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Resistance to American Foulbrood in Honey Bees
X. Comparative Mortality of Queen, Worker, and Drone Larvae[1]

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Queen, worker, and drone honey-bee (*Apis mellifera*) larvae of similar ages and genotypes were given, in their food, a constant dosage of water-suspended *Bacillus larvae* spores, or water only, in 10 experimental replications. Pooled data from all replications showed that spore treatment resulted in about 93% mortality of female larvae reared as queens, 82% mortality of those reared as workers, and 68% mortality of the male (drone) larvae. Control treatment mortality was less than 5% for each caste. Chi square analyses of mortality associated with control treatments showed no significant differences between any two of the three castes, whereas mortality differences between castes following spore treatments were highly significant and are interpreted as resulting from differences in resistance. These differences in resistance are attributed to differences in food supplied by adult bees to the larvae.

INTRODUCTION

Woodrow (1942) demonstrated that younger honey-bee larvae are highly susceptible to *Bacillus larvae*, the cause of American foulbrood, while older larvae are completely resistant. Recently, several investigations have established that larvae of certain genetic lines are more resistant to the bacillus than are larvae of other genetic lines (Rothenbuhler and Thompson, 1956; Bamrick, 1960; Lewis and Rothenbuhler, 1961; Bamrick and Rothenbuhler, 1961). The present study was designed to learn whether or not the three castes of the honey bee are equally susceptible to American foulbrood. The only previous studies on this question (Woodrow, 1942; Katznelson and Jamieson, 1950), done years ago, and in the latter case only indirectly pertinent, would indicate that susceptibility is the same for all castes. Further consideration of previous work is presented in the discussion.

MATERIALS AND METHODS

The overall experimental approach to this investigation involved four major steps. (a) Both male and female larvae of similar age and genotype were obtained from a single queen. (b) Some larvae were fed spores of *B. larvae* in their food, while other larvae were fed water as a control treatment. (c) All larvae were then transferred to different cells (females to queen cells and worker cells, and males to drone cells) which contained noninoculated food, where the larvae of each caste underwent further development. (d) Survivors and nonsurvivors of each caste were recorded from 10 replications of this procedure.

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A single, artificially inseminated queen of an inbred line supplied all larvae tested. Since this genetic line has been inbred for a number of years, both male and female larvae (from unfertilized and fertilized eggs, respectively) can be expected to be genetically similar. Similar ages of male and female larvae were assured by caging a queen over both drone and worker cells on a specially prepared comb. After oviposition during a 12-hr period, the queen was removed from the comb, which was then put into a rearing colony where the eggs hatched.

When the larvae of a test sample were an average age of 9 hr, the food of each larva, which had been supplied by adult nurse bees, was given a spore or water treatment. Spore treatment consisted of placing, by the use of a microsyringe, a 0.287-mm^3 droplet of a water suspension of spores into the food, in which each recipient larva was lying. The spore suspension was prepared so as to contain 3650 spores/dose as determined by the use of a Petroff-Hausser bacteria-counting chamber. Water treatment consisted of an identical procedure in which sterile water was added to the larval food. Rothenbuhler and Thompson (1956) discuss the method of food inoculation in greater detail.

Twenty-four hours after inoculation, when the larvae of the test sample were an average of 33 hr old, they were transferred to brood cells containing spore-free food from which larvae the same age as the test larvae had been removed. Three classes of transfers, each involving two sets of larvae, were made: both spore-fed and water-fed females to queen cells; both spore-fed and water-fed females to worker cells; and both spore-fed and water-fed males to drone cells. Twenty-four hours after transfer, a base count was made on all six sets, and from this count, subsequent calculations were made. Randomized sampling of treated larvae for transfer to pathogen-free cells was used to eliminate bias.

Two colonies containing commercial hybrid bees were used for rearing larvae. One of these nurse colonies, supplied with sealed brood, reared queens; the other colony, supplied with unsealed brood, reared workers and drones. After each successive test the rearing functions of nurse colonies were alternated.

After the cells containing the sample of test larvae were sealed by adult bees, the larvae were placed in an incubator to complete their development. The day before the expected emergence of the adult bees in each cast, the cells were opened and examined. Survivors and nonsurvivors were recorded for both the water and the spore series.

Results and Analyses

The data are presented in Tables 1 and 2. Table 1 shows numbers of water-fed queens, drones, and workers surviving and not surviving in all 10 tests, as well as percentages of survival and nonsurvival calculated from pooled data. Percentages of nonsurvival are 4.51%, 3.51%, and 3.88% for queens, workers, and drones, respectively. A similar classification of spore-fed individuals appears in Table 2. Of those larvae fed spores, 92.79% of queens, 81.68% of workers, and 68.35% of drones did not survive.

Each statistical analysis involved several different chi squares. Chi squares were calculated for each of the 10 experimental replications and for the pooled data from all 10 replications. In addition, a sum of chi squares was obtained by adding the chi-square values from all replications. An interaction (heterogeneity) chi square was obtained by subtracting the pooled chi square from the sum of chi squares. Since addition theorems do not apply to adjusted chi squares, no adjustments were made for small numbers (less than 5) present in some of the replicates, so that sums of chi squares and interaction chi squares could be com-

TABLE 1

INTERCASTE MORTALITY COMPARISONS OF CONTROL INDIVIDUALS

Test replicate number	Classification of control individuals							Chi-square comparisons		
	Queens		Workers		Drones			Queens to workers	Queens to drones	Workers to drones
	Dead	Alive	Dead	Alive	Dead	Alive	D.F.			
1	1	15	1	16	1	13	1	0.00	0.01	0.02
2	0	8	0	12	0	17	1	0.00	0.00	0.00
3	1	9	0	16	0	12	1	1.66	1.26	0.00
4	1	16	1	27	0	34	1	0.13	2.04	1.23
5	1	16	0	31	1	15	1	1.86	0.00	1.98
6	0	20	2[a]	44	1	27	1	0.90	0.73	0.03
7	0	12	1	22	2	29	1	0.54	0.12	0.11
8	0	5	1	41	2	24	1	0.12	0.41	1.07
9	1	17	2	34	1	20	1	0.00	0.00	0.02
10	1	9	2	32	1	32	1	0.21	0.84	0.32
Sum of chi squares	—	—	—	—	—	—	10	5.42	5.41	4.78
Pooled data and chi square	6	127	10	275	9	223	1	0.24	0.09	0.05
Caste percentage	4.51	95.49	3.51	96.49	3.88	96.12	—	—	—	—
Interaction chi square	—	—	—	—	—	—	9	5.18	5.32	4.73

[a] One of these individuals was a larva dead of American foulbrood. All other dead control larvae were removed by adult bees between the base count and the final count.

TABLE 2

INTERCASTE MORTALITY COMPARISONS OF SPORE-FED INDIVIDUALS

Test replicate number	Classification of spore-inoculated individuals						D.F.	Chi-square comparisons[b]		
	Queens		Workers		Drones			Queens to workers	Queens to drones	Workers to drones
	Dead[a]	Alive	Dead	Alive	Dead	Alive				
1	39	6	43	12	23	7	1	1.21	1.26	0.03
2	24	0	27	5	24	8	1	4.12	7.00[b]	0.87
3	29	1	29	9	29	12	1	5.54	7.79[b]	0.31
4	24	2	46	10	47	16	1	1.47	3.58	0.99
5	33	2	52	14	20	16	1	4.12	14.07[b]	6.06
6	37	5	49	9	35	25	1	0.26	10.54[b]	9.83[b]
7	17	1	43	10	50	21	1	1.82	4.45	1.86
8	15	0	48	16	50	21	1	4.70	5.87	0.35
9	42	4	54	6	35	16	1	0.52	7.60[b]	7.92[b]
10	23	1	46	7	26	15	1	1.45	8.57[b]	7.05[b]
Sum of chi squares	—	—	—	—	—	—	10	25.21[b]	70.73[b]	35.27[b]
Pooled data and chi square	283	22	437	98	339	157	1	19.56[b]	65.44[b]	24.58[b]
Caste percentage	92.79	7.21	81.68	18.32	68.35	31.65	—	—	—	—
Interaction chi square	—	—	—	—	—	—	9	5.65	5.29	10.69

[a] Dead and missing larvae and pupae.

[b] Significant at the 1% level of probability.

puted (Snedecor and Cochran, 1967). No individual replicate chi-square value is indicated as significant, however, unless significance is obtainable by use of the adjustment technique where applicable.

The results of water feeding are interpreted to be homogeneous, for the three nonsignificant interaction chi squares provide no evidence to the contrary (Table 1). Each individual replicate chi square, sum of chi squares, and pooled data chi square value tests the null hypothesis that no difference in mortality of controls exists between the pair of castes considered. Not one chi-square value is significant at even the 5% level of acceptance, and thus there is no evidence that differences in mortality existed among the controls of the three castes.

Analyses for intercaste mortality differences of spore-fed individuals have interaction chi-square values which are not significant, and homogeneity among the 10 tests is concluded (Table 2). Both the sum of chi-squares value and the pooled data chi-square values are significant at the 1% level of probability for each of the three caste-to-caste comparisons. Thus, each of the three null hypotheses, that no significant difference in mortality exists between castes, is rejected. These differences are interpreted to indicate real differences in resistance among the three castes. Thus, it is concluded that queen larvae are least resistant; drone larvae, most resistant; and worker larvae, intermediately resistant to the disease.

Discussion

The results presented diverge from those presented by Woodrow (1942). Woodrow concluded, "Infection was produced in queen and drone larvae as readily as in worker larvae." Two major differences exist between Woodrow's work and our investigation. The first involves variation in age of larvae tested for resistance. At the time of Woodrow's experimentation, the effect of maturation on resistance, which he himself was in the process of discovering, was unknown. Woodrow's queens were confined for oviposition ". . . for about 16 hours to one day," and separate samples of brood (from separate queen cagings) provided larvae for individual caste tests. The second major difference involved genetic variation in brood tested. Woodrow presumably had no inbred stock available, nor did he state that he used a single queen to produce all test larvae. As a consequence of information acquired during and since Woodrow's work, our investigation utilized materials and methods which reduced both genetic and larval-age variation.

Much of the literature cites the work of Katznelson and Jamieson (1950) as evidence that no difference in resistance between castes exists. They conclude, however: "Considering the possible variation in age of larvae . . . there was no significance between the ages of susceptibility of queen and worker larvae. . . ." Thus, they address themselves not to variation in resistance throughout the larval period, but rather to variation in length of larval life required to achieve maturation resistance (Katznelson, 1961).

Since both the ages and the genotypes of the three test groups were similar, the source of variation in resistance must have been environmental. The most striking environmental difference concerns the quality and quantity of food. Townsend and Shuel (1962) review many differences reported between the food of queen larvae and worker larvae. The food of drone larvae differs significantly from the food of the other two castes (see, e.g., Haydak, 1957). Such differences in diet may result in differences in resistance. Various investigators (Schulz-Langner, 1960; Bamrick, 1964) have suggested that addition of honey to the larval diet might explain maturation resistance

and even differential genetic resistance if larvae actually grow at a faster rate, as Sutter's (1963) work suggests. Differing amounts of honey or pollen present in the food of the three castes may be the source of differential survival among castes. These differences in food may be directly related to the difference in mortality; or, they may interact with the genetic elements of sex and caste, lead to developmental differences, and thereby indirectly result in differences in resistance.

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