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Estimating the genetic variance of group characters: social behaviour of honeybees (*Apis mellifera* L.)

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Summary. A new approach is presented to estimate the genetic variance of social behaviour of groups. Honeybees (*Apis mellifera* L.) are used as an example for highly social organisms. Most characters of economic importance strongly rely on collective group characters of honeybee colonies. The average relatedness between small groups of workers of one honeybee colony can be estimated using a discrete multinomial distribution. The genetic variance of a social behaviour (alarm behaviour) of groups of honeybee workers is estimated with the intraclass correlation between groups within a colony. In two populations tested, the coefficient of genetic determination was high (0.96–0.98) indicating that the metabolic bio-assay used was only weakly affected by environmental effects.

Key words: Genetic variance – Group characters – Alarm behaviour – *Apis mellifera* L.

Introduction

Estimates of genetic variance are based on the analysis of individual organisms in classical quantitative genetics (Weber 1978; Falconer 1981). In populations which are highly socially structured, however, this constraint causes problems when genetic effects on social characters are tested. Groups often show a collective behaviour which cannot be found in the individual organism. Interactions and relationships between the different genotypes of the group members will be important genetic components which determine the actual phenotype of the group.

The present study will focus mainly on the honeybee (*Apis mellifera* L.) as a eusocial insect of economic importance. Though heritabilities of individual worker characters for honeybees have been estimated repeatedly (Oldroyd and Moran 1983; Moritz 1985) estimates of the genetic variance of social behaviour lack an underlying theoretical model. Also because of the polyandrous mating system (queens mate with up to 17 males (Adams et al. 1978)) and the resulting variance in intracolonial relationships between workers of one colony (Laidlaw and Page 1984), the honeybee is of particular interest for studying this problem. Most characters of economic importance, such as honey production, disease resistance (hygenic behaviour), and defensive behaviour or brood production result from a collective social behaviour of groups of honeybees within the colony (Collins and Rothenbuhler 1978; Milne 1980, 1982). A single worker will not show any of these characters and it is not possible to assign a certain phenotype or genotype for group behaviour to individuals.

Relationship between groups

The average relationship between two groups of workers from the same honeybee colony will be dependent on the group size. The probability of finding genes, identical by descent, in two large groups will be one, since all the queen's and drones' genes will be equally represented in each group. The smaller the group size, the larger will be the genetic diversity between any two groups. The probabilities for certain allelic combinations in a group will be given by discrete binomial distributions for the queen and multinomial distributions for drone genes.

The probability Φ' for identical genes from the queen in two different groups A and B, with n members each, will be

$$\Phi' = \sum_{i}^{2^n} \sum_{j}^{2^n} p(i,n) \cdot p(j,n) \cdot s_q(i,j) \qquad (1)$$

where

n = group size
$p(i, n)$ = probability of allelic combination *i* in group A
$p(j, n)$ = probability of allelic combination *j* in group B
$s_q(i, j)$ = probability of idential alleles by descent transmitted by the queen in allelic combinations *i* and *j*.

The terms $p(i, n)$ and $p(j, n)$ are dependent on the group size *n* and will follow a binomial distribution.

A similar equation can be used to estimate the probability, Φ' of male alleles identical by descent in both groups.

$$\Phi = \sum_{i}^{d^n} \sum_{j}^{d^n} p(i, n) \cdot p(j, n) \cdot s_d(i, j) \qquad (2)$$

where

n = group size
d = number of drones per mating
$p(i, n)$ = probability of allelic combination *i* in group A
$p(j, n)$ = probability of allelic combination *j* in group B
$s_d(i, j)$ = probability of identical alleles by descent transmitted by the drones in allelic combinations *i* and *j*.

Both $p(i, n)$, and $p(j, n)$ are dependent on the group size and will follow a multinomial distribution if more than two drones have mated the queen.

Covariance between groups

Similar to the definition of additive relationship for individuals, *r* (Falconer 1981), we now can define the additive relationship between groups, r^*, as follows

$$r^* = (\Phi + \Phi')/2 \,. \qquad (3)$$

In addition to additive effects, non-additive interactions between genes and also between genotypes will affect the phenotype of the group. The following simple classical model for the phenotype of an individual, Y_{ijk}, with mother, i, father, j, and population mean μ

$$Y_{ijk} = \mu + \alpha_i + \alpha_j + \delta_{ij} + \varepsilon_{ijk} \qquad (4)$$

with α = additive, δ = dominance, and ε = remainder effects, in which epistatic and maternal effects are not considered, can be extended for groups.

The genotypic value for Y_{ijk} is

$$y_{ij} = \alpha_i + \alpha_j + \delta_{ij} \,. \qquad (5)$$

Given a group *r* with *n* workers of queen *q* inseminated with semen mixture *d*, the phenotype X_{dqr} of this group is determined by the sum of all individual phenotypes and the interactions between the different members of the group. Thus, using (5) and (4) we get

$$X_{dqr} = \mu + \sum_{i}^{n} y_{idq} + \sum_{i}^{n} \sum_{j}^{n} \beta_{ijdq} + \sum_{i}^{n} \varepsilon_{idqr} \qquad (6)$$

where:

$\sum_{i}^{n} y_{idq}$ = sum of additive genotype effects;
$\sum_{i}^{n} \sum_{j}^{n} \beta_{ijdq}$ = sum of nonlinear genotype interactions;
$\sum_{i}^{n} \varepsilon_{idqr}$ = sum of environmental effects.

The following variance components are to be considered:

$$S_P^2 = S_A^2 + S_D^2 + S_{GI}^2 + S_E^2 \qquad (7)$$

where

S_P^2 = total phenotypic variance
S_A^2 = additive genetic variance

$$\left.\begin{array}{l} S_D^2 = \text{dominance variance} \\ S_{GI}^2 = \text{variance due to genotype interaction} \end{array}\right\} S_I^2$$

S_E^2 = environmental variance.

For honeybees a sib analysis as suggested by Rinderer (1976) enables us to estimate most of these variance components. The non-additive genetic variation, however, cannot be separated into S_D^2 and S_{GI}^2 with classical methods. This could be tested by experimentally arranging groups with certain genotype frequencies. For further considerations we will refer to, S_I^2, as the total of non-additive genetic variance.

Groups may covary because of these non-additive effects if male and female allele-pairs are identical by descent in these groups. In contrast to the model for individuals, these identical genes do not have to be in the same individual. This probability, which covers all non-additive possibilities of covariance due to gene and genotype interactions is $\Phi \Phi'$. Combining this with equation (6) we get (8), which is similar to the equation for individuals (Weber 1978)

$$\mathrm{Cov}[x, y] = [(\Phi + \Phi')/2] \cdot S_A^2 + \Phi \Phi' \cdot S_I^2 \qquad (8)$$

where

$\mathrm{Cov}[x, y]$ = Covariance between two groups X and Y
$[(\Phi + \Phi')/2]$ = r^* = additive relationship
$\Phi \Phi'$ = dominance relationship
S_A^2 = additive genetic variance
S_I^2 = non-additive genetic variance.

Mating types

We have to distinguish two common mating types in honeybee breeding. Case 1 is shown in Fig. 1 a, where all the drones which inseminate the queen are offspring from one drone mother queen ("sire" queen). This method can only be performed with the aid of artificial insemination. Since all drones are brothers, Φ cannot be less than 0.5 in this case.

The other case is natural mating, where matings with unrelated males are assumed. In this case the limit of Φ for an indefinite number of drones is 0 (Fig. 1 b). The graph for two males in Fig. 1 b is identical to the binomial case and also represents the dependence of Φ' on group size. Even for fairly large groups there is still considerable genetic variation between groups.

Genetic variance of alarm behaviour

A study on the alarm reaction of honeybees of two different populations may serve as an example for the estimate of genetic variance of a group character.

Five European honeybee colonies of *Apis mellifera carnica* and 10 colonies of *A. m. intermissa* from North Africa were tested in this study. The African bees were from an unselected feral population northwest of Tunis (Sejenane). The *A. m. intermissa* queens were naturally mated with an unknown number of drones and there was no evidence of any inbreeding in the population. The European colonies were from non-inbred commercial stock of the Institut für Bienenkunde in Oberursel (FRG). The *A. m. carnica* queens were artificially inseminated with a set of eight drones of one unrelated sire queen each. The sire queens for the different test colonies were also unrelated.

Newly emerged workers of these colonies were kept in an incubator at 30 °C, 60% RH and fed with water, pollen and sugar ad libitum. At the age of 4 days, 40 workers of the same colony were grouped and placed into a glass container (200 ml) connected to an air flow-through system (flow rate: 200 ml/min). A sensor measured the oxygen concentration of the air which had passed through the test container with the bees, a second flask contained 20% NaOH to absorb CO_2, and a third one had $CaCO_3$ to absorb water vapor in the air. A control channel recorded spontaneous changes of oxygen concentration in air which was not pumped through the system. An electronically timed T-valve allowed for the introduction of either fresh air, or air saturated with iso-pentyl-acetate (IPA, a major compound of the alarm pheromone of honeybees) into the test system. After reaching resting metabolic activity the test bees reacted to a fice second IPA-flush with a typical increase in their metabolism (Moritz et al. 1985). This was measured as a decrease of oxygen concentration in the system. The amount of oxygen metabolized (µl/bee/min) was calculated and the difference between oxygen consumption (ΔVO_2) before and after IPA application was used to measure the metabolic response to the pheromone. This bio-assay shows a typical group behaviour, since individual bees do not respond in the test and only in the social context of a group do they perform the typical reaction (Southwick and Moritz 1985).

Results

Table 1 shows the average metabolic reactions of the tested colonies. Fifteen groups of 40 workers were tested per colony. There is a significant difference between the samples from the two populations tested in this study. The workers of *A. m. intermissa* reacted with an average ΔVO_2 of 24.5 ± 0.84 µl/bee/min whereas *A. m. carnica* workers showed a significantly lower reaction of 16.59 ± 0.8 µl/bee/min ($t = 6.83$, $P < 0.01$).

Table 1. Average values for ΔVO_2 ± SE of the tested colonies. Fifteen groups of 40 workers (4 days old) were tested per colony

Colony number	*A. m. carnica* ΔVO_2 (µl/bee/min)	*A. m. intermissa* ΔVO_2 (µl/bee/min)
1	10.12 ± 0.33	30.13 ± 1.64
2	14.18 ± 0.26	36.93 ± 0.99
3	14.72 ± 0.26	11.13 ± 0.75
4	14.97 ± 0.91	29.10 ± 1.70
5	29.56 ± 0.26	30.81 ± 1.20
6		35.53 ± 0.67
7		26.73 ± 0.75
8		17.69 ± 0.91
9		17.67 ± 0.48
10		9.33 ± 0.48
Average	16.58 ± 0.80	24.50 ± 0.84

Table 2. ANOVA table for *A. m. carnica* (**a**) and *A. m. intermissa* (**b**). The standard deviations for the intraclass correlation, *t*, were calculated according to equation 13.6.2 of Snedecor and Cochran (1980)

a

Source of variance	Degrees of freedom	Mean of squares
Colony	4	832.1
Test group	70	4.1
Total	74	48.1
Intraclass correlation: $t = 0.93 \pm 0.10$		

b

Source of variance	Degrees of freedom	Mean of squares
Colony	9	1,459.1
Test group	140	18.5
Total	149	105.5
Intraclass correlation: $t = 0.84 \pm 0.13$		

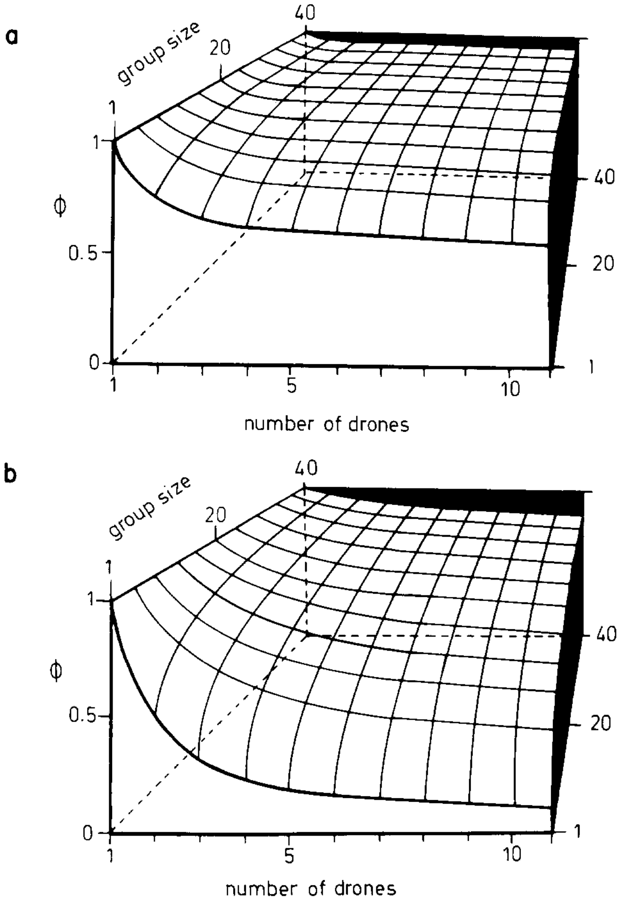

Fig. 1 a, b. Estimates for Φ (probability for a male gene to be identical by descent in two groups of workers) dependent on the number of workers in the group (group size) and the number of males which mated the queen. **a** all drones are offspring from one drone mother ("sire" queen); **b** all drones which mate the queen are unrelated. The case for two drones in **b** represents the course of Φ' (probability for a queen gene to be identical by descent in two groups of workers)

In order to determine the coefficient of genetic determination, h_b^2, of the metabolic reaction, the within-colony-test-group intraclass correlation, t, was estimated from an ANOVA (Table 2). The standard error was determined using the method of Snedecor and Cochran (1980). The average additive relationship between two test groups was estimated according to Fig. 1 with $r^* = (0.8 + 0.95)/2 = 0.875$ for the natural mated *A. m. intermissa* queens and $r^* = (0.94 + 0.95)/2 = 0.945$ for the artificial inseminated *A. m. carnica* queens.

Similar to the equation given by Falconer (1981) for individuals, we can define the following for the estimate of the heritability in groups

$$\mathrm{h}_b^2 = \mathrm{t}/\mathrm{r}^* \tag{9}$$

obtaining 0.96 ± 0.16 ($h_b^2 \pm$ SD) for *A. m. intermissa* and 0.98 ± 0.11 for *A. m. carnica* as estimates for the coefficient of genetic determination. Using these values as estimates for h^2 in the narrow sense (additive effects only), they may be biased up to 46% and 48% ($\mathrm{S}_\mathrm{I}^2/\mathrm{S}_\mathrm{P}^2$), respectively, due to non-additive effects, as we can see from equation (8).

Discussion

The different metabolic reactions to IPA in the two tested subspecies may well have practical importance. Since this behaviour is closely correlated to the defensive behaviour of honeybee colonies in the field (Moritz et al. 1985) the African bees are likely to be more aggressive than the European stock tested. It is not surprising to find preselected European material less reactive than bees from feral colonies. As there is a large genetic variance for this behaviour in the *A. m. intermissa* population, selection for low defensive behaviour should be successful.

The present model for estimating genetic variance of group characters can be used for other social behaviour in honeybees, such as longevity, hoarding behaviour or hygenic behaviour (Kulincevic and Rothenbuhler 1973; Milne 1980, 1982, 1983). With a more sophisticated pedigree than the one used in the present study, it would be possible to analyse the different genetic variance components more accurately. The present data show that a first rough estimate of h_b^2 of group characters is possible without performing any breeding prior to testing. If the estimates of h_b^2 are close to zero the tested character is unlikely to be selectable. This method, therefore, is a helpful tool to test the genetic variance of group characters before a sophisticated selection pedigree is performed as it allows more accurate estimates of the selectability, as suggested by Rinderer (1976). On the other hand, high estimates of h_b^2 do not necessarily predict high heritabilities in the narrow sense because of the large bias by non-additive gene and genotype interactions. Also, maternal effects and similarities in hive environment prior to eclosion may increase the intra-class correlation in the present data set. The results show high estimates for h_b^2 of the tested behaviour, considerably higher than for defensive behaviour found in field studies (Collins et al. 1984). This may be due to a bias in the estimate. However, the extremely controlled environment and the highly accurate bio-assay, might result in larger values for the heritability than in field studies. Collins et al. (1984) and Rinderer et al. (1983) found larger estimates for alarm behaviour in laboratory tests ($h^2 = 0.3 - 1.3$) than in field tests ($h^2 = 0.1$ to 0.93). Estimates of h^2 for group behaviour in previous studies do not consider genetic variation within the group. This may lead to drastic underestimation of the genetic variance, especially when there is a large variance in the numbers of males per mating and the group size is small.

A large variance in the numbers of males per mating may also affect the estimator for h_b^2 in the present study. This problem rises when workers from natural mated queens, with an unknown number of drones, are tested. In Fig. 1 we can see that this effect is particularly critical in small groups. For groups with more than 20 individuals, however, the effect on the estimate of Φ and r^* becomes less significant.

Several authors have shown that there is a non-homogeneous distribution of semen in the spermatheca of the queen, and each drone will contribute to the offspring at a different frequency (Laidlaw and Page 1984; Moritz 1983, 1986). In the present model, however, equal contributions of each drone are assumed, since the contribution of males in natural matings is unknown. Though these effects may not be very strong (Laidlaw and Page 1984), they may lead to underestimates of Φ and the genetic variance may be overestimated.

Nevertheless, the basic estimators in quantitative genetics, originally defined for characters of individuals can be easily redefined and used in the quantitative genetic analysis of collective group behaviour.

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