

Heritability of Worker Characters in the Honeybee (*Apis mellifera*)

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Abstract

Heritability of characters (e.g. hamuli number) of honeybee workers can be estimated from data obtained from a random sample of colonies from a single population without the need for controlled matings. Formulae are developed for the estimation of relatedness among workers within colonies as a function of number of drone matings with the queen and the relatedness among these drones. Heritability is then estimated by dividing the intraclass correlation of the character by the relatedness among the workers within colonies. Estimates obtained by this method are shown to be considerably less biased than those obtained by analysing data from single-drone inseminations because of the contribution of dominance variance to the covariance of sib workers.

The heritability of hamuli number of workers in a population from the Sydney region (mean number 21.14, s.e. 0.07), estimated from an intraclass correlation based on 20 bees from each of 39 colonies and from published estimates of the average number of drone matings with each queen, was shown to be 0.68, s.e. 0.18.

Introduction

Difficulties of Heritability Estimation

Estimates of heritability of characters in the honeybee *Apis mellifera* are difficult to obtain, but their importance cannot be over-emphasized when developing selection programs. Various techniques for measuring heritability have been described and discussed by Rinderer (1977). These methods take account of the haplo-diploid method of sex determination and the caste system. Conventional methods of heritability determination (particularly parent-offspring regression) are inappropriate. This is because the characters of parents (queens and drones) cannot be regressed on those of workers belonging to another caste, which have a quite different pattern of development. Further, because of multiple mating and haplo-diploidy, the conventional half-sib and full-sib analyses approaches (Falconer 1981, p. 151) to the estimation of heritability cannot be applied since the half- and full-sib relationships are not appropriate. As Rinderer (1977) points out, the problems of sib analysis can be overcome by the use of artificial insemination. Where queens are inseminated with the homogenized pooled semen of some 20 unrelated drones, the relatedness of sib groups approaches that of diploid-diploid half-sibs (0.25), and allows the use of conventional methods of sib analysis for estimating heritability. [The technique described by Kaftanoglu and Peng (1980) would seem most appropriate for collecting semen for this purpose.] Similarly, the use of single-drone

inseminations produces worker offspring of defined relatedness (0.75). Rinderer (1977) gives formulae for calculating heritability in both of the above cases. However, these methods have the disadvantage that artificial insemination is an expensive and time-consuming process. Further, as will be shown, the use of inseminations by a single drone causes an upward bias in heritability estimates if the character displays non-additive genetic variation.

Clearly, if a simpler technique can be found which eliminates the need for controlled matings and queen rearing, then estimation of heritability would be considerably facilitated. Such a technique would exploit the fact that workers occupying a normal mature colony are 'sisters'. If an estimate of the average relationship among these sisters could be obtained, then it should be possible to estimate heritability from an intraclass correlation (t) calculated from samples taken from the population for which the heritability estimate is required. The estimate $\hat{h}^2$ is then simply (Falconer 1981, p. 151):

$$\hat{h}^2 = t/r, \quad (1)$$

where r is the average relatedness of sib groups.

A review of the literature has enabled us to obtain an estimate of r . Using this estimate we have applied the proposed method to a set of data pertaining to hamuli number in a randomly mating population of honeybees in the Sydney region. Hamuli (i.e. wing hook) number was chosen as a character for study since it is readily measured, is variable (Phillips 1929), and there is evidence that this variation is heritable (Goncalves 1972). Hamuli are located on the proximal end of the anterior margins of the posterior wings. Their biological function is to secure the wings in flight (Snodgrass 1956, p. 116).

Paternity of Worker Offspring of Naturally Mated Queens—Effective Number of Matings

If a queen mates with a number of drones, it does not follow that in a sample of workers taken from her hive at any one time that all the drone subfamilies will be represented equally. Six factors will affect the paternity of a sample of offspring, and thus their average relatedness. These are now presented in what we consider decreasing order of significance.

First, there are the claims or assumptions by some sociobiologists (Orlove 1975; Trivers and Hare 1976; Charnov 1978) that the semen of male Hymenoptera remains clumped in the spermatheca, thus raising the average relatedness of diploid offspring at any one time to 0.75. This hypothesis finds some support in the data of Taber (1955) who found highly significant temporal variations in the frequency of mutant progeny types from multiple-inseminated queens. Woyke (1963) demonstrated less temporal variation in a similar genetic marker experiment. However, he sought to interpret the fact that neither the first nor the last drone in the insemination were consistently favoured to indicate that the semen is essentially mixed.

Secondly, the number of drones in the mating is of critical importance in determining the paternity of the offspring. The present point of interest, however, is not the absolute number of drones which mate with honeybee queens, but rather the number which contribute to the subsequent fertilization of the queen's eggs just prior to the sampling period.

Thirdly, there are the relative amounts of semen contributed by each drone which may differ because of differences in ejaculate volume (Kerr *et al.* 1962) and also expulsion of semen after mating (Woyke 1964).

Fourthly, if the drones involved in matings are related to each other or to the queen, the average relatedness of sib workers will be raised. This seems unlikely to occur in most areas. Many hundreds of drones gather in drone-congregation areas, and these are drawn from a wide area (Ruttner 1972). Also, virgin queens tend to search out distant congregation areas (Pechhacker, personal communication).

Fifthly, sperm-competition could cause certain gametes to be over-represented in the offspring, and hence may raise their average relatedness. However, Parker (1970) noted that no conclusive evidence has been produced for the effect in *Apis mellifera*.

Finally, if the range of number of matings is high, then there may be a heterogeneous or even bimodal pattern of relatedness, with workers within some hives being closely related and workers within other hives being less closely related. Again, it does not seem likely that the range is high, since queens which are not sufficiently inseminated tend to fly again until they are (Woyke 1964) or they are rapidly superseded and are not likely to be found heading mature colonies.

Despite the above possibilities, current opinion is that semen is contributed roughly equally to fertilization by each semen load in the spermatheca (Crozier and Brückner 1981). Re-interpretation of data of Taber (1955) by Page and Metcalf (1982) suggests that while the effect of sperm-clumping may be quite high initially, it declines with time, so that one-year-old queens would produce offspring with each drone represented more or less equally. In their electrophoretic study they were able to show that in the cases studied there was 'a near consistent mixture of sperm'.

Estimates of the average number of drones involved in matings range from as low as 7 in temperate regions (Peer 1956) to as high as 17.3 in tropical regions (Adams *et al.* 1977). Indirect measurements of the number of matings of naturally mated queens are of greatest interest to the current discussion, rather than direct observations on mating behaviour such as those of Gary (1963) or the dissection approach of Woyke (1955). This is because we are concerned with the 'effective number of matings' defined here as 'the equivalent number of unrelated drones contributing equal amounts of semen to fertilization at any one time', rather than the actual number of copulations. This is equivalent to the 'effective promiscuity' coined by Starr (1979, p. 54).

Adams *et al.* (1977) estimated average 'number of matings' to be 17.3, whereas Taber and Wendel (1958), re-analysing the data of Peer (1956), obtained a value of 9.8. We are interpreting these as estimates of 'effective number of matings'. There are a number of possible reasons for the difference. Firstly, the truncated Poisson estimation technique used in both studies requires that the frequency of the genetic markers be known. Peer's data may fail in this respect if the cordovan marker drones were less competitive than the wild-type. On the other hand, sex alleles as used by Adams *et al.* rapidly approach equilibrium at equal frequencies in a panmictic population (Yokoyama and Nei 1979). Secondly (as claimed by Adams *et al.*), the climate may influence number of matings. Thirdly, there may be differences in the mating behaviour of the 'Africanized' bees analysed by Adams *et al.* and the bees studied by Peer.

We take the view that the published data is not sufficient to demonstrate the effective number of matings unequivocally in *A. mellifera*, particularly as it may vary between regions and races. We have arbitrarily chosen an average of the above values and conclude that a reasonable approximation of effective number of matings is 13. It will be shown later that increase in effective number of matings beyond 8 decreases relatedness by only small amounts.

Theory

Estimation of Heritability

One-way analysis of variance is used for obtaining estimates of heritability. The analysis is based on the model:

$$Y_{ik} = \mu + \alpha_i + \varepsilon_{ik},$$

where Y_{ik} is the observation on the k th worker from the i th colony, μ is the population mean, α_i is the effect of the i th colony and ε_{ik} is the effect of uncontrolled genetic and environmental deviations. Mean-square estimates from the analysis of variance are used to estimate the intraclass correlation (t) (Falconer 1981, p. 135), and then heritability from equation (1).

The approximate standard error of the heritability estimate is obtained from the analysis of variance as follows:

$$\text{S.E. } (h^2) = [\text{var}(\sigma_c^2)]^{1/2} / [r(\sigma_c^2 + \sigma_w^2)],$$

where

$$\text{var } (\sigma_c^2) = (2/k^2)\{[(k\sigma_c^2 + \sigma_w^2)^2/(c+1)] + [(\sigma_w^2)^2/(n-c+2)]\},$$

and

$$\begin{aligned} k\sigma_c^2 + \sigma_w^2 &= \text{between-colony mean square} \\ \sigma_w^2 &= \text{within-colony mean square} \\ c &= \text{number of colonies} \\ r &= \text{average relatedness of sib groups} \\ k &= \text{a coefficient to take account of unequal sample sizes} \\ &= [1/(c-1)][n - (\sum n_i^2/n.)] \end{aligned}$$

where

$$\begin{aligned} n. &= \text{total number of workers sampled, and} \\ n_i &= \text{number of bees sampled from the } i\text{th colony.} \end{aligned}$$

Determining the Average Relatedness of Workers

The relatedness of the workers in a single, non-inbred colony can be calculated as follows (Malecot 1948):

$$r = \frac{1}{2}(\phi + \phi'), \quad (2)$$

where

$$\begin{aligned} r &= \text{relatedness or coefficient of additive relationship,} \\ \phi &= \text{probability that the paternal allele in one worker is identical to that in a sibling worker, and} \\ \phi' &= \text{probability that the maternal allele in one worker is identical to that in a sibling worker.} \end{aligned}$$

Note that the above formula is only appropriate when the parents are not inbred.

Also of interest here is the probability that sibs will share genotypes as well as genes (i.e. the probability of correlated dominance deviations contributing to the sib intraclass correlation). This probability has the value $\phi \cdot \phi'$ and is also known as the dominance relationship. Taking this into account, the intraclass correlation t is comprised as follows:

$$t = (r\sigma_A^2 + \phi \cdot \phi' \sigma_D^2) / \sigma_P^2 \quad (3)$$

(ignoring other sources of covariance, such as additive by additive affects, etc. which would make only a very small contribution), the resultant estimate of heritability is:

$$\hat{h}^2 = t/r = (\sigma_A^2 / \sigma_P^2) + (\phi \cdot \phi' \sigma_D^2 / r \sigma_P^2). \quad (4)$$

Thus the bias in an estimate of heritability in the narrow sense obtained from sib analysis is: $(\phi \cdot \phi' \cdot \sigma_D^2) / r \sigma_P^2$. Values for r and $\phi \cdot \phi'$ are now evaluated for the various matings of interest.

Case 1: Single Drone Insemination

The situation is best illustrated thus:

Drone (haploid)	Queen (diploid)	Parents
a	$a_1 a_2$	
$p_1 m_1$	$p_2 m_2$	Random diploid progeny

where p = paternal allele and m = maternal allele. Now ϕ (the probability that $p_1 = p_2$) = 1 since $p_1 = p_2 = a$ and ϕ' (the probability that $m_1 = m_2$) = $\frac{1}{2}$ since the probability that $m_1 = m_2 = a_1 = \frac{1}{4}$ and the probability that $m_1 = m_2 = a_2 = \frac{1}{4}$. Hence $r = \frac{2}{3}$ and $\phi \cdot \phi' = \frac{1}{2}$. Thus the bias in heritability estimates is $\frac{2}{3} (\sigma_D^2 / \sigma_P^2)$.

Case 2: Queen Mated with n Unrelated Drones

In this case assume equal contributions of sperm from each drone, which are completely mixed, i.e. n = effective number of matings.

Drones	Queen	
$a_1, a_2, \dots, a_n$	$a_n + 1, a_n + 2$	
$p_1 m_1$	$p_2 m_2$	Random diploid progeny

The probability (ϕ) that $p_1 = p_2 = (1/n \cdot 1/n) + (1/n \cdot 1/n) + \dots + (1/n \cdot 1/n)$ for alleles a_1 with a_1 ; a_2 with a_2 ; up to a_n with $a_n = 1/n$. Now $\phi' = \frac{1}{2}$ (as previously). Therefore

$$r = (1/2n) + \frac{1}{4} \quad (5)$$

and

$$\phi \cdot \phi' = \frac{1}{2} (1/n), \quad (6)$$

and the upward bias in heritability will therefore be

$$[2/(2+n)] \cdot [\sigma_D^2 / \sigma_P^2]. \quad (7)$$

Equations (5) and (6) are shown in Figs 1(a) and 1(b) respectively. Note that for large n , r approaches $\frac{1}{4}$ —the situation in diploid half-sibs. The value of $\phi \cdot \phi'$ approaches 0 for large n . For $n = 13$, the value of $\phi \cdot \phi' / r$ is 0.13. Hence the bias in the

heritability estimate caused by the inclusion of dominance deviation will be relatively small in naturally mated queens.

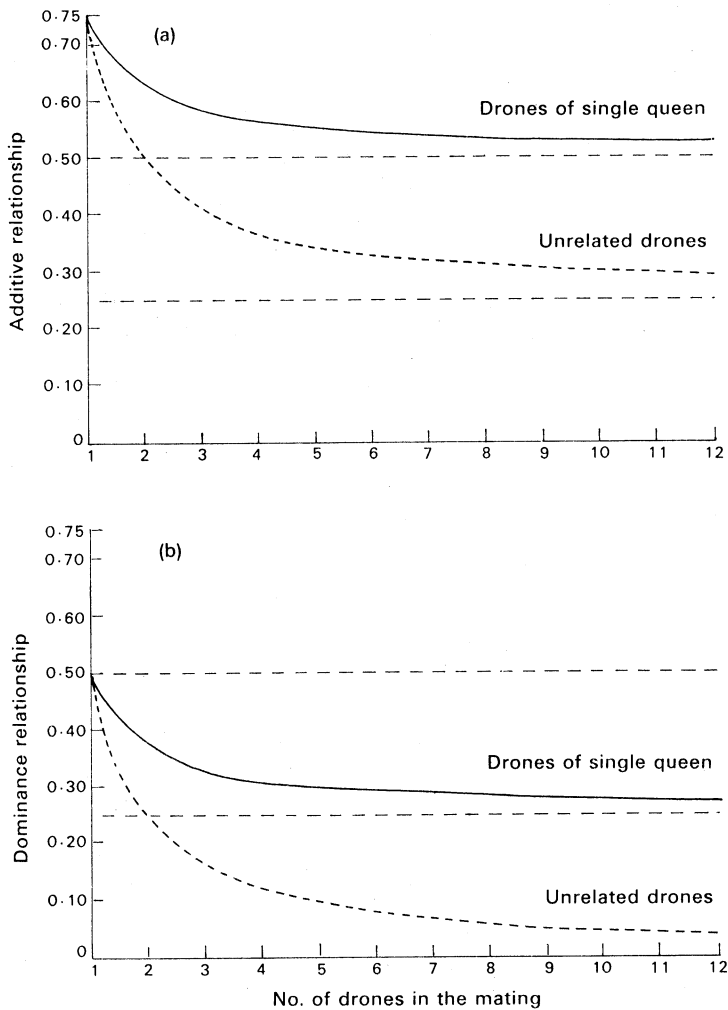


Fig. 1. (a) Additive relationship between the workers of a colony for from 1 to 12 effective matings: — relationship when the drones are of a single queen (equation 8); - - - relationship when the drones are unrelated (equation 5). (b) Dominance relationship for from 1 to 12 effective matings: — relationship when the drones are of a single queen (equation 9); - - - relationship when the drones are unrelated (equation 6).

Case 3: Queen Mated with n Drones Unrelated to her but Derived from a Single Non-inbred Drone Mother (e.g. Controlled Mating Carried Out with Artificial Insemination)

Drones	Queen	
a_1, a_2, a_1, a_2	$\times a_3, a_4$	
$p_1 m_1$	$p_2 m_2$	Random diploid progeny

There are two ways in which the paternal alleles of workers can be identical in this case. Firstly, both can be derived from the contributed semen of a single drone. The probability of this event is $(1/n) \cdot (1/n)$. However, there are n different ways in which this can occur. Thus the probability that both paternal alleles, p_1 and p_2 , are of the same drone is $1/n$, and the probability of identity given this is 1. Secondly, the gametes may be derived from different brother drones. The probability of this event is: $1 - (\text{probability that alleles are from the same drone}) = (n-1)/n$. Given that the gametes are derived from different brother drones, the probability that they are identical is $\frac{1}{2}$. Therefore,

$$\phi = (1/n) + \frac{1}{2}(n-1)/n,$$

and

$$\phi' = \frac{1}{2} \text{ (as previously).}$$

Thus:

$$r = (1/2n) + [(n-1)/4n] + \frac{1}{4} \quad (8)$$

and,

$$\phi \cdot \phi' = (1/2n) + \frac{1}{4}(n-1)/n. \quad (9)$$

The upward bias in heritability is:

$$(n+1)\sigma_D^2 / (2n+1)\sigma_P^2.$$

Equations (8) and (9) are shown graphically in Figs 1(a) and 1(b) respectively.

Note that as n tends to infinity, r tends to $\frac{1}{2}$, the relationship of diploid full-sibs. Also, Fig. 1(b) shows that considerable bias due to dominance deviation will occur in this case and that the dominance relationship asymptotically approaches $\frac{1}{4}$, the value for diploid-diploid full-sibs.

A summary of this theory is presented in Table 1.

Table 1. Summary of the relatedness of diploid offspring and bias due to dominance deviation for the matings of interest

Case No.	ϕ	ϕ'	Additive relationship $r = \frac{1}{2}(\phi + \phi')$	Dominance relationship $\phi \cdot \phi'$	Coefficient of dominance bias, $\phi \cdot \phi' / r$
1	1	$\frac{1}{2}$	$\frac{3}{4}$	$\frac{1}{2}$	$\frac{2}{3}$
2	1	$\frac{1}{2}$	$(1/2n) + \frac{1}{4}$	$\frac{1}{2} \cdot (1/n)$	$2/(2+n)$
3	$(1/n) + \frac{1}{2}(n-1)/n$	$\frac{1}{2}$	$(1/2n) + [(n-1)/4n] + \frac{1}{4}$	$(1/2n) + \frac{1}{4}(n-1)/n$	$(n+1)/(2n+1)$

Materials and Methods

Twenty worker bees were taken randomly from each of 39 colonies. Only colonies so situated that drift of foragers would be minimal were utilized.

An effort was made to sample unrelated colonies only. Twenty-four colonies were situated in a semi-commercial apiary, and these were mainly hived swarms of unknown origin collected in the Sydney region. Five of the colonies were from a small apiary that had been neglected for many years. There were four feral colonies, and the remaining six were in another semi-commercial apiary. In our opinion, it is reasonable to assume that these are random samples from a single population. To the extent that this assumption is not true, the present estimation of heritability serves only as an illustration of the proposed method.

The bees were collected in May 1980. Since May is the end of the season in Australia, the colonies and queens should have been mature. Each bee was killed by dropping it into 20% (v/v) ethanol as it was taken from the hive. Later the samples were scored by removing the right hindwing and counting the number of hamuli under $\times 20$ magnification. Where the right wing was mutilated or lost, the left was substituted if no alternative bee was available. This should not have disturbed the analysis, since high correlations have been reported between hamuli number on the left and right wing (Phillips 1929), even in highly inbred lines (Clarke 1982, personal communication). A total of 764 bees were scored. The optimum number of bees per sample and number of colonies, required in order to obtain minimum standard error of the heritability estimates, were determined using formulae presented by Falconer (1981, p. 168), assuming that heritability was about 0.3.

Results

The mean number of hamuli per bee was 21.14 with a variance of 3.42. The following analysis of variance was performed using the program 'Nesreg' (Hammond *et al.* 1972).

Source of variation	d.f.	Sum of squares	Mean square
Between colonies	38	606.61	15.96
Progeny with colonies	725	2003.23	2.76

The intra-colony correlation is 0.19606. Therefore, from equation (1), the heritability estimate is 0.68 with an approximate standard error of 0.183. The bias due to dominance deviation is $0.13 \sigma_D^2 / \sigma_P^2$.

Discussion

Heritability is high in the population sampled. While no direct comparisons can be made, it is of interest to contrast our results with three others. Roberts (1961) found an intra-class correlation of 0.29 for number of hamuli. It is not possible to calculate a heritability from this value since the relatedness of his sib-groups varied widely. However, the result seems to indicate a lower heritability than for our population, and since only two drones were used in the matings, average relationship was higher than in the present investigation.

By recalculating the analysis of variance presented by Lee (1974), we were able to obtain an intra-class correlation of 0.19. Again, because within-sib group relationship varied widely and also because some sib groups were related to each other, it was not possible to estimate heritability, although it would be seen to lower than that observed for the present population, since within-sib group relatedness was generally higher than in our case.

Goncalves (1972, 1976, p. 117) presents regressions of offspring on parents for hamuli number. However, since that author selected across castes, it is not valid to estimate heritability from the data presented, although it is obviously high for his population.

While with open-mated queens, upward bias in heritability estimates due to the inclusion of dominance deviation (see Fig. 1(b)) is small, there are other sources of methodical bias. Since the sib groups are raised in a common environment, covariance within sib groups may be higher than that expected from common genes alone. Techniques used by Roberts (1961) in which eggs were reared to pupation in specially prepared feeder colonies in order to standardize rearing conditions could reduce this effect.

Finally, we appreciate that the present method of estimating heritability is based on conflicting interpretations of the nature and magnitude of 'effective number of matings' in honeybees. While it now appears certain that hypotheses of absolute sperm clumping are incorrect, and the average relatedness of workers within hives is closer to $\frac{1}{4}$ than to $\frac{3}{4}$ (Page and Metcalf 1982), the issue is not fully resolved. Since the effect of sperm clumping is reduced with increasing age of queens, the effect in the present case would have been small, since only mature queens were used.

With naturally mated queens, it is highly likely that effective number of matings is at least 8.5 (Taber and Wendel 1958). If this number is substantially higher, then average relatedness of diploid progeny will remain relatively unaffected. For example, the present heritability estimate is 0.63, using the Taber and Wendel (1958) value for effective number of matings and 0.70 for the Adams *et al.* (1977) value.

Hopefully further data will be produced in the future to clarify the effective number of matings in open-mated queens. If and when this is done, the present method should become even more useful for the calculation of heritability of characters in worker honeybees. Even as it stands, it appears to be more appropriate that the method of insemination by a single drone, since it is much less biased by dominance variance.

Furthermore, techniques have recently been developed for empirical estimation of within-colony relatedness using electrophoretic markers (Pamilo and Crozier 1982). Application of these techniques to honeybees may provide more direct and accurate estimates of within-colony relationship, which can then be used for heritability estimation.

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