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Additive and Heterotic Genetic Effects in the Haplo-diploid Honeybee *Apis Mellifera*

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Abstract

Nine lines of honeybees were used to form a 9 × 9 partial diallel cross. Hamuli number was determined for samples of worker offspring. One set of workers was reared in non-maternal colonies which had been made uniform, as far as possible, with respect to colony strength (number of workers), while another set was sampled directly from the combs of each maternal colony. Combining ability analysis of variance revealed significant additive and non-additive genetic effects for both sets of data, regardless of whether inbred parentals were included or excluded from the analysis. Uniform rearing removed average heterosis and reciprocal effects.

The results demonstrate that there was both additive and, somewhat surprisingly, non-additive genetic variation in this haplo-diploid species, among the lines studied. A complex interaction between colony strength and hamuli number was evident from the fact that average heterosis was eliminated by uniform rearing, which removed the effect of variation in colony strength between inbred and hybrid colonies.

Introduction

Honeybees (*Apis mellifera*) are haplo-diploid. Hoshiba and Kusanagi (1978) demonstrated that in euploid tissue, there are 16 non-homologous chromosomes in males, and 16 pairs of homologous chromosomes in females. Sex in the species is determined by a series of balanced sex-limited lethal alleles (Crozier 1975). Individuals heterozygous at the sex locus are female. Individuals which are homozygous or hemizygous are male. However, homozygous diploids are eliminated by the workers at the first larval instar (Woyke 1963*a*, 1963*b*).

It has been varyingly estimated that there are from 6 to 19 sex alleles at equilibrium in outbred populations of honeybees, giving high brood viability (reviewed by Page and Laidlaw 1982). However, where inbreeding has occurred, the frequency of homozygous diploids in a colony will increase, to a maximum of 50%. The consequence of the balanced, lethal, sex-determining mechanism is that the detrimental effects of mating between relatives are more serious than in similar diploid species. This is because reduced brood viability reduces hive populations. When this occurs, the brood may not be properly cared for, with a consequential adverse effect on colony and individual performance. Thus crosses between highly inbred lines of honeybees might be expected to show high levels of heterosis for any characters causally related to colony population size, since such crossing will tend to eliminate sex-allele homozygosity, and will consequently lead to larger and stronger colonies.

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Conversely, characters expressed in both sexes (such as hamuli number) are expected to show less heterosis than in similar diploid species. This is because deleterious recessive alleles should be more rapidly eliminated in the haploid male caste, and overdominance obviously cannot operate in haploids. Thus the sex-allele system of honeybees might be seen as increasing the expression of heterosis in this species, whereas male haploidy might be seen as a factor decreasing the expression of heterosis, relative to diploid species.

It is difficult to separate the effects of sex-allele homozygosity and conventional inbreeding depression on honeybee characters. However, by rearing inbred and hybrid individuals together in the same hive, Brückner (1975, 1976, 1977, 1979, 1980) demonstrated inbreeding depression for several characters of honeybees, exclusive of sex-allele effects.

Hamuli are the hooks which hold honeybee wings together in flight (Snodgrass 1956). They are a convenient character for genetical study as they are variable in number (Alpatov 1929; Phillips 1929), and readily counted. Hamuli number is highly heritable (Oldroyd and Moran 1983) and has been shown to display moderate heterosis (Roberts 1961).

In this study, the concept of combining ability (Griffing 1956) is used to estimate the relative importance of additive and non-additive genetic effects for hamuli number, and to investigate the confounding influence of the sex-determining mechanism.

Materials and Methods

The Lines

Nine lines of honeybees, selected on the basis of their presumed genetic diversity, were chosen for study (see Table 2). Most of the lines consisted of the descendants of a single imported queen, maintained by brother–sister matings. All the lines were thought to have coefficients of inbreeding between 0·75 and 1 (the exact values cannot be estimated due to the inadequate pedigree records prior to importation), and were assumed for the purposes of this study to be totally inbred.

Experimental Procedure

A 9 × 9 partial diallel cross was formed between the lines using artificial insemination (Mackensen and Ruttner 1976). The queens, once inseminated, were kept in small hives (nuclei).

Daughter workers were sampled from each queen. In the first experiment, they were taken directly from the combs of the maternal colony (the maternally reared group). In the second experiment, the queens were induced to lay in small combs, which were then simultaneously removed to large colonies just after the eggs had hatched. In the latter case, therefore, all the larvae were reared in a uniform environment (the uniformly reared group). It was not possible to rear all the genotypes under uniform conditions contemporaneously. However, analyses of variance showed that no time or genotype-by-time interaction effects could be detected among the four sampling periods used. A small number of crosses were not available for uniform rearing, as there were losses of queens during the experiments.

For both experiments, the bees were killed, and the number of hamuli were counted by removing the right wing from each bee, and placing it between two microscope slides. Counting was performed under ×20 magnification. For the uniformly reared group, there were 14 replicates per cross, and for the maternally reared group, there were 20 replicates per cross.

Analysis

Fixed-effects (model 1, Griffing 1956) diallel analyses were performed on both sets of data, using the method of Pederson (1980), which adjusts for missing plots. These procedures produced a combining ability analysis of variance, and estimates of general combining ability for each line under both rearing conditions. General combining ability is approximately equivalent to the additive genetic merit or breeding value as defined by Falconer (1981). Heterotic effects were estimated for both sets of rearing conditions by comparing the average phenotype of first-cross workers with the average phenotype of contemporaneous workers of the two relevant parental lines. Reciprocal effects were estimated as the difference between reciprocal matings (Griffing 1956).

Results

Table 1 presents the combining ability analyses of variance for both sets of rearing conditions. In each case, the experiments have been analysed including and excluding the parental data (Griffing's 1956 methods 1 and 3). The exclusion of parentals from the analysis allows estimation of genetic effects free from inbreeding depression and sex-allele effects.

Table 1. Combining ability analyses of variance of hamuli number of honeybees under different rearing conditions

** Significant at the 0·1% level; n.s., not significant

Source of variation	Parentals included D.F.	Parentals included M.S.	Parentals excluded D.F.	Parentals excluded M.S.
		Maternally reared		
Genotype	55	14·99**	46	13·67**
General	8	52·27**	8	46·42**
Specific	33	8·34**	24	5·29**
Reciprocal	14	9·34**	14	9·34**
Error	1064	2·10	893	2·14
		Uniformly reared		
Genotype	40	11·28**	34	10·77**
General	8	35·98**	8	28·79**
Specific	24	6·51**	18	7·15**
Reciprocal	8	0·90$^{n.s.}$	8	0·90$^{n.s.}$
Error	533	2·06	455	1·98

Table 2. Estimates of general combining ability effects in honeybee races

The variance of the difference of any two general effects was obtained from the variance–covariance matrix after each element had been multiplied by the error-mean-square (Pederson 1980). The square root of this variance was used as an estimate of the standard error of the difference of these two general effects. Significance was determined using a two-tailed *t*-test. Where two effects are followed by a different letter, they are significantly different at the 5% level. The rank correlations of the combining abilities between the two rearing conditions are 0·84 and 0·87 ($P < 0·05$) for the inclusion and exclusion of parentals respectively

Line No.	Race	Parentals: Included	Parentals: Excluded	Parentals: Included	Parentals: Excluded
		Maternally reared		**Uniformly reared**	
1	*ligustica*	−0·26^{e}	−0·33^{d}	−0·01^{bc}	0·03^{bc}
2	*ligustica*	−0·20de	−0·21^{cd}	−0·05^{c}	−0·04^{cd}
3	*carnica*	−0·16^{d}	−0·10^{bc}	0·15^{bc}	0·22^{bc}
4	*carnica*	−0·01^{c}	−0·22^{d}	−0·34^{d}	−0·38^{d}
5	*caucasica*	0·36^{b}	0·28^{b}	0·31^{b}	0·37^{b}
6	*caucasica*	0·91^{a}	1·08^{a}	0·85^{a}	0·91^{a}
7	Synthetic	0·15^{bc}	0·14^{bc}	0·17^{bc}	0·23^{bc}
8	Synthetic	−0·04^{cd}	0·11^{bc}	0·27^{bc}	0·11^{bc}
9	Synthetic	−0·73^{f}	−0·74^{f}	−1·35^{e}	−1·44^{e}

In all analyses, genotype effects were highly significant. These were partitioned into general, specific and reciprocal effects. General and specific effects were highly significant for both sets of rearing conditions and with and without the inclusion

of parentals in the analysis. Reciprocal effects were significant only when the bees were maternally reared.

Table 2 presents the general combining abilities of each line. The combining abilities of the two *A. mellifera caucasica* lines were significantly higher than lines of the other two races.

Table 3 gives the values of the reciprocal effects (Griffing 1956) for the maternally and uniformly reared bees. Where the bees were maternally reared, the direction of mating caused significant differences in phenotype for eight crosses. Except for the cross between lines 4 and 5, this effect was removed by uniform rearing, and the overall reciprocal effects were removed by uniform rearing (Table 2).

Table 3. Reciprocal effects for uniformly and maternally reared honeybees

Cross	Maternally reared	Uniformly reared	Cross	Maternally reared	Uniformly reared
12	0·77[A]	0·18	25	0·45[A]	–
13	−0·67[A]	0·11	26	0·02	−0·21
14	−0·45[A]	–	34	0·85[A]	0·04
15	−0·22	−0·11	35	−0·55[A]	–
16	0·42[A]	–	45	−0·12	−0·29[A]
23	−0·42	–	46	−0·42[A]	0·25
24	−0·35	0·11	56	0·22	–

[A] Effect significantly different from 0 using the variance estimator presented by Griffing (1956).

Table 4. Comparison of mean hamuli number of intra- and interracial honeybee hybrids (lines 1–6 only)

Within rearing conditions, two means followed by a different letter are significantly different at the 5% level (two-tailed *t*-tests based on the error variance obtained from analysis of variance of the three genotypic groups)

Rearing condition	Mean hamuli number of: Intra-racial hybrids	Parentals	Inter-racial hybrids	Overall
Maternally reared	21·92[a]	22·08[ab]	22·42[bc]	22·24
% of parental mean	99·27	100·00	101·54	
Uniformly reared	21·53[a]	21·96[ab]	22·12[bc]	21·97
% of parental mean	98·04	100·00	100·73	

A comparison of the degree of heterosis in inter- and intra-racial crosses is shown in Table 4. Because of the unknown racial affinity of lines 7, 8 and 9, this comparison is only made for lines 1–6. For both rearing conditions, inter- and intra-racial crosses were not significantly different from the parentals. However, the interracial crosses had significantly more hamuli than the intra-racial crosses. The overall (average) heterosis may be estimated by comparing the mean of all F_1's, with the mean of all parentals, including the three lines excluded from Table 4. For the maternally reared group, the parental mean was 21·70 hamuli (s.e. = 0·12) and that of all F_1's 22·23 hamuli (s.e. = 0·05), the difference being

significant ($P < 0{\cdot}01$, $t = 3{\cdot}91$, d.f. = 1118). For the uniformly reared group, the parental mean was 21·83 hamuli (s.e. = 0·20), and the F_1 mean was 21·74 hamuli (s.e. = 0·07). These are not significantly different ($P = 0{\cdot}625$, $t = 0{\cdot}62$, d.f. = 572). Hence the average heterosis was reduced to an insignificant level by uniform rearing.

Discussion

Hamuli number was variable among the lines studied, and the contribution of genetic effects to this variation was substantial as demonstrated by the significant general and specific combining ability effects (Table 2). General combining ability effects contributed about 2·5 as much variation among lines as did specific effects. Reciprocal effects were significant where the bees were maternally reared.

Because offspring samples were reared in separate hives in the maternally reared group, genotype effects may have been inflated by common within-genotype environmental variance. Hence the combining abilities of this group should be treated with caution. However, any common environmental effect was removed by uniform rearing, and the general combining ability results were highly correlated for both rearing conditions.

General combining ability effects were significant in all analyses. This indicates that there were additive genetic differences in hamuli number among the lines studied. The *A. m. caucasica* lines (5 and 6) had the highest general combining abilities. The *A. m. ligustica* and *A. m. carnica* lines were considerably lower in their combining ability than the *A. m. caucasica* lines. They were not significantly different from each other. This observation is in agreement with Ruttner (1975), who considers that the *A. m. carnica* and *A. m. ligustica* races are closely related. Further, Alpatov (1929) demonstrated that in *A. m. caucasica* specimens from southern Russia, hamuli number is high.

The reason for conducting the experiments under the different feeding regimes was to compare the effect of the two environments on heterotic and reciprocal effects. Reciprocal effects were highly significant in the maternally reared group, but not significant in the uniformly reared group. This suggests that the feeding environment was significant in influencing phenotype. The fecundity of the dam influences the number of bees in a colony. Hence, in the maternally reared group, certain crosses may not have had sufficient nurse bees available for optimal feeding of larvae, while others would have had an ideal feeding environment. Given that reciprocal effects were not significant in the uniformly reared group, conditions in the maternal hive appear the most likely cause of the significant reciprocal effects observed in the maternally reared group. There is some suggestion in the work of Roberts (1961) that maternal effects influence morphological characters. He showed differences between a number of uniformly reared reciprocal crosses between eight inbred lines of honeybees, for a suite of morphological characters, including hamuli number. Further, Moritz (1980, 1982) has demonstrated cytoplasmically determined maternal effects for enzyme activity in honeybees. The elimination of reciprocal effects by uniform rearing in this study implies that there are no cytoplasmically transmitted factors influencing hamuli number.

Since specific effects were significant when the inbred parentals were excluded from the analyses, non-additive genetic effects have been demonstrated, unrelated to inbreeding depression, either of the conventional kind or consequent to the sex

allele system. It should be stressed that Griffing (1956) proposed exclusion of inbred parentals from diallel analyses specifically in order to detect such non-additive effects. Although the effects were small (accounting for about 2% of the total variance), non-additive (heterotic) effects, exclusive of sex-allele effects have been demonstrated for a character expressed in both males and females, in a haplo-diploid species. This demonstrates that conventional heterosis can exist in a haplo-diploid species, despite the fact that it will be restricted to only one sex. Since, average heterosis was significant only in the maternally reared group, it must have been largely due to the maternal hive environment. Queen honeybees mated to related drones have reduced brood viability due to cannibalism of diploid eggs homozygous at the sex locus (Woyke 1963*a*, 1963*b*). Thus, a reasonable interpretation of the present results is that the nuclei headed by queens mated to closely related drones lacked general 'vigour' because of reduced brood viability and a consequential depletion in the number of individuals per colony, and were thus unable to maintain adequate larval feeding. The uniformly reared group were not affected by such variation in the feeding environment, and hence the observed average heterosis was reduced to an insignificant level. The fact that, in the uniformly reared group, average heterosis was not significant, but specific effects were highly significant, indicates that the direction of heterosis varied between lines and crosses, and may further indicate that the optimal number of hamuli is intermediate.

In both the maternally and uniformly reared groups, significant differences between the two offspring groups could be detected, the interracial hybrids having a significantly higher number of hamuli than the intra-racial hybrids (Table 4). This observation accords with the expectation of greater heterosis arising from greater genetic divergence between the lines crossed.

In conclusion, this investigation of hamuli number has shown considerable additive genetic variation among the lines studied, and has indicated that this was mainly attributable to differences between races. The importance of the maternal environment in determining average heterosis of a morphological character has been demonstrated. A degree of non-additive genetic variation was found, and this was somewhat surprising since the major models of heterosis and inbreeding predict reduced importance of non-additive genetic effects in haplo-diploid organisms such as honeybees, for characters like hamuli number which are expressed in both sexes. These non-additive effects were shown to be exclusive of the effects of the sex-determining mechanism, since they were still detected after uniform rearing, and the exclusion of the parentals from the analysis.

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