

GENETIC AND WITHIN-COLONY ENVIRONMENTAL COMPONENTS OF VARIATION IN THE HAMULI NUMBER OF AFRICANIZED HONEY BEES (*Apis mellifera* L.)

José Alexandre Felizola Diniz-Filho¹, Osmar Malaspina² and Daniela Honorato Cavalheri²

ABSTRACT

We analysed the genetic and within-colony components of variation in the hamuli number of an Africanized honey bee population, using the methodology proposed by Oldroyd and Moran (*Aust. J. Biol. Sci.* 36: 323-332, 1983), modified to account for within-colony environmental variation. Estimated heritability was equal to 0.6768 ± 0.2991 , assuming that queens were inseminated by 17 unrelated drones, and the within-colony environmental variance component was non significant ($F=0.02$; $P=0.996$). This indicates that heritabilities of hamuli number obtained using the methodology proposed by Oldroyd and Moran (*Aust. J. Biol. Sci.* 36: 323-332, 1983) are not seriously affected by within colony environmental effects, at least in this population of Africanized honey bees.

INTRODUCTION

Oldroyd and Moran (1983) proposed a very simple method for estimating the heritabilities (h^2) of characters measured in honey bee workers. It consists of performing a single classification Analysis of Variance (ANOVA) between colonies, using workers within colonies as replicates, with the model

$$Y_{ij} = \mu + C_j + \epsilon_{ij}$$

where μ is the grand mean, Y_{ij} is the i th-worker measured in the j th-colony, C_j is the j th-colony (the queen effect) and ϵ_{ij} is the residual term associated with each observation. The intraclass correlation coefficient (t) (Sokal and Rohlf, 1981), derived from ANOVA, is used to estimate h^2 as

$$h^2 = t / r$$

where r is the average genetic relatedness within the colony. Because of polyandry, *Apis mellifera* colonies can be viewed as sib groups, mixing super-sisters, daughters of the same drone, with $r=0.75$, and half-sisters, daughters of distinct (unrelated) drones, with $r=0.25$. Assuming that there is a mixing of sperm in the queen's spermatheca (Page, 1986), the probability of sampling super-sisters in a colony should be proportional to the degree of polyandry ($P=1/n$, where n is the number of drones that inseminate each queen) (Rinderer, 1977). The average r within a colony, therefore, is also a function of n . For naturally inseminated queens, we assume thorough mixing of sperm, and that drones mating with the queen are unrelated and contribute with a similar amount of sperm, in such a way that

$$r = 1/2n + 0.25.$$

Despite some possible upward bias in h^2 , related to the absence of precise information about the number of matings in the population (when the number of matings increases, h^2 estimates also increase; when this number decreases, the bias due to dominance effects increases), the methodology proposed by Oldroyd and Moran (1983) has been used in several studies (Milne, 1985; Moritz, 1985; Oldroyd *et al.*, 1991). Recently, Oldroyd *et al.* (1991) applied this methodology to obtain h^2 estimates for 25

¹ Departamento de Biologia Geral, Instituto de Ciências Biológicas, Universidade Federal de Goiás, Caixa Postal 131, 74001-970 Goiânia, GO, Brasil. Send correspondence to J.A.F.D.-F.

² Departamento de Biologia, Instituto de Biociências, UNESP, Caixa Postal 199, 13506-900 Rio Claro, SP, Brasil.

characters used to discriminate Africanized and European honey bees (Daly and Balling, 1978). Their estimates were usually higher than 1.0, an unusual value for a ratio. They attribute these values to sampling problems or underestimates of genetic relatedness within colonies. They also showed, by reanalysing data from Rinderer *et al.* (1986), that h^2 decreases by an average of 41% when workers are reared in a non-maternal environment. This indicates that upward bias in h^2 may be due to a confusion between additive and within-colony environmental effects, since the sib group (sisters in a colony) share the same within-colony environmental conditions. Similar results were found in the heterosis of hamuli number by Oldroyd and Moran (1987), comparing diallelic crosses among nine lines of *A. mellifera* L.

MATERIAL AND METHODS

We investigated the effect of microenvironment on hamuli number in Africanized honey bees reared in the Apiary of UNESP at Rio Claro, São Paulo, Brazil. We changed the original design proposed by Oldroyd and Moran (1983), and instead of sampling distinct colonies, we simultaneously reared brood from a total of 16 distinct colonies in four larger (randomly chosen) "nursing-colonies" (four distinct colonies in each nursing-colony). The brood was transferred to nursing-colonies 24 hours after oviposition and, after pupation combs were covered with a metal screen (to avoid mixture of newborn bees). Hamuli were then counted in 15 workers from each comb. This nested design was analysed with the linear model

$$Y_{ijk} = \mu + B_j + C_k(B_j) + \varepsilon_{ijk}$$

where B_j is the j th-nursing-colony and $C_k(B_j)$ is the k th-brood comb placed in the j th-nursing-colony. Heritabilities were estimated as $h^2 = t/r$, where t is the intraclass correlation obtained from variance components derived from the nested ANOVA design. Variance components were estimated using the VARCOMP procedure of SAS-Pc (SAS Institute Inc., 1988). Standard errors of heritability were calculated following Oldroyd and Moran (1983), and a range of h^2 values (with standard errors) were then estimated, assuming various numbers of matings.

RESULTS AND DISCUSSION

The ANOVA results are given in Table I. Nursing-colony effects were not significant ($P=0.996$) through there was a very significant effect of variation between brood donor colonies within nursing colonies. Variance components are shown in Table II. Nursing

colonies showed a small negative variance component, which could be attributed to the small number of degrees of freedom at this level (3). The real value must be close to zero, indicating no microenvironmental effects on the hamuli number. Based on Table II and following Moritz (1985), we found an intraclass correlation equal to 0.1891, and it was possible to estimate h^2 for distinct number of matings (Table III). As shown in our previous paper using this method (Diniz-Filho *et al.*, 1993), changes in h^2 are small when the number of matings is large (more than 10). If we assume that queens are inseminated by an average of 17 drones (Adams *et al.*, 1977; Lobo, 1991), we obtain $h^2 =$ approximately 0.68 ± 0.30 . This value is close to the values obtained by Oldroyd and Moran (1983) and Oldroyd *et al.* (1991), using a similar methodology, and confirm that, although non-additive effects act on the distribution of hamuli number, they are small if compared with additive effects. Gonçalves (1977) also obtained elevated realized heritabilities for hamuli number, but these values were estimated by regression of offspring on mid-parent after 22 generations of directional selection.

Oldroyd *et al.* (1991) estimated $h^2 = 0.87$ for hamuli number in Africanized bees reared in a maternal environment, and $h^2 = 0.55$ for bees reared in non-maternal conditions. This reduction indicates the existence of a larger environmental variance acting on the character when workers are reared in other colonies. However, the non-maternal rearing experiment analysed by Oldroyd *et al.* (1991) involved the development of Africanized bees in European colonies, in such a way that it may be difficult to distinguish between within-colony effect and racial

Table I - Results of nested ANOVA applied to hamuli number in workers from sixteen colonies of Africanized honey bees raised in four nursing colonies.

Source	SS	DF	MS	F
Nursing-colony	0.746	3	0.249	0.02ns
Combs (donor-colony)	139.750	12	11.646	4.50**
Error	580.000	224	2.589	

ns - non significant at 5% level, ** - $P < 0.01$

Table II - Variance components derived from ANOVA of Table I by VARCOMP procedure of SAS-Pc

Source	Variance component
Nursing-colony	-0.189953
Combs (donor-colony)	0.603770
Error	2.589286

Table III - Relatedness (r) within colonies and heritabilities estimated for hamuli number ($h^2 \pm$ standard error) as a function of number of matings (drones).

Number of matings	r	$h^2 \pm$ S.E.
1	0.7500	0.2521 \pm 0.1114
2	0.5000	0.3782 \pm 0.1671
3	0.4160	0.4539 \pm 0.2005
4	0.3750	0.5043 \pm 0.2228
5	0.3500	0.5403 \pm 0.2387
6	0.3335	0.5673 \pm 0.2507
7	0.3214	0.5883 \pm 0.2599
8	0.3125	0.6052 \pm 0.2674
9	0.3055	0.6189 \pm 0.2734
10	0.3000	0.6304 \pm 0.2785
11	0.2954	0.6401 \pm 0.2828
12	0.2917	0.6484 \pm 0.2865
13	0.2885	0.6556 \pm 0.2897
14	0.2857	0.6618 \pm 0.2925
15	0.2833	0.6674 \pm 0.2949
16	0.2812	0.6724 \pm 0.2971
17	0.2794	0.6768 \pm 0.2991
18	0.2777	0.6808 \pm 0.3008
19	0.2763	0.6844 \pm 0.3024
20	0.2750	0.6876 \pm 0.3039

differences in nursing capacity or even physical differences in the colony environment. On the other hand, our null negative component for between nursing colony effect may be attributed to a small variance in the nursing colonies, compared to the larger differences between the European and Africanized colonies used by Rinderer *et al.* (1986). Of course, other morphological characters, especially those related to size variation, may be more affected by the quantity of food supplied by nursing workers and by colonial climatic conditions. Our results indicate that, at least within a single local population of Africanized bees, hamuli number is not affected by within-colony environmental effects and that h^2 estimates of this character, using the method of Oldroyd and Moran (1983), are not seriously upwardly biased by this effect.

ACKNOWLEDGMENTS

The authors are grateful to Sérgio Pascon for performing the experimental work. This work was partially supported by Doctoral (J.A.F.D.F.) and Research (O.M.) Fellowships provided by CNPq and by FUNDUNESP.

Publication supported by FAPESP.

RESUMO

Neste trabalho foram analisados os componentes genéticos e microambientais (dentro de colônias) atuando sobre o número de hâmulos em uma população de abelhas africanizadas, utilizando a metodologia de Oldroyd e Moran (*Aust. J. Biol. Sci.* 36: 323-332, 1983), modificada para avaliar componentes de variação ambiental dentro das colônias. A herdabilidade estimada foi igual a $0,6768 \pm 0,2991$ (assumindo rainhas inseminadas por 17 machos, em média), e o componente de variação microambiental não foi significativo a nível de 5% ($F=0,02$; $P=0,996$). Isso indica que as estimativas de herdabilidade do número de hâmulos utilizando a metodologia de Oldroyd e Moran (*Aust. J. Biol. Sci.* 36: 323-332, 1983) não são sobrestimadas em função da variância ambiental atuando dentro dos grupos de irmãos (colônias), pelo menos em uma população local de abelhas africanizadas.

REFERENCES

- Adams, J.E., Rothman, E.D., Kerr, W.E. and Paulino-Simões, Z.L. (1977). Estimation of the number of sex alleles and queen matings from diploid male frequencies in a population of *Apis mellifera*. *Genetics* 86: 583-596.
- Daly, H.V. and Balling, S.S. (1978). Identification of Africanized honey-bees in the Western Hemisphere by discriminant analysis. *J. Kansas Entomol. Soc.* 51: 857-869.
- Diniz-Filho, J.A.F., Bueno, O.C., Chaud-Netto, J. and Malaspina, O. (1993). Heritability of the number of ovarioles in honey bee workers (*Apis mellifera* L.) (Hym.: Apidae). *Rev. Brasil. Genet.* 16: 917-921.
- Gonçalves, L.S. (1977). Estimativas de variabilidade, herdabilidade e progresso de seleção em duas linhagens selecionadas de *Apis mellifera* L. (seleção direcional). *Ciência & Cultura* 29: 736.
- Lobo, J.A. (1991). Modelos para análise de frequências alélicas e mistura racial em *Apis mellifera*. Doctoral Thesis, FMRP-USP, Ribeirão Preto.
- Milne, C.P. (1985). An estimate of the heritability of the corbicular area of the honeybee. *J. Apic. Resear.* 24: 137-139.
- Moritz, R.F.A. (1985). Heritability of the postcapping stage in *Apis mellifera* and its relation to varroaosis resistance. *J. Hered.* 76: 267-270.
- Oldroyd, B. and Moran, C. (1983). Heritability of worker characters in the honeybee (*Apis mellifera*). *Aust. J. Biol. Sci.* 36: 323-332.
- Oldroyd, B. and Moran, C. (1987). Additive and heterotic genetic effects in the haplo-diploid honeybee, *Apis mellifera*. *Austr. J. Biol. Sci.* 40: 57-63.
- Oldroyd, B., Rinderer, T.E. and Buco, S. (1991). Heritability of morphological characters used to distinguish European and Africanized honeybees. *Theor. Appl. Genet.* 82: 499-504.
- Page, R. (1986). Sperm utilization in social insects. *Ann. Rev. Entomol.* 31: 297-320.
- Rinderer, T.E. (1977). Measuring the heritability of characters in honeybees. *J. Apic. Resear.* 16: 95-98.
- Rinderer, T.E., Sylvester, H.A., Collins, A.M. and Pesante, D. (1986). Identification of Africanized and European honey bees:

effects of nurse bee genotype and comb size. *Bull. Entomol. Soc. Amer.* 32: 150-152.

SAS, Institute Inc. (1988). *SAS/STATS User's Guide*. Release 6.03 Edition. Cary, NC.

Sokal, R.R. and Rohlf, F.J. (1981). *Biometry*. 2nd edn. W.H. Freeman, NY, pp. 859.

(Received August 23, 1993)