The value of single-drone inseminations in selective breeding of honey bees

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Abstract

By mating queens with spermatozoa from a single drone, it was possible to increase the expression of a colony trait that was found only at low levels in colonies with queens mated to many drones. Nonreproduction of varroa mites averaged 22% in colonies with queens mated to one drone but only 6% in colonies with queens mated to six drones. Mating with a single drone increased the phenotypic variance among a group of colonies, thus making it possible to detect a colony characteristic that was masked by multiple mating. The survival and fecundity of singly mated queens that were less than four months old were not different from those of sister queens that were each mated to six drones. However, singly mated queens produced fewer progeny in a test that began with queens that were 7 months old. Therefore, the technique of using singly mated queens may be useful in field testing and selective breeding as long as the queens are less than about six months old during the evaluation.

Introduction

Plans for the selective breeding of honey bees are slightly different from those of most other plants and animals because all spermatozoa produced by a male honey bee are genetically identical. A normal male honey bee (drone) is haploid, and in the production of spermatozoa a drone produces about 10 million replicates of the gamete (an unfertilized egg) from which he developed.

The technique of inseminating a queen with a single drone is not new. It was made possible by the development of instrumental insemination of queen bees and was described by Mackensen & Roberts (1948). When a queen is mated to one drone, worker bees in the colony all have identical genetic material from their father, who is represented by identical spermatozoa that are now in the sperm storage organ (spermatheca) of the queen. Thus the worker bees are more closely related than normal sisters and have a relatedness of 0.75. A group of closely related sisters in a colony (the daughters of the same drone) is called a subfamily.

Walter Rothenbuhler made extensive use of the single-drone insemination and was probably its strongest advocate. "If a colony is composed of only one subfamily, it expresses the full intensity of each genetically determined behavior characteristic... unmodified by social environmental factors imposed by bees of other subfamilies" (Rothenbuhler 1960).

In nature a queen mates with many drones. A queen probably retains some of the spermatozoa from each of her 10-20 matings, and the resulting colony of bees consists of many subfamilies, one from each of the drones that mated with the queen. Therefore, when a queen is mated to many drones, the worker bees in that colony have much more genetic diversity than do the workers in a colony with a queen inseminated with a single-drone. In most cases this diversity is probably beneficial for the colony (Oldroyd *et al.* 1992, Fuchs and Schade 1994). Page *et al.* (1995) conclude that colonies with greater genetic diversity are more average with respect to most characteristics (such as bee population, cell size, defense behavior, diseases, mortality rate, etc.) and that being average is better only because it may reduce the probability for colony failure.

Genetic diversity within a colony poses two problems for bee breeding. First, a colony with a genetically diverse population of worker bees may mask the expression of genetic characteristics that are present at low frequencies. Thus colony characteristics that occur at a low frequency (such as resistance to varroa mites) may not be detectable. Second, a queen produced from a colony with a multiply mated queen is not a good genetic representative of the colony. She will have only about 0.25 (half sister) relatedness to the worker bees in that colony. Thus, it is possible that a heritable characteristic that exists in a colony will not be present in a daughter queen. In contrast, a queen produced from a single-drone mating will have 0.75 relatedness to the worker bees in the colony. Therefore, the use of single-drone mating makes it easier to both find and then retain heritable characteristics in bees.

Unfortunately, the productive life of a queen inseminated with semen from a single drone is shorter than that of a queen that is inseminated with more semen. Queens mated to a single drone usually survive less than one year (Mackensen 1964, Camargo & Gonçalves 1971). Therefore, the period of evaluation and propagation needs to be brief. Colony evaluations are traditionally conducted for an entire beekeeping season, and such projects do not use queens inseminated with a single drone because most queens would not survive the duration of the test. However, a field test that lasts only 10-14 weeks (Harbo 1996) provides an opportunity to test colonies with queens mated to single drones.

The purpose of this paper was to show that selective breeding of honey bees can be more effective when test colonies consist of only one subfamily of worker bees (colonies with queens mated to one drone). A second purpose was to define the conditions wherein singly mated queens can be used successfully in field testing.

Materials and methods

The experiment consisted of 24 colonies that were established with uniform packages of bees and a test queen. The test queens consisted of two groups of sister queens that were randomly inseminated with semen from either one or six drones. The queens began laying about 10 April and the colonies were evaluated for populations of bees and mites on 27 June.

Bees and mites for the test were collected from normal colonies of bees into a single large cage on 29 March. The following day, they were subdivided into 24 smaller cages that were small versions of commercial packages of bees. Each package with 375 ± 31 g (mean ± SD) of bees was placed in a hive with 5 combs and a caged virgin queen. To minimize drift, screens confined the bees to their colonies until after dark on 31 March. Queens were given 3 minutes of CO_2 narcosis on 4 April just before they were released from their cages and on 5 April while they were inseminated. To prevent queens from leaving their hive to mate, one wing was clipped and queen excluders were placed over the entrances.

Queens were randomly assigned a treatment (one or six drones) and then inseminated with drones that had been collected from the entrances of >40 colonies at three different apiaries. The drones were mixed and recollected into cages so that each drone

Table 1. Analysis of variance of the percent nonreproduction of mites in brood cells. Independent variables were stock (queens were from 2 different sources) and insemination (queens were inseminated with either 1 or 6 drones). Data were log transformed because of unequal variances and skewed distributions.

	df	*mean square*	*F*	*P > F*
Stock	*1*	*0.002*	*0.12*	*0.77*
Insemination	*1*	*0.11*	*8.1*	*0.01*
*Stock * Insemination*	*1*	*0.002*	*0.15*	*0.71*
Error	*20*	*0.013*		

Table 2. Means ± SD of the four variables measured in the 24 colonies that began on 29 March with 375 ± 31 g of bees and 90 mites. The effect of a one- or six-drone insemination was the same in both stock types, so the 4 combinations of insemination and stock type are not listed .

Variable	1 drone(n = 13)	6 drones(n = 11)	Stock A(n = 11)	Stock B(n = 13)
Nonreproduction[1]	0.22 ± 0.16	0.06 ± 0.07	0.16 ± 0.17	0.14 ± 0.14
Cells of brood (26 May)	5286 ± 1515	5665 ± 934	5667 ± 1173	5285 ± 1369
Weight of bees (g) (27 June)	1217 ± 467	1212 ± 280	1301 ± 397	1142 ± 373
Mite population (27 June)	428 ± 168	565 ± 254	503 ± 203	481 ± 239

[1] The analysis of this variable is described in Table 1. None of the other variables showed any statistical differences.

selected for insemination was a random selection from the population that had been collected. Queens began laying about 10 April when they were 18 days old.

Colonies were evaluated for queen performance by measuring the amount of capped brood on 26 May and the weight of the bee populations on 27 June. Capped brood was measured with a wire grid with 1 inch (2.54 cm) squares. The weight of the bees in each colony was estimated by screening the entrances of all the colonies after dark on 26 June and then weighing the colonies with and without bees on the following morning (Harbo 1986).

Mite populations were measured on 27 June. The entire mite population in each colony was on the adult bees on 27 June because the queens had all been caged on 6 June, leaving no brood in the colonies. While the hive parts and frames were being weighed without bees (as mentioned in the paragraph above), a sample of ca. 150 grams of bees was taken from the population of bees that had been brushed from the combs into an empty hive body that was temporarily placed at the normal location of the colony. Thus each sample was taken from its colony as the total weight of bees was being estimated. The total number of mites in each colony could then be calculated by knowing the weights of the bees in the colony, the weight of the bees in the sample, and the number of mites in the sample.

Nonreproduction of mites in brood cells was evaluated by counting 300 cells of brood in the purple-eyed pupal stage. 10.0 ± 4.3 (mean ± SD) cells with mites were evaluated in each colony.

In a second test, a group of 18 queens was evaluated when they were 7–10 months old. Ten queens were inseminated with 1 drone and 8 with 6 drones. The queens were reared in May, inseminated in June, and allowed to lay eggs in small colonies for the rest of the season. Colonies were moved to a remote apiary on 29 December and the number of bees in each colony was calculated on 5 January by weighing the bees and counting a subsample of bees from each colony (as described above). Capped brood was measured on 23 February and 6 March and the bee populations were estimated again on 7 March.

Data were analyzed with general linear model analysis of variance. The treatment variables were insemination (one or six drones) and stock type (queens were from two different stocks). Four analyses were conducted with each of the following serving as the dependent variable: (1) nonreproduction of mites in brood cells, (2) change in mite population, (3) change in bee population, and (4) amount of capped brood. The second experiment evaluated older queens and only the last two variables.

Data of percent nonreproduction of mites were skewed, so nonparametric statistics were used to determine if the variability of nonreproduction of mites was different within each of the two groups of colonies (colonies with 1 vs colonies with 6 sub-

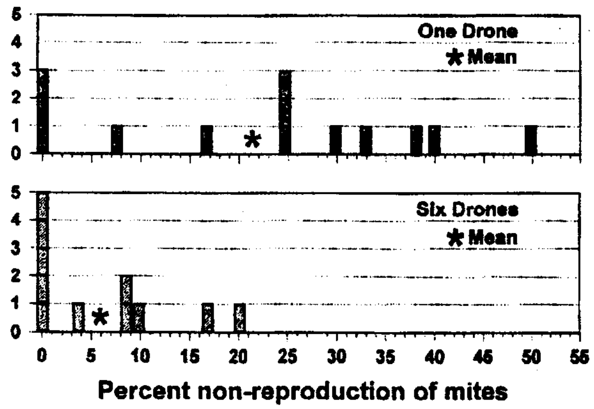

Figure 1. Comparing the phenotypic variation among 24 colonies with queens mated to one or six drones. Each rectangle represents the percentage of varroa mites in a colony that did not produce progeny while in a brood cell. Numbers at the left indicate the number of colonies that have the same frequency of nonreproducing mites.

families of bees (data in Figure 1). Stock type was not analyzed because it had no significant effect on nonreproduction in this model (Table 1). Observations were divided into 3 classes (0-15, 16-30, and >30% nonreproduction). The 2 x 3 contingency table was analyzed with Fisher's exact test to determine if the distributions of the observations in the two groups were unequal.

Results and discussion

The single drone insemination made a marked difference in the ability to detect nonreproduction of mites in brood cells (Table 1). The other 3 variables did not show significant effects from different inseminations or different stocks of bees (Table 2). I conclude that single-drone inseminations are valuable and perhaps necessary for detecting the range of variation that can exist when measuring certain characteristics of bees at the colony level.

Comparing the distribution of the variable nonreproduction of mites in brood cells is probably more important than comparing the means (Figure 1). Based on Fisher's exact test, there was a 0.03 probability that the two groups had equal variability for nonreproduction of mites. Therefore, I conclude that the distributions were not equal and that insemination with one drone probably caused an increase in the variability among colonies.

Although it is not surprising that there was greater variability among colonies with queens mated to single drones than among colonies with queens mated to six drones, I expected the means to be equal. The means were not equal (Table 2, Figure 1). The geometric means (perhaps more valid for these data because of the skewed distribution) were also quite different (11.8 and 2.8%). In these results it appears that when most of the bees in a colony did not possess the characteristic for nonreproducing mites, the expression of the characteristic was repressed.

Results from the first test suggest that queens mated to single drones can be used in field testing if the queens are young. When queens were less than four months old, colonies with queens mated to single drones produced as many progeny as colonies with mated to six drones (Table 2).

The age limit for using singly mated queens in field tests is somewhere between 4 and 7 months. Thus a field test needs to be short if singly mated queens are to be used. The second test was conducted with older queens, and it suggested that queens mated to single drones may not be acceptable for field testing if the queens are over 7 months old. In that test, the bee population in ten colonies with queens mated with one drone grew by a factor of 1.36 from 5 January to 7 March, whereas the population in eight colonies with queens mated to six drones grew by a factor of 2.06 ($F = 11.1$, $P = 0.005$). Thus, the groups were different, and the colonies with singly mated queens were unsatisfactory.

In most cases, queens should be inseminated and laying before they are put into uniform colonies for field testing. I did not do that in the first experiment because I was testing an insemination procedure and did not want to show bias toward either treatment by subjectively choosing queens for the test. Fortunately only three of the original 27 queens did not survive (two inseminated with one drone and one inseminated with six drones). However, in most cases the insemination procedure is not being tested, and in those cases it is helpful to cull the poor queens (for example those that have been injured and those that are not laying well).

In conclusion, colonies containing only one subfamily of bees (with queens inseminated with semen from a single drone) are valuable and perhaps necessary at times for detecting the range of variation that can exist among colonies of honey bees. This study showed that insemination with a single drone was important for detecting nonreproduction of mites in brood cells, but it may also be important for detecting other characteristics that are present in a population at low frequencies. It appears that Rothenbuhler (1960) was correct in stating that when there are many subfamilies of worker bees in a colony (a colony with a multiply mated queen), the social environmental factors imposed by bees of other subfamilies can modify the full expression of a characteristic that may appear in only one of the subfamilies.

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