POLYANDRY IN HONEY BEES (*APIS MELLIFERA* L.): SPERM UTILIZATION AND INTRACOLONY GENETIC RELATIONSHIPS

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ABSTRACT

Sperm usage by queen honey bees was examined by progeny analyses using six phenotypically distinct genetic markers. No evidence was found for sperm displacement or precedence. All queens used the sperm of all males that inseminated them during all sampling periods. Sperm usage, as measured by phenotypic frequencies, did fluctuate nonrandomly but did not result in abnormally high representation of a single phenotype or the elimination of other phenotypes as has often been suggested. The genetic relationships of workers within honey bee colonies are estimated from the data presented. Average genetic relatedness is shown to be low among colony nestmates and probably approaches 0.25 in colonies with naturally mated queens. There is no evidence for elevated relatedness among colony subfamilies due to nonrandom fluctuations in sperm usage by queens or for numerical dominance of any subfamilies.

HAMILTON (1964) introduced the concepts of inclusive fitness and kin selection to explain the evolution of altruistic behavior. His hypothesis received considerable support from studies of social hymenopteran insects where the evolution of complex social behavior and sterile worker castes was explained to be a consequence of high genetic relationships among sibling nestmates, and kin selection. HAMILTON (1964, 1972) recognized that polyandry should lead to reduced genetic relationships among nestmates and weakens arguments for the evolution of eusociality by kin selection.

The highly eusocial honey bee (*Apis mellifera* L.) is extremely polyandrous. Estimations of the average number of mates for honey bee queens (*Apis mellifera* L.) range up to 17.25 males (TRIASKO 1951, 1956; TABER and WENDEL 1958; WOYKE 1960, 1964; KERR *et al.* 1962; ADAMS *et al.* 1977). Matings take place while in flight over a period of several days following emergence (WOYKE 1960, 1964). A queen receives about 6 million spermatozoa into her oviducts from each male (KERR *et al.* 1962) from where a *total* of approximately 5.3–5.7 million are transported to the spermatheca by active and passive mechanisms over a period of up to 40 hours (MACKENSEN and ROBERTS 1948; WOYKE 1960; WOYKE 1983). These spermatozoa will last her egg laying life of 1 to 2 years.

Theoretical treatments of kin selection have assumed single matings or invoked sperm clumping or sperm displacement for polyandrous species (ORLOVE 1975; TRIVERS and HARE 1976; CHARNOV 1978). These assumptions are based primarily upon the conclusions of TABER (1955) for sperm utilization by multiple-inseminated queen honey bees. Taber proposed that sperm utilization by honey bee queens is non-uniform and that the spermatozoa from an individual male "clump" together and do not mix appreciably with the sperm of other males during mating or within the spermatheca. CROZIER and BRÜCKNER (1981) and PAGE and METCALF (1982) pointed out that the data presented by Taber do not support his own conclusions. Page and Metcalf presented allozyme data and theoretical arguments showing that assumptions of sperm clumping and elevated relatedness are not justifiable. MORITZ (1984) showed that 5 instrumentally inseminated queens from his study each used the spermatozoa of all 8 of their mates during a 21 day sampling period and that the relative contribution of each male did not vary significantly over sampling periods. However, TABER'S data continue to be used to invoke assumptions of at least temporary, numerical superiority of given intracolony subfamilies (GETZ, BRÜCKNER and PARISIAN 1982).

KERR, MARTINHO and GONCALVES (1980) suggested that the spermatozoa of individual mates tend to "aggregate" or "agglomerate" within spermathecae leading to a high proportion of a single male's spermatozoa being used at any given time. They concluded that the sperm of only 1 or 2 males is used at a time and that this leads to high genetic relationships among worker nestmates, thus supporting kin selection hypotheses. However, their data, like those of Taber, do not support their conclusions. Furthermore, PAGE, KIMSEY and LAIDLAW (1984) studied by histological sectioning the migration of spermatozoa into queen spermathecae. They concluded that spermatozoa migrate into the spermatheca over time and readily diffuse throughout the available space. They found no evidence for agglomerations, aggregations, clumps, layers, or wads of spermatozoa within spermathecae.

In this paper we present new data that furthers our understanding of sperm utilization by queen honey bees. We then discuss the genetic relationships among worker nestmates based on these data.

MATERIALS AND METHODS

Three experiments were conducted during 1982–1984 at the Bee Biology Facility, University of California, Davis, and the North Central States Bee Research Unit, Madison, Wisconsin. To test sperm usage in honey bee queens, we used genetic markers at two gene loci that allowed us to determine six distinct worker progeny phenotypes corresponding to six distinct male phenotypes (see Table 1). For this study we assume that instrumental insemination yields a fair representation of sperm usage under conditions of natural mating. This assumption is supported by reported findings that the males ejaculate into the medium oviduct of the queen and do not directly fill the spermatheca (BISHOP 1920; LAIDLAW 1934, 1939, 1944). It is also supported *ex post facto* by the results reported here; they do not differ significantly from those of other investigators using naturally mated queens with fewer phenotypic markers (TABER 1955; KERR, MARTINHO and GONCALVES 1980; PAGE and METCALF 1982). If a bias exists, it should be toward *less* mixing of semen with instrumental insemination since the mixing effect of the forceful male ejaculation into the median oviduct of the queen is eliminated.

TABLE 1

Test genotypes

Queens	Drones	Type	Progeny	
			Integument	Eye color
	+, +	1	Wild type	Wild type
	+, s^t	2	Wild type	Tan
	+, s	3	Wild type	Red
cd/cd, s^t/s^t				
	cd, +	4	Cordovan	Wild type
	cd, s^t	5	Cordovan	Tan
	cd, s	6	Cordovan	Red

All test queens were homozygous for recessive cordovan integument (cd) and tan eye color (s^t). Tan and snow (s) eye colors are allelic and both recessive to the black, wild type (+), however, they are codominant to each other and in combination produce red-colored eyes (s/s^t). Drones all clearly express the appropriate genotype, allowing easy selection.

Experiment 1: This experiment was designed to determine (1) the minimum number of males that contribute spermatozoa to the spermatheca of a queen, (2) the effect of the order of injection of the semen of individual males upon the proportion of worker progeny produced, and (3) patterns of intraspermathecal sperm usage.

Five double homozygous recessive, virgin test queens (daughters of two sister queen mothers) were each instrumentally injected with semen from six different males representing each of the six phenotypic classes. (Here we make the distinction between injection and insemination because of the uncertainty associated with spermatozoa entering the female reproductive tract and effecting insemination by entering the spermatheca.) Eye mutant queen and drone mothers were distantly related with $G < 0.0625$ (pedigree coefficient of relationship, CROZIER 1970). Some brother drones were, by necessity, used for some inseminations. Due to the nonavailability of some classes of males at times, two additional test queens were injected with semen from five different males, one of each of five phenotypic classes. Semen for each injection was taken into the syringe in serial order from one male at a time. The order of collection of semen from males of each phenotype was randomized to minimize any confounding biases that might occur as a consequence of sex allele homozygosity (MACKENSEN 1951).

After each injection, queens were returned to their five-frame nucleus colonies and, after a few days, commenced egg laying. Worker progeny from surviving queens (mortality occurred throughout the test period) were analyzed periodically over 22 months by removing a comb containing sealed and emerging brood. Combs were placed into individual comb emergence cages in an incubator maintained at approximately 33° and 50% relative humidity. Emerged workers from each comb were counted as they were classified by phenotype at 1- to 3-day intervals over several days (representing subsamples). Total counts for each comb represented one sample. This procedure allowed us to evaluate sperm usage at three levels: (1) day-to-day egg laying, as measured among small subsample groups; (2) seasonal trends, measured among samples of individual queens; and (3) sperm usage over the expected egg-laying life of a queen (about 1 yr).

Experiment 2: The purpose of this experiment was to determine the effect of insemination order on sperm usage when subsequent inseminations take place over a period of several days and to determine whether spermatozoa are mixing *within* the spermathecae of queens. Four sister virgin test queens were each injected with the semen from four different phenotypically distinct drones, one on each of 4 consecutive days. The order of injection for each phenotype was randomized. Worker progeny were sampled as in experiment 1.

Experiment 3: This experiment was conducted to determine the effect of a full spermatheca on subsequent inseminations. Four double homozygous virgin test queens (three of which were sisters, one a cousin) were each injected with approximately 7 mm^3 of semen from wild-type males on each of two occasions, 2 days apart, for a total of 14 mm^3 each. Two days later (4 days after the

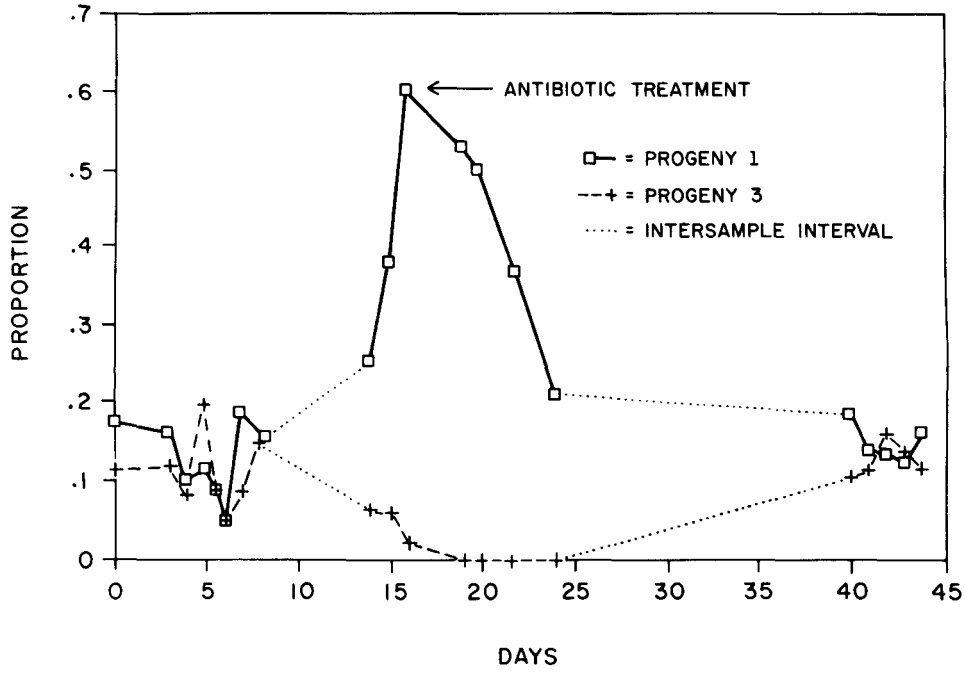

FIGURE 1.—The relative proportions of worker individuals belonging to patrilineal subfamilies 1 and 3 for three sampling periods for queen 517-822. An infection of AFB disease was detected in this colony during sampling period 1. Subfamily 1 increased dramatically relative to the other five, whereas subfamily 3 decreased to extinction. Antibiotic treatment restored subfamily frequencies to preinfection levels by sampling period 3. The steep decline in frequency for subfamily 1 immediately following treatment cannot be ascribed to the antibiotic treatment but may instead suggest that subfamily 1 had a higher resistance to the disease at low levels of innoculum relative to the others.

first injection) each queen was injected with approximately 2–2.5 mm^3 (represents two drones) of cd,s^t genotypes. Worker progeny were sampled as in experiments 1 and 2.

RESULTS AND DISCUSSION

The results of this study show that (1) honey bee queens use the spermatozoa of all mates during all sample periods in fluctuating, but representative, proportions; (2) there is no evidence of sperm precedence or displacement; (3) spermatozoa do mix within the spermatheca even when the spermatheca is already "packed." All queens tested used the sperm of all representative drones in significant proportions during all sampling periods (Table 2), however, some *subsamples* were missing some phenotypic classes. In most cases, this corresponded to a small subsample and is best explained by sampling error. However, queen 517-822 showed a dramatic decrease in the frequency of phenotype 3 and a corresponding increase in phenotype 1 during sampling periods 1 and 2 (Figure 1). During this time, the colony suffered an increasing infection of American Foul Brood (AFB) disease resulting in considerable brood mortality. Antibiotics were fed to this colony during sample period 2 resulting in the disappearance of AFB symptoms and the restoration of preinfection

TABLE 2

Proportion of individuals of each of six progeny phenotypes for each sampling period

Queen 510-822							
Phenotype	1	2	3	4	5	6	Count
Injection order	6	5	2	3	4	1	
1982							
Sample 1 (7/26–8/2)	0.105	0.153	0.184	0.123	0.208	0.226	1176
Sample 2 (10/17–10/26)	0.172	0.127	0.158	0.119	0.113	0.311	488
1983							
Sample 3 (3/10–3/17)	0.145	0.178	0.145	0.084	0.131	0.318	214
Total	0.127	0.149	0.173	0.118	0.175	0.259	1878
Queen 517-822							
Phenotype	1	2	3	4	5	6	Count
Injection order	4	5	3	1	6	2	
1982							
Sample 1 (8/3–8/11)	0.148	0.272	0.120	0.183	0.134	0.142	640
Sample 2 (8/17–8/27)	0.430	0.058	0.020	0.215	0.204	0.073	604
Sample 3 (9/12–9/16)	0.150	0.280	0.121	0.185	0.121	0.143	1116
Sample 4 (10/18–10/26)	0.127	0.278	0.137	0.175	0.127	0.156	212
Total	0.213	0.226	0.098	0.191	0.144	0.128	2572
Queen 519-822							
Phenotype	1	2	3	4	6	6	Count
Injection order	3	2	4	1	6	5	
1982							
Sample 1 (7/26–8/2)	0.147	0.217	0.121	0.124	0.107	0.284	1859
Sample 2 (9/5–9/11)	0.217	0.244	0.058	0.123	0.141	0.216	1762
Total	0.181	0.230	0.090	0.123	0.123	0.251	3621
Queen 548-822							
Phenotype	1	2	3	4	5	6	Count
Injection order	2	5	1	4	3	6	
1982							
Sample 1 (8/3–8/11)	0.074	0.205	0.134	0.106	0.255	0.225	1544
Sample 2 (10/17–10/20)	0.225	0.110	0.156	0.179	0.069	0.260	173
1983							
Sample 3 (3/19–4/2)	0.122	0.143	0.177	0.204	0.109	0.245	441
1984							
Sample 4 (6/3–6/6)	0.175	0.097	0.165	0.165	0.136	0.262	103
Sample 5 (6/11–6/19)	0.114	0.062	0.270	0.139	0.111	0.304	352
Total	0.101	0.165	0.162	0.134	0.194	0.243	2613
Queen 567-822							
Phenotype	1	2	3	4	5	6	Count
Injection order	1	5	4	3	6	2	
1982							
Sample 1 (7/26–8/2)	0.077	0.127	0.289	0.194	0.071	0.241	2700
1983							
Sample 2 (3/8–3/12)	0.116	0.131	0.208	0.236	0.072	0.238	475
Sample 3 (3/26–4/2)	0.110	0.103	0.265	0.212	0.070	0.239	1142
Sample 4 (4/17–4/19)	0.196	0.087	0.350	0.165	0.034	0.168	668
Sample 5 (9/22–9/27)	0.099	0.131	0.183	0.168	0.203	0.217	595
Total	0.104	0.118	0.273	0.195	0.081	0.229	5580

Order of injection represents the reverse order of semen taken into the syringe.

TABLE 3

G-test of heterogeneity for each queen over all subsamples and sample periods

	No. of phenotypes	No. of subsamples	Count	*G*	d.f.
Queen 510-822	6				
Sample					
1		6	1176	35.86	25
2		1	488		
3		3	214	8.19	10
Total		3	1878	54.76**	10
Queen 517-822	6				
Sample					
1		7	640	45.80*	30
2		7	604	103.54*	30
3		5	1116	25.46	20
4		1	212		
Total		4	2572	388.24**	15
Queen 519-822	6				
Sample					
1		6	1859	19.50	25
2		7	1762	47.29*	30
Total		2	3621	28.78**	5
Queen 548-822	6				
Sample					
1		7	1544	260.46**	30
2		1	173		
3		7	441	35.56	30
4		2	103	9.33	5
5		5	352	20.38	20
Total		5	2613	236.49**	20
Queen 567-822	6				
Sample					
1		7	2700	31.75	30
2		5	475	21.57	20
3		5	1142	74.44**	20
4		2	668	15.15**	5
5		6	595	41.57*	25
Total		5	5580	251.96**	20
Queen 534-822	5				
Sample					
1		3	1260	19.74*	8
2		6	1597	20.78	20
3		1	177		
4		7	2222	57.38**	24
5		7	352	14.16	24
Total		5	5608	266.82**	16
Queen 556-822	5				
Sample 1		10	762	28.43	18

G-test of heterogeneity from SOKAL and ROHLF (1969). Queen 556-822 had expected frequencies of progeny types 3 and 5 of less than 5, therefore, counts for those groups were lumped with adjacent progeny counts for the statistical analysis.

* Statistically significant nonrandom deviations in a series of observations at the 0.05 level; ** the 0.01 level of significance.

TABLE 4

Proportion of offspring from each ordered injection over all sample periods for each queen inseminated with semen of six phenotypically distinct males

	Injection order					
Queen no.	1	2	3	4	5	6
510-822	0.259	0.173	0.118	0.175	0.149	0.127
517-822	0.191	0.128	0.098	0.213	0.226	0.144
519-822	0.123	0.230	0.181	0.090	0.251	0.123
548-822	0.162	0.101	0.194	0.134	0.165	0.243
567-822	0.104	0.229	0.195	0.273	0.118	0.081
Mean ± SE	0.168 ±0.0274	0.172 ±0.0261	0.157 ±0.0205	0.177 ±0.0316	0.182 ±0.0247	0.144 ±0.0269

$F = 0.294$ ($P > 0.05$) for one-way analysis of variance of the angular transformed data.

TABLE 5

Proportion of offspring of each phenotype over all sample periods for each queen injected with semen of six phenotypically distinct males

	Phenotype					
Queen no.	1	2	3	4	5	6
510-822	0.127	0.149	0.173	0.118	0.175	0.259
517-822	0.213	0.226	0.098	0.191	0.144	0.128
519-822	0.181	0.230	0.090	0.123	0.123	0.251
548-822	0.101	0.165	0.162	0.134	0.194	0.243
567-822	0.104	0.118	0.273	0.195	0.081	0.229
Mean ± SE	0.145 ±0.0222	0.178 ±0.0219	0.159 ±0.0329	0.152 ±0.0169	0.143 ±0.0198	0.222 ±0.0240

$F = 1.506$ ($P > 0.05$) for one-way analysis of variance of the angular transformed data.

phenotypic frequencies in sample period 3. This suggests that at least some observed fluctuations in progeny phenotypes may be due to different susceptibilities to the many honey bee brood diseases.

Phenotypic frequency fluctuations within colonies, among sampling periods, were greater than that expected due to chance sampling alone (Table 3). Heterogeneity tests show statistically significant deviations for all queens from which multiple samples were taken. These fluctuations were not of sufficient magnitude, however, to results in a preponderance of 1 male's sperm being used at any given time. Fluctuations among subsamples (short term) tend to be less than those of samples (long term), suggesting localized homogeneity within a heterogeneous spermatheca.

There is no evidence for sperm precedence or displacement, or for differential fitness of individual phenotypes. The order of injection of spermatozoa has no measurable effect upon the frequency of progeny phenotypes (Tables 4 and 5). Differences in phenotypic frequency totals were probably the result of differences in the amount of semen produced by individual drones, possible

TABLE 6

G-test for heterogeneity for queens inseminated on 4 consecutive days (experiment 2)

Queen no.	Count	*G*	d.f.
1982 569-82			
Sample 1 (8/23–8/28)	1935	9.75	9
1982 577-82			
Sample 1 (8/23–8/26)	1573	21.37*	9
Sample 2 (10/17–10/20)	63		
Total	1636	14.60**	3
1982 587-82			
Sample 1 (8/23–8/26)	1854	59.85**	6
1983			
Sample 2 (3/4–3/12)	275	4.83	6
Sample 3 (4/12–4/16)	689	10.02	6
Total	2818	573.13**	6
1982 595-82			
Sample 1 (8/25–9/2)	2463	32.99	24
1983			
Sample 2 (3/26–4/2)	1080	17.69	15
Sample 3 (9/21–9/27)	255	8.22*	3
Total	3798	86.19**	6

Data are presented for each sampling period.
* $P < 0.05$.
** $P < 0.01$.

TABLE 7

The proportion of offspring from each ordered insemination for each queen injected with a single drone of a different phenotype on each of 4 consecutive days

	Injection order			
Queen no.	1	2	3	4
569-82	0.017	0.751	0.212	0.020
577-82	0.178	0.331	0.112	0.380
587-82	0.540	0.085	0.266	0.109
595-82	0.245	0.284	0.118	0.353
Mean ± SE	0.245 ±0.1093	0.363 ±0.1400	0.177 ±0.0374	0.216 ±0.0892

$F = 0.541$ ($P > 0.05$) for one-way analysis of variance of the angular transformed data.

lethal effects due to homozygosity of sex alleles (MACKENSEN 1951) and the mechanism of filling the spermatheca.

The order of insemination has no effect upon progeny phenotypic frequencies even when four successive, single drone inseminations occur at intervals of 1 day. Spermatozoa from the last (fourth) insemination are still able to migrate into the spermatheca, diffuse and be utilized by the queen in proportions not significantly different from the previous inseminations (Tables 6 and 7).

TABLE 8

Count and proportion of marker (eye mutant) progeny for queens from experiment 3

	Count	Proportion marker progeny	Proportion marker semen	Ratio
Queen 509-822				
Sample 1 (9/12–9/17)	1280	0.074	0.152	0.487
1982 Queen 516-82				
Sample 1 (9/12–9/17)	1851	0.068	0.125	0.544
1983				
Sample 2 (3/8–3/12)	1042	0.121	0.125	0.968
Sample 3 (3/19–3/25)	474	0.095	0.125	0.760
Sample 4 (9/21–9/27)	655	0.084	0.125	0.672
Total	4022	0.087	0.125	0.696
	$G = 23.55$**	d.f. = 3		
1982 Queen 521-822				
Sample 1 (9/12–9/17)	1876	0.076	0.152	0.50
1983				
Sample 2 (3/4–3/12)	514	0.134	0.152	0.882
Sample 3 (3/19–3/21)	100	0.040	0.152	0.263
Total	2490	0.086	0.152	0.623
	$G = 19.12$**	d.f. = 2		
1982 Queen 546-82				
Sample 1 (8/28–9/2)	3130	0.047	0.152	0.309
1983				
Sample 2 (3/8–3/19)	799	0.071	0.152	0.467
Total	3929	0.052	0.152	0.342
	$G = 7.12$	d.f. = 1		

The proportion of marker semen of the total volume of semen used for injections is shown next to the ratio of the proportion of marker progeny counted to the proportion of marker semen used. The ratio is a measure of transfer efficiency of marker semen into a packed spermatheca. *G* values and degrees of freedom are given for queens from which multiple progeny counts were made.

Spermatozoa are able to enter the spermatheca even when multiple inseminations have occurred over a period of 6 days. Furthermore, these spermatozoa are mixed within the spermatheca and are used in significant, near constant proportions (Table 8). The spermathecae of these queens should contain a dense mass of spermatozoa after the first two injections of 7 mm^3 (representing more than 14 drones total) and are considered to be full (WOYKE 1960; MACKENSEN 1964). It should be noted that the proportion of progeny contributed by the last injection (experiment 3) is approximately one-half (mean 0.539) the relative contribution of total semen. This may suggest that the spermathecae of these queens were sufficiently full to reduce migration efficiency of the spermatozoa, however, the results may be confounded by differences in transfer efficiency of spermatozoa as a consequence of injection volume (MACKENSEN 1964). No such relationship was found with the last (fourth) insemination from experiment 2 in which only four males total were used.

Relatedness: PAMILO and CROZIER (1982) discussed the different models and methods used to measure genetic relationships (relatedness) of individuals and

populations based upon pedigree analyses and gene frequency data. For our analyses, we use two measures of nestmate relatedness. We assume no inbreeding, that all males inseminating a given queen are unrelated to each other (this assumption is not strictly valid for our data), and we consider only the genetic relationships of diploid progeny.

Subfamily relatedness, R_i, is defined as the average relatedness of a member of subfamily i (having father i in common) to the sample of progeny under consideration. Using the pedigree coefficient of relationship values of ¾ for members of the same subfamily and ¼ for members of different subfamilies (CROZIER 1970),

$$R_i = 0.75\ p_i + 0.25(1 - p_i) \tag{1}$$

where p_i = the frequency of members of the ith subfamily.

Colony relatedness, R_c, is the average relatedness of all workers in the colony based on a given progeny sample, or sums of samples;

$$R_c = \sum_{i}^{k} p_i R_i. \tag{2}$$

We assume that the colony consists of a very large number of individuals at the same subfamily frequencies as the progeny sample(s) under consideration. Expected colony relatedness ($\hat{R}_c$) is defined as the relatedness value when all subfamilies are at equal frequency ($1/k$) and represents a minimum value for a given number of subfamilies. Any deviation in subfamily frequencies results in an increase in R_c over the expected $\hat{R}_c$. R_c can be expressed in terms of the expected colony relatedness and the variance in frequencies among subfamilies (σ_p^2):

$$\hat{R}_c = ¼ + ½k, \tag{3}$$

and

$$R_c = \hat{R}_c + \frac{k\sigma_p^2}{2}. \tag{4}$$

Number of matings: The number of matings for each queen can be expressed in three ways. First, there is the *actual* number of males that copulate with the queen or are used for instrumental insemination, $\hat{m}$. The number of males that *effectively* inseminate the queen is a function of sperm usage over the life of the queen or, in this case, over all sampling periods. We assume that the total progeny count over all sample periods accurately estimates the relative contribution of each male. The *insemination effective number*, m_1, is the number of matings that results in a value of $\hat{R}_c$, from equation 3 (assuming each mating results in a subfamily of at least one member), equal to the measured value from equation 4. The extent of the deviation between $\hat{m}$ and m_1 is a function of the deviations in subfamily frequencies from the expected ($1/k$):

$$m_1 = \frac{k}{k^2\sigma_p^2 + 1}. \tag{5}$$

TABLE 9

Insemination effective number (m_1) *and short-term effective number* (m_2) *of matings for queens calculated using equations 5 and 6*

Queen no.	m_1	m_2	d_t	% m_1
510-822	5.6	5.2	0.8	50
517-822	5.6	4.8	1.2	33
519-822	5.3	5.2	0.8	88
548-822	5.6	5.2	0.8	67
567-822	5.1	5.0	1.0	90
Mean	5.4	5.1	0.9	66
SD	0.23	0.18	0.18	24.5

d_t, The total difference in number of matings ($\hat{m} - m_2$, $\hat{m} = 6$); % m_t, the percent of the total difference (d_t) contributed by m_1. Sample means and standard deviations are given for the tabulated values.

The third measure of the number of matings takes account of fluctuations in subfamily frequencies among sampling periods. This *short-term effective number*, m_2, is the number of matings that results in a value of $\hat{R}_c$ (from equation 3) equal to colony relatedness averaged over all sampling periods. Where the value of m_1 (equation 5) was dependent upon the variance in subfamily frequencies based on total counts (σ_p^2), m_2 is dependent upon the total variance over all k subfamilies and n sample periods (σ_t^2):

$$\sigma_t^2 = \sum_j^n \sum_i^k \frac{(p_{ij} - \bar{p})^2}{nk},$$

$$m_2 = \frac{k}{k^2\sigma_t^2 + 1}. \tag{6}$$

The average *colony* relatedness, taken from total progeny counts of five queens inseminated with the semen of six males each (experiment 1) is 0.342 ± 0.0038 SD. The average *short-term* relatedness (calculated from the average of individual sampling periods for each queen) is 0.348 ± 0.0038. The average *insemination effective number* is 5.4 ± 0.23 compared with a *short-term effective number* of 5.1 ± 0.18 (see Table 9).

The deviations of both m_1 and m_2 from the actual value ($\hat{m} = 6$) is surprisingly small compared to the possible range and reflects very small variance in subfamily frequencies. It is meaningful to look at these two measures separately. The *insemination effective number* primarily reflects differences in the insemination abilities of different males and events that take place during sperm migration to the spermathecae. It can be considered mostly extraspermathecal. Variability in amounts of spermatozoa produced by each male, viscosity and concentration of semen, physical displacement of spermatozoa within the oviducts by subsequent copulations, intraoviductal sperm competition, and variability in queen control of flow of semen past the spermathecal duct will lead to variability in representation of spermatozoa of males within queen spermathecae. Differences in intraspermathecal sperm viability and survivor-

ship among groups of like sperm will also add to this variance, however, the data presented suggest that these effects are probably minimal. Differential survivorship of progeny can affect the variance in progeny frequencies and confound our estimations.

The *short-term effective number* reflects both extra- and intraspermathecal events, as well as sampling error. Intraspermathecal events include incomplete mixing of spermatozoa within the spermatheca and differential survival, viability, and motility of spermatozoa. The variance in subfamily frequencies (σ_p^2) used to calculate m_1 is an additive component of the total variance (σ_t^2) used to calculate m_2. Therefore, with large samples, the difference between m_2 and m_1 reflects primarily the contribution of intraspermathecal events to the decrease in the effective number of queen matings. Table 9 suggests that most of the observed variability in subfamily frequencies can be attributed to extraspermathecal events.

It is clear from these analyses that the genetic relationships among worker nestmates are low for honey bees. Considering that queens normally mate on average more than 17 times (ADAMS *et al.* 1977), nestmate relatedness is probably very close to 0.25. It is likely that deviations in sperm usage that lead to the numerical dominance of one subfamily, or high instantaneous levels of relatedness, are extremely rare.

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LITERATURE CITED

ADAMS, J., E. D. ROTHMAN, W. E. KERR and Z. L. PAULINO, 1977 Estimation of the number of sex alleles and queen matings from diploid male frequencies in a population of *Apis mellifera*. Genetics **86:** 583–596.

BISHOP, G. H., 1920 Fertilization in the honey-bee. II. Disposal of the sexual fluids in the organs of the female. J. Exp. Zool. **31:** 267–286.

CHARNOV, E. L., 1978 Sex-ratio selection in eusocial Hymenoptera. Am. Nat. **112:** 317–326.

CROZIER, R. H., 1970 Coefficients of relationship and the identity of genes by descent in the Hymenoptera. Am. Nat. **204:** 216–217.

CROZIER, R. H. and D. BRÜCKNER, 1981 Sperm clumping and the population genetics of Hymenoptera. Am. Nat. **117:** 561–563.

GETZ, W. M., D. BRÜCKNER and T. PARISIAN, 1982 Kin structure and the swarming behavior of the honey bee *Apis mellifera*. Behav. Ecol. Sociobiol. **10:** 265–270.

HAMILTON, W. D., 1964 The genetical evolution of social behavior, I, II. J. Theor. Biol. **7:** 1–52.

HAMILTON, W. D., 1972 Altruism and related phenomena, mainly in social insects. Annu. Rev. Ecol. Syst. **3:** 193–232.

KERR, W. E., M. R. MARTINHO and L. S. GONCALVES, 1980 Short communication: kinship selection in bees. Rev. Bras. Genet. **111:** 339–344.

KERR, W. E., R. ZUCCHI, J. T. NAKAKAIRA and J. E. BUTOLO, 1962 Reproduction in the social bees. J. NY Entomol. Soc. **70:** 265–270.

LAIDLAW, H. H., 1934 The reproductive organs of the queen bee in relation to artificial insemination. Masters Thesis, Louisiana State University.

LAIDLAW, H. H., 1939 The morphological basis for an improved technique of artificial insemination of queen bees of *Apis mellifica* Linnaeus. Ph.D. Dissertation, University of Wisconsin, Madison.

LAIDLAW, H. H., 1944 Artificial insemination of the queen bee (*Apis mellifera* L.): morphological basis and results. J. Morphol. **3:** 429–465.

MACKENSEN, O., 1951 Viability and sex determination in the honey bee (*Apis mellifera* L.). Genetics **36:** 500–509.

MACKENSEN, O., 1964 Relation of semen volume to success in artificial insemination of queen honey bees. J. Econ. Entomol. **57:** 581–583.

MACKENSEN, O. and W. C. ROBERTS, 1948 *A Manual for the Artificial Insemination of Queen Bees*, ET-250. United States Bureau of Entomology and Plant Quarantine, Washington, D. C.

MORITZ, R. F. A., 1984 Homogeneous mixing of honeybee semen by centrifugation. J. Apic. Res. **22:** 249–255.

ORLOVE, M. J., 1975 A model of kin selection not invoking coefficients of relationships. J. Theor. Biol. **49:** 289–310.

PAGE, R. E., R. B. KIMSEY AND H. H. LAIDLAW, 1984 Migration and dispersal of spermatozoa in spermathecae of queen honeybees (*Apis mellifera* L.). Experientia **40:** 182–184.

PAGE, R. E. and R. A. METCALF, 1982 Multiple mating, sperm utilization, and social evolution. Am. Nat. **119:** 263–281.

PAMILO, P. and R. H. CROZIER, 1982 Measuring genetic relatedness in natural populations: methodology. Theor. Pop. Biol. **21:** 171–193.

SOKAL, R. R. and F. J. ROHLF, 1969 *Biometry.* W. H. Freeman and Company, San Francisco.

TABER, S., 1955 Sperm distribution in the spermathecae of multiple-mated queen honey bees. J. Econ. Entomol. **48:** 522–525.

TABER, S. and J. WENDEL, 1958 Concerning the number of times queen bees mate. J. Econ. Entomol. **51:** 786–789.

TRIASKO, V. V., 1951 Sign indicating the mating of queens. (In Russian) Pchelovdstvo **11:** 25–31 (Abstracted in Apicultural Abstracts 5/53).

TRIASKO, V. V., 1956 Polyandry in honeybees. In: XVIth International Beekeeping Congress Preliminary Science Meeting (Abstracted in Apicultural Abstracts 233/56).

TRIVERS, R. L. and H. HARE, 1976 Haplodiploidy and the evolution of the social insects. Science **191:** 249–263.

WOYKE, J., 1960 Natural and artificial insemination of honeybees. (In Polish) Pszczel. Zes. Nauk. **4:** 183–273. Summarized in Bee World **43:** 21–25 (1962).

WOYKE, J., 1964 Causes of repeated mating flights by queen honeybees. J. Apic. Res. **3:** 17–23.

WOYKE, J., 1983 Dynamics of entry of spermatozoa into the spermatheca of instrumentally inseminated queen honeybees. J. Apic. Res. **22:** 150–154.

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