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Selection and estimation of the heritability of sunflower (*Helianthus annuus*) pollen collection behavior in *Apis mellifera* colonies

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ABSTRACT. We selected honey bee colonies (*Apis mellifera* L.) with a high tendency to collect sunflower pollen and estimated the heritability of this trait. The percentage of sunflower pollen collected by 74 colonies was evaluated. Five colonies that collected the highest percentages of sunflower pollen were selected. Nineteen colonies headed by daughters of these selected queens were evaluated for this characteristic in comparison with 20 control (unselected) colonies. The variation for the proportion of sunflower pollen was greater among colonies of the control group than among these selected daughter colonies. The estimated heritability was 0.26 ± 0.23, demonstrating that selection to increase sunflower pollen collection is feasible. Such selected colonies could be used to improve sunflower pollination in commercial fields.

Key words: Honey bee, Sunflower, Pollen collection, Selection, Heritability

INTRODUCTION

Commercial hybrid sunflower (*Helianthus annuus* L.) seed is produced by plants with cytoplasmic male sterility. This line is known as male-sterile (MS); female flowers are fertilized with male lines denoted as male-fertile (MF), allowing recovery of fertility in the hybrid (F1). In the seed fields, the MS-MF lines are planted in separate rows. In order to obtain seed production, pollen has to be transferred from the MF to the MS line. Honey bees (*Apis mellifera* L.) are used in sunflower fields (McGregor, 1976) to ensure adequate seed production; these bees should visit both parental lines. However, honey bees are rarely observed collecting sunflower pollen; they confine their activities to gathering nectar (Tepedino and Parker, 1982). Low visit frequencies to MF have been reported in Argentinean sunflower fields (Bedascarrasbure, 1983). Attractivity of the pollen for honey bees can be affected by lipid content. Satyabir et al. (1999) demonstrated that honey bees prefer *Brassica campestris* pollen, which has a high lipid content, over *Helianthus annuus* pollen, which has a low lipid content. Alternative pollen sources can reduce pollination and consequently hybrid-seed production in sunflowers (Basualdo et al., 1994). Many efforts have been made to diminish the effect of competitive flowering species on commercial crop pollination.

Basualdo et al. (2000) found that Africanized honey bees collected a significantly greater proportion of sunflower pollen than did European honey bees. Variability among honey bee colonies in their preferences to collect *Medicago sativa* (Nye and Mackensen, 1965), *Vaccinium macrocarpon* (Shimanuki et al., 1967) and *Trifolium pratense* (Palacio, 1987) pollen have also been reported. Free and Williams (1973) found that progeny from different queens differed in their pollen preferences. Nye and Mackensen (1965) and Mackensen and Nye (1966, 1969) selected lines of honey bees with high and low preferences for pollen of alfalfa (*Meticago sativa*) and suggested that this trait is inherited and that stock lines of bees could be used for pollination of commercial crops. However, heritability for this trait has not been reported. This is difficult to estimate in bees because of biological characteristics, such as the haplo-diploid system and polyandry.

We selected honey bee colonies (*Apis mellifera* L.) for a high tendency to collect sunflower pollen and estimated the heritability of this trait.

MATERIAL AND METHODS

The study was conducted over a two-year period in large commercial sunflower seed-production fields (65 ha) in General Villegas (35°S, 63°W), Buenos Aires province, Argentina. Plots contained three rows of MF plants, followed by 10 rows of MS plants, with a distance of 0.7 m between rows and a density of five seeds per linear meter. The first year, flowering lasted from January 7 to 16. The second year, flowering was from January 29 to February 8.

All the colonies were kept in single hive bodies, in standard Langstroth hives; these colonies were approximately equalized, by adding or removing frames with bees, to reach an average strength of five to six frames of brood. The colonies had little stored pollen, and they had the standard colony strength used in commercial pollination of agricultural crops in Argentina. Seventy-four colonies (initial population), headed by *A. m. ligustica* queens of a commercial apiary of Buenos Aires province, were moved to the fields when 15% of the flowers had

opened. Colonies were placed at the edge of the fields in groups of 10, and were located with their entrances facing the crops. Frontal pollen traps were fitted to the entrances. The first pollen samples were taken from the traps on January 8 (day one after the colonies were placed in the field); seven samples were collected. Total pollen was removed from the traps each sample day and was frozen at -20°C until analyzed.

Conditioning pollen samples

Pollen samples of each colony were diluted with hot distilled water. Twenty milliliters of distilled water was added to each gram of pollen sample. The solution was homogenized and a 5-mL aliquot was centrifuged at 3,500 rpm for 5 min (Bedascarrasbure and Bailéz, 1987). The supernatant was poured off and the sediment was acetolyzed according to Erdtman (1986). The number of sunflower pollen grains among 200 pollen grains (on two slides) was counted in each sample. Data are reported as a percent of sunflower pollen.

On the basis of results of these samples in the first year, we selected the five colonies that collected the highest percentages of sunflower pollen. The queens from the selected colonies were wintered successfully in an apiary of the Field Station of the Universidad Nacional del Centro de la Provincia de Buenos Aires; they headed the breeder colonies used for queen production. Twenty daughter queens were produced and all introduced into four-frame nucleus colonies. Of these, 19 were successfully mated naturally and began laying. When the colonies reached full size (filled a deep 10-frame Langstroth brood chamber), they were placed together with another 20 colonies (control group), headed by queens from a commercial apiary in Buenos Aires province, in sunflower fields in General Villegas. The management of the colonies was the same as in the previous year. Sunflower pollen collection was monitored with the same methodology.

Statistical analysis

The percentage of sunflower pollen collections at the beginning, middle and end of flowering was compared between selected daughter colonies and control colonies with a *t*-test for unequal variances, using the PROC *t*-test of SAS Institute (1989). A square-root transformation was made of the data before this analysis.

For estimation of heritability, an analysis of variance was made of the data of colonies headed by daughter queens (F1) and mother colonies. The percentage of sunflower pollen collected by each colony at the beginning, middle and end of flowering was considered (Time effect). Variance components were estimated by minimum squares, for maternal half-sib populations. Phenotypic variance was estimated with a fixed effect model (Eler et al., 1994):

$$Yij = \mu + Fj + eij \qquad \text{(Eq. 1)}$$

where: μ = overall mean, Yij = proportion of sunflower pollen collected by a daughter colony of the ith mother in the jth time, Fj = effect of time, eij = error.

In this model, the mean square error estimates the phenotypic variance.

Additive variance was estimated by:

$$Yijk = \mu + Fi + Mj + eijk \quad \text{(Eq. 2)}$$

where: μ = overall mean, Yijk = proportion of sunflower pollen collected by the kth colony in the ith time, daughter of the jth mother, Fi = fixed time effect, Mj = effect of the jth mother, eijk = error.

Heritability h^2 was calculated as:

$$h^2 = \frac{68}{19}\frac{\sigma^2 A}{\sigma^2 P} \quad \text{(Eq. 3)}$$

The 68/19 ratio in the equation is a correction for σ^2A as a consequence of haplo-diploidy and mating of the queen to an estimated 17 unrelated drones.

Standard error was calculated as:

$$S^{h2} = 4\sqrt{\frac{2(1 - t)^2[1 + (n - 1)t]^2}{n(n - 1)(s - 1)}} \quad \text{(Eq. 4)}$$

where: t = 1/4 of the h^2, n = function of daughter number, S = queen number.

RESULTS

In the first year, sunflower pollen collection was greater at the beginning of flowering, and then there was a progressive reduction in percentage of sunflower pollen collected in the middle and at the end of flowering (Figure 1). Colonies selected as mothers had the greatest sunflower pollen collection rates in all flowering periods (Figure 1).

In the second year, sunflower pollen collection increased during flowering (Figure 2). Colonies headed by daughter queens (F1) again collected more sunflower pollen than control colonies (Figure 2), but these differences were significant only at the beginning of flowering (t = 2.73; d.f. = 20; P = 0.01).

The variation in the proportion of sunflower pollen collected in the three periods was greater among colonies of the control group than among colonies headed by daughters of selected queen mothers (Table 1). Colonies headed by sister queens, daughters of the same mother had ranges of variation between 5.3 and 19.9%.

Phenotypic variance ($\sigma^2 p$) estimated by mean square error was 154.217 (Table 2). The mother effect was considered in the model used for estimating the additive variance ($\sigma^2 A$; Table 3). The $\sigma^2 A$ was 11.146 (Table 4) and the estimated heritability was 0.26.

DISCUSSION

The pollen collection patterns were different in the two years. In the first, the percentage sunflower collection was highest at the beginning of flowering, with a strong decrease thereafter (Figure 1); in the second, the highest sunflower pollen collection was at the end of the flowering period (Figure 2). The flowering dates were different in these two years and alternative competing flowering species could have been available at different times. If more attractive

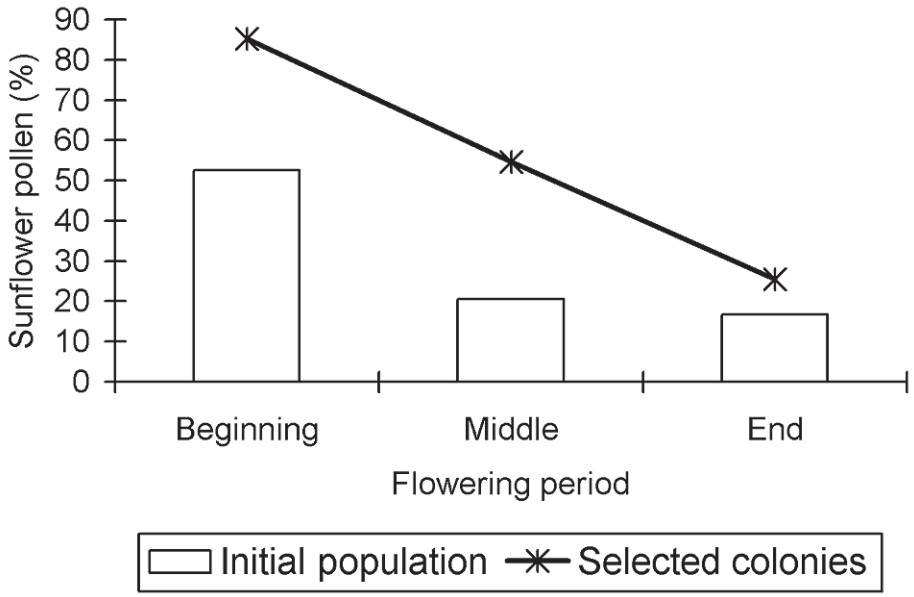

Figure 1. Average percentage of sunflower pollen collection at the beginning, middle and end of the flowering period of 74 honey bee colonies (initial population) and of five selected colonies.

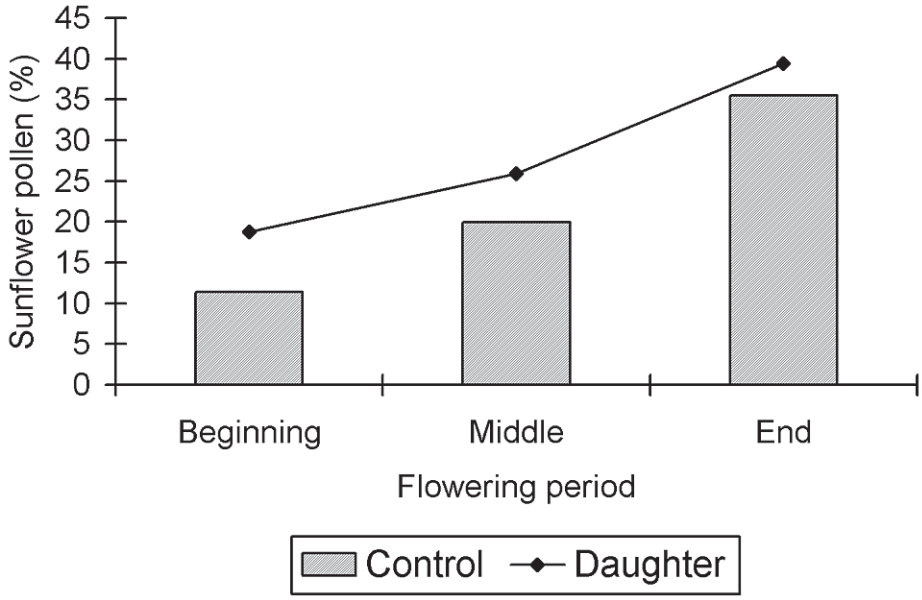

Figure 2. Average percentage of sunflower pollen collection at the beginning, middle and end of the flowering period for 19 daughter (F1) and 20 control honey bee colonies.

Table 1. Mean percentage sunflower pollen collection at the beginning, middle and end of the flowering period for 19 selected daughter colonies and 20 control colonies.

Flowering period	Daughters	Control
Beginning	18.8 ± 1.86 (28.5)	11.4 ± 3.02 (33.6)
Middle	25.9 ± 2.38 (42.6)	19.9 ± 3.9 (65.7)
End	39.4 ± 3.06 (49.0)	35.5 ± 4.72 (64.4)

Data are reported as means ± SE and range in parentheses.

Table 2. Analysis of variance for sunflower pollen collection (percentage) at the beginning, middle and end of the flowering period (time effect), for 19 colonies headed by daughter queens (F1) of selected mothers (fixed effect model 1).

Source of variation	Degrees of freedom	Sum of squares	Mean square	F value	P value
Time	2	4920.127	2460.063	15.95	0.0001
Error	54	8327.716	154.217		
Total	56	13247.844			

Table 3. Analysis of variance for sunflower pollen collection (percentage) at beginning, middle and end of the flowering period (time effect), for 19 colonies headed by daughter queens (F1) and the five selected mother colonies (model 2).

Source of variation	Degrees of freedom	Sum of squares	Mean square	F value	P value
Time	2	3365.8321	1682.9161	2.68	0.0069*
Mother	4	758.2167	189.5542	10.10	0.0003*
Time x mother	8	573.0945	71.6368	1.14	0.3519
Error	42	6996.4057	166.5811	0.43	0.8963
Total	56	13247.844			

Table 4. Estimations of phenotypic variance ($\sigma^2 p$), additive variance ($\sigma^2 A$) and heritability (h^2) for sunflower pollen collection by *Apis mellifera* L. colonies.

Parameter	Theorical mean square*	Value estimated
σ_p^2	*MS (error)*	154.217
σ_A^2	$\frac{MS\ (mother) - MS\ (time \times mother)}{k}$	11.146

*From statistical model. k = 10.579. MS = mean square; k is a constant based on the degrees of freedom.

alternative sources of pollen and nectar become available, the flow of information within the colony redirects bees to these sources. In fact, we observed pollen of *Eucalyptus* sp, *Cardus* sp, *Centaurea* sp, *Taraxacum* sp, Brassicaceae, and others in the samples.

Colonies selected as mothers had a high frequency of collection of sunflower pollen in all sampled periods when compared with the other colonies. Considering the entire flowering period, mother colonies collected (approximately) 30% more sunflower pollen and showed "fidelity" to sunflowers even during the days that sunflower pollen collection was low for the control colonies. This differential pollen collection among colonies has been reported for other species (Nye and Mackensen, 1965; Shimanuki et al., 1967; Free and Williams, 1973; Palacio, 1987) and could be attributed to genetic differences among colonies.

Although significant differences were detected in sunflower pollen collection between daughter and control colonies only at the beginning of flowering, the daughter colonies had a higher sunflower pollen collection during the three periods that were evaluated (4 to 7% more). Colonies headed by sister queens tended to collect sunflower pollen to a more similar degree than those headed by unrelated queens; this suggests heritability of this trait. Daughter queens were mated naturally; the preferences of pollen of particular species might be controlled by several genes with additive effects (Mackensen and Nye, 1966, 1969); nevertheless, the colonies headed by daughter queens had as much "fidelity" to sunflowers as the mother colonies. The reasons for this preference were not elucidated. Colonies in the same location often differ qualitatively and quantitatively in the way that they exploit the flora. Greater foraging distances of pollen collectors relative to nectar foragers have been reported (Gary et al., 1972; Danka et al., 1993). There could be differences between colonies in their exploitation of pollen sources; scouts in some colonies are quick to discover alternative pollen sources, consequently they have less fidelity to the crop. On the other hand, Free and Williams (1973) suggested that innate preferences are also involved.

Estimated heritability was high, considering that the trait that we evaluated is behavioral and is considerably influenced by environment, as demonstrated by the high degree of phenotypic variance. The accuracy of the estimation of heritability, given by a low standard error, depends more on the number of mothers than on the number of daughters of each mother. In this study, only five mothers were selected; this could be the reason for the high value of the standard error.

Our results show that selection to increase sunflower pollen collection can be made. These colonies could be used in commercial sunflower fields to improve pollination. If most pollen collectors were collecting sunflower on MF lines, the proportion of foragers that move between rows would be greater. On the other hand, if abundant sunflower pollen is entering the hive, the "chance" that it could be transferred among nest mates and then transferred to MS (DeGrandi-Hoffman and Martin, 1993) would be greater.

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