1966

Selecting and Breeding Honey Bees for Collecting Alfalfa Pollen

Otto Mackensen

William P. Nye
Utah State University

Follow this and additional works at: https://digitalcommons.usu.edu/piru_pubs

Part of the [Entomology Commons](#)

Recommended Citation

Mackensen, O., and W. P. Nye. 1966. Selecting and Breeding Honey Bees for Collecting Alfalfa Pollen. J. Apic. Res. 5(2):79-86, fig., tables.

This Article is brought to you for free and open access by the Pollinating Insects Research Unit at DigitalCommons@USU. It has been accepted for inclusion in All PIRU Publications by an authorized administrator of DigitalCommons@USU. For more information, please contact digitalcommons@usu.edu.

Journal of Apicultural Research 5(2) : 79-86 (1966)

SELECTING AND BREEDING HONEYBEES FOR COLLECTING ALFALFA POLLEN*

OTTO MACKENSEN and WILLIAM P. NYE

*Entomology Research Division, Agricultural Research Service, U.S.D.A.,
Baton Rouge, Louisiana, U.S.A.*

and

*Entomology Research Division, Agricultural Research Service, U.S.D.A.,
Logan, Utah, U.S.A.*

Manuscript received for publication 23rd May, 1966

SUMMARY

Selection within lines of honeybees showing high and low preference for collection of alfalfa pollen was continued through the third and fourth generations. In the line showing high preference the average percentage of pollen-collecting bees that were collectors of alfalfa pollen increased from 39.8% in the second generation to 49.8% in the third, and to 66.4% in the fourth. In the line showing low preference the corresponding percentages were 26.2%, 14.8% and 7.6%. Hybridization of the two lines resulted in an intermediate F₁. Although the value of these bees in increasing seed production has not been tested, results suggest that commercially useful strains or hybrids could be developed.

INTRODUCTION

Nye and Mackensen (1965) showed that preference for the pollen of alfalfa (*Medicago sativa*) is inherited in honeybees (*Apis mellifera*). In 1962, out of 356 colonies we selected 3 that ranked high in their preference for pollen of alfalfa and 3 that ranked low. In the second generation, reared and tested in 1963, we made brother-sister matings among queens and drones reared from these 6 colonies to form 6 test groups. The colonies headed by sister queens were more similar in the proportion of alfalfa pollen collected than those headed by unrelated queens, suggesting heritability of the factor studied. On the other hand, statistically, we were unable to show a correlation between these colonies and those headed by the mother queens, or between those headed by the mother queens in the first and second years. In this report we present results of testing two additional generations for the tendency, and results from hybridizing the two lines.

MATERIALS AND METHODS

Our test unit was the entire colony representing two generations: the queen and all her worker progeny. Although preference for alfalfa pollen can only be expressed in the workers, in this paper the queen of the colony will identify the generation (the third and fourth generations of queens and their colonies, tested in 1964 and 1965).

The bees were bred at Baton Rouge, Louisiana. Testing and selection were carried out at Logan, Utah. At Baton Rouge each queen was instrumentally

* In cooperation with the Louisiana Agricultural Experiment Station, and Utah Agricultural Experiment Station.

inseminated twice with 4–5 μ l. semen, receiving sperm from an average of 10.8 drones. To estimate the sperm content in the spermathecae of test queens, we counted the sperm present in the spermathecae of 22 queens instrumentally inseminated but not used in the tests. The mean number of spermatozoa was 4.945 ± 0.353 million. In 1964, 154 queens were inseminated; of these 94% began laying eggs. In 1965, 211 queens (including those of the hybrid group) were inseminated; of these 92% began laying eggs.

Each year there were several mating groups of sister queens. Within each group queens with the highest viability were selected for further testing. With few exceptions the viability of the brood of test queens of the inbred lines ranged from 60 to 85%; that of the hybrid group was 90% or higher. Each group of queens was identified by a different colour painted on the thorax. The designations used to identify a queen consisted of the year, the first letter of the colour used to mark her, and an individual number. The identity of the lines was not revealed to the testing personnel until all data had been recorded. At least 14 queens from each group were shipped to the laboratory at Logan, to provide a minimum of 12 test queens per group.

Information regarding the mating scheme of the third and fourth generations of test queens and the colours used to mark them are provided in Table 1. From the 6 mating groups of the second generation queens tested in 1963, queens 63-Y-1, 63-Y-5 and 63-Y-13 were selected from the highest of the 3 high-ranking groups (those collecting most alfalfa pollen) for continuation of the line, and queens 63-W-13, 63-W-16 and 63-W-17 from the lowest of the 3 low-ranking

TABLE 1. Matings of queens of the third and fourth generations reared and tested for tendency to collect alfalfa pollen in 1964 and 1965, and the colour used to identify the test queens (Baton Rouge, Louisiana)

Rank in collecting alfalfa pollen of line or lines mated	Mating		Colour mark of test queens
	Daughters of	Sons of	
Third-generation queens, 1964			
high	63-Y-1	× 63-Y-13	pink
	63-Y-13	× 63-Y-5	orange
	63-Y-5	× 63-Y-1	yellow
low	63-W-17	× 63-W-16	blue
	63-W-16	× 63-W-13	white
	63-W-13	× 63-W-17	green
Fourth-generation queens, 1965			
high	64-P-9	× 64-P-10	white
	64-P-7	× 64-Y-16	orange
	64-Y-23	× 64-P-9	red
low	64-B-11	× 64-W-8	yellow
	64-B-21	× 62-B-2	green
	64-B-2	× 64-W-26	blue
low and high	64-B-2	× 64-P-10	pink

groups (those collecting least alfalfa pollen) for continuation of that line. Since each of the groups chosen had originated from a single initially selected colony, each line was descended from the queen of that colony and the drones she met in natural mating. In the spring of 1964, sons and daughters of the selected queens were reared and mated as shown in Table 1.

In 1965 sons and daughters of queens chosen from the 1964 groups marked pink and yellow were reared for continuation of the high-ranking line, and from the 1964 groups marked blue and white for continuation of the low-ranking line. Matings were made as shown in Table 1, which also shows the cross made to produce the hybrid colonies containing inbred queens and hybrid worker bees.

In 1964 third-generation queens were received in Logan in 5 shipments arriving between 6th and 27th May. During the week of 6th July, the colonies of 12 queens from each mating group were distributed equally to 4 test locations (near West Smithfield, Barker's Hollow, Howell and Petersboro, all in Utah) in selected fields of alfalfa grown for seed. In addition 3 colonies with unrelated, naturally mated queens (unselected) were placed at each location as controls. Beginning on 14th July, 6 samples of returning pollen foragers were taken about twice a week by the method we developed earlier (Nye & Mackensen, 1965). The percentage of pollen-collecting bees that were collecting alfalfa pollen was calculated for each colony on each sampling date.

In 1965 the fourth-generation test queens were received in Logan between 4th and 25th May. For these tests 6 colonies of each of the 6 inbred groups and 6 colonies of hybrids were placed at each of 2 locations near Fielding and Howell in Utah. From 12th July until 3rd August, bees were sampled for their pollen loads, as in 1964.

Upon completion of sampling, the technician recorded for each colony the amounts of sealed and unsealed brood, amounts and kind of pollen stored, number of frames of bees, and behaviour of bees and queens on the combs. These data were used in selecting queens for qualities other than the tendency to collect alfalfa pollen. In particular, selection for qualities of handling was necessary; in the earlier generations, while selections were being made exclusively on the basis of collection of alfalfa pollen, the worker bees became steadily more restless.

Our method of breeding and preliminary selection has held inbreeding to less than 25% in both lines, and retarded loss of sex alleles—as shown by the viability maintained.

Data were analysed statistically on a split-plot basis with each location representing a split plot. For the analysis of variance we used the arc sines of the percentages of alfalfa pollen collectors, because of unequal numbers of total pollen collectors within samples. We did not include the 1964 control group and the 1965 hybrid group in this analysis.

RESULTS

In 1964 conditions were favourable. The third-generation colonies built up rapidly and contained populations of 30–40 thousand bees at the time they were moved to the test locations. However, July and August were exceptionally dry months. At West Smithfield the alfalfa dried up about 10 days after the bees were

moved in. At the other locations collection of alfalfa pollen was affected by applications of an insecticide, which killed some of the bees. At Barker's Hollow application of insecticide caused heavy losses of bees from 15th to 22nd July. At Howell, losses of bees from application of an insecticide occurred the week before sampling started, and at Petersboro such losses were noted on the second and fourth sampling dates. In 4 colonies queens were superseded by new queens, but only after the tests had been completed.

Results of the 1964 tests of the third generation are summarized in Table 2 and Fig. 1. The percentages of alfalfa pollen collectors of both the high- and low-ranking lines covered as wide a range as that observed in the second generation, but unlike results for the second generation, results for the third showed that most of the low-ranking colonies were concentrated at the low end of the scale. The means were further apart than the means for the second generation, which were 39.8% for the high-ranking line and 26.2% for the low-ranking line. Although in the third generation some of the means for colonies of the 2 lines overlapped, the group means for the low-ranking line did not overlap those of the high-ranking line at any location individually or over all locations.

TABLE 2. Percentage of alfalfa pollen collected by third-generation test colonies in 1964 (Mean of 6 samples, Logan, Utah)

Location	Groups of high-ranking line			Groups of low-ranking line			Control
	pink	orange	yellow	green	white	blue	
West Smithfield	45.6	3.5	1.4	1.0	0.0	7.7	11.3
	38.0	1.1	11.0	0.9	2.9	2.2	3.4
	0.6	33.3	40.5	1.3	11.0	1.5	4.2
	Mean	29.8	8.9	14.6	1.0	4.1	5.1
Barker's Hollow	95.5	11.1	25.2	6.2	0.0	0.5	20.5
	43.8	37.2	12.2	2.9	2.5	0.0	3.0
	69.9	3.2	22.1	12.9	1.9	0.8	5.3
	Mean	66.2	17.0	20.0	7.3	1.4	0.5
Howell	75.5	76.0	81.0	53.9	32.0	21.3	53.1
	98.2	93.0	92.5	56.2	46.0	13.9	88.8
	92.9	80.1	78.1	81.0	19.7	26.4	11.0
	Mean	89.3	83.3	83.2	64.9	30.9	18.9
Petersboro	95.7	58.4	50.5	1.5	1.6	5.3	31.1
	85.2	47.3	31.4	3.7	5.2	1.2	7.6
	23.1	15.2	28.2	0.0	17.5	5.2	2.2
	Mean	68.2	38.7	36.0	1.9	5.5	4.4
Group mean	63.0	44.0	41.9	26.5	11.3	7.5	19.0
Line mean		49.8			14.8		

Analysis of variance of the 1964 data (Table 3) showed that the difference between lines was highly significant (at the 1% level), even though the variance among dates and that among locations were also highly significant. The interactions between dates $\times$ lines and between locations $\times$ lines were also highly significant. The disrupting factors were largely due to the normal seasonal effects of alfalfa coming into bloom and then disappearing as the seed was set, plus the

COLONIES

PERCENT ALFALFA POLLEN COLLECTORS
(CLASS CENTERS)

FIG. 1. Distribution of the percentage of alfalfa pollen collectors in honeybee colonies of high and low alfalfa pollen preference lines for 3 generations, and in hybrid colonies

detrimental effects of insecticide poisoning and drought. The variance among groups within lines was also highly significant, but the interactions of locations $\times$ groups within lines and those of dates $\times$ groups within lines were not significant, an indication that such variance was probably genetic. The colonies of the control group collected a little more alfalfa pollen than those of the low-ranking line.

TABLE 3. Analysis of variance of third and fourth generations of colonies tested in 1964 and 1965 for their tendency to collect the pollen of alfalfa (Logan, Utah)

Source of variance	d.f.	Mean square	d.f.	*Mean square
	Third-generation test colonies, 1964		Fourth-generation test colonies, 1965	
Locations	3	10.713 261 93**	1	0.011 899 65
Lines	1	17.636 782 86**	1	50.815 107 42**
Groups within lines	4	0.981 898 79**	4	0.031 300 02
Locations $\times$ lines	3	1.270 046 60**	1	0.058 266 88
Locations $\times$ groups within lines	12	0.283 520 31	4	0.168 607 38
Pooled error (a)	48	0.187 048 44	60	0.141 507 29
Dates	5	0.602 557 85**	5	1.251 203 52**
Dates $\times$ lines	5	1.713 928 10**	5	2.110 233 17**
Dates $\times$ groups within lines	20	0.163 188 22	20	0.030 085 24
Dates $\times$ locations	15	0.793 931 11**	5	0.159 408 76
Dates $\times$ locations $\times$ lines	15	0.126 844 56	5	0.188 914 89*
Dates $\times$ locations $\times$ groups within lines	60	0.105 246 98	20	0.079 869 84
Pooled error (b)	240	0.103 425 03	300	0.074 979 82

* Significant at the 5% level

** Significant at the 1% level

In 1965 the fourth-generation test colonies were in good condition when moved into the test locations, but during the first 8 days at Fielding some field bees were lost because of the application of an insecticide. At Howell, collection of alfalfa pollen was affected after 27th July by application of an insecticide and cutting of the alfalfa for hay. Three of the fourth-generation queens were superseded by new ones, but only after the tests were completed.

Results of the 1965 tests are summarized in Table 4 and Fig. 1. The means calculated for corresponding groups and the overall means for the 2 locations were very similar. The difference between means for the 2 lines became greater in 1965, and variability within lines was very much less than that for the 1964 tests. All the means for the 3 groups of the high-ranking line were definitely high, and those for the 3 groups of the low-ranking line were definitely low. Group means for the 2 lines did not overlap, nor did those for individual colonies. The mean of the hybrid group of colonies was roughly intermediate.

The analysis of variance of the 1965 tests (Table 3) showed that the difference between lines was highly significant. Variance among dates and the interaction between dates $\times$ lines were both highly significant, owing no doubt to the normal seasonal changes in the alfalfa crop and to poisoning from the insecticide. The lack of significant variation among groups of sister queens within lines suggests that probably little genetic variance remained within the lines. The overall mean calculated for the fourth generation of the high-ranking line was 66.4%, compared with 49.8% for the third generation. The overall mean for the low-ranking

line was 7.6%, compared with 14.8% for the third generation. The variance among inbred sister queens within groups was considerably less in the fourth generation than in the third.

TABLE 4. Percentage of alfalfa pollen collected by the fourth-generation test colonies in 1965 (Mean of 6 samples, Logan, Utah)

Location	High-line groups			Low-line groups			Hybrid pink
	red	white	orange	yellow	green	blue	
Fielding	91.6	79.8	75.3	6.1	1.4	1.8	25.1
(location	60.9	59.6	66.1	2.0	8.7	10.5	27.9
mean 35.0)	52.4	74.6	65.5	8.4	17.3	2.4	16.8
	34.7	72.9	40.6	8.3	5.2	0.8	11.3
	71.1	88.5	86.5	5.9	0.7	20.7	34.1
	63.0	65.9	86.1	2.3	2.3	6.8	15.7
Mean	64.8	73.6	72.2	5.5	6.1	5.9	23.0
Howell	68.3	54.0	50.2	10.1	8.8	4.1	27.5
(location	51.6	59.1	64.7	10.9	17.7	11.0	31.7
mean 37.5)	52.7	65.0	42.4	7.6	8.8	4.0	48.6
	73.9	65.8	56.7	9.1	0.4	5.2	43.7
	78.4	56.7	88.9	8.0	26.8	6.7	51.1
	79.2	66.8	58.5	12.2	5.0	5.1	65.7
Mean	66.9	61.2	61.5	9.6	12.3	5.9	44.0
Group mean	65.8	66.9	66.4	7.5	9.4	5.9	34.1
Line mean		66.4			7.6		

The colonies of the high- and low-ranking lines were about the same in population and in production of honey.

DISCUSSION

Steady progress in separating high- and low-ranking lines of alfalfa pollen collectors through 3 generations of selection, coupled with the achievement of complete separation of the 2 lines, proved that the tendency to collect the pollen of alfalfa is heritable. This result opens up the possibility of developing special high-quality strains or hybrids of honeybees for commercial use in pollinating alfalfa, particularly since a comparatively small number of colonies were used in the selection experiments. Our lines have deteriorated in their handling qualities, because we selected only for or against collection of alfalfa pollen. In a long-range breeding programme this deterioration could be prevented, and other characteristics of economic importance could be retained and developed simultaneously. Results of our work suggest the possibility of breeding strains of bees with a preference for other specific crops for which pollination is a problem.

At the present time we are unable to make an estimate of the number of genes involved. The gradual separation of the high- and low-ranking lines and the tendency of the hybrid worker populations to be intermediate, do however suggest that alfalfa pollen preference might be controlled by several genes with mainly additive effects.

Since the colonies of the high- and low-ranking lines had about equal populations and produced about the same amounts of honey, it is evident that a prefer-

ence for alfalfa nectar is not associated with a preference for collecting alfalfa pollen.

Many questions remain to be answered. Are bees of the high-ranking line better collectors of alfalfa pollen than those of the general run of commercial stock? The pollen preference of the one small unselected control group we tested in 1964 is hardly a reliable index of how our high-ranking line compares with other stocks of bees. Can our line bring about a better seed set than other stock? Is the preference specific for alfalfa, or does it extend to some of the other legumes such as clover? The work will be continued in an effort to answer these questions and to learn more about the inheritance of this valuable characteristic. We need to determine the behavioural, physiological and morphological differences between the two lines. Such information will provide valuable assistance in further selection for the preference to collect alfalfa pollen.

ACKNOWLEDGEMENTS

We wish to express our thanks to Dr. Donald V. Sisson, Utah State University, and Dr. B. R. Farthing, Louisiana State University, for statistical assistance, and to Dr. W. C. Rothenbuhler for suggestions about the manuscript. We also thank Messrs. M. W. Peterson, Max Rasmussen and Earl Wood for providing test locations.

REFERENCE

- NYE, W. P. & MACKENSEN, O. (1965) Preliminary report on selection and breeding of honey-bees for alfalfa pollen collection. *J. apic. Res.* 4(1) : 43-48