ESTIMATION OF THE NUMBER OF SEX ALLELES AND QUEEN MATINGS FROM DIPLOID MALE FREQUENCIES IN A POPULATION OF *APIS MELLIFERA*[1]

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ABSTRACT

The distribution of diploid males in a population of *Apis mellifera* was obtained by direct examination of the sexual phenotypes of the larvae. Using these data, estimates are derived for the number of sex alleles and the number of matings undergone by the queen. The number of sex alleles is estimated to be 18.9. The estimate is larger than previous ones, which have ranged between 10 and 12. However, the increase in the number of sex alleles can be explained by the large effective population number for our data. The best estimator of the number of matings by a queen is a maximum likelihood type that assumes a prior distribution on the number of matings. For the data presented here, this estimate is 17.3. This estimate is compared to others in the literature obtained by different approaches.

SEX determination in *Apis mellifera* is controlled by a sex locus, first described by MACKENSEN (1951). A multiallelic series exists at this locus such that all heterozygotes are females (workers or queens) and all homozygotes develop into diploid males which are usually eaten in the larval state 72 hours after eclosion by workers (WOYKE 1963). Interestingly, the homozygotes are behavioral lethals, but if allowed to develop to maturity are completely viable, fertile males (WOYKE 1965a) similar in appearance but slightly larger than normal haploid males or drones (CHAUD-NETTO 1975).

Diploid males have also been detected in other genera of Hymenoptera, specifically *Bombus atratus* (GAROFALO 1973), *Bracon hebetor* (= *Habrobracon juglandis*; WHITING 1943), *Melipona quadrifasciata* (KERR 1975), *Nasonia vitripennis* (= *Mormoniella vitripennis*, SAUL *et al.* 1965), *Neodiprion nigroscutum* (SMITH and WALLACE 1971), and *Tetragonula quadrangula* (TARELHO 1973). In Melipona a second set of genes has been identified, acting in the early prepupal stage, that is responsible for the sexual phenotype of the adult. These genes have been shown to control the level of juvenile hormone (KERR 1975; CAMPOS, VELTHUIS and VELTHUIS 1975; KERR, AKAHIRA and CAMARGO 1975).

In *Apis mellifera*, matings where the drone sex allele is identical to one of the queen's alleles result in the production of 50% viable brood, identified by a random distribution of empty cells (see LAIDLAW, GOMES and KERR 1956). Such broods with a lowered viability are known to beekeepers as "shot broods." The lowered viability and the likely consequent queen supersedure are both factors that contribute to a reduction in yield (LAIDLAW, GOMES and KERR 1956). In this paper we use the distribution of the frequency of diploid males in a population of *Apis mellifera* to estimate (i) the number of sex alleles at the sex locus in this population, and (ii) the number of matings undergone by the queen.

MATERIALS AND METHODS

The bee population studied was that of the apiary owned by Mr. A. CARITA located in Horto Navarro de Andrade de Rio Claro in the state of Sao Paulo, Brasil. This apiary consisted of 90 hives, and the colonies studied were hybrids between *A. mellifera adansonii* and *A. mellifera ligustica*. The queens are considered to mate freely, and within 5 km of the apiary there are at least 500 other hives. Larvae were obtained during one day from each of the 90 hives, fixed in Dietrich's solution, preserved in 70% ethanol and sexed according to the method described by KERR and NIELSON (1967). Sample size varied between 40 and 92.

RATIONALE

The sex allele system is similar to self-incompatability systems found in many flowering plants (WOYKE 1976). The deterministic (*e.g.*, NAGYLAKI 1975) and stochastic (WRIGHT 1939, 1965) behavior of such systems is well known, and the symmetry of these systems dictates that a locally stable equilibrium will exist at $q_i = 1/K$ where q_i is the frequency of the ith allele and K is the number of sex alleles. It is easy to see that any allele with a frequency greater than $1/K$ will be selected against and its frequency lowered. Similarly, any allele with a frequency less than $1/K$ will be selected for and increase in frequency. NAGYLAKI (1975) gives the exact ranges of attraction for this equilibrium point.

Two types of mating can be delineated for this system:

(i) Mating where the allele of the drone is identical with one of the alleles of the queen, *e.g.*,

$$X_i \, X_j \times X_j$$

where X_i and X_j denote different alleles at the sex locus. These matings which we call the "matched" type would be expected to produce 50% diploid males in their offspring.

(ii) Mating where the allele of the drone is different from either of the alleles of the queen, *e.g.*,

$$X_i \, X_j \times X_{j'} \, j' \neq i, j \ .$$

This type of mating ("unmatched") will produce all workers and no diploid males. Clearly the distribution of the frequency of the diploid males in a sample will be determined by the frequency of matings of each type, which is in turn determined by the number of sex alleles.

It is less obvious, however, that the distribution of the frequency of diploid males from queen to queen will also depend on the *total* number of drones that inseminate a queen. Consider, for example, that a queen mates only once. Matings are either "matched," in which case 50% of the offspring from that queen would be expected to be diploid males, or the matings are "unmatched" in which case no diploid males will be produced. Thus the distribution of the frequency of diploid males between colonies can be bimodal. As the number of matings/queen increases, the variance of this distribution decreases to a minimum at the extreme of an infinite number of matings, namely $V\left(\dfrac{Y}{N}\right) = \dfrac{\theta(2-\theta)}{4N}$, where θ is the probability of a matched mating and Y is the total number of diploid males in a sample of size N.

Thus the distribution of the number of diploid males in a sample depends not only on the number of sex alleles in the population, but also on the number of different drones which inseminate a queen.

DISTRIBUTION OF THE NUMBER OF DIPLOID MALES IN THE SAMPLE

In this section we obtain a distribution for the number of diploid males produced by a queen in terms of the number of matings, M, and the probability of a "matched mating", θ, the only type which may produce diploid males.

Assuming random mating, the parameter θ is related to the frequencies of the sex alleles (q_i) by the following equation,

$$\theta = \frac{2 \sum\limits_{i=1}^{K} q_i^2 (1 - q_i)}{1 - \sum\limits_{1}^{K} q_i^2} \ . \tag{1}$$

When the population is at equilibrium, (1) reduces to

$$\theta = \frac{2}{K} \ . \tag{2}$$

If Z_1 is the number of offspring in the sample resulting from matings of type (i); then the distribution of Y, the number of diploid males derived from these matings, is described by the binomial distribution with parameters $(Z_1; \pi)$ where π is the segregation ratio,

$$P(Y|Z_1) = \binom{Z_1}{Y} (\pi)^Y (1 - \pi)^{Z_1 - Y} \ . \tag{3}$$

In the absence of disturbing forces, such as viability differences π is equal to 1/2 and so equation (3) reduces to

$$P(Y\,|Z_1) = \binom{Z_1}{Y}(1/2)^{Z_1} \ . \tag{4}$$

Now if T denotes the total number of offspring produced in the population, Z the number of offspring resulting from all matings of type (i) in the population and n the size of the sample (taken without replacement), then Z_1 has a hypergeometric distribution,

$$P(Z_1|T,n,Z) = \frac{\binom{Z}{Z_1}\binom{T-Z}{n-Z_1}}{\binom{T}{n}} \ . \tag{5}$$

Similarly, the distribution of the number of offspring produced by matings of type (i), out of a total of T offspring, is also binomial with parameters $(T; R/M)$, where R is the number of matched matings and M is the total number of matings,

$$P(Z|T,R,M) = \binom{T}{Z}\left(\frac{R}{M}\right)^Z\left(1-\frac{R}{M}\right)^{T-Z} \ . \tag{6}$$

It follows, by a simple combination of equations (4), (5) and (6), that the distribution of the total number of diploid males in the sample, Y, is also binomial with parameters $(n; R/2M)$,

$$P(Y|R,n,M) = \binom{n}{Y}\left(\frac{R}{2M}\right)^Y\left(1-\frac{R}{2M}\right)^{n-Y} \ . \tag{7}$$

The distribution of the number of matched matings, R, is again binomial with parameters $(M; \theta)$,

$$P(R|M,\theta) = \binom{M}{R}\theta^R(1-\theta)^{M-R} \quad R = 0,1,2,\ldots,M \ . \tag{8}$$

Therefore, the distribution of Y given θ, found by combining (7) and (8), yields

$$P(Y|M,n,\theta) = \sum_{R=0}^{M} P(Y|R,n,M)\ P(R|M,\theta) \ . \tag{9}$$

This equation treats the number of matings M as a fixed number for any one sample, but can vary from sample to sample. If the distribution of M were known to be $P(M|\lambda)$, where λ denotes the expected number of matings, then the distribution of Y given θ and λ is

$$P(Y|\lambda,\theta) = \sum_{M=1}^{\infty}\sum_{R=0}^{M} P(Y|R,M)\ P(R|M,\theta)\ P(M|\lambda) \ . \tag{10}$$

The assumption of a truncated Poisson distribution for M is probably reasonable, that is

$$P(M|\lambda) = \frac{\lambda^M e^{-\lambda}}{M! \, (1 - e^{-\lambda})} \, . \tag{11}$$

It is worthy of mention that the assumptions involved in the derivation of equation (9) are few. We assume that the numbers of diploid males amongst offspring from each matched mating are independent, that the probability of a matched mating is constant for all matings, and that the sample of offspring observed is a random sample taken without replacement from all offspring. All of these assumptions can be easily justified from the Mendelian behavior of the sex locus and from the observed lack of any viability differences amongst homozygotes and heterozygotes (WOYKE 1965b).

ESTIMATION

In this section we describe procedures to derive estimates of the number of matings and the number of sex alleles.

The data we use to estimate the parameters of our model are of the form $(Y_1, n_1), \ldots, (Y_s, n_s)$ where Y_i represents the number of diploid males observed in the ith sample of size n_i and s is the number of samples. One sample was taken from each hive.

First we obtain an estimate of θ, then treating the parameter θ as fixed, and equal to this estimate, we determine the number of matings using the procedures described in detail below.

Estimation of the number of alleles at the sex locus: A reasonable estimate of θ is given by

$$\hat{\theta} = \frac{2 \sum_{i=1}^{s} Y_i}{\sum_{i=1}^{s} n_i} \, . \tag{12}$$

This estimator is unbiased and by standard procedures has variance

$$\text{Var} \, (\hat{\theta}) = \frac{\theta(1-\theta)}{N^2} \sum_{i=1}^{s} \frac{n_i(n_i-1)}{M_i} + \frac{\theta(2-\theta)}{N} \, , \tag{13}$$

where N is the sum of the n_i, and M_i is the number of matings producing the ith sample. If we assume that the number of matings has a truncated poisson distribution (see equation 11) then the variance of $\hat{\theta}$ is approximately

$$\text{Var} \, (\hat{\theta}) \simeq \frac{\theta(1-\theta)}{N^2(\lambda-1)(1-e^{-\lambda})} \sum_{i=1}^{s} n_i \, (n_i - 1) + \frac{\theta(2-\theta)}{N} \, . \tag{14}$$

This approximation is reasonable for λ large, which is the case for our data.

Although the variance of $\hat{\theta}$ is unknown, it can be estimated by replacing the unknown parameters by their estimates (see RESULTS section). The estimate of

the variance obtained by this approach is 1×10^{-4}, and thus the standard error of $\hat{\theta}$ is 0.01. Therefore any loss in precision by the use of a two-step process is minimal.

Assuming an equilibrium exists in the population, we combine (3) and (12) to obtain an estimator of the number of sex alleles, given by

$$\hat{K} = \frac{\sum\limits_{i=1}^{s} n_i}{\sum\limits_{i=1}^{s} Y_i} \ . \tag{15}$$

The expectation of this estimator is infinite because there is a positive probability that $\sum\limits_{i=1}^{s} Y_i = 0$, namely that no diploid males are produced in all matched matings. Although this possibility is small, we investigated the sampling distribution of $\hat{K}$ to determine the suitability of this estimator for our data.

The sampling distribution of $\hat{K}$ was obtained empirically by computer simulation. Fifty replicates of the 90 samples were generated based on model (11) with the parameters θ and λ replaced by their estimates (see RESULTS section). An estimate of $\hat{K}$ was then calculated using equation (15) for each of the 50 replicates. The mean of the 50 replicates was 18.94 which is identical to the estimate obtained using (15) (see RESULTS section). Thus we conclude that the estimator given by (15) is satisfactory for our data.

Generation of the sampling distribution of $\hat{K}$ also allowed a determination of an empirical confidence interval for estimated number of alleles. The values obtained from the replicates ranged from a low of 15 to a high of 24. However, 82% of the estimates were in the range of 17 to 21, while 90% of the estimates were in the range 16 to 22.

Estimation of the number of matings: maximum likelihood, assuming a distribution for M. While it is possible to formulate a single-step maximum likelihood estimation process based on equation (10), this becomes exceedingly cumbersome and expensive in computation time. As mentioned before we use a two-step process which is considerably simpler and for our data results in little loss of precision. We obtain $\hat{\theta}$ from equation (12) and $\hat{\theta}$ is substituted for θ in the likelihood equation,

$$L(\lambda, \theta) = \prod_{i=1}^{s} \sum_{M=1}^{\infty} \sum_{R=0}^{M} P(Y_i | \lambda, \theta) \ . \tag{16}$$

This equation is impossible to evaluate numerically owing to the limits of summation on the M. For practical considerations we therefore set the upper limit of of summation on M as 100. This limit of 100 was determined empirically. Larger values did not affect the estimate of λ. The maximum likelihood estimate of λ is found by determining the value of λ for which $L(\lambda, \theta)$ is a maximum.

Estimation of the number of matings: without the assumption of a distribution for M. Little reliable information is available on the detailed mating behavior of Apis and consequently the assumption of any distribution for M may be

somewhat arbitrary. The assumption of a truncated Poisson distribution does not take into account genetic and environmental factors which can affect the number of matings. For example, the probability of identity by descent at other loci may affect fitness of the queens and consequently reduce the number of inseminations. In addition WOYKE (1964) mentions that disease and inclement weather conditions may sharply reduce the number of matings. Accordingly we present estimators of λ using equation (9) which assumes that the M_i are fixed numbers varying from sample to sample.

(i) An estimate of the number of matings, assuming no prior distribution can be obtained quite easily using a Maximum Likelihood approach. The number of matings for each sample is estimated using model (9) and setting $\hat{\theta}$ equal to $\hat{\theta}$. The maximum of equation (9) is calculated for all M_i and the value of M_i which maximizes this function is then the estimate of M_i, that is:

$$L(M) = \sup_{M} \sum_{R=0}^{M_i} P(Y_i|R,M) \, P(R|M_i,\theta) \ . \tag{17}$$

Undoubtedly for some samples or hives the estimate of the number of matings will be large. In particular cases where the Y_i/n_i are close to $\hat{\theta}/2$ the variances of the estimates of the number of matings are extremely large. Hence a weighted average of the $\hat{M}_i$ must be developed to obtain a reasonable estimate of λ. An intuitively reasonable approach is to use weights for the M_i which depend on both the variance within each hive $\sigma^2_{W_i}$ and the variance between hives σ^2_B. In fact, if these variances were known the following estimator is suitable

$$\hat{\lambda} = \frac{\sum\limits_{i=1}^{s} (\sigma^2_{W_i} + \sigma^2_B)^{-1}\hat{M}_i}{\sum\limits_{i=1}^{s} (\sigma^2_{W_i} + \sigma^2_B)^{-1}} \ . \tag{18}$$

Since these variances are unknown, we suggest that their sum be estimated using $(\hat{M}_i - \hat{\lambda})^2$. An estimate of λ can therefore be obtained from the following equation using standard iterative techniques:

$$\hat{\lambda} = \frac{\sum\limits_{i=1}^{s} (\hat{M}_i - \hat{\lambda})^{-2}\hat{M}_i}{\sum\limits_{i=1}^{s} (\hat{M}_i - \hat{\lambda})^{-2}} \ . \tag{19}$$

In this model, excessively large values of $\hat{M}_i$ would be expensive to compute and would contribute little to the overall estimate of $\hat{\lambda}$. For practical considerations, we therefore set a maximum value of $\hat{M}_i$ to be $5\tilde{\lambda}$ where $\tilde{\lambda}$ is an *a priori* estimate of $\tilde{\lambda}$. Thus the maximum of model (17) is defined within the range $M\varepsilon(1,5\tilde{\lambda})$.

(ii) An estimator of the number of matings also is obtained using a method of

moments type estimator. The variance of Y_i/n_i can be obtained exactly from (9) and is

$$V(Y_i/n_i) = \frac{\theta(1-\theta)}{4M_i} \left(\frac{n_i-1}{n_i}\right) + \frac{\theta}{4n_i}(2-\theta) \ . \tag{20}$$

If we assume that the variance of the number of matings between hives is small (σ_B^2 close to zero) we may use the sample variance of the Y_i/n_i, v^2, to obtain an estimator of $\lambda, \overline{\lambda}$ given by

$$\overline{\lambda} = \frac{\dfrac{1}{s}\,\theta\,(1-\theta)\,\sum\limits_{i=1}^{s}\dfrac{n_i-1}{n_i}}{4\left[v^2 - \dfrac{\theta}{2}\left(1-\dfrac{\theta}{2}\right)\dfrac{1}{s}\sum\limits_{i=1}^{s}\dfrac{1}{n_i}\right]} \ . \tag{21}$$

Estimation of the number of matings: using the frequency of the null class. We can also estimate the number of matings from the proportion of samples or hives which contain no diploid males. Such an estimator will be analogous to that derived for the mutation rate using the frequency of cultures containing no mutations (*e.g.*, LEA and COULSON 1949). Although in our case the estimator is not based on a sufficient statistic, data for this estimator could conceivably be gathered more simply. For example, pieces of comb could be incubated away from the parent hive, and protected from workers. The larvae hatching could then be scored for just presence or absence of diploid males after about 5 days. WOYKE (1969) describes methods for rearing diploid males.

Hives containing no diploid males can occur in either of two ways: (i) none of the matings are of the matched type and (ii) one or more of the matched type but no diploid males are produced. Equation (10) then reduces to the form

$$P(Y=0) = (1-\theta)^\lambda + P(Y=0|R>0)\,P(R>0) \tag{22}$$

If the contribution of each drone is substantial, the second part of equation can be ignored, reducing the probability of obtaining no diploid males to the probability that no matched matings occur. Thus by equating the proportion of cases in which no diploid males were observed, with $(1-\theta)^\lambda$, a second estimator λ^* is obtained, namely the solution of

$$P(Y=0) = (1-\theta)^{\lambda^*} \tag{23}$$

RESULTS

The frequency of diploid males averaged over the samples from 90 hives was 0.0503, with standard deviation 0.0309. The estimate of the probability of a "matched" mating obtained from (12) is 0.1056, with standard error 0.0100. Under the assumption of gene frequency equilibrium, the actual number of alleles is equal to the effective number of alleles (CROW and KIMURA 1970) and this number can be obtained from (15). For these data the number of sex alleles is estimated to be 18.9. An empirically derived 90% confidence intervals is 16–22.

TABLE 1

Estimates of the expected number of matings

Method	Equation	Estimate	Log_e likelihood calculated from (16)
Maximum likelihood—Truncated Poisson distribution of the number of matings	16	17.25	$-.20 \times 10^3$
Maximum likelihood—fixed number of matings for each hive	19	1.72	$-.54 \times 10^3$
		3.46	$-.38 \times 10^3$
		4.25	$-.34 \times 10^3$
		11.60	$-.22 \times 10^3$
		49.32	$-.10 \times 10^4$
		54.97	$-.13 \times 10^4$
		63.88	$-.18 \times 10^4$
Method of moments	21	61.75	$-.16 \times 10^4$
Frequency of the null class	23	30.48	$-.34 \times 10^3$

Estimates of the number of matings obtained from the four different approaches described earlier are shown in Table 1. The natural logarithm of the likelihood function (16) is also provided for each estimate to indicate the shape of the likelihood function. It can be seen that the different procedures yield widely different estimates. The maximum likelihood estimate of 17.25, assuming a truncated Poisson distribution of the number of matings, is the most efficient from a statistical standpoint. Procedures that do not incorporate a distribution for M give biologically unrealistic estimates for the mean number of matings. This is hardly surprising, because any estimate incorporating information on the distribution of the number of matings (assuming this information is reasonable) will be more efficient and therefore more realistic.

Equation (19) assumes that the number of matings for each have is fixed, but that this number is not necessarily the same for each hive. Equation (19) is a polynomial and therefore possesses multiple roots. For our data seven roots were found that were real and greater than zero, and these are given in Table 1. The existence of a multiplicity of solutions for these data effectively discredit this estimation procedure.

The method of moments procedure assumes that there is no variation in the number of matings between hives (σ_B^2 close to zero). However, it is likely that the number of matings between hives will vary considerably, and the unreasonably high estimate obtained from this procedure probably reflects the violation of this assumption for our data.

Estimation based on the number of samples containing zero diploid males also yielded an exceptionally high estimate for the number of matings. While this method is intuitively easy to understand, it is an approximation and is suitable only if the contribution of each drone in the *sample* is substantial. For our data, samples ranged from 40–92, which are probably too small for this estimator.

In summary therefore, the method of maximum likelihood assuming a prior distribution of the number of matings is most satisfactory for these data.

DISCUSSION

Estimation of the number of sex alleles: The estimate of the number of sex alleles in the population described here is considerably higher than those obtained for other populations of *Apis mellifera*. Two estimates for a population in Piracicaba, Brasil were 12.4 (LAIDLAW, GOMES and KERR 1956) and 11 (KERR 1967), and an estimate for a smaller population in Guarapari, Brasil was 8.5 (KERR 1967). In a U.S. population MACKENSEN (1955) estimated the number of sex alleles to be 11. With the exception of MACKENSEN's estimate, all figures require the assumption of gene frequency equilibrium, namely that all alleles occur in equal frequency. It is unlikely, though, that the difference between the estimate reported here and previous figures can be explained simply on the basis of the assumption of gene frequency equilibrium. If the population is not at equilibrium, any estimate of the number of alleles will be biased downwards. In population genetic terms the effective number of alleles in the population, n_e, is reduced, whereas the actual number, n_a, is of course unchanged. Thus the estimate in this paper and those obtained by LAIDLAW, GOMES and KERR (1956) and KERR (1967) must be considered minimum estimates. It is possible that the Piracicaba and Guarapari populations were not at equilibrium. However, the observed and expected numbers of alleles for these populations are in good agreement, arguing for the equality of allelic frequencies and the existence of an equilibrium (KERR 1967). Furthermore, the discrepancy between the estimate reported here and that obtained by MACKENSEN (1955) still would remain to be explained. We may safely assume therefore that the differences in the number of alleles found in the various populations are real and not a manifestation of an inequality of allele frequencies.

The number of alleles which can be maintained at one locus by a population ultimately depends on the rate of introduction of new alleles and the random extinction of alleles from the population. The rate of introduction of new alleles will be determined by the mutation rate at this locus. The rate of loss of alleles from the population will be determined by the effective size of the population. It is extremely unlikely that the mutation rate for the sex locus will differ from population to population. Consequently, we conclude that the most likely explanation for an increased number of sex alleles in the population reported here lies in an increased effective population number.

For our data two factors contribute to an increased effective population number.

(i) The most obvious explanation is of course that, *ceteris paribus*, actual population size is larger. The Piracicaba population containing approximately 200 hives is larger than the Guarapari population (KERR 1967) and also contains more sex alleles (11–12.4 *versus* 8.5). Available data for the population reported here suggest that population size is approximately 500 hives, which may completely account for the increased number of alleles.

(ii) Effective population number is increased substantially if the population is not isolated, but receives immigrants from other populations. The Horto

Navarro population described here has hybridized extensively with the African race of bee, *Apis mellifera adansonii* introduced into Brazil in 1956. This would serve to increase the effective population number significantly. Earlier estimates of the number of alleles in populations of *Apis mellifera* (LAIDLAW, GOMES and KERR 1956) were made either prior to the introduction of the African bee or just subsequent to it. Thus the large number of alleles in the Horto Navarro population can be explained by a large effective population number caused by a large actual population size and hybridization with the African bee.

Estimation of the number of matings: The mating behavior of the honey bee, *Apis mellifera*, has been the object of a considerable amount of study. In spite of the fact that as early as 1814 (HUBER 1814) multiple mating of the queen was observed, the idea that the queen mates only once was widely accepted until as late as 1940. The work of OERTEL (1940) and ROBERTS (1944) firmly established that multiple mating is the rule rather than the exception. Since that time a number of studies have been designed to estimate the number of drones mating with a single queen. Our estimate of the number of matings is 17.25, based on a maximum likelihood approach assuming a truncated Poisson distribution for the number of matings. This is higher than previous estimates, and it is worthwhile to compare this estimate with previously reported results.

In the past, three approaches have been used to estimate the number of matings.

(i) The first method, which may be called the direct method, involves the direct observation of the number of drones mating with a single queen. This type of data certainly indicates that many matings are possible since the entire mating process only takes a few seconds (GARY 1963); whereas the length of the mating flight has been measured at between 13 (TABER 1954) and 21 minutes (ALBER *et al.* 1955; ROBERTS 1944; WOYKE 1960). Furthermore, it has been observed that after a number of matings the sting chamber is held open by a mucus plug and this may provide a stimulus for future matings (GARY 1963). Two experiments with tethered queens, one where drones were removed manually, and one where the sting chamber was artificially left open by removing the terminal segments, have shown 25 and 37 drones respectively mating with a single queen (GARY 1963). These experiments, however, are generally criticized as representing an artificial situation, and it is unlikely that this number of matings/flight occurs often. In addition, these and other direct estimates of the number of matings only estimate the number of matings/mating flight. It is well known that queens can make up to five mating flights (ROBERTS 1944) and experiments on the artificial release of queens show that many more flights are also feasible (GARY 1971). Thus the total number of drones mating with a queen may be estimated by direct observation to be quite high. Such estimates are quite imprecise, of course, and no meaningful comparison with the results presented in this paper is possible.

(ii) The second approach, which may be called the indirect approach, involves a comparison of the semen found in the oviducts of the queen with the semen produced by a drone. Using this method, TRIASKO (1951, 1956) made the earli-

est estimates of the number of matings and he concluded that, on the average, a queen mates with 5, 10, or 13 drones depending on whether the calculations were made using the weight of semen, number of spermatozoa or volume of semen. WOYKE (1960), using the same procedure, measuring the volume of semen, estimated that a queen mates with between 1 and 17 drones, the average being 8–9. KERR *et al.* (1962) concluded that a queen mates with 7–12 drones based on the number of spermatozoa found in the oviduct of a queen. Although all of these figures are in reasonably good agreement, they estimate the number of matings/mating flight only. Only 10% of the sperm is retained in the spermathecae (RUTTNER, WOYKE and KOENIGER 1973), the remainder being expelled from the oviducts within 10–20 hours of mating (WOYKE 1960). There is no correlation between the amount of sperm in the spermathecae and the number of matings (WOYKE 1971), and the subsequent matings usually take place at least 24 hours later (WOYKE 1960; PARKER 1970). Therefore, this indirect approach is not able to estimate the total number of drones mating with a queen. However, judging by the frequency of queens flying more than once (68%; WOYKE 1960) the total number of matings is likely to be considerably more than the number for any single flight. Estimates of the number of matings obtained from this method are not, therefore, in conflict with the estimates presented in this paper, although no critical comparisons are possible.

(iii) A third approach, which may be called the mutant queen approach, involves the use of virgin queens homozygous for a recessive allele of a gene which is polymorphic in the surrounding population. The distribution of the phenotypes of the workers between the hives can then be used to obtain an estimate of the average number of drones mating with a queen. In contrast to the previous two approaches, this method can yield an estimate of the total number of drones mating with a queen, and so these estimates can be compared directly with those presented in this paper. Under the assumption that the number of matings follows a truncated Poisson distribution, TABER and WENDEL (1958) estimated the number of matings to be between 7 and 10 for four different populations in the U.S. and Canada. Thus their estimates of the number of matings are considerably lower than the estimates reported here. These estimates may be criticized on the basis that the method requires the used of mutant (in this case *cordovan*) queens, and these may mate less often than wild-type queens. In addition, these figures must be regarded with some suspicion because they are slightly lower than the estimates for a single mating flight, and under normal conditions 68% of queens fly more than once, although not all mate on succeeding flights. A possible resolution of the discrepancy between TABER and WENDEL's results and the results reported here may lie in the different environments in which the populations were located. Under inclement weather conditions queens will fly only once, and the U.S. and Canadian environments often provide weather highly unsuitable for queen mating. On the other hand, the invironments in which the Brazilian populations were located are subtropical and a much more amenable environment for apiaries. Thus the difference between the

two sets of estimates may reflect a difference in the number of mating flights undergone by bees in temperate and subtropical zones.

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