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William P. Nye
Utah State University

Otto Mackensen

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SELECTIVE BREEDING OF HONEYBEES FOR ALFALFA POLLEN: FIFTH GENERATION AND BACKCROSSES*

WILLIAM P. NYE AND OTTO MACKENSEN

Entomology Research Division, Agricultural Research Service, U.S.D.A., Logan, Utah, and Baton Rouge, Louisiana, U.S.A.

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Summary

Selection within lines for honeybees showing strong and weak tendencies toward collection of alfalfa pollen was continued through the fifth generation. In the line with a strong tendency (high line), the average percentage of pollen collectors collecting alfalfa pollen increased from 66% in the fourth generation to 85% in the fifth. In the line with the weak tendency (low line), the corresponding percentages were 8% and 18%. Backcrosses of the two lines resulted in an intermediate mean. At Howell, Utah, where other pollen than alfalfa was scarce, the percentages were 99% and 53% for the high and low lines respectively, and at Fielding, Utah, where other pollen was abundant, they were 54% and 2%. Although we have not compared the productive ability of the high line with that of commercial bees, results suggest that commercially useful strains could be developed.

Introduction

In 1966 we achieved complete separation of inbred lines of honeybees (*Apis mellifera*) that ranked high and low as collectors of alfalfa pollen, and proved that the tendency to collect the pollen of alfalfa is heritable. We were unable to estimate the number of genes involved, but the gradual separation of the two lines, and the tendency of the hybrid worker population to be intermediate in collecting, suggest that the preference might be controlled by several genes with additive effects.

The present paper reports on: (1) progress in the fifth generation of selection; (2) the performance of backcrosses; (3) the behaviour of bees of the two lines in an alfalfa field; (4) the distance pollen collectors will go in search of alfalfa pollen.

Methods

Inbreeding and backcrosses

Our breeding and testing methods for honeybees were much as described previously (Nye & Mackensen, 1965; Mackensen & Nye, 1966). The queens were bred at Baton Rouge, Louisiana, and the selections and other observations made at Logan, Utah. Selection was based on good handling qualities, high brood viability, and the strong and weak tendencies to collect alfalfa pollen.

The breeding system was similar to that used for the last few generations. Each line is descended from one of the original colonies and, in each generation after the original selection, we have tested three groups of sister queens from each line by rearing virgins or drones from the selected queens, and mating them in three combinations, to form the new test groups of sister queens. Mother–daughter matings were

* In co-operation with Utah and Louisiana Agricultural Experiment Stations

made to establish the two inbred lines, but there were no further matings between virgins and drones from the same colony.

Backcrosses were made to study segregation, by mating drones of a hybrid queen individually with virgin queens of the two lines. One generation was produced each year, and each group of queens was identified by paint of a different colour on the thorax. Queens were identified by the year (a letter or letters representing the colour) and by individual number. The 1966 matings of queens of the fifth generation, and the backcrosses produced, are listed in Table 1. From this Table and Table 1 of our 1966 paper, the ancestry of any queen can be traced.

TABLE 1. Matings of queens of the fifth generation reared and tested for tendency to collect alfalfa pollen in 1966, 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*	
high	65-W-29	×	65-O-6	white
	65-R-43	×	65-W-19	red
	65-O-1	×	65-R-33	orange
low	65-B-1	×	65-G-2	green
	65-G-11	×	65-B-14	grey
	65-B-8	×	65-G-10	blue
high backcross	65-O-1	×	hybrid*	
low backcross	65-G-11	×	hybrid*	

* Daughter of cross-mated queen 65-P-30

The bees in the line that ranked high in pollen collection have light body colouration, and those from the line that ranked low are dark. This difference was useful in checking colonies for drifting, and for observations of pollen foragers in the field.

The fifth-generation queens were received in Logan between 10th and 20th May 1966. Conditions in late May and June were unfavourable for the development of colonies, but they developed rapidly after they were moved to alfalfa. Originally they were placed at Howell, Utah, on first-crop alfalfa where other sources of pollen were scarce. Later, they were moved to a second-crop alfalfa field at Fielding, Utah, where other sources were plentiful and more similar to those of previous years. From 11th to 26th July, four samples of bees from each colony were taken at Howell; from 1st to 12th August, four samples were taken from each colony at Fielding.

The method of collecting the samples was modified to include both nectar and pollen foragers (Nye & Mackensen, 1965). In 1966 we used a small household vacuum cleaner operated by a petrol-driven portable generator (120v a-c), but we collected the bees in cloth bags instead of the paper ones supplied with the vacuum cleaner. These bags were later placed in a can where the bees were anaesthetized with carbon dioxide. The nectar collectors were counted and released, and the pollen collectors killed and refrigerated so that the floral sources of the pollen could be determined.

Standard statistical procedures were applied in the analysis of the data; the arc sines of the percentages of pollen collectors were used. The number of colonies was adjusted in a random manner to provide equal numbers for comparison.

Results

At Howell, the colonies ranking as collectors of alfalfa pollen collected this pollen almost exclusively, but the colonies ranking low obtained more than half their pollen from other sources, despite their scarcity. Thus there were more pollen collectors returning to hives of the high-ranking line than to hives of the low-ranking line. At Fielding, where other sources of pollen were relatively abundant, the low-ranking colonies almost ignored alfalfa and—probably as a consequence—these colonies had more pollen collectors than colonies of the high-ranking line.

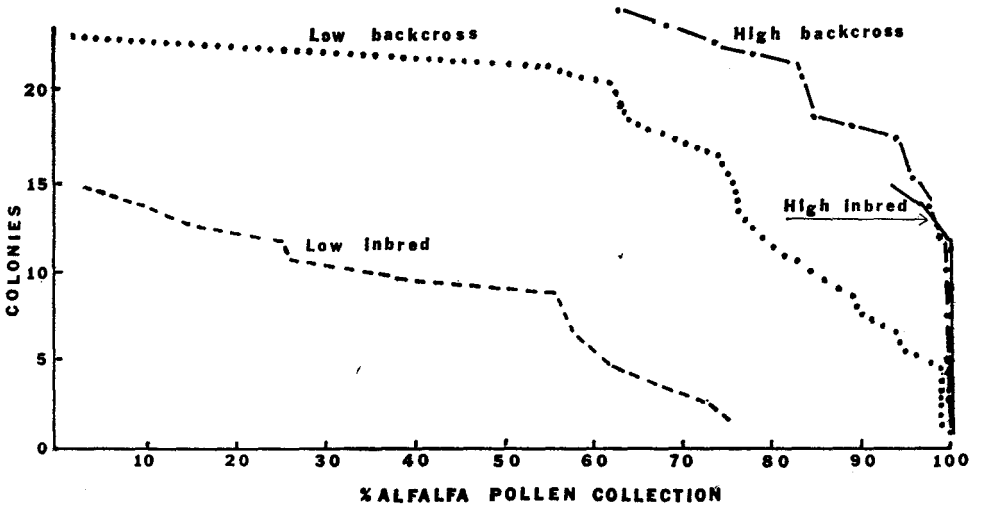

FIG. 1. Alfalfa pollen collection at Howell for the inbreds and backcrosses (July 1966)

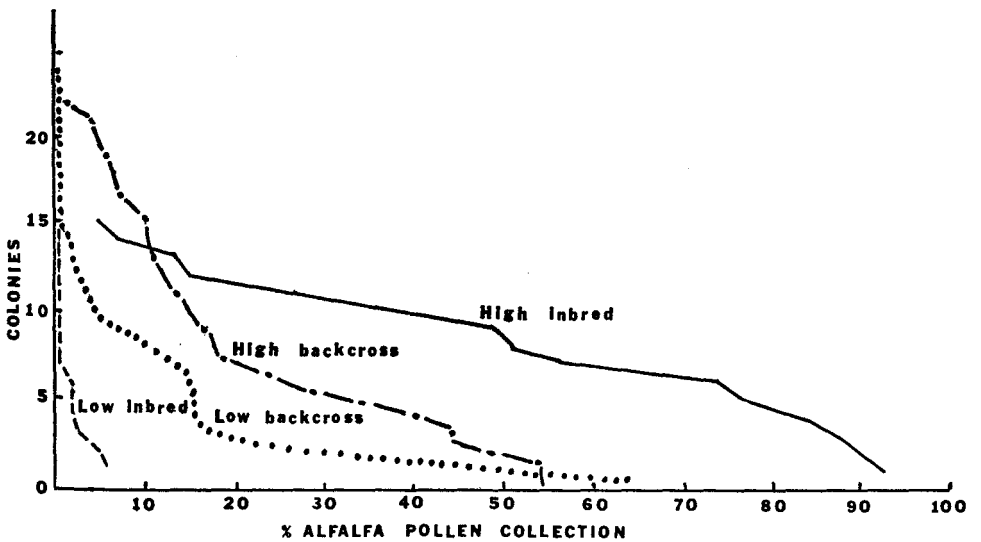

FIG. 2. Alfalfa pollen collection at Fielding for the inbreds and backcrosses (August 1966)

The numbers of collectors of alfalfa pollen in the colonies of the high-ranking line were definitely high; in the colonies of the low-ranking line they were definitely low. Group means for the two lines did not overlap, and little overlapping of means of individual colonies occurred. Also, the means for the two backcrosses fell between the means of the inbred lines, each backcross being nearer to its corresponding inbred line. The same was true for the percentage of pollen collectors that collected alfalfa pollen (Fig. 1, Fig. 2).

TABLE 2. Analysis of variance of fifth generation of colonies tested in 1966 for their tendency to collect pollen of alfalfa (Logan, Utah)

Source of variance	*d.f.*	*Mean square*	
		(Howell)	(Fielding)
Pollen collectors / total bees			
Lines (high and low)	1	0·086048†	0·179892*
Matings within lines	4	0·018291	0·058483
Queens within matings	24	0·012441*	0·048305†
Error	90	0·007295	0·018939
Alfalfa pollen collectors / pollen collectors			
Lines (high and low)	1	28·029808†	12·619796†
Matings within lines	4	0·971066†	0·850457*
Queens within matings	24	0·205825	0·303339†
Error	90	0·166447	0·104402
Pollen collectors / total bees			
Lines (backcrosses)	1	0·000999	0·083378*
Queens within lines	44	0·022349†	0·028025
Error	138	0·004896	0·022600
Alfalfa pollen collectors / pollen collectors			
Lines (backcrosses)	1	3·718233†	0·978327†
Queens within lines	44	0·392798	0·192051
Error	138	0·297887	0·134218
Pollen collectors / total bees			
Lines (inbreds and backcrosses)	3	0·029303	0·077451
Queens within lines	56	0·021923†	0·039061†
Error	180	0·006392	0·020826
Alfalfa pollen collectors / pollen collectors			
Lines (inbred and backcrosses)	3	10·538587†	4·725404†
Inbreds vs. backcrosses	1	0·694	1·344*
High line + backcrosses vs. low line + backcrosses	1	24·499†	8·041†
High line, low backcrosses vs. low line, high backcrosses	1	6·453†	4·774†
Queens within lines	56	0·342165*	0·280278†
Error	180	0·239196	0·111898

Difference in variance (higher variance in numerator)

High line, high backcross	$\frac{\text{High backcross}}{\text{High line}}$	$\times\times$	$\frac{\text{High line}}{\text{High backcross}}$
Low line, low backcross	$\frac{\text{Low backcross}}{\text{Low line}}$	$\times\times$	$\frac{\text{Low backcross}}{\text{Low line}}$†
Backcrosses	$\frac{\text{Low}}{\text{High}}$	$\times\times$	$\frac{\text{High}}{\text{Low}}$†

* Significant at the 5% level of confidence
† Significant at the 1% level of confidence

The analysis of variance (Table 2) shows significant differences between the percentages of pollen collectors that were collectors of alfalfa pollen at both locations for the two inbred lines, between the two backcrosses, and between each inbred line and its respective backcross. Also, a significant variance among mating groups within lines suggests that some genetic variance may remain.

A comparison was also made between the variances within the backcrosses and the inbred lines (Table 2). Because of more segregation, we could expect the backcrosses to be more variable than the corresponding inbreds. This was true at Howell (but not at Fielding) for the high-ranking line and for the backcrosses; for the low-ranking line and these backcrosses, it was true at Fielding but not at Howell. Perhaps the difference occurred because at Howell, where little except alfalfa pollen was present, the high-ranking line was collecting alfalfa pollen at a maximum, whereas at Fielding, where other pollen was abundant, the low-ranking line was collecting the other pollens at a maximum. The same seemed true to a lesser extent of the backcrosses: at each location, the backcross to the line that preferred the less abundant pollen had the lower variance.

No definite pattern of segregation was recognizable in the backcrosses. Thus, the data support our earlier conclusion that a preference for alfalfa pollen is probably controlled by several genes that have a mainly additive effect.

Collection of alfalfa pollen in the field

Honeybees were observed in two alfalfa seed fields and in one irrigated alfalfa hay field, and the numbers of light and dark bees collecting alfalfa pollen were recorded (Table 3). In the alfalfa seed field at Howell, where pollen other than alfalfa was scarce, 75% of pollen collectors were collecting alfalfa pollen. As the season advanced and other sources became available, this percentage decreased; the average percentage of pollen collectors collecting alfalfa pollen on this field was 27·4%. On the irrigated alfalfa hay field across the road, the percentage was only 3·6%, though the alfalfa in this field had the same "competition" from other floral sources as the seed field.

TABLE 3. Number of bees observed, and numbers of light and dark bees collecting alfalfa pollen, for two seed fields and one hay field (Logan, Utah, 1966)

Location	*No. bees observed*	*Alfalfa pollen collectors*	
		Light	*Dark*
Howell seed field	500	118	19
Howell hay field	500	17	1
Fielding seed field	557	49	5

At Fielding, where pollen other than alfalfa pollen was plentiful, only 9·7% of pollen collectors were collecting alfalfa pollen. In all three areas, the light bees (high-ranking line) greatly predominated over the dark bees (low-ranking line) among the pollen collectors (Table 3). This visual record thus confirmed the greater tendency to collect alfalfa pollen of the high-ranking line.

Effect of distance on collection of alfalfa pollen

In our examination of preference for alfalfa pollen, the test colonies had been placed in alfalfa grown for seed. The question therefore arose as to whether we were breeding a bee that worked close to the hive and collected alfalfa pollen because of its abundance nearby. We had obtained some evidence to the contrary in 1965, when our test colonies at Fielding showed the effect of pesticides applied to an alfalfa seed

field 1½ miles [2½ km] from the test colonies, and again at Howell when pesticide was applied to a seed field one mile away.

In 1966, we placed groups of the two inbred colonies (used previously in the alfalfa seed field) to select for preference for alfalfa pollen in fields that were 1½ and 4½ miles [2½, 7½ km] from the same alfalfa field. The colonies were in hives fitted with pollen traps, and five samples of pollen were collected from each hive from 11th July to 8th August 1966. Then the pollen was refrigerated until the floral sources could be determined.

Of the colonies placed in alfalfa at Howell, where other pollens were scarce, the high-ranking line collected an average of 77·3% of its pollen from alfalfa; the low-ranking line collected 16·5%. Colonies of the high-ranking line placed 1½ miles away collected an average of 75·7%; those of the low-ranking line collected 2·1%. For colonies of the high-ranking line placed at the greatest distance (4½ miles) the average was 10·5%; for those of the low-ranking line it was only 1·5%. At this farthest location, two colonies of the high-ranking line collected 31·6% and 23·0% of their pollen from alfalfa, though one colony collected only 2%.

In all locations, colonies of the low-ranking line collected a greater total amount of pollen than those of the high-ranking line, whose bees probably spent more time trying to find alfalfa, so that there were fewer pollen collectors foraging. Another important reason for the higher pollen collection by colonies of the low-ranking line was their larger size—an average of 2·5 frames of bees and 310 sq cm of sealed brood more than colonies of the high-ranking line.

Discussion

The steady progress in separating inbred lines that rank high and low as collectors of alfalfa pollen through five generations of selections, and the complete separation of the two lines reported by Mackensen and Nye (1965), prove that the tendency to collect alfalfa pollen is heritable. The variability of our backcrosses in the absence of any definite pattern of segregation confirms our earlier conclusion that this characteristic is probably dependent on many genes, each having an additive effect. Our failure to find a ratio of segregation may be caused by a great nongenetic variability and by insufficiently precise testing methods.

The inheritance of the characteristic raises the possibility of developing special strains or hybrids of honeybees for commercial use in pollinating alfalfa. Also, since we were able to achieve significant results in selections from a rather small number of colonies, selections for other crop preferences might be successful.

The greater number of pollen collectors of the high-ranking line in alfalfa fields, and the higher percentage of alfalfa pollen collectors in that line from hives as far as 7½ km from alfalfa fields, confirm the greater tendency of the high-ranking line to collect alfalfa pollen. The results show that we are not breeding a bee that works only close to the hive, but one that ranges over a wide area in search of alfalfa.

Although we have not compared the seed-setting ability of the high-ranking line with that of commercial honeybees, it is reasonable to suppose that it would be superior. In 1963 and 1964, we tested an unselected group of colonies against the inbred lines; we found that the average percentage of collectors of alfalfa pollen from the unselected colonies fell below the midpoint between the high- and low-ranking lines. Since pollen collectors are much more efficient than nectar collectors in tripping and cross-pollinating alfalfa flowers, and since increased cross-pollination benefits seed production, the greater value for alfalfa seed production of a line that ranks high in collecting alfalfa pollen seems indubitable.

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