Effects of Intracolonial Genetic Diversity on Honey Bee (Hymenoptera: Apidae) Colony Performance

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Ann. Entomol. Soc. Am. 85(3): 335–343 (1992)

ABSTRACT Honey bee colonies having varied genetic diversity were produced from five inbred lines. One line was used as a queen mother of 62 experimental colonies. These queens were inseminated with various combinations of semen obtained from single colonies of the remaining four lines. In estimating colony performance, the seasonal weight gain and mean brood area of colonies comprising two or three subfamilies were compared with those of colonies comprising a single subfamily. Some specific combinations of subfamilies reduced colony performance, whereas others enhanced it. The results suggest that present methods for estimating quantitative genetic parameters in honey bees may be inexact approximations because they fail to take into account the effects of interactions among subfamilies, which may be quite large. Some consequences of these subfamily interactions for honey bee breeding programs are discussed.

KEY WORDS Insecta, *Apis mellifera*, quantitative genetics, breeding

A HONEY BEE, *Apis mellifera* L., queen mates with 6–17 drones early in her life (reviewed by Laidlaw & Page [1984]). The semen of these matings is stored in the queen's spermatheca and released as required to fertilize eggs. Mating occurs well away from the nest in locales known as drone congregation areas, where males from many different colonies aggregate (Zmarlicki & Morse 1963). This behavior means that all drones mating with a honey bee queen are likely to be unrelated, leading to colonies that are rich in genetic diversity for many traits.

Sex in this species is determined by a series of balanced alleles (Mackensen 1951). Individuals heterozygous at the sex locus are female. Hemizygous (i.e., haploid) individuals are male. Individuals diploid but homozygous at the sex locus are male but are eaten by nurse bees shortly after they hatch (Woyke 1963). Shaskolsky (1976) and Page (1980) suggest that honey bee polyandry may have evolved to compensate for the reduction in fitness resulting when a queen mates exclusively with a drone carrying one of the same sex alleles as herself. Such a mating results in much reduced brood viability and a large reduction in queen fitness.

Another explanation for the evolution of this multiple mating is that a colony has increased fitness because of greater genetic variance within its worker population (Crozier & Page 1985). Haplodiploidy and polyandry create honey bee colonies made up of several subfamilies of worker bees, each sired by a different drone. Workers within a subfamily are super sisters and have a coefficient of relatedness of 0.75. However, workers from different subfamilies share no genes from a common father and have a coefficient of relatedness of only 0.25 (Page & Laidlaw 1988). Thus, there is a very high degree of genetic relatedness among bees within subfamilies but a high degree of genetic diversity among subfamilies.

These genetic differences among subfamilies within colonies may increase colony fitness and productivity in several ways (Crozier & Page 1985). First, behavioral specialization for particular tasks may increase overall colony efficiency. For example, colonies composed of groups with different floral type predilections might operate effectively over a broader range of environments than a colony composed of genetically uniform individuals. Second, genetically diverse colonies may be more resistant to pathogens than genetically uniform colonies (Sherman et al. 1988). Some plant breeding techniques specifically aim to produce genetically diverse varieties, which are less likely to be catastrophically susceptible to new pathogens (e.g., Jeger et al. 1981), for this reason.

Differences do occur in the average probable behavior of members of different subfamilies (Calderone & Page 1991). Subfamilial differences within colonies of unselected bees have now been demonstrated for a broad range of honey bee activities (Kolmes et al. 1989). These

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include guarding the hive entrance, removing dead bees from the hive (Robinson & Page 1988), defensive behavior (Breed et al. 1990), brood care (Noonan 1986, Noonan & Kolmes 1989, Page et al. 1989), grooming of nestmates (Frumhoff & Baker 1988), and tendency to collect nectar or pollen (Calderone et al. 1989, Oldroyd et al. 1991).

The widespread existence of intracolonial genetic variance in behavior intimately related to colony welfare suggests that this variance may not be selectively neutral, merely resulting from polyandry selected as a consequence of the sex determining mechanism. It may in fact be helpful or essential to colony function. If intracolonial genetic variance increases colony fitness, it should be possible to estimate its importance experimentally by comparing the performance of genetically diverse colonies with that of genetically uniform colonies. Colonies of bees composed of mixed lines should outperform groups of bees from constituent lines acting alone for traits related to fitness. Using analogous terminology from diploid quantitative genetics (Falconer 1981), if genetically mixed groups of bees behave differently from the mean behavior of constituent groups, we would have evidence of nonadditive intracolonial genetic interactions between groups (Moritz 1988, Moritz & Hillesheim 1989). On the other hand, where the performance of mixed groups can be predicted by the average performance of the constituent groups, we would have additive genetic interaction between groups.

We propose (and adopt here) the following terminology to describe such interactions. The terminology is analogous to definitions of general and specific combining ability as defined by Griffing (1956) for quantitative traits. The performance of a group of honey bees of defined size and average genetic relatedness of 0.75 we call the general subfamily effect (GSE). The specific subfamily effect (SSE) we define as the deviation of the performance of combined groups from that predicted from their combined GSEs.

There are several reports of the genotype of a group of bees affecting the behavior of other individuals through the group's social environment. Winston & Katz (1982) found that in cross-fostered bees, the genotype of the host colony influenced the age at onset of foraging of the introduced bees. Moritz & Southwick (1987) found that the alarm reaction of genetically mixed groups of bees sometimes exceeded that of the constituent groups that made up the mixture, demonstrating a significant positive SSE. Moritz & Hillesheim (1989) used instrumental insemination to produce colonies with varying levels of genetic variance. Samples of bees from these colonies where then used in a Kulinčevic & Rothenbuhler (1973) hoarding test. Bees from the genetically diverse colonies tended to hoard less than genetically uniform groups of bees, demonstrating a significant negative SSE.

The existence of SSEs has profound consequences for the design of honey bee breeding programs (Moritz & Hillesheim 1989). These programs have often aimed to reduce intracolonial genetic variance by using just a few drones, often from a single inbred drone-mother queen. More recent designs (Page et al. 1982, Moran & Oldroyd 1983) produce unnaturally large intracolonial genetic variance by using homogenized semen collected from a wide variety of drone mothers for the insemination of queens. If intracolonial genetic variance is desirable and subfamilial combinations often have large positive SSEs, traditional breeding programs may in fact be counterproductive. Conversely, if intracolonial genetic diversity is undesirable, modern programs, with their emphasis on the reduction of rates of inbreeding by maintaining extraordinarily high levels of intracolonial genetic diversity, may be counterproductive. Furthermore, methods for estimating the heritability of colony characters and predicting response to selection will need to be modified to account for SSEs if they are found to be important.

In this report, we describe an experiment in which colonies of low genetic diversity were compared with colonies of higher genetic diversity for the characters weight gain and brood area, which are important both economically and to colony fitness.

Materials and Methods

Five colonies were randomly selected from separate apiaries in the Baton Rouge area. Queens heading these colonies were naturally reared, open-mated, and not subjected to artificial selection of any kind. They therefore represented a random sample of the local honey bee population. Inbred lines were established from each of these colonies by "selfing." In this process, virgin queens are induced to lay parthenogenetic eggs by repeated treatments with carbon dioxide (Harbo 1986). The queens are then instrumentally inseminated with semen collected from these offspring males, which are genetically the queen's own gametes (Laidlaw & Page 1986). Lines A–D were inbred for one generation. A daughter queen was then reared from each line and allowed to open mate. These four queens had inbreeding coefficients, F, of 0.5 and were used as drone mothers. Line E was inbred for two generations of selfing. A queen was reared from the $F = 0.75$ generation and inseminated with the semen of a single brother drone. This queen was used as the mother of all experimental queens. Thus, experimental queens had an average coefficient of relatedness, G, of 0.83 and an F of 0.75. Fig. 1 shows the breeding scheme used to produce the inbred lines.

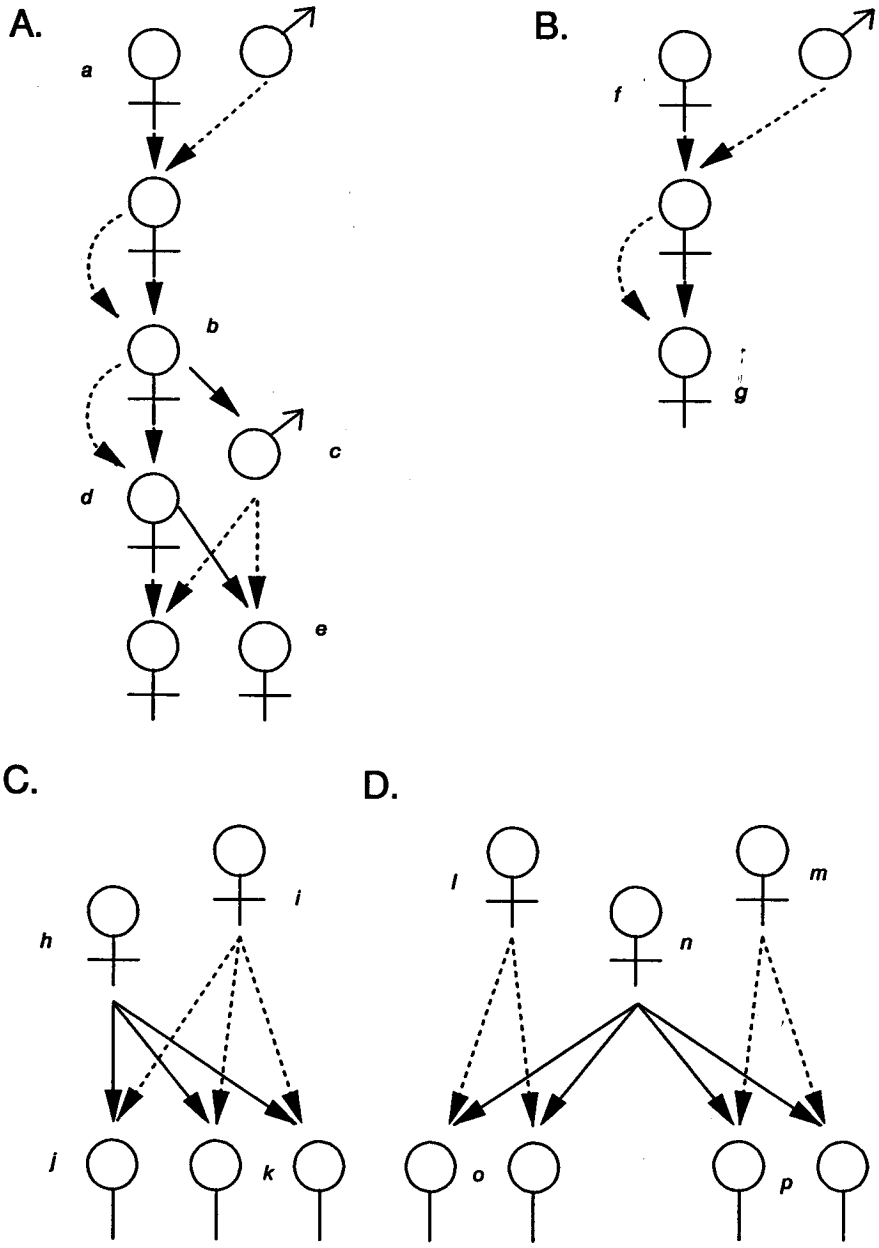

Fig. 1. Breeding scheme used to produce the experimental colonies. (A) Scheme used to produce the inbred queen mother. Queen *a* was a queen selected at random from the Baton Rouge population. This line was inbred by selfing for two generations. The queen *d* was inseminated by a single drone, *c*, obtained from queen *b*. Daughters of this mating, *e*, were used in experimental colonies. (B) Production of inbred drone mothers. Queens such as *f* were randomly selected from the Baton Rouge population. They were inbred for one generation to produce queens such as *g* with coefficients of inbreeding equal to 0.5. (C) Genetic constitution of single-subfamily colonies. Drones were collected from a colony headed by queen *i*, which is equivalent to queen *g*. Semen of these drones was homogenized. Queen *h*, equivalent to queen *e*, was inseminated with this semen. Workers *j* and *k* have zero inbreeding but a coefficient of relationship of 0.81. (D) Genetic constitution of two-subfamily colonies. Queens *l* and *m* are equivalent to queens *g* and *i*. Queen *n* is equivalent to queens *e* and *h*. The workers *o* and *p* have a coefficient of relationship of 0.44. Methods of computing coefficients of inbreeding and relatedness in honey bees are given by Laidlaw & Page (1986).

Experimental queens were reared and instrumentally inseminated with 8 μl of semen using standard methods (Harbo 1986) during a period of uniformly favorable environmental conditions in April and May 1990. Various semen mixtures were prepared from drones obtained from colo-

Table 1. Genotypes and numbers of colonies at commencement of experiment

Colonies					
Single-drone line		Two-drone line		Three-drone line	
Line[a]	*n*	Lines	*n*	Lines	*n*
A	6	AC	6	ABD	3
B	5	AD	7		
C	7	BC	2		
D	13	BD	7		
		CD	5		

[a] A, B, C, and D indicate drone mother queens ($F = 0.5$) used to provide drones to inseminate the experimental queens.

nies A–D to produce colonies of varying genetic constitutions. The actual crosses made are listed in Table 1 and the genetic constitution of the experimental colonies is given in Fig. 1. During any particular insemination session, semen from at least 25 drones was collected into a 50-μl capillary tube. Semen was then expelled into a microcentrifuge tube containing 500 μl of buffered saline solution (2.43 g sodium citrate, 0.04 g potassium chloride, 0.21 g sodium bicarbonate, 0.3 g glucose, 0.018 g benzylpenicillin, 0.02 g dihydrostreptomycin in 100 ml of 50 m*M* Tris buffer to give pH 8.5 at 25°C) (J. L. Williams, personal communication). Where a semen mixture was being prepared, equal amounts of semen from the different drone mother colonies were expelled into the saline solution. The semen and saline solution were thoroughly mixed by vortexing. Semen was then reconcentrated by centrifugation at 6,000 × *g* for 10 min. The supernatant was discarded and the semen taken up into a clean syringe. This procedure results in mixed spermatozoa (Moritz 1983) and a high rate of successful inseminations (Kühnert et al. 1989).

After queens had been laying for at least 1 mo (to ensure partial if not complete replacement of bees by offspring of the experimental queens), colonies were standardized to about four frames of bees and brood by removal of excess bees from stronger colonies. Colonies were then randomly allocated to two similar locations and given hive space for honey storage on 29 June 1990. Colonies were weighed at ≈10-d intervals using a clock-faced scale accurate to ±0.25 kg and the area of brood (eggs, larvae, and pupae in square centimeters) was estimated regularly by placing a grid on the surface of all brood combs and counting the number of squares that covered brood. To help eliminate observer bias, colonies were identified by a number rather than by treatment. However, differing integument colors of workers made paternally mixed colonies quite obvious. Hives were enlarged with additional equipment to accommodate expanding colony populations and honey stores when necessary and equipment was removed when populations declined. Appropriate adjustments to colony weights were made for these manipulations.

If any drone mother line carried the same sex allele as the queen mother line, a reduction in brood viability and colony performance would occur (Woyke 1986). Therefore, brood viability of each drone mother line in combination with the queen mother line was checked. An area of 100–200 newly laid eggs was chosen and their precise locations recorded with the aid of a plastic strip fixed with thumb tacks to the comb. The proportion of eggs present as larvae 3 d later, was taken as a measure of brood survival (Woyke 1976).

Results

Brood Area. Colonies with three paternities had significantly larger brood nests than colonies with only one or two paternities throughout most of the summer (Fig. 2). Colonies with two paternities were not significantly different from those with only one for this character.

To explore further the data for drone genotype effects, we first excluded two measurements. The initial measurement (June 29) was excluded because colonies had been recently made uniform with respect to brood area. The last measurement (November 16) was also excluded because all the colonies had virtually ceased brood rearing at that time (Fig. 2). Variances were found to differ among times within treatment groups, a violation of the assumptions of repeated-measures designs. Therefore, the data were analyzed as a multivariate analysis of variance (ANOVA), treating brood area at each time as a separate variable. The main effect for block and all interactions of blocks with other effects were not significant (variance ratios were less than one). Therefore the block effect and its interactions were removed from the model to produce a completely random design. This improved the balance of the experiment.

Independent of the number of subfamilies, significant drone effects were detected (Table 2, Fig. 3). Colonies with line A or C sires alone tended to have larger brood nests than colonies with line B or D sires. This means that drone genotype can affect colony performance. Fig. 3 also suggests drone genotype by environment interactions for the brood area phenotype. For example, ABD paternity colonies maintained large brood nests until the end of the season, when their brood nests were of average size.

The effects of drone genotype were partitioned into components of interest required to detect the presence of SSEs using linear contrasts (Table 2). Comparisons of two-subfamily colonies with the mean of colonies composed of constituent lines did not reveal any significant differences, although colonies with AC paternity had marginally smaller brood nests than the mean of

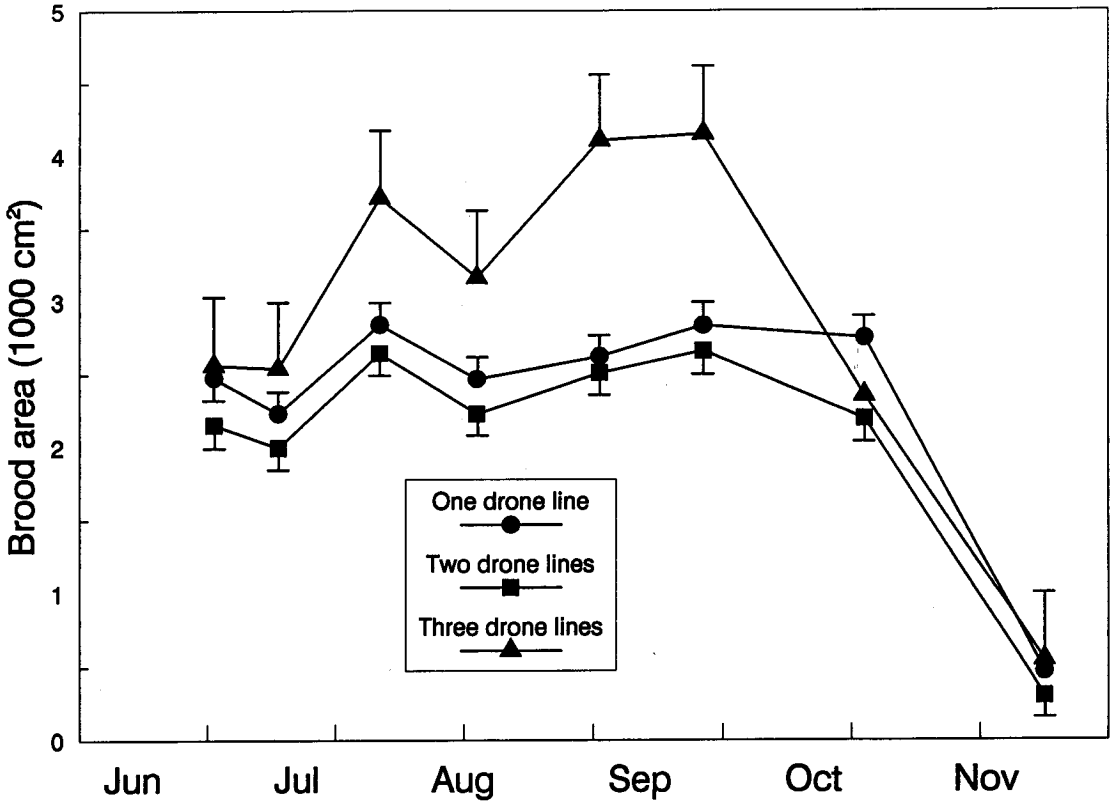

Fig. 2. Least-square means of brood area (1,000 cm²) of colonies composed of one, two and three subfamilies. Bars indicate standard errors of means, estimated from a repeated-measures ANOVA.

colonies of A and C paternity ($P = 0.096$). The three colonies sired by three drone lines (ABD) had marginally larger brood nests than the mean of colonies sired by the three constituent lines ($P = 0.098$) and the mean of mixed AD and pure B colonies ($P = 0.074$). This suggests the presence of two significant positive SSEs for brood area, with these three lines combining to produce colonies that maintained larger brood nests than the mean performance of colonies composed of various mixtures of the constituent lines.

Table 2. Subfamily combining effect multivariate ANOVA for brood area (cm²) of colonies of honey bees of various paternities[a]

Source of variation	Hotelling-Lawley trace	*F* Equivalent	*P*
Drone genotype	2.28	6.1	0.04
Mixed ABD vs. mean of A, B, and D	0.37	1.98	0.10
Mixed ABD vs. mean of mixed AD and B	0.40	2.15	0.07
Mixed ABD vs. mean of mixed BD and A	0.18	0.95	n.s.
Mixed AC vs. mean of A and C	0.37	1.99	0.10
Mixed AD vs. mean of A and D	0.05	0.28	n.s.
Mixed CD vs. mean of C and D	0.32	1.71	n.s.
Mixed BD vs. mean of B and D	0.17	0.92	n.s.

[a] Contrasts are not orthogonal.

Colony Weight Gains. Colony weight gain was estimated as the weight of each colony at the end of the season minus the colony's weight at the beginning of the season, corrected for any hive equipment added during the season. Two-way analysis of variance of drone genotype and location again revealed no location effect or interaction of location with drone genotype. Therefore, location effects were removed from the model for the analyses reported below.

Paternity affected colony weight gain (Fig. 4). Colonies with line A or C paternity had significantly greater weight gain than colonies with line B or D paternity. ANOVA was used to explore the data for the presence of SSEs using nonorthogonal contrasts (Table 3). Two significant negative SSEs were identified. Colonies with mixed A and C paternity had significantly reduced weight gain from what would be predicted from the performance of colonies with their constituent paternities. Similarly, lines C

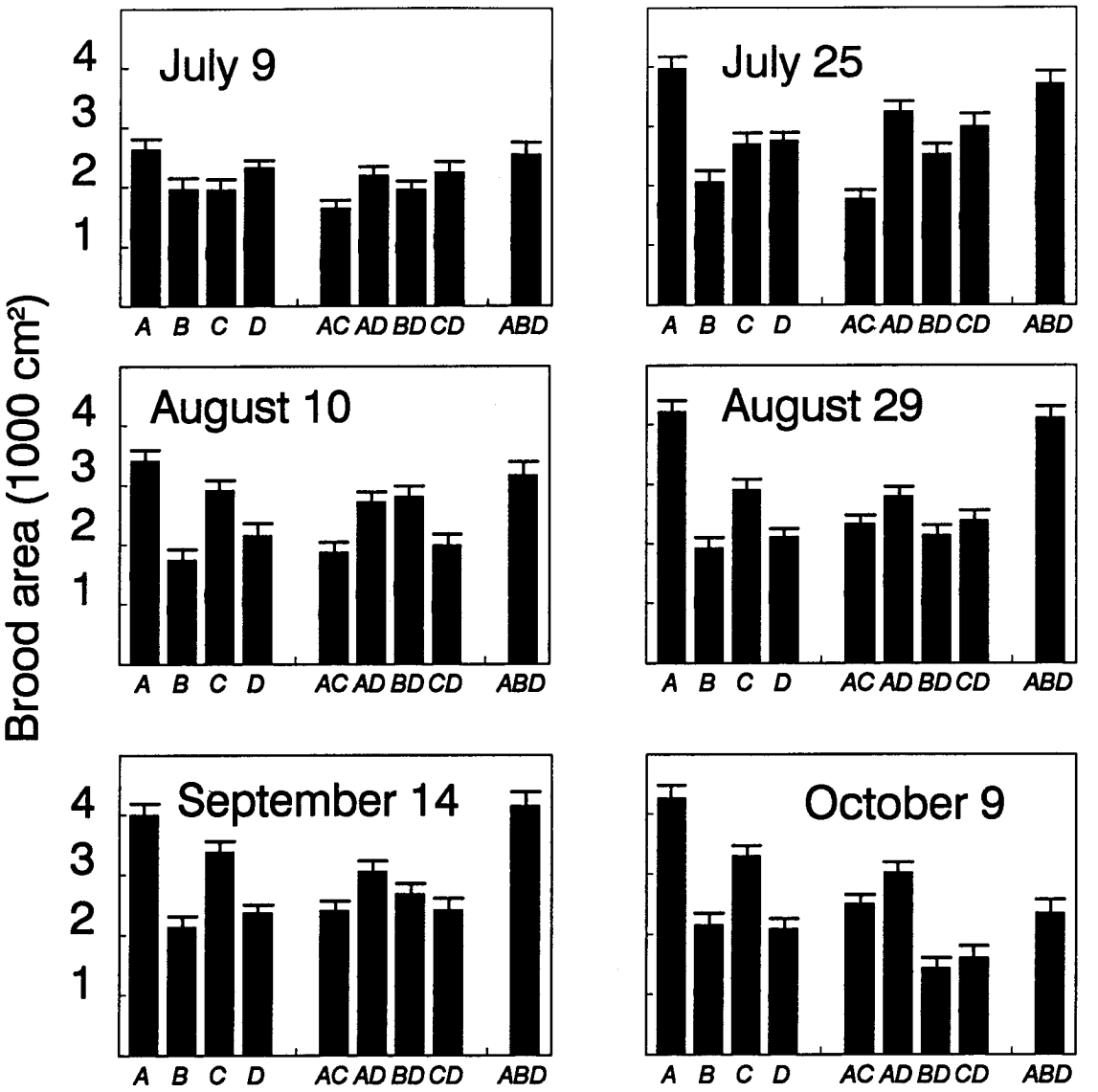

Fig. 3. Least-square means of brood area of each paternity group (cm^2) at each measurement (except the first). Bars indicate standard errors of means.

and D combined poorly. Such colonies gained less weight than would be predicted from the performance of colonies of constituent paternities acting alone. However, the three-paternity ABD colonies did not differ significantly from what would be predicted from the mean of colonies with their constituent paternities.

Brood Viability. Brood viability exceeded 95% for all combinations of drone lines with the queen line. Therefore, it was assumed that each drone mother line carried different sex alleles from the queen mother line and differences in colony performance were not due to sex allele homozygosity.

Discussion

All queens in this experiment were highly related, reared under uniform environmental conditions and inseminated with an equal volume of semen. They were placed in similarly sized colonies, and their progress was observed for a season. Differences in the performance of colonies was attributable largely to the paternity of the workers. Contrary to the findings of Oldroyd & Goodman (1990), worker paternity is shown here to have a significant effect on colony performance. Worker paternity can affect both colony weight gain and brood area (Tables 2 and 3).

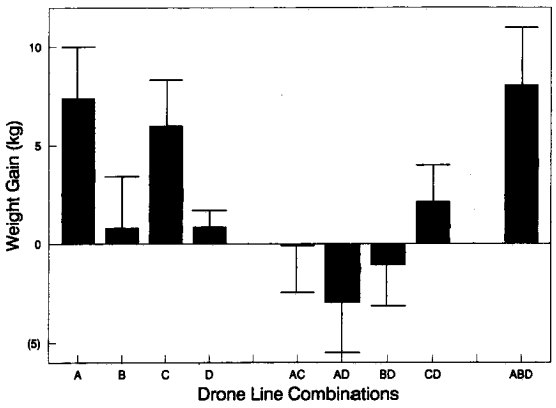

Fig. 4. Least-square means of seasonal colony weight gain (kg) for the various paternity groups. Bars indicate the standard errors of means.

Significant phenotypic correlations between brood area and colony weight gain have often been reported (reviewed by Oldroyd & Goodman [1990]). The present data also demonstrate such a correlation. Colonies with A and C paternity performed best for both the measured characters.

Of greater interest, however, is the demonstration that colony weight gain is not always a linear function of the performance of the individual subfamilies acting in isolation. Certain combinations of subfamilies produced significant deviations from that predicted by the additive combination of their GSEs. This result is consistent with the findings of Moritz & Hillesheim (1989), who found a significant negative correlation between the number of subfamilies in a group of bees and their performance in the Kulinčevic & Rothenbuhler (1973) hoarding test. We found two negative SSEs, in which combining two paternities in one colony resulted in a reduction in colony weight gain. However, we also found two positive SSEs which approached significance despite the small sample size. Colonies with combined ABD paternity had greater weight gain than the mean of AD + B colonies for both colony weight gain and brood area (Tables 2 and 3).

Table 3. Subfamily combining effects ANOVA for seasonal weight gain (kg) of colonies of honey bees of various paternities[a]

Source of variation	df	Mean square	*F*	*P*
Drone genotype	8	61.6	2.2	0.047
Mixed ABD vs. mean of A, B, and D	1	62.9	2.3	n.s.
Mixed ABD vs. mean of mixed AD and B	1	96.9	3.5	0.07
Mixed ABD vs. mean of mixed BD and A	1	53.6	2.0	n.s.
Mixed AC vs. mean of A and C	1	148.7	5.4	0.03
Mixed AD vs. mean of A and D	1	13.7	0.5	n.s.
Mixed CD vs. mean of C and D	1	125.5	4.6	0.04
Mixed BD vs. mean of B and D	1	12.7	0.5	n.s.
Error	35	27.3	—	—

[a] Contrasts are not orthogonal.

We have shown that certain combinations of paternities within a colony reduce colony performance, whereas certain combinations can increase it. These nonlinear interactions have implications for the design of bee breeding programs and for the estimation of heritability in honey bees. Published estimates of heritability of characters expressed by groups of honey bees (e.g., Soller & Bar Cohen 1967, Collins et al. 1984, Oldroyd et al. 1987, Bienefeld & Pirchner 1990) assume that subfamily interactions do not affect the performance of group characters. Our data demonstrate that these effects can be extremely large and cannot be validly ignored.

Our experiment was limited in scope and did not contain colonies of high genetic diversity. Thus, we cannot generalize our results or recommend simplifying assumptions for the calculation of quantitative genetic parameters for honey bees. That is, the fact that we found negative SSEs in two of four comparisons for colony weight gain does not mean that all two-subfamily colonies will tend to exhibit negative SSEs. We suspect that positive SSEs may exist in certain two-subfamily colonies. Similarly, our three-subfamily colonies were generally superior to colonies with less intracolonial genetic variability, but that does not imply that increasing genetic variance is generally beneficial to honey bee colonies. It is as likely that this specific combination of three subfamilies had a high SSE.

Rothenbuhler (1960) recommended the use of inbred queens mated to single drones for the study of group characters such as honey production. Such a technique allows the accurate estimation of GSEs. However, our study shows the genetic variance within a natural population of honey bees is such that SSEs can exceed GSEs. Hence, GSEs cannot be used to make meaningful predictions about colony performance. Indeed, adaptive genetic architecture of honey bee colonies may be founded on fortuitous SSEs. If so, breeding programs that maximize positive SSEs for economically important characters by optimizing the number and kind of subfamilies in commercial colonies may lead to substantial improvements in honey bee genotypic merit. Further, significant SSEs may influence the maintenance of polyandry in the species. If colony fitness is improved by multiple mating (re-

sulting in positive SSEs), then polyandry should be selected.

Acknowledgment

We thank J. Baxter, G. Perrone, and D. Pursifull for dedicated technical assistance; and R. Page and A. Collins for stimulating discussion and comments on the manuscript. The work was done in cooperation with the Louisiana Agriculture Experiment Station.

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Received for publication 8 July 1991; accepted 7 November 1991.