

## Genetic breeding on the bee *Melipona scutellaris* (Apidae, Meliponinae)

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### ABSTRACT

A selection of queens of *Melipona scutellaris* through the most productive colonies were carried out during eight months in an orange honeyflow. Each of the colonies was evaluated by its production, that is, the gross weight production (pollen, brood, geopropolis and wax of each hive). With this data a coefficient of repeatability was estimated by the intraclass correlation method, obtained  $r = 0.835 \pm 0.071$ . The repeatability is very high showing that the analysed data (production) is repeatable. Selection was then carried out using the regression coefficient of each colony and the respective production gain. Using these data the colonies were divided into three groups according to the method Vencovsky and Kerr (1982): **a** with the colonies of highest productivity, **b** of least productivity, and **c** of intermediary productivity. Colonies with the highest production (**Group a**) gave their queens to those of the lowest production (**Group b**) after their queens were taken out and killed; while those of intermediate (**Group c**) stayed with the same queens during the entire experiment both before and after the selection. The modifications in weight, that is, the genetic response was  $(\hat{R}) = 7.98$  gr per day which indicated a selection gain. The estimate of the realized herdability is twice the rate of the response to selection  $(\hat{R})$  by the selection differential ( $S_2$ ). That is  $\hat{h}_R^2 = 2(\hat{R} / S_2)$  then  $\hat{h}_R^2 = 0.166$

### PALAVRAS-CHAVE

Melhoramento, abelha, *Melipona scutellaris*, Apidae.

## Melhoramento genético na abelha *Melipona scutellaris* (Apidae, Meliponinae)

### RESUMO

Foi feita uma seleção de rainhas durante oito meses com 10 colônias aproveitando uma florada de laranjeiras. Cada colônia teve sua produção total (mel, pólen, crias, geoprópolis e cera) avaliada. Estimamos com estes dados o coeficiente de repetibilidade por meio de uma correlação intraclasse e obtivemos  $r = 0.835 \pm 0,071$ . Esse valor é alto e mostra que a produção de cada colônia é repetível. Executamos uma seleção usando o coeficiente de regressão de cada colônia e o respectivo ganho de produção. No uso desses dados, as colmeias foram divididas em três grupos, de acordo com o método de Vencovsky e Kerr (1982): **a** contendo as colônias de maior produtividade; **b** com as colônias que apresentaram menor produtividade e **c** com as colônias de produção intermediárias. As colônias com maior produção (grupo **a**) deram suas rainhas para aquelas com menor produção (grupo **b**) cujas rainhas foram retiradas e mortas. As colônias do grupo **c** permaneceram com suas próprias rainhas durante todo o experimento tanto antes como depois da seleção. As modificações no peso, quer dizer, a resposta genética ocorrida foi  $(\hat{R}) = 7.98$  gramas por dia, indicando um ganho proveniente da seleção. A estimativa da herdabilidade é o dobro da resposta à seleção dividida pela seleção diferencial ( $S_2$ ), ou seja:  $\hat{h}_R^2 = 0,166$ .

### KEYWORDS

Breeding, selection, *Melipona*, bee.

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## INTRODUCTION

Many papers have been produced using populations of the honey bee *Apis mellifera* L. (Collins, 1986; Kulincevic, 1986; Laidlaw and Page, 1986; Rinderer, 1986; Vencovsky and Kerr, 1982). There are about 100 Brazilian species of stingless bees that produce excellent honey, but had never been subjected to direct genetic improvement. Three of these species have been domesticated by pre-Columbian human populations: *Melipona beecheii*, Bennett the Yucatanian cab; *Melipona compressipes Fabricius*, the tiuba of Maranhão; and *Melipona scutellaris* Latreille the Northeastern Brazilian bee urussú. The present work is part of a larger study being carried out on the stingless bees (Meliponinae).

## MATERIAL AND METHODS

This work was conducted at the State University of São Paulo (UNESP), on the campus of Jaboticabal, State of São Paulo. Among the 21 colonies of *Melipona scutellaris*, only 10 colonies were used for this specific experiment. No information on either resistance to migration or on the behavior of these bees in orange flowers were available. The evaluation period of the hives extended from August, 10, 1992 to March 3, 1993. All hives were in the same environment: in the absence of nectar flow, colonies were fed syrup (50% water plus 50% commercial sugar and a pill of Teragran - M) or 50% commercially rejected honey of *Apis mellifera* L., because it was collected by the honey bees in sugar cane stacks produced after burning the straws.

In order to discover the production of a specific colony at different months, a coefficient of repeatability was estimated, using data obtained on the weight of the all hives with *M. scutellaris* bees. This coefficient set the superior limit of herdability and includes additive effects of genes plus non-additive effects and the permanent environmental differences existent among colonies. The method used was intraclass correlation (Fisher 1954). The following statistical model was used:

$$Y_{ij} = u + a_i + e_{ij} \quad \text{where:}$$

$Y_{ij}$  = Production j within the hive i (j = 1, 2, ..., 8; i = 1, 2, ..., 10).  
u = general media effect  
 $a_i$  = colony i effect (i = 1, 2, ..., 10)  
 $e_{ij}$  = aleatory error.

Table 1 shows the variance analysis model of the variance components used to estimate repeatability.

Production repeatability of colonies was estimated from variance components obtained using the above model followed by:

$$r = \frac{\sigma_c^2}{\sigma_c^2 + \sigma_{ev}^2} \quad \text{where:}$$

**Table 1** - Variance analysis model for obtaining the components used for estimating the repeatability in colonies of *Melipona scutellaris*.

Sources of variation	D.F.	Q.M.	E (Q.M.)
Between colonies	C - 1	QM <sub>2</sub>	$\sigma_{ev}^2 + k\sigma_c^2$
Within colonies	N - C	QM <sub>1</sub>	$\sigma_{ev}^2$

$r$  = repeatability coefficient

$\sigma_c^2$  = colony variance coefficient

$\sigma_{ev}^2$  = environment variance component

The standard error of the repeatability coefficient

( $\delta_r$ ) was obtained by the formula (Fisher 1954):

$$\delta_r = \sqrt{\frac{2(1-r)^2[1+(k-1)r]^2}{k(k-1)(n-1)}} \quad \text{where:}$$

$r$  = repeatability coefficient

$k$  = number of colonies

$n$  = total number of observations

## DIRECTIONAL SELECTION (S)

The directional selection was made based on the weight of the hives. A regression coefficient was estimated for the gross weight (in grams) of each hive, relative to time (x = 0, 30, 60, ..., 210 days).

Based on the preliminary data obtained from these estimates, colonies were divided into three groups:

**a** = Three colonies with the largest regression coefficients.

**b** = Three colonies with the smallest regression coefficients.

**c** = Control group made up of 4 colonies with intermediate regression coefficients.

The method of selection used, developed by Vencovsky and Kerr (1982), runs as follows:

About 25% of the best colonies (as in this present case the aim was total colony weight; the "best colonies" were the 3 heaviest) have their three queens (**Group a**) removed and introduced in the 25% worst colonies (the 3 lightest ones) **Group b** - that previously had their 3 queens removed and killed. The workers of the three best colonies each choose a virgin queen that takes the nuptial flight, and usually begins egg laying within 14 days. This selection process (and queen

supersedure) was carried out on the 3rd of April, 1993, after 210 days (7 months) migration of the bees to and from an enormous orange honey flow.

The selection differential was estimated by the difference of the mean regression coefficient of **Group a** and the mean linear regression coefficient of the population.

### ANSWER TO SELECTION ( $\hat{R}$ )

The answer to selection was quantified through the estimates of the linear regression coefficients, and was considered as being the result of the difference of the mean linear regression coefficients of the colonies of the groups **a** and **b** after and before the selection, through the linear mean regression coefficients of **Group c** (= control group), after and before selection.

$$\hat{R} = (\hat{b}_{ab1} - \hat{b}_{ab0}) - (\hat{b}_{c1} - \hat{b}_{c0})$$

$\hat{R}$  = Estimate genetic answer to selection

$\hat{b}_{ab0}$  = Estimate of the mean linear regression coefficient of the colonies belonged to **Group a** and **b** before selection.

$\hat{b}_{ab1}$  = Estimate of the mean linear regression coefficient of the colonies of the **Group c** (control) before selection.

$\hat{b}_{c1}$  = Estimate of mean linear regression coefficient of the colonies that belong to **Group c** (control) after selection.

$\hat{b}_{c0}$  = Estimate of mean linear regression coefficient of the colonies that belong to **Group c** (control) before selection.

### REALIZED HERDABILITY ( $h_R^2$ )

The realized herdability was estimated as being two times the rate of the selection ( $R$ ) by the selection differential ( $S$ ); both were estimated based on the regression. The result was multiplied by 2, due to the data which was based on the mother effect, since all mates were random.

## RESULTS AND DISCUSSION

### Genetic Selection

The data based on to the gross weight production of the 10 colonies of *Melipona scutellaris* are in Table 2 and 3.

The results of the variance analyses obtained from the data of the gross weight of the hives (table 2 and 3) allowed the estimation of the repeatability coefficient, as presented in table 4.

According to the analysis of variance (see Table 4) a significant effect is indicated ( $P < 0.01$ ) which may infer that the colonies are different as far as weight is concerned.

### REPEATABILITY ( $R$ )

The estimate of repeatability for production for the experimental colonies was  $r = 0.8346 \pm 0.71$ . This is a very high value and means that the observations present the real capacity of production of the colonies in order to carry out a selection program.

### SELECTION OF COLONIES

Queens of meliponids are usually inseminated by one male. The data obtained recently by Carvalho (2001) for this species shows that 8% of their queens are inseminated by two males. The selection of queens must be made through the performance of the colonies.

Table 5 shows the estimates of the regression coefficients of 10 colonies of *Melipona scutellaris* before and after selection of the queens.

As can be seen in Table 5, the constitution of the groups according to the regression coefficients is:

**Group a:** superior colonies, with the greatest regression coefficients: k 419, k 309, k 430.

**Table 2** - Gross weights in grams of 10 colonies of *Melipona scutellaris*, with respective means ( $\bar{x}$ ) and standard deviation ( $s$ ) before selection.

Days	Hives									
	k426	k427	k419	k309	k436	k418	k295	k431	k428	k430
0	4420	5360	5280	4460	5160	6200	4580	5200	5080	4880
30	4400	5360	5580	4680	5120	6320	4700	5180	5160	5000
60	4340	5320	5900	4800	5120	6300	5000	5180	5280	5120
90	4320	5320	5900	4960	4860	6300	4820	5120	5300	5000
120	4280	5040	5600	4900	4960	6100	4660	5000	5280	4960
150	4380	5060	5680	5240	4880	6100	4720	4940	5500	5160
180	4340	5120	5900	5540	4940	6240	4820	4920	5500	5620
210	4340	5180	5940	5800	5300	6400	4960	4900	5400	5900
X	4352.5	5220.0	5722.5	6687.5	5042.0	6245.0	4782.5	5055.0	5312.0	5205.0
S	45.2729	135.6466	231.3779	846.7754	155.4487	106.7709	145.5777	128.1740	150.0238	361.3862

**Table 3** - Gross weights in grams of 9 colonies of *Melipona scutellaris*, with respective means ( $\bar{x}$ ) and standard deviation (s) after selection.

Days	Hives								
	k426	k427	k419	k309	K436	k418	k295	k431	k428
0	4380	4960	6500	5600	5000	6280	4880	4900	5400
30	4600	5000	6580	5680	5060	6000	4840	5020	6460
60	5000	6340	6540	6000	5200	6080	5200	5700	5860
90	5300	6060	7080	6220	5560	6940	5500	6880	7080
120	5900	6060	7620	6320	5560	7760	5800	6960	7980
X	5036.00	5684.00	6864.00	5978.00	5235.00	6612	5240	5892	6556
S	599.5665	652.9012	483.8181	334.0958	225.6546	740.4863	409.9756	987.1778	1016.4054

**Table 4** - Summary of variance analysis of the gross weights of ten hives of *Melipona scutellaris* (from 10 Aug 92 to 10 Mar 93).

Source of variation	DF	MS	F
Between colonies	9	207968.89	41.37**
Within colonies	70	50267.14	

**Group b:** inferior colonies with the smallest regression coefficients: k 426, k 427, k 431.

**Group c:** control colonies with intermediate regression coefficients: k 436, k 418, k295, k 428.

## DIFFERENTIAL SELECTION

There are two considerations to be made as far as the differential selection obtained through the estimation of the mean linear regression coefficients are concerned.

a) The selection differential ( $S_1$ ) that takes in account all colonies involved in the bee reproduction before selection:

$$S_1 = \hat{b}_{a0} - \hat{b}_p$$

$$S_1 = 121.11 - 33.90$$

$$S_1 = 87.21 \text{ grams}$$

b) The selection differential ( $S_2$ ) utilized in the estimation of realized herdability, took in account only nine colonies (since hive K430 died due to the production of diploid males):

$$S_2 = \hat{b}_{a0} - \hat{b}_p$$

$$S_2 = 120.24 - 24.02$$

$$S_2 = 96.22 \text{ grams}$$

The size of the two selection differential shows the phenotypic superiority of queens selected even if one colony died (due to diploid drone production). Evidently, any selection event must take into account that some colonies will die or at least be weakened due to sex-alleles.

**Table 5** - Estimates of the regression coefficients before ( $\hat{b}_0$ ) and after ( $\hat{b}_a$ ) the selection of queens of *Melipona scutellaris*.

Rank	Colonies	$\hat{b}_0$	$\hat{b}_a$
01	k426	-9.28 ± 6.52	374 ± 36.13
02	k427	-41.90 ± 14.78	326 ± 146.33
03	k431	-50.48 ± 5.63	598 ± 103.61
04	k419	62.62 ± 28.87	274 ± 78.65
05	k309	177.86 ± 18.70	205 ± 29.57
06	k430	122.86 ± 33.35	
07	k436	-6.43 ± 25.77	140 ± 16.00
08	k418	2.38 ± 17.77	390 ± 149.70
09	k295	26.90 ± 21.64	250 ± 39.71
10	k428	54.52 ± 11.39	578 ± 162.43
Mean ( 10 colonies)		33.90 ± 1.84	
Mean (9 colonies)		24.02 ± 1.86	348.33 ± 9.40

## QUEENS' SUBSTITUTION

Queens of **Group a** were introduced into colonies of **Group b** with no extra precautions, as mentioned by Monteiro and Kerr (1990), and all were accepted. The queenless colonies of **Group a** had new queens emerge and accepted, and all carried out their respective nuptial flight on May, 1992. The new queen of colony k 430 was inseminated by a male with one *xo* sex allele equal to one of the two of the queens which lead to a segregation not different from 1:1 ( 1/2 females to 1/2 diploid males). Usually two events of diploid male production caused the colony to die out.

## RESULT OF THE SELECTION ( R )

The estimates of the selection results were obtained by the differences between the mean linear regression coefficients of the **Groups a** and **b** before and after the selection by the estimate of the mean linear regression coefficient of the control **Group c** also after and before the selection for nine colonies of *Melipona scutellaris*

**Table 6** - shows the values of the estimates of the mean coefficient of regression of groups a, b, a + b and c, before and after selection of queens.

GROUPS	BEFORE	AFTER
a	$\hat{b}_{a0} = 120.24 \pm 11.89$	$\hat{b}_{a1} = 239.5 \pm 27.05$
b	$\hat{b}_{b0} = -33.89 \pm 2.99$	$\hat{b}_{b1} = 432.66 \pm 31.78$
a + b	$\hat{b}_{ab0} = 27.76 \pm 2.98$	$\hat{b}_{ab1} = 355.4 \pm 15.77$
c	$\hat{b}_{c0} = 19.34 \pm 4.78$	$\hat{b}_{c1} = 339.5 \pm 22.99$

The queens of control **Group c** remained the same from the beginning of the experiment. Therefore, the difference between the mean linear regression coefficients of these four colonies, before and after selection, provides the non-genetic variation that occurred during this entire experiment.

The phenotypic variation that occurred in the colonies of **Groups a and b** are made of both genetic and non genetic components, since there were chances of queens from **Group a to Group b**. The new queens of **Group a** are daughters of the queens that headed each **Group a** colony and, therefore, they have half of the additive inheritance:

$$\hat{R} = (\hat{b}_{ab1} - \hat{b}_{ab0}) - (\hat{b}_{c1} - \hat{b}_{c0})$$

$$\hat{R} = 7.98 \text{ grams / day}$$

Another important component to be considered is the environmental effect (*E*) that was obtained through the difference of the estimates of the mean linear regression coefficients of the colonies of the control group after and before selection.

$$E = \hat{b}_{c1} - \hat{b}_{c0}; E = 319.66 \text{ grams/day.}$$

The size of this value indicates a great environmental variation during the period of the experiment. In the beginning of the evaluation the ten colonies were taken from Jaboticabal to an orange plantation (Monte Alto city), from August to October, 1992; in October they were returned to Jaboticabal where they remained until July 1993, and went to Bebedouro/SP to use the orange honey flow.

### REALIZED HERDABILITY ( $h_R^2$ )

The estimate of the realized herdability is twice the rate of the answer to selection ( $\hat{R}$ ) by the selection differential ( $S_2$ )

$$\hat{h}_R^2 = 2(R/S_2); \hat{h}_R^2 = 0.166$$

The value of the realized herdability was inferior to those cited by Collins (1986) for *Apis mellifera* L. according to the following works:

a) Soller and Bar-Cohen (1967) found a value of  $b^2 = 0.36$

for orange honey production in one generation of selection;

b) Rothenbuhler *et al.* (1979) found values of realized herdability for rapid line syrup collection of  $h_R^2 = 0.553$  and for slow line syrup collection of  $h_R^2 = 0.324$ ;

c) Hellmich *et al.* (1985) encountered values for pollen collection in the high line of  $b^2 = 0.556 \pm 161$  and  $b^2 = 0.063 \pm 0,190$  for the low line. The selection in the above items a and b was disruptive, that is, in two directions.

Pirchener (1969) considers that disruptive selection allows a better control of the environment. It is important to notice that our experiment had a control group of colonies.

### CONCLUSION

The repeatability value was  $r = 0.8343 \pm 0.07$ , which is a high value and indicates that there are more variations between colonies than within, suggesting that the method was robust.

The superiority of the selected colonies was shown through the selection differential with

$$S_1 = 87.21 \text{ grams and } S_2 = 96.22 \text{ grams.}$$

The answer to selection ( $\hat{R}$ ) in the three groups involved in queen substitution was = 7.98 grams per day and indicated that there was a genetic improvement in these groups. The three exceptional colonies that had their queens taken out produced new laying queens quickly, but one produced diploid males which shows that 10 hives make up a very small group and this suggests that experiments of this type should be made with 50 or more hives.

The value for realized herdability for honey production was = 0.164, what is inferior those found in literature for *Apis mellifera*.

### SUMMARY

Selection of queens of more productive colonies was carried out in 10 hives of *Melipona scutellaris* that were evaluated for eight months through its gross weight. The repeatability coefficient (*r*) was estimated with these data using the intraclass correlation method. The production is a repetitive character of  $r = 0.8346 \pm 0.071$ .

Selection was made with mean regression coefficient of each hive and of production gains. The colonies then constituted three groups: **Group a** of colonies with greater, **Group b** with colonies with less growth and **Group c** with hives of intermediate growth. Hives with greater growth (**Group a**) had their queens taken and introduced into those with lesser growth (**Group b**) that had its queens removed and killed; while the colonies of **Group c** remained with its respective queens during all periods before and after selection.

The genetic answer occurred in the group related to queens substitution and was = 7.6 grams/day which indicates that there was an improvement in these groups.

Realized heritability was estimated as being the rate between the answer to selection by the selection differential (S), then = 0.164.

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