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PRELIMINARY REPORT ON SELECTION AND BREEDING OF HONEYBEES FOR ALFALFA POLLEN COLLECTION*

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SUMMARY

Some colonies of honeybees on alfalfa collect a much higher percentage of alfalfa pollen than others. The possibility of genetic differences between colonies was investigated. Colonies collecting high and low percentages of alfalfa pollen were first selected. Daughters of queens from three 'high' and three 'low' colonies were inseminated from their brothers, and colonies headed by queens of these six lines were tested. Colonies headed by sister queens were more similar in the proportion of alfalfa pollen they collected than were those headed by unrelated queens. This suggests heritability of the factor studied. On the other hand no correlation was found with colonies headed by the mother queens, nor between those headed by the mother queens in the first and the second year. Queens from 'high' and 'low' lines were selected for mating and testing in 1964.

INTRODUCTION

When colonies of honeybees (*Apis mellifera*) are located by a field of alfalfa (*Medicago sativa*), some collect a much higher percentage of alfalfa pollen than others. The differences could be due to recruitment by scout bees which in separate colonies become oriented to different sources, or possibly to genetic differences among colonies. Akerman and Lesins (1949) noted a difference between a colony of Italian bees and one of northern dark bees. The first did not trip the blossoms when caged with them, even after they were given young brood and eggs to increase their need for pollen. The colony did not even work second-crop alfalfa for pollen, whereas a colony of northern dark bees began to trip the flowers and collect pollen on their third day in the cage, and continued until all blossoms were tripped and had set seed.

In Petersen's six years of experiments (1954) *Apis mellifera mellifera* tripped about 2.8% of the alfalfa blossoms visited, whereas *A. m. ligustica* (all types with light abdominal circles) tripped only about 2.1%.

Hunkeler (1943) reported that Carniolan bees were ousting northern dark bees in red clover districts in Germany, because they produced more honey from red clover and did a better job of pollination. Pedersen (1945) found that Italian bees produced more honey on second-crop red clover than northern dark bees, and that Italian bees less often took nectar from holes cut in the corolla tube, on both first and second crop red clover. Gubin (1947) studied the relative increase in red clover seed yield as one approaches apiaries of different races. He calculated that Carniolan bees with slightly longer tongues than the northern dark bees were slightly better pollinators of red clover, and that Caucasian bees (with even longer tongues) were nearly twice as valuable.

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Goetze (1953) reported results similar to those of Gubin, and he stated that a relative constancy is inherited and can be increased by inbreeding and selection for foraging activity and length of proboscis.

The purpose of the present research is to determine whether the tendency to collect alfalfa pollen is inherited, and if so, to develop lines with high and low pollen-collecting characteristics, and to determine how the tendency is inherited. Selection and testing are done at Logan, Utah, and breeding at Baton Rouge, Louisiana.

So far, we have sought to obtain at least preliminary data on the following questions: (1) Do colonies headed by the same queens retain their affinity or lack of affinity for collecting alfalfa pollen from year to year? (2) Do colonies headed by daughter queens tend to retain the degree of affinity of colonies headed by their mothers? (3) Do colonies headed by sister queens tend to collect alfalfa pollen to a similar degree, and does this differ from that of colonies headed by unrelated queens?

MATERIALS AND METHODS

Pollen traps provide a good means of collecting samples of pollen from a small number of colonies, for determining the plant sources of the pollen. To study a large number of colonies, we used a device similar to that described by Jaycox (1960). Returning pollen foragers were collected at the hive entrance by suction from a direct-drive, multi-vane high-pressure wheel blower, run by a 6-volt D.C. motor. The sampler head was connected to the air intake of the blower by a piece of flexible vacuum tubing, and bees sucked into a plastic centrifuge tube which held about 100 bees. In the field the sampler was connected to a 12-volt car battery.

In order to delay returning foragers until they could be collected, a narrow strip of burlap was temporarily pushed well into the hive entrance. The samples of bees were put into paper bags, which were placed into friction-top cans containing cloth bags of potassium cyanide. The samples were left in a refrigerator until they were examined under a microscope to determine the percentage of bees with alfalfa pollen loads.

In 1962 pollen-collecting bees were sampled on 3 dates from a total of 356 colonies in 5 apiaries in 4 locations. On the basis of these samples we selected the 5 colonies collecting the highest and the 5 collecting the lowest percentages of alfalfa pollen. The 'high' colonies collected 30–100%, and the 'low' 0–30%. The percentages of pollen collectors working alfalfa on the 3 dates (4 individual colonies) varied as follows:

	July 10	July 12	Aug. 10	Average
USDA	100.0	100.0	100.0	100.0
Cox	81.5	100.0	77.3	86.4
Budge	0.0	0.0	0.0	0.0
Cox	4.6	2.0	0.0	2.2

The queens from the 10 selected colonies were shipped to Baton Rouge where they wintered successfully.

Instrumental brother-sister inseminations were made between sons and daughters of queens from 3 'high' and 3 'low' colonies. Each queen was inseminated twice, with approximately 5 ml. semen each time. Of 95 queens thus inseminated, 89 started laying.

A rough estimate of viability was obtained by placing an empty comb in the brood nest and later determining the number of young larvae in one or two 100-cell areas. Most of the brood fell within the range 60–85% viability. Theoretically, with multiple brother-sister matings, the viability should range around 75%. The queens with the highest brood viability were selected for testing at Logan. Altogether 79 of the inseminated queens and 4 of the original queens were sent there in 5 lots, all posted by air mail before noon; 3 arrived the next morning, one the next afternoon, and one on the second day after mailing. All queens were introduced into 4-frame nuclei which were combined 3 weeks later with full-size colonies. They were introduced without attendants in small round screen cages with candy plugs. The resulting colonies were moderately large and each produced about 25 kg. surplus honey.

During the first week in July 2 colonies of each of the 6 lines and 2 control colonies were placed in each of 5 selected alfalfa seed fields. Colonies headed by the 4 original queens selected in 1962 were also placed at one of the 5 locations. There were thus 74 colonies in the test.

Samples of bees were collected from the colonies for three weeks, starting on July 11 in 3 locations, and on July 12 in the other 2. Samples were collected twice weekly, alternate collections from each colony being made in the morning (10–12hr.) and afternoon (12–15hr.).

The percentage of alfalfa pollen collectors was determined from the total number of pollen collectors. The variation in the different samples collected for individual colonies varied with the amount of alfalfa in flower at the different locations. Below is an example for one 'high' and one 'low' colony:

	a.m.	p.m.	a.m.	p.m.	a.m.	p.m.	
<i>Queen line</i>	<i>July 11</i>	<i>16</i>	<i>23</i>	<i>25</i>	<i>30</i>	<i>Aug. 2</i>	<i>Average</i>
High Cox ..	100.0	100.0	97.67	96.61	27.27	27.45	74.83%
Low Cox ..	4.76	17.39	9.09	0.0	3.77	0.0	5.84%

In general, colonies headed by sister queens tended to collect alfalfa pollen (regardless of location) in amounts which reflected the progress of flowering with the season. The time of day did not seem to affect the amount collected.

RESULTS

The colonies headed by the 4 original queens failed completely to retain their original ranking as alfalfa pollen collectors in the second year. The percentages of alfalfa pollen collectors for the four original queen stocks were:

	1962	1963
Y-1 USDA ..	100.0	46.7
W-5 Budge ..	0.0	52.0
W-5 USDA ..	25.0	39.1
W-3 Cox ..	9.5	35.7

Colonies headed by daughter queens mated to their brothers did not retain the rankings of the colonies headed by their mothers the preceding year. Percentages of alfalfa pollen collectors for the 6 original colonies in 1962 and for the 60 progeny colonies in 1963 are given in Table 1. This shows that there was a tendency for colonies headed

by sister queens to behave similarly with regard to the percentage of pollen collectors working alfalfa, regardless of location. Differences between queen lines were significant ($P < 0.01$), the 3 Budge lines, the USDA and Cox (low) lines, and the Cox (high) line, forming three significantly different norms.

TABLE 1. Summary of the percentage of alfalfa pollen collectors for queen lines at 5 locations

	ORIGINAL STOCK: PERCENTAGE OF ALFALFA POLLEN COLLECTORS 1962							
	USDA 100	Cox 86	Budge 54	Budge 30	Budge 0	Cox 2	Control	Average
	PROGENY: PERCENTAGE OF ALFALFA POLLEN COLLECTORS 1963							
Howell	48.23	71.26	42.15	43.98	66.82	39.80	22.49	47.82
Petersboro ..	24.18	58.28	52.43	51.32	48.16	23.41	32.35	41.45
Barker's Hollow	14.86	59.87	44.73	14.77	22.91	5.30	23.57	26.57
Fielding.. ..	8.26	60.48	18.16	34.96	20.84	19.53	17.46	25.67
Newton.. ..	11.58	47.41	30.31	23.78	15.10	12.98	13.85	22.14
Average.. ..	21.42	59.46	37.56	33.76	34.77	20.21	21.94	32.73

The interaction locations $\times$ queens (Table 2) shows that colonies headed by sister queens from parents high in alfalfa pollen collection tended to collect more alfalfa pollen in all locations (regardless of the amount of bloom present) than did those from parents of low alfalfa pollen collection. The queen lines from 'high' colonies performed similarly in all locations. The main interactions occurred principally in the 'medium' colonies, and mainly in locations where little alfalfa bloom was available and where insecticides affected the field force of colonies in the collection of alfalfa pollen.

TABLE 2. Summary of means for interaction locations $\times$ queens

Locations	1	2	3	Queens 4	5	6	7	Average
Howell	71.26	48.23	42.15	43.98	66.82	39.80	22.49	47.82
Petersboro	58.28	24.18	52.43	51.32	48.16	23.41	32.35	41.45
Barker's	59.87	14.86	44.73	14.77	22.91	5.30	23.57	26.57
Fielding	60.48	8.26	18.16	34.96	20.84	19.53	17.46	25.67
Newton	47.41	11.58	30.31	23.78	15.10	12.98	13.85	22.14
Average	59.46	21.42	37.56	33.76	34.77	20.21	21.94	

LSD 0.05 = 16.20

The interaction dates $\times$ queens (Table 3) shows that colonies headed by sister queens of the 'high' line collected alfalfa pollen on different dates in amounts associated with the amounts of bloom available at all locations. The main interactions occurred between the 'medium' lines on different dates, in amounts of bloom available and the effect of insecticide poisoning on different dates at all locations. This is a seasonal effect.

TABLE 3. Summary of means for interaction dates $\times$ queens

Dates	Queens							Average
	1	2	3	4	5	6	7	
1	23.48	13.41	21.82	5.34	38.76	20.12	1.36	17.76
2	51.73	35.71	21.59	38.62	44.56	35.83	9.90	33.99
3	80.04	33.57	58.19	48.46	44.67	20.38	29.63	44.99
4	78.62	16.36	48.05	57.05	29.29	24.10	34.49	40.85
5	63.79	19.86	41.40	28.81	32.74	11.42	32.82	32.98
6	59.08	9.61	36.29	24.28	18.57	9.38	23.46	25.81
Average	59.46	21.42	37.56	33.76	34.79	20.21	21.94	

LSD 0.05 = 32.95

Sampling date also interacted with location. In general, alfalfa pollen collection was best at each location when the alfalfa bloom was most abundant at that location, but it fell at Newton and Fielding on the fifth date, and at Petersboro on the third date, following insecticide applications. At Howell the drop after the third date was associated with declining bloom, but a light rain just before the fifth date brought about a recovery in bloom reflected by the increase in pollen collection on the sixth date.

Analysis of variance also indicated a three-way interaction between locations, dates and queens.

DISCUSSION

The fact that colonies headed by sister queens tended to collect alfalfa pollen to a more similar degree than those headed by unrelated queens certainly suggests heritability of the factor studied. On the other hand, the lack of correlation between alfalfa pollen collection by the progeny and the original stock, and by colonies headed by the same mother queens from one year to the next, throws some doubt on heritability of this factor.

PLANS FOR FUTURE WORK

It is proposed to breed and test only one 'high' and one 'low' line in 1964. The three best queens of each line will be selected, and three-way matings made for this work. Analysis of the components of variance in 1963 indicates that 3 instead of 2 queens of each line at each location would increase the precision of testing, even if the number of locations is reduced to 4. In addition to progeny testing, as many as possible of the original queens of the 'highest' and 'lowest' lines will be retested at each location.

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