Malaysia was 0.27 mm^3. These spermathecae contained a mean of 1.03×10^6 spermatozoa, whereas 5 drones sampled in the same area had a mean of $0.13 \times 10^6 \pm 0.01$ spermatozoa in their seminal vesicles. These findings suggested that these two queens mated about eight times. However, the number may be much higher than this because, at least in other species of honey bee (i.e. *A. mellifera* and *A. cerana*: Koeniger et al. 1991; *A. florea*: Oldroyd et al. 1995b; *A. dorsata*: Moritz et al. 1995; Oldroyd et al. 1996) queens expel much of the semen they receive during mating.

Here we report on the number of matings in *A. andreniformis* determined from microsatellite allele distributions in workers and queens.

Materials and methods

Four colonies of *A. andreniformis* were caught in tea plantations near the village of Chiang Dao (19°22′ N, 98°58′ E) in northern Thailand. A sample of pupae was obtained from colonies 1, 2 and 3, whereas only adult bees were available from the fourth colony which was combless. Queens were also obtained from colonies 2 and 4. Colonies were separated by more than 1 km, so drifting bees were unlikely to have affected the results for colony 4. The species of the sampled bees was determined from the color of the scutellum and the nest morphology (Wongsiri et al. 1996). Voucher specimens are lodged at the United States Department of Agriculture's Honey Bee Breeding, Genetics, and Physiology Research Laboratory in Baton Rouge Louisiana. All samples were frozen in liquid nitrogen for transport to the laboratory, where they were stored at −70°C.

DNA was extracted from the antennae of individual bees by boiling the ground tissue in 1 ml 5% Chelex 100 resin for 15 min (Walsh et al. 1991). DNA extractions were then amplified using the polymerase chain reaction (PCR) using 11 primer sets specific to the microsatellite loci specified in Table 2. For each primer pair, the reverse primer was radio actively end-labelled, except for locus B124, where the forward primer gave a clearer result. The γ-phosphate from ^{33}P-ATP (Bresatec) was transferred to the 5′-terminus of the primer, using T4 polynucleotide kinase (Pharmacia). PCRs were then conducted in 10 μl volumes, which were composed of 5 μl of the Chelex DNA extraction, 0.4 μM of each primer, 20 μM of each dNTP, 1.0–1.5 mM $MgCl_2$[1], 1 × reaction buffer, and 0.45 units of *Taq* polymerase (Biotech International). The precise Mg^+ concentrations and cycling conditions used for each locus are given in Table 2.

Table 2 Primer sequences and PCR conditions for 11 microsatellite loci from *A. mellifera* and *Bombus terrestris* used and use of which was attempted to amplify microsatellite loci in *A. andreniformis*. Primer sequences were taken from Estoup et al. (1994, 1995). Loci A88 and B124 were usually multiplexed, i.e., both sets of primers were provided in the one reaction

Locus	Primers	Annealing temperatures (°C)	$MgCl_2$ (mM)	Number of cycles	Number of alleles observed
A8	5′CGAAGGTAAGGTAAATGGAAC 5′GGCGGTTAAAGTTCTGG	51–60	1.5	30	No product
A14	5′GTGTCGCAATCGACGTAACC 5′GTCGATTACCGATCGTGACG	58	1.5	30	2
A28	5′GAAGAGCGTTGGTTGCAGG 5′GCCGTTCATGGTTACCACG	51–58	1.2	30	No product
A29	5′AAACAGTACATTTGTGACCC 5′CAACTTCAACTGAAATCCG	51–57	1.0	30	No product
A35	5′GTACACGGTTGCACGGTTG 5′CTTCGATGGTCGTTGTACCC	51–57	1.2	30	No product
A43	5′CACCGAAACAAGATGCAAG 5′CCGCTCATTAAGATATCCG	51–55	1.2	30	No product
A76	5′GCCAATACTCTCGAACAATCG 5′GTCCAATTCACATGTCGACATC	58	1.2	30	2
A88	5′GCGAATTAACCGATTTGTCG 5′GATCGCAATTATTGAAGGAG	57	1.2	35	4
A107	5′CCGTGGGAGGTTTATTGTCG 5′GGTTCGTAACGGATGATGACACC	58	1.2	30	2
A113	5′CTCGAATCGTGGCGTCC 5′CCTGTATTTTGCAACCTCGC	51–60	1.2	30	No product
B124	5′GCAACAGGTCGGGTTAGAG 5′CAGGATAGGGTAGGTAAGCAG	57	1.2	35	7

PCR products were run on 6% polyacrylamide sequencing gels with M13 control DNA sequencing reactions run on the same gel as size standards. Microsatellite alleles were scored as fragment lengths in base pairs.

The queen genotype was determined for each locus in each colony. For colonies 2 and 4, this was determined directly from queen DNA. Where queens were unavailable (colonies 1 and 3), queen genotypes were inferred from worker genotypes in the following way: when an allele was present in every worker, the queen was considered homozygous for that allele. When every worker carried one of two alleles, the queen was assumed heterozygous for those two alleles (Estoup et al. 1994). Paternal alleles of each worker were then deduced by subtraction (Estoup et al. 1994). That is, the paternal allele of each worker is that allele not carried by the queen.

Where a queen carries two alleles (say A_1 and A_2) at a particular locus, paternity is uncertain for all workers with the same genotype as the queen at that locus. That is, for these A_1A_2 workers, one cannot tell if a particular allele is paternal or maternal in origin. Our approach to these workers was as follows. Homozygous workers of genotype A_1A_1 and A_2A_2 were unambiguously of different subfamilies X and Y and were allocated to their appropriate paternity groups accordingly. The ambiguous heterozygous workers, A_1A_2, were then allocated to the two alternative paternity classes based on the proportion of the two homozygous classes. That is, if the number of A_1A_1 workers was x and A_2A_2 workers y, then the A_1A_2 workers would be allocated to subfamily X in the proportion x and to Y in the proportion y (Oldroyd et al. 1996).

The average coefficient of relatedness, g_{ww}, weighted according to the relative proportions of each subfamily in our samples, was computed from:

$$g_{ww} = \sum_{i=1}^{k} \{([0.75p_i] + [0.25(1-p_i)])p_i\} \quad (1)$$

(Laidlaw and Page 1984), where p_i = the relative frequency of the i^{th} subfamily and k is the number of subfamilies. The effective number of matings (m) was computed from:

$$m = \frac{1}{\sum_{i=1}^{k} p_i^2} \quad (2)$$

(Starr 1984).

Results

We attempted to amplify 11 microsatellite loci identified by Estoup et al. (1994, 1995) from *A. mellifera* and *Bombus terrestris* genomic libraries. All these loci have shown useful allelic variation in *A. mellifera*. Of these loci, six were not sufficiently conserved to permit successful PCR amplification in *A. andreniformis*, under the wide variety of amplification conditions we attempted (Table 2). Five primer pairs were sufficiently conserved to permit PCR amplification, but only four provided sufficient allelic variation to assist in paternity identification (Table 3).

Using these four polymorphic loci, we found that the *A. andreniformis* queens heading the colonies sampled this study mated at least 10–20 times, producing an average relatedness, g_{ww}, of workers of 0.30 ± 0.007, and an average effective number of matings of 9.1 ± 0.83 (Tables 3 and 4). These results may slightly underestimate the actual number of subfamilies, due to finite sample sizes. Consider a colony of k equally frequent subfamilies, from which a sample of size n workers is drawn, and their paternity determined. The probability that a particular subfamily will be absent from this sample is $(1-1/k)^n$, and therefore the expected number missing is $k(1-1/k)^n$ and the expected number of subfamilies, $E(k)$, present in the colony is:

$$k - \left[k\left(1-\frac{1}{k}\right)^n\right] \quad (3)$$

(Cornuet and Aries 1980).

By substituting our observed numbers of subfamilies for $E(k)$, and our actual sample sizes for n, we numerically evaluated k, and found that the estimated values of k were very similar to those observed (Table 4). [We also estimated k for the data of Moritz et al. (1995) and found estimates of the actual number of matings almost identical to those found by their completely different procedure.]

There is no evidence for polygyny in the four colonies examined. Because queens can only transmit one of two alleles to worker offspring at any one locus, the presence of more than two homozygous classes of workers would be evidence for polygyny (provided no

Table 3 Genotypes (microsatellite length in base pairs) of queens and drones for 4 microsatellite loci in 4 colonies of *A. andreniformis*

	Microsatellite loci				
	A88	B124	A14	A107	Number of worker bees
Colony 1					
Queen allele 1	136	201	–	108	
Queen allele 2	142	201	–	108	
Drone 1	134	203	–	108	5
Drone 2	134	207	–	108	9
Drone 3	136	201	–	108	2
Drone 4	136	203	–	108	1
Drone 5	136	207	–	108	6
Drone 6	140	201	–	108	4
Drone 7	140	203	–	108	9
Drone 8	142	201	–	108	4
Drone 9	142	203	–	108	2
Drone 10	142	205	–	108	10
Drone 11	142	207	–	108	8
				Total	60

Table 3 (Continued)

	Microsatellite loci				
	A88	B124	A14	A107	Number of worker bees
Colony 2					
Queen allele 1	136	201	199	108	
Queen allele 2	140	203	199	108	
Drone 1	134	209	199	108	1
Drone 2	136	201	198	108	3
Drone 3	136	201	199	108	14
Drone 4	136	205	198	108	6
Drone 5	136	205	199	108	3
Drone 6	136	209	198	108	1
Drone 7	136	211	199	108	4
Drone 8	140	201	198	108	4
Drone 9	140	201	199	108	8
Drone 10	140	203	199	108	2
Drone 11	140	205	199	108	1
Drone 12	142	201	199	108	9
Drone 13	142	203	199	108	3
Drone 14	142	205	198	108	1
Drone 15	142	205	199	108	1
Drone 16	142	209	198	108	1
Drone 17	142	211	198	108	1
Drone 18	142	211	199	108	4
Drone 19	142	Q	198	108	10
Drone 20	Q	211	198	108	1
				Total	78
Colony 3					
Queen allele 1	136	203	198	108	
Queen allele 2	136	203	199	108	
Drone 1	134	201	198	108	6
Drone 2	134	201	199	108	11
Drone 3	134	203	198	108	2
Drone 4	134	203	199	108	5
Drone 5	134	209	198	108	4
Drone 6	136	201	199	108	5
Drone 7	136	203	199	108	4
Drone 8	136	207	199	108	4
Drone 9	136	209	199	108	7
Drone 10	140	201	198	108	1
Drone 11	140	203	Q	108	3
Drone 12	142	201	198	108	6
Drone 13	142	201	199	108	2
					60
Colony 4					
Queen allele 1	134	201	198	108	
Queen allele 2	136	217[a]	199	108	
Drone 1	134	203	199	108	6
Drone 2	134	207	199	108	1
Drone 3	136	201	199	108	9
Drone 4	136	203	199	108	3
Drone 5	140	201	199	106	1
Drone 6	140	201	199	108	2
Drone 7	142	201	199	108	6
Drone 8	142	203	199	108	7
Drone 9	142	207	199	108	2
Drone 10	142	207	199	106	4
					41

[a]In colony 4, when a worker bee carried the 217 base length allele of microsatellite B 124, it was assumed, because of the rarity of the allele in this study, to be a queen allele.

Table 4 Observed, estimated and effective (m) number of matings and average coefficient of relatedness (G_{ww}), for four colonies of *A. andreniformis*. The observed number of matings is the actual number of different paternities seen in the sample. The estimated number is the number of matings estimated from Eq. 3 that would be present in an infinite sample from that colony. The effective number of matings is estimated from Eq. 2

Colony	Number of matings			Average relatedness
	Observed	Estimated	Effective	
1	11	11.04	8.4	0.31
2	20	20.41	10.8	0.30
3	13	13.11	10.1	0.30
4	10	10.14	7.1	0.32

null alleles were carried by the queen at the loci studied). In the two colonies where queens were available for analysis, all workers carried one of the two queen alleles. In all colonies, no more than two classes of homozygous workers were detected in any one colony.

Discussion

These results demonstrate that *A. andreniformis* queens mate about 10–20 times, a similar number of times to *A. mellifera*, but more often than *A. florea*, and considerably less often than *A. dorsata* (Table 1). They show that the number of matings estimate for *A. andreniformis* from sperm counts (7–8) by Koeniger et al. (1990) was conservative. Indeed, our estimate of levels of polyandry in *A. andreniformis* may also be conservative. First, our finite sample sizes may have left some subfamilies undetected. Second, the limited number of variable microsatellites identified, and the limited allelic diversity of some of these, may mean that some drones were not uniquely identified by the panel of microsatellites used.

The extent of the first bias is probably low. The expected number of matings in an infinite sample size are similar to the actual number of matings observed (Table 4). However, the calculation of the expected number of matings assumes an equal number of individuals per subfamily, an assumption which is probably violated (Table 3). Therefore, we may have failed to detect all rare subfamilies.

The extent of the second bias is difficult to assess, but is probably quite low. Fifteen alleles were available for analysis across four polymorphic loci, meaning that up to 112 genotypes could be potentially identified, whereas only 54 were actually identified (Table 3). However, the most frequent patrilines in colony 2 also carry the most frequent alleles, suggesting that some subfamilies remain undetected in this colony at least.

In *A. florea* (Oldroyd et al. 1995b), *A. dorsata* (Moritz et al. 1995; Oldroyd et al. 1996) and *A. mellifera* (Koeniger and Koeniger 1991) the total amount of semen present in copulating males exceeds the volume found in mated queens, thus indicating that queens expel excess semen after mating. These observations support the hypothesis, first articulated by Koeniger and Koeniger (1990, 1991), that a form of sexual selection operates in the genus whereby drones are selected to produce more spermatozoa in order to increase their share of paternity of potential offspring, whereas queens are selected to mate many times and expel excess semen (Oldroyd et al. 1995b). In *A. andreniformis*, males contain about 0.13×10^6 spermatozoa, whereas queens have about 1×10^6 spermatozoa in their spermathecae (Koeniger et al. 1990). Therefore, given that they mate at least 10–20 times, queens must also expel excess semen in *A. andreniformis*. Thus despite the comparitively low semen volume in drones in this species, the genus-wide phenomenon of selection for higher semen volume in males, and semen expulsion in females, appears to hold, even if to a reduced degree.

Queens of all *Apis* species so far examined with microsatellite analysis mate more than six to ten times. The number six is significant, because beyond six matings there is no consequential effect on intracolonial genetic relatedness (Page and Metcalf 1982; Oldroyd and Moran 1983) or the variance of diploid male distribution among colonies (Page 1980; Page and Metcalf 1982). Therefore explanations for the evolution of polyandry which are based on arguments relating to optimal sex allocation or enhanced brood viability cannot explain the extreme levels of polyandry (defined here as more than six matings) observed in *Apis* (Moritz et al. 1995; Oldroyd et al. 1996).

Extreme polyandry cannot evolve unless benefits outweigh the costs (Pamilo 1991), and we argue that because of reproductive behavior in the genus, the potential costs of polyandry are significant. All species of honey bee mate on the wing (Koeniger and Koeniger 1991). *A. mellifera* (Loper et al. 1987; Pechhacker 1994) and *A. dorsata* (Koeniger et al. 1994) queens mate at leks known as drone congregation areas (DCAs), and it appears likely that the other *Apis* species would also have particular mating domains, the physiography of which is likely to be species-specific. DCAs can remain fixed in location for many years. For example, an *A. mellifera* DCA at number 2 sports oval at Sydney University has been present for at least 15 years (B.P. Oldroyd, personal observations). Because DCAs are virtually permanent, and are occupied by large numbers of nutritious, stingless insects at specific times of day (Rowell et al. 1986; Underwood 1990a; Rinderer et al. 1993), rates of predation by birds and insects (and possibly bats in the case of *A. dorsata*) at DCAs would be expected to be extremely high. Thus there may be significant time-dependent risk of mortality for queens while on mating flights. Other potential risks of additional matings include sexually transmitted diseases, and inclement weather (Moritz et al. 1995; Oldroyd et al. 1996). Repeated mating flights would

substantially increase risks to the queen (Moritz et al. 1995).

Crozier and Page (1985) and Page (1986) suggested that it is unlikely that extreme polyandry occurs because of an inability of queens to avoid additional copulations. This is because (at least in *A. mellifera*) queens must open their sting chamber before successful copulation can occur (Gary 1963). However, in *A. cerana* and *A. mellifera*, drones place a mating sign in the sting chamber during copulation, which can not be removed by the queen in flight, and may assist subsequent matings (Koeniger 1990). This may suggest that queens of these species do not have complete control over the number of their partners, although we would speculate that they could fly in a way that would prevent copulation. In *A. dorsata*, *A. florea* and *A. andreniformis*, no mating sign is present (Koeniger and Koeniger 1991), suggesting that queens have full control over mating. Thus, the fact that extreme polyandry has evolved in all *Apis* species so far examined, including those where queens almost certainly have complete control over mating, despite the potential for significant costs, suggests that substantial benefits of polyandry accrue. These benefits are generally thought to arise from increased intracolonial genetic diversity (Crozier and Page 1985; Oldroyd et al. 1995b, 1996; Moritz et al. 1995).

The benefits of intracolonial diversity probably relate to increased disease resistance (Hamilton 1987; Sherman et al. 1988; Shykoff and Schmid-Hempel 1991a,b; Dreller et al. 1995), increased colony efficiency due to genetically influenced task specialization (Calderone and Page 1988, 1991; Robinson and Page 1988; Calderone et al. 1989; Page et al. 1989; Oldroyd et al. 1991, 1992a,b, 1993, 1994; Page and Robinson 1991; Fewell and Page 1993; Fuchs and Schade 1994; Giray and Robinson 1994; Guzmán-Novoa et al. 1994 a, b; Robinson et al. 1994; Dreller et al. 1995; Page and Fondrk 1995), or increased ability to buffer environmental perturbations (Crozier and Page 1985; Page et al. 1995). In all cases, these mechanisms must involve some kind of behavioral overdominance (or positive specific subfamily effects, Oldroyd et al. 1992) to explain the evolution of extreme polyandry.

Predictions, with respect to mating biology, of these three hypotheses are:

1. *Polyandry is an adaptive response to parasite and pathogen loads.* In some ways, this hypothesis is a modification of the "Red Queen" hypothesis for the maintenance of sex, which proposes that sexual recombination generates rare host genotypes that will be more likely to be resistant to frequent pathogen genotypes (Jaenike 1978; Hamilton 1980; Lively 1987; Lively et al. 1990). Lively (1987) showed that in the facultatively sexual snail *Potamopyrgus antipodarum*, the frequency of sexual reproduction increased with parasite load. Similarly, in social insects, those species with greater exposure to parasites and pathogens are predicted to mate more often than other species, generating diverse worker genotypes which are less likely to catastrophically succumb to infection (Sherman et al. 1988).

Although other species of *Apis* may have a tendency to aggregate (Oldroyd et al. 1995a), *A.dorsata* is extreme in this regard (Table 1). Aggregations of 2–120 colonies on a single tree or building are quite common (Seeley et al. 1982; Ruttner 1988; personal observations). Aggregations and frequent drifting of workers among colonies (Moritz et al. 1995), probably contributes to higher pathogen loads in this species than others. Therefore, the parasite hypothesis would predict that *A. dorsata* would mate more frequently than other species, which is what is observed (Table 1). A further testable prediction of this hypothesis is that detailed surveys of pathogen loads within a species should show a negative correlation between pathogen load and level of polyandry.

2. *Polyandry increases possibilities for genetically based task specialization.* On theoretical grounds it has been assumed that, overall, groups of specialised individuals acting together perform more efficiently than generalists (Oster and Wilson 1978). In *Apis* there is a great deal of evidence that there is a strong genetic component to task specialisation (reviewed in Robinson 1992), and emerging theoretical (Page et al. 1989) and empirical (Oldroyd et al. 1992b; Fuchs and Schade 1994) support for the idea that polyandry and task specialisation increases colony fitness, and it is easy to postulate that behavioral dominance could occur by task specialisation (but see Woyciechowski and Warakomska 1994). It is not easy to see how one species would have a greater requirement for behavioral polyethism than another. So the only prediction of this hypothesis is that all species should show high levels of polyandry.

3. *Polyandry produces a genetically diverse worker population which is more able to buffer environmental extremes and reach a phenotypic norm, even under varied environmental conditions.* Page et al. (1995) advanced the novel idea that polyandry promotes phenotypic homeostasis. This hypothesis would predict that species which face extremely varied environments would be more polyandrous than species which exist in more uniform environments. *A. dorsata* and the related *A. laboriosa* undergo frequent long-distance migration, in response to adverse environmental conditions (Morse and Laigo 1969; Koeniger and Koeniger 1980; Roubik et al. 1985; Underwood 1990b; Dyer and Seeley 1994), whereas there is no evidence that *A. florea* or *A. andreniformis* migrate more than a few kilometres, and then only in response to seasonal cues, not localised nectar dearths (Wongsiri et al. 1996). Thus hypothesis 3 might also predict that *A. dorsata* would mate more frequently than *A. florea* and *A. andreniformis*, as is observed.

African subspecies of *A. mellifera* are reputed to undertake frequent long-distance migrations (Fletcher 1978), whereas this behavior is rare or absent in European subspecies (Seeley 1985). Thus higher levels of polyandry in African subspecies compared with European subspecies might be predicted under hypothesis 3. On the other hand, seasonal extremes present in some parts of Europe may be greater than those in much of Africa.

The similar number of matings in *A. mellifera* and *A. andreniformis* (Table 1) is significant because the data exclude the possibility that mating frequency in *Apis* is proportional to body size: the smallest species, *A. andreniformis*, has been demonstrated to have a mating frequency similar to that of *A. mellifera*, a medium-sized species.

We conclude that while genetic variance hypotheses remain front runners in explaining the evolution of extreme polyandry in *Apis*, there is no reason to prefer any of the sub-hypotheses. This does not exclude the hypothesis (Shaskolsky 1976; Page 1980) that the initial selective force for polyandry was consequential reduction in variance of diploid male production.

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