Relationship Between Hygienic Behavior and *Varroa destructor* Mites in Colonies Producing Honey or Royal Jelly

by

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ABSTRACT

Genetic and phenotypic parameters for hygienic behavior, invasion and infestation rates, total and effective reproduction of *Varroa destructor* in Africanized honeybee colonies producing honey (20 hives) or royal jelly (30 mini-hives) were analyzed. The significance of monthly fixed effects, type of product (honey and royal jelly) and their interactions were verified through generalized linear model procedures. Software WinBugs (Bayesian Inference Using Gibbs Sampling) with Bayesian inference was employed for (co)variance estimates. The average values for colonies producing honey or royal jelly were 74.38 and 71.40% for hygienic behavior in 24 hours; infestation rates 8.30 and 11.40%; invasion rates 9.50 and 7.50%; total reproduction 1.02 and 0.55%; effective reproduction was 0.62 and 0.33%, respectively. The additive genetic variance for invasion (0.16), total reproduction (0.25) and effective reproduction (0.94) rates of the mite were higher than estimates for hygienic behavior in 24 hours (0.05) and infestation rate (0.04). Mean heritability for hygienic behavior, infestation and invasion rates, total and effective reproduction of the mite were

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0.58, 0.54, 0.56, 0.63 and 0.61, respectively. The genetic correlation of -0.48 for hygienic behavior with total reproduction rate of *Varroa destructor* shows that hygienic behavior may be the most interesting trait for selection. Besides a heritability of high magnitude, when combined with the total reproduction rate of the mite, it has a high and antagonistic correlation. Consequently, in cases of high infestation of *Varroa destructor*, the selection for hygienic behavior would decrease the reproduction rate of the mite.

Key-words: bee breeding, heritability, genetic correlation, phenotypic correlation, Bayesian inference

RESUMO

Foram analisados os parâmetros genéticos e fenotípicos para o comportamento higiênico e as taxas de invasão, infestação e reprodução total e efetiva do ácaro *Varroa destructor* em colônias de abelhas *Apis mellifera* africanizadas produtoras de mel (20) ou geleia real (30). Foi verificada a significância dos efeitos fixos de mês, tipo de produto e suas interações, utilizando o procedimento de modelos lineares generalizados. Para a estimação das (co)variâncias foi utilizado o software WinBUGS por meio de inferência Bayesiana. As médias para colônias produtoras de mel ou geleia real, respectivamente, foram: para o comportamento higiênico em 24h, 74,38 e 71,40%; taxas de infestação 8,30 e 11,40%; taxas de invasão 9,50 e 7,5%; taxas de reprodução total 1,02 e 0,55 e reprodução efetiva 0,62 e 0,33. As herdabilidades médias para comportamento higiênico, taxa de infestação, taxa de invasão, taxa de reprodução total e efetiva do ácaro foram 0,58, 0,54, 0,56, 0,63 e 0,61, respectivamente. A correlação genética encontrada de -0,48 para a característica comportamento higiênico com taxa de reprodução total do ácaro *Varroa destructor* indicou que o comportamento higiênico pode ser considerado a característica mais interessante para seleção, pois além de apresentar herdabilidade de alta magnitude, quando associada à taxa de reprodução total do ácaro apresenta uma correlação alta e

antagônica, sendo assim, em casos de alta infestação do ácaro *Varroa destructor*, selecionando-se para comportamento higiênico estaríamos diminuindo a taxa de reprodução do ácaro.

Palavras-chave: melhoramento de abelhas, herdabilidade, correlação genética, correlação fenotípica, inferência bayesiana

INTRODUCTION

Africanized honeybees are specifically significant owing to their positive adaptation in Brazil and to their honey production. Since the honey bee has high genetic variability between queens from the same geographical region (Winston *et al.* 1983) with regard to economical features, the honeybees are interesting examples within a genetic improvement program.

The peculiarity of honeybees makes genetic improvement difficult due to environmental influences and genetic differences in mating level (Bienefeld *et al.* 2007). However, researchers in many countries are undertaking genetic improvement with some success. The best honeybee production selection programs in the USA necessarily include the selection of hygienic honeybees.

Hygienic behavior is an efficient resistance mechanism against brood diseases and has been well-documented since the 1940s when much research was undertaken in the area (Message 2006). This hygienic behavior includes the colony capacity to inhibit infestation by mites of the genus Varroa.

The mite *Varroa destructor*, which causes varroatosis in *Apis cerana* and *Apis mellifera*, was introduced in Brazilian apiculture at the beginning of the 1970s. Called *Varroa jacobsoni*, the genus was introduced in Brazil through Paraguayan beekeepers (Morse & Gonçalves 1979). In turn, the queens bought from Paraguay had been imported from Japan.

Population dynamics of the mite Varroa have varied by region (Calderón *et al.* 2010). In Brazil were greatly different from those reported in other varroatosis infested regions. Over 20% rates, initially reported,

brought concern to researchers and beekeepers (Moretto *et al.* 1991). However, as the mite spread itself throughout Brazilian regions, it became clear that infestation rates, although initially high, declined some years after the first infestation.

A balance seems to have been reached between the mite *Varroa destructor* and Africanized honeybees in climate conditions of Brazil (Moretto & Mello 2001). In some regions of the country the varroatosis infestation rates reached the very low percentage of 2% (Moretto *et al.* 1993, Carneiro *et al.* 2007, Calderón *et al.* 2010).

This research was carried out to estimate phenotypic and genetic parameters which report hygienic behavior with the population dynamics of *Varroa destructor* in Africanized honeybee colonies which produce royal jelly or honey. The possible inclusion of these traits in genetic improvement programs in Brazil may be achieved.

MATERIAL AND METHODS

The assay was undertaken at the Central Apiary of the Iguatemi Experimental Farm of Universidade Estadual de Maringá, Maringá PR Brazil, from February 2009 to November 2009. Fifty colonies of Africanized honeybees, 30 in mini-hives which produced royal jelly, and 20 in Langstroth hives with supers, which produced honey which was used.

Production of queens

Queen production for the production of their daughters from honey-producing colonies to replace the queens of royal jelly-producing colonies was undertaken in October 2008, April 2009 and August 2009. Modified Doolittle (1889) method for queen production, or rather, the grafting of worker larvae from their original cell to acrylic cups with royal jelly diluted in distilled water (1:1), was employed.

Whereas ten queens were randomly replaced in January 2009 in the 30 royal jelly-producing colonies, nine queens were once more randomly replaced in April and 12 in August.

Production of royal jelly

A modified Doolittle (1889) method was employed for royal jelly production whilst its grafting and collection were undertaken twice a week. So that appropriate larvae for royal jelly production could be obtained, grafting was previously programmed with the introduction of an empty comb four days prior to grafting and placed in the center of the different colonies in the ten-frame nests or five-frame nuclei in Langstroth hive model. After larvae grafting, the cup bars were carefully placed in their respective Langstroth hive or mini-hive.

Hygienic behavior

Rothenbühler (1964) and Newton *et al.* (1975), revised by Spivak & Downey (1998) reported that the hygienic behavior test establishes the time spent by honeybee colonies to detect, uncap and remove dead brood worker honeybees by freezing (-20ºC during 24h) from one section of the 5 x 6 cm comb (with approximately 100 capped brood - pupae of the comb), separated from the colony nest for evaluation, and to determine whether the colony is or not hygienic.

Royal jelly- and honey-producing colonies underwent hygienic behavior test (Taber 1982, Gramacho 1995). A comb with capped brood worker bees, aged 17-18 days, or rather, during the rose-colored pupa phase, placed on both sides, A and B, was removed from each colony. The central section with 5 x 10 cm had been removed from the comb, was photographed, and frozen at -20ºC for 24h. After that, the comb section was then conditioned in an incubator at 34ºC and 60% humidity during 4h to dry and establish the same internal temperature of the colonies. After restored to their respective colonies, the sections were again photographed 24h for counting of the uncapped cells.

Hygienic behavior percentages were obtained by calculating the number of alveoli of capped brood at zero hour as a function of the number of alveoli of the remaining capped brood in 24 h.

Further, 15, 11, 12, 14, 11 and 7 out of the 20 honey-producing matrix colonies were evaluated for hygienic behavior respectively in February,

March, April, May, June and July 2009. Similarly, 14, 18, 11 and 13 out of 30 royal jelly-producing colonies were evaluated respectively in February, March, April and September 2009.

Estimates for hygienic behavior were obtained by calculating:

$CH_x = (TO_{zero\ hour} - AO_x)/ TO_{zero\ hour})$

Where,

CH_x is the relationship between the number of cleaned alveoli and the total number of capped brood cells in which $_x$ reaches 24h;

$TO_{zero\ hour}$ is the total number of capped brood cells at zero hour;

AO_x is the total number of capped brood cells in which $_x$ reaches 24h.

The number of cells with partially removed pupae (crp), pointed (cp) and uncapped (cd) which, as a rule, demonstrate the hygienic behavior corresponding to uncapping activities (pointing and destruction of the opercula or uncapping).

Variables analyzed with regard to *Varroa destructor* consisted of:

Infestation rate of adult worker honeybees

The mite infestation rate, as proposed by Stort *et al.* (1981), was undertaken to verify the number of mites in worker honeybees. The method retrieves approximately one hundred adult worker honeybees from a comb at the centre and placed in a dish with alcohol 70% and the contents stirred. Total number of worker honeybees and mites was counted to evaluate each colony infestation percentage.

Invasion rate in worker pupae

Observation of the number of *Varroa* mites, as proposed by De Jong & Gonçalves (1981), was performed to estimate the number of mites in the worker pupae. The method included the retrieval of a comb of capped brood from each colony. Opercula were removed with insect pin from 100 (50 from each side of the comb) pupae. Female mites and their offspring in pupae and alveoli were analyzed by attached light.

Tests were undertaken in the twenty honey-producing colonies: five colonies were tested during February 2009; six in March; 11 in April; eight in May; 15 in June and 10 in July 2009.

In the 30 royal jelly-producing colonies the test was undertaken in 11 colonies during February 2009; seven in March; nine in April and nine in September.

Reproduction rates of the mite *Varroa destructor*

Mite total reproduction rate was calculated as follows:

TRT = total number of descendents/ (number of adult females)

Mite effective reproduction rate was calculated as follows:

TRE = number of deutonymphs + young adults / (number of female adults)

Whereas total reproduction rate (TRT) is the total number of descendents produced by the mite, effective reproduction rate is the number of viable descendants. Since worker pupae under analysis were approximately 18 – 19 days old, there was no sufficient time for the final development of the mite immature forms, in Africanized honeybees. Therefore, only deutonymphs, female adults and young females could parasitize the forthcoming worker honeybee one to two days after analysis. This fact actually contributed towards the mite reproduction.

When Toledo & Nogueira-Couto (1996) investigated Africanized honeybees and hybrids from Caucasian, Italian and Carniolian sister queens, they found no statistical difference for *Varroa* total and effective reproduction rates.

Data analysis

R Development Core Team statistic program (2009) gave a previous analysis of data to verify the significance of fixed month effects, type of product (royal jelly or honey) and their interactions. Since Shapiro-Wilk test showed non-normality of data, Generalized Linear Models (GLM) procedures were used, in which it was assumed the Gama distribution and inverse link function, for variables. Data were corrected when any significant effect occurred.

Since worker honeybees that performed the hygienic behavior are middle-aged, averaging 15.2 days old (Arathi *et al.* 2000), worker groups

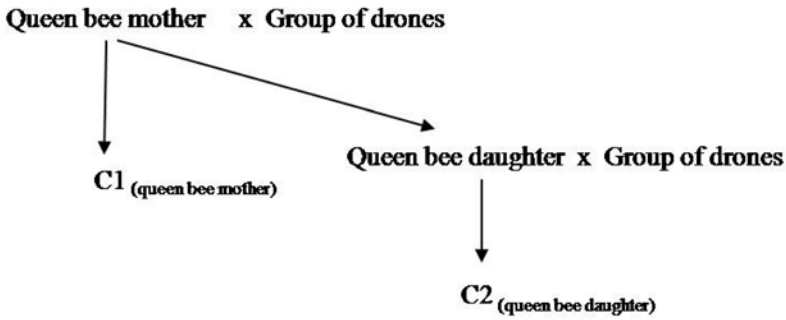

Fig.1. Relationship among honey-producing (C1) and royal jelly producing colonies (C2).

executing the same task in the colony are changed approximately at every 35.2 days.

Fig. 1 shows the relationship among colonies of queen bee mothers and those of the queen bee daughters amounted to 0.25 since fatherhood is unknown.

Corrected covariance among colonies of queen bee mothers and those of queen bee daughters amounts to 0.25 of additive genetic variance.

The two colonies may be described as featuring aunt-niece relationships, or rather; the queen bee from which C1 derived is the queen mother that produced C2.

Analysis strategy took the traits in pairs while considering that the same characteristic in the queen bee mother colonies was another characteristic when evaluated in the queen bee daughter colony.

Data were organized through the pairing of recording of the queen bee mother colonies and those of the queen bee daughter colonies. The archive in each analysis with two traits was formed by four columns: the first refers to recording of trait 1 in the queen bee mother colonies; the second refers to recording of trait 2 in queen bee mother colonies; the third refers to recording of trait 1 in the queen bee daughter colonies; the fourth refers to recording of trait 2 in the queen bee daughter colonies. Employing the following multivariated structure, we have:

$$Y_{ijk} \sim NMV\,(\mu_k; \Sigma_k), \text{ or rather,}$$

$$\begin{bmatrix} y_1 \\ y_2 \\ y_3 \\ y_4 \end{bmatrix} \quad NMV\left\{ \begin{bmatrix} 0 \\ 0 \\ 0 \\ 0 \end{bmatrix}, \begin{bmatrix} \sigma^2_{y_1} & \sigma_{y_1y_2} & \sigma_{y_1y_3} & \sigma_{y_1y_4} \\ \sigma_{y_2y_1} & \sigma^2_{y_2} & \sigma_{y_2y_3} & \sigma_{y_2y_4} \\ \sigma_{y_3y_1} & \sigma_{y_3y_2} & \sigma^2_{y_3} & \sigma_{y_3y_4} \\ \sigma_{y_4y_1} & \sigma_{y_4y_2} & \sigma_{y_4y_3} & \sigma^2_{y_4} \end{bmatrix} \right.$$

In the case of matrix Σ_k, the variance matrix and (co)variance in the reports, inverted Wishart distribution, was taken up by $\Sigma_k \sim IW(R_k,J)$ with $R_k=I_J=I_4$, in which I is the identity matrix and the scale parameter is equal to four.

Marginal distribution for parameters was obtained *a posteriori* by software WinBUGS 1.4.2 (Spiegelhalter *et al.* 1994) which precedes Bayesian analysis. One million rates were generated for each parameter in a Monte Carlo Markov Chain (MCMC) process, declining initial discarding period and with all sampled rates inserted within a final chain. Chain convergence was tested by criteria proposed by Heidelberger & Welch (1983) and Geweke (1992), implemented in a CODA library, available in the computer system R Development Core Team (2009).

Based on estimated rates for Σ_k and employing a program developed by computer system R Development Core Team (2009), phenotypic ($\sigma^2_{y\text{C1}}$ and $\sigma^2_{y\text{C2}}$) and additive genetic ($\sigma^2_{a\text{C1}}$ and $\sigma^2_{a\text{C2}}$) variance for the two traits and phenotypic ($\sigma^2_{y\text{C1}y\text{C2}}$)and additive genetic ($\sigma^2_{a\text{C1}a\text{C2}}$) covariance between the two was calculated as follows:

$$\sigma^2_{y\text{C1}} = \frac{\sigma^2_{y_1} + \sigma^2_{y_3}}{2}$$

$$\sigma^2_{y\text{C2}} = \frac{\sigma^2_{y_2} + \sigma^2_{y_4}}{2}$$

$$\sigma^2_{yC1yC2} = \frac{\sigma_{y_1y_4} + \sigma_{y_2y_3}}{2}$$

$$\sigma^2_{aC1} = 4\sigma_{y_1y_3}$$

$$\sigma^2_{aC2} = 4\sigma_{y_2y_4}$$

$$\sigma^2_{aC1aC2} = 4\sigma_{yC1yC2}$$

So that heritability and correlation would be within the parameter space established for them, namely, from 0 to 1 and from -1 to 1, respectively, samples that failed to satisfy the above mentioned conditions were discarded from the Markov chain. The new chain underwent convergence tests proposed by Heidelberger & Welch (1983) and Geweke (1992).

Mean distribution rate, median, standard error, credibility interval and high density region were set at 95% for each variance component and genetic parameter.

RESULTS AND DISCUSSION

Percentage mean for hygienic behavior in 24h (CH24), adult infestation rate, invasion rate in the pupae, total and effective reproduction rate for honey and royal jelly-producing colonies were respectively 74.38 and 71.40%; 8.30 and 11.40%; 9.50 and 7.5%; 102.40 and 54.60%; 62.00 and 33.00%.

Previous data analysis did not show effects of production types for hygienic behavior in 24h (CH24).

Interaction existed for product and period in the case of mite infestation in adult honeybees. This fact shows that during the cold months there was an increase in infestation rates for honey-producing colonies. The number of colony individuals during this period decreased and an increase in mite concentrations occurred (Nogueira-Couto 1991).

Product type, honey and royal jelly, was significant for the pupa invasion rate, respectively with means of 9.5 and 7.5%. According to Segndo Lee *et al.* (2010) the mite prefers the nurse bees, which tend to stay in the brood frame. Thus, the colonies submitted to royal jelly production, which need a greater amount of nurse bees responsible for feeding the queen larvae, are probably more affected by *Varroa* mites.

Moretto *et al.* (1991) verified invasion rates in pupae in three regions and concluded that Africanized honeybees were more resistant to *Varroa* than Italian honeybees bred with Africanized honeybees. In Brazil, Africanized honeybees tolerate the mite, and high swarming rates contribute to this tolerance (Boecking & Ritter 1993).

Total and effective reproduction rates of *Varroa* mites were not affected by period or product type.

Table 1 shows estimates of addictive genetic and phenotypic covariance in a two-trait analysis for variables under analysis. Additive genetic variance for invasion (0.16), total (0.25) and effective (0.94) reproduction rates of the mite are higher than those estimated for hygienic behavior in 24h (0.05) and infestation (0.04). Estimates for phenotypic variance had a similar behavior. Higher additive genetic variance proportion for the mite reproduction rates may be due to higher precision of tye method employed when compared to infestation, invasion and hygienic behavior.

Table 2 shows estimates of heritability and the respective credibility intervals and high density regions in a two-trait analysis.

Mean heritability for hygienic behavior, infestation rate, invasion rate, total and effective reproduction rate of mites were respectively 0.58, 0.54, 0.56, 0.63 and 0.61. Although Spivak & Gilliam (1993) said that this behavior is genetically determined, but not always expressed, it seems to be dependent on environmental factors of the colony. Harbo & Harris (1999) recorded heritability estimates of 0.65 for such behavior in 24h. In the following year, Boecking *et al.* (2000) estimated heritability at 0.36 for the removal of capped brood perforated between 13 and 15h.

Table 1. Estimates of components of addictive genetic and phenotypic covariance with respective intervals of credibility and high density regions, at 95%, in a two-trait analysis for hygienic behavior in 24h infestation, invation and total and effective reproductive rates of *Varroa destructor* in Africanized honeybees.

Components*	Estimates	Credibility interval	High density region
σ^2_{a1}	0.05	0.005 – 0.12	0.0004 – 0.10
$\sigma_{a1\,2}$	-0.001	-0.06 – 0.05	-0.06 – 0.05
σ_{a1a3}	-0.001	-0.10 – 0.10	-0.10 – 0.10
σ_{a1a4}	-0.21	-0.65 – 0.21	-0.63 – 0.23
σ_{a1a5}	0.004	-0.35 – 0.35	-0.35 – 0.35
σ^2_{a2}	0.04	0.004 – 0.10	0.0005 – 0.10
σ_{a2a3}	0.001	-0.04 – 0.04	-0.04 – 0.04
σ_{a2a4}	-0.07	-0.47 – 0.28	-0.45 – 0.30
σ_{a2a5}	0.07	-0.29 – 0.41	-0.29 – 0.41
σ^2_{a3}	0.16	0.02 – 0.38	0.002 – 0.33
σ_{a3a4}	0.07	-0.08 – 0.23	-0.08 – 0.23
σ_{a3a5}	0.01	-0.10 – 0.12	-0.10 – 0.12
$\sigma^2_{a\,4}$	0.25	0.05 – 0.52	0.02 – 0.47
σ_{a4a5}	0.26	-0.36 – 0.82	-0.32 – 0.85
σ^2_{a5}	0.94	0.18 – 2.31	0.06 – 1.98
$\sigma^2_{y\,1}$	0.09	0.05 – 0.15	0.05 – 0.15
σ_{y1y2}	-0.0003	-0.01 – 0.01	-0.01 – 0.01
$\sigma_{y1y\,3}$	-0.0002	-0.02 – 0.02	-0.02 – 0.02
$\sigma_{y1y\,4}$	-0.05	-0.16 – 0.05	-0.16 – 0.05
σ_{y1y5}	0.001	-0.08 – 0.08	-0.08 – 0.08
σ^2_{y2}	0.08	0.05 – 0.13	0.05 – 0.12
$\sigma_{y2y\,3}$	0.0002	-0.01 – 0.01	-0.01 – 0.01
$\sigma_{y2y\,4}$	-0.02	-0.12 – 0.07	-0.11 – 0.08
σ_{y2y5}	0.02	-0.07 – 0.10	-0.07 – 0.10
σ^2_{y3}	0.29	0.16 – 0.52	0.14 – 0.47
$\sigma_{y3y\,4}$	0.02	-0.02 – 0.05	-0.02 – 0.05
σ_{y3y5}	0.002	-0.02 – 0.02	-0.02 – 0.02
σ^2_{y4}	0.37	0.21 – 0.66	0.18 – 0.59
$\sigma_{y4y\,5}$	0.07	-0.09 – 0.20	-0.08 – 0.21
σ^2_{y5}	1.37	0.65 – 3.01	0.54 – 2.59

* a and y represent the addictive genetic and phenotypic effects, respectively, for all components; indexes 1, 2, 3, 4 and 5 correspond to the hygienic behavior in 24h, infestation, invasion, total and effective reproduction rate of the mite, respectively.

Table 2. Estimates of heritability *(h^2)* in a two-trait analysis and respective credibility intervals and high density regions at 95%, and mean heritability for hygienic behavior in 24h, infestation, invasion and reproduction rates of mite *Varroa destructor* in Africanized honeybees.

Characteristic	Two-trait analysis	Estimates	Credibility interval	High density region
	h^2_{a1a2}	0.52	0.06 – 0.97	0.09 – 0.99
1	h^2_{a1a3}	0.57	0.07 – 0.98	0.12 – 0.99
	h^2_{a1a4}	0.56	0.07 – 0.98	0.12 – 0.99
	h^2_{a1a5}	0.66	0.12 – 0.99	0.19 – 0.99
Mean	H^2	0.58	–	–
	h^2_{a2a1}	0.55	0.07 – 0.98	0.11 – 0.99
2	h^2_{a2a3}	0.52	0.06 – 0.97	0.09 – 0.99
	h^2_{a2a4}	0.47	0.05 – 0.96	0.03 – 0.93
	h^2_{a2a5}	0.64	0.13 – 0.98	0.19 – 0.99
Mean	H^2	0.54	–	–
	h^2_{a3a1}	0.56	0.07 – 0.98	0.11 – 0.99
3	h^2_{a3a2}	0.55	0.07 – 0.98	0.11 – 0.99
	h^2_{a3a4}	0.56	0.07 – 0.98	0.11 – 0.99
	h^2_{a3a5}	0.57	0.07 – 0.98	0.12 – 0.99
Mean	H^2	0.56	–	–
	h^2_{a4a1}	0.67	0.17 – 0.99	0.23 – 0.99
4	h^2_{a4a2}	0.59	0.09 – 0.98	0.15 – 0.99
	h^2_{a4a3}	0.58	0.08 – 0.98	0.13 – 0.99
	h^2_{a4a5}	0.68	0.16 – 0.99	0.23 – 0.99
Mean	H^2	0.63	–	–
	h^2_{a5a1}	0.68	0.16 – 0.99	0.24 – 0.99
5	h^2_{a5a2}	0.56	0.07 – 0.98	0.12 – 0.99
	h^2_{a5a3}	0.54	0.06 – 0.98	0.11 – 0.99
	h^2_{a5a4}	0.66	0.15 – 0.99	0.21 – 0.99
Mean	H^2	0.61	–	–

Costa-Maia (2009) estimated heritability of 0.28 for this component by Bayesian inference.

Credibility intervals for heritability estimates were not only broad and positive but also coincided with high density regions.

Falconer (1987) reported on the importance of heritability due to the fact that it helps in the estimate of addictive genetic variance associated to a trait within a specific population.

Table 3 shows estimates of genetic and phenotypic correlation among the traits under analysis. The positive correlations are: adult infestation

Table 3. Estimates of genetic (r_g) and phenotypic (r_y) correlation coupled to their respective credibility intervals and high density regions at 95%, within a two-trait analysis, for hygienic behavior in 24h, infestation, invasion and total and effective reproduction rates of the mite *Varroa destructor* in Africanized honeybees

Components*	Estimates	Credibility interval	High density region
$r_{g1,2}$	-0.02	-0.95 – 0.94	-0.99 – 0.88
$rg_{1,3}$	-0.007	-0.95 – 0.94	-0.99 – 0.89
$rg_{1,4}$	-0.48	-0.99 – 0.69	-0.99 – 0.50
$rg_{1,5}$	0.01	-0.93 – 0.94	-0.88 – 0.99
$rg_{2,3}$	0.02	-0.93 – 0.94	-0.88 – 0.99
$rg_{2,4}$	-0.19	-0.96 – 0.89	-0.99 – 0.80
$rg_{2,5}$	0.21	-0.87 – 0.97	-0.75 – 0.99
$rg_{3,4}$	0.46	-0.73 – 0.99	-0.52 – 0.99
$rg_{3,5}$	0.06	-0.93 – 0.95	-0.87 – 0.99
$rg_{4,5}$	0.43	-0.65 – 0.98	-0.44 – 0.99
$ry_{1,2}$	-0.003	-0.15 – 0.14	-0.15 – 0.15
$ry_{1,3}$	-0.001	-0.16 – 0.15	-0.16 – 0.16
$ry_{1,4}$	-0.08	-0.19 – 0.07	-0.21 – 0.07
$ry_{1,5}$	0.002	-0.17 – 0.17	-0.17 – 0.17
$ry_{2,3}$	0.003	-0.14 – 0.15	-0.14 – 0.15
$ry_{2,4}$	-0.03	-0.16 – 0.11	-0.17 – 0.11
$ry_{2,5}$	0.03	-0.13 – 0.18	-0.12 – 0.18
$ry_{3,4}$	0.07	-0.07 – 0.19	-0.06 – 0.20
$ry_{3,5}$	0.01	-0.14 – 0.16	-0.14 – 0.16
$ry_{4,5}$	0.07	-0.09 – 0.19	-0.08 – 0.20

*Indexes $_1$, $_2$, $_3$, $_4$ and $_5$ represent hygienic behavior in 24h, infestation, invasion, and total and effective reproduction rate, respectively.

rates and the effective reproduction rate (0.21); invasion rate in pupae and the total reproduction rate (0.46); total reproduction rate and effective reproduction rate (0.43). The other correlations are only slightly genetically and phenotypically related owing to their low rates.

The most important negative genetic correlation is hygienic behavior and the mite total reproduction rate (-0.48). The above antagonism, namely, the selection of Africanized honeybees for hygienic behavior, decreases *Varroa destructor* mite total reproduction rate.

Since credibility intervals for all broad estimates included a zero value in their intervals, no correlation lies among the analyzed traits. However, when data symmetry is investigated, their probability in fact exists with higher and lower rates than zero value. Figs. 2 to 11 (see appendix) show estimates of phenotypic and genetic correlation to demonstrate probabilities and whether one trait affects or not another trait.

Figs. 2 (hygienic behavior with infestation), 3 (hygienic behavior with invasion), 4 (hygienic behavior with effective reproduction rate), 5 (infestation with invasion) and 6 (invasion with effective reproduction rate) show that phenotypic and genetic correlation have graphic symmetries and highest frequencies close to zero. This fact shows that correlation is very low and thus traits are genetically only slightly related.

No correlation exists between hygienic behavior and *Varroa* infestation rates, since these traits are not interdependent (Fig. 2). When colonies with hygienic behavior are selected, there may not be any impact on the mite invasion rates (Fig. 3). This fact is shown in Table 3 by correlation -0.007 with a credibility interval ranging between -0.95 and 0.94.

Fig. 5 shows that infestation rate is not correlated with the *Varroa* invasion rate. This may be explained by worker grooming, which may prevent the mites from invading the cells of the capped brood. Grooming may have an important role in the maintenance of low infestation rates (Junkes *et al.* 2007).

Figs. 7 (hygienic behavior with total reproduction rate), 8 (infestation with total reproduction rate), 9 (infestation with total reproduction rate), 10 (invasion with total reproduction rate) and 11 (total reproduction rate with the mite effective reproduction rate) show that phenotypic and genetic correlation with a high probability. In fact, these are asymmetrical figures displaced from zero rates. Figs. 4 and 7 show the highest frequencies of less than zero rates and represent great probability that genetic correlations exist and are negative.

Hygienic behavior may be antagonistic to total reproduction rate of the mites (Fig. 7). This fact may be explained by the characteristics'

independence. In fact, the mite may reproduce itself regardless of the colonies' efficient hygienic behavior. Conversely, the mite may be in the colonies without reproducing itself (Fig. 8).

However, if the infestation rate increases, the mite effective reproduction rate may increase too (Fig. 9). This fact may occur since mite reproduction rates may increase owing to age with a possible introduction of a new recently occurring haplotype of the mite in Brazil. The reproductive capacity of the mite may be thus increasing (Carneiro *et al.* 2007). Increasing the invasion rate might also increase the total reproduction rate of the mite (Fig. 10).

Fig. 11 shows that there may be a correlation among reproduction rates of the mite when above zero rates occur. This is due to the fact that rates are mutually dependent.

Since criteria for selection should be chosen so that selected honeybees do not lose their adaptive characteristics, such as their relative resistance to diseases (Toledo & Mouro 2005), a higher negative correlation for hygienic behavior with total reproduction rate, as that found in the current research, should be taken into account and analyzed with great care. Actually, hygienic behavior associated with all the other traits had low correlation and all traits associated with total reproduction rates had significant correlation. This may occur because hygienic behavior simulates problematic brood and total reproduction rate might be a highly precise measurement since the pupae analyzed for this rate (brown eyed pupae) indicate the precise phase of the mite reproduction.

It should be also emphasized that the infestation rate of the mite *Varroa destructor* in Brazil is relatively low. The rate interferes with the mite reproduction rate since when no infestation occurs, no reproduction rate exists.

However, at low infestation rates the hygienic behavior trait is still the most recommended selection criterion. Selections for the above trait would solve the reproduction problem of the mite *Varroa destructor* in the context of brood diseases.

When high infestation rates of the mite exist, the most important criterion is hygienic behavior. In fact, it presents high level heritability and negative correlation with total reproduction rate of the mite *Varroa destructor*.

ACKNOWLEDGMENTS

The authors would like to thank the National Research Council (CNPq, process no. 301943/2005-2 and Araucaria Foundation 37/2005 4025 for financial support.

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APPENDIX

Figures 2-11 begin on page 19

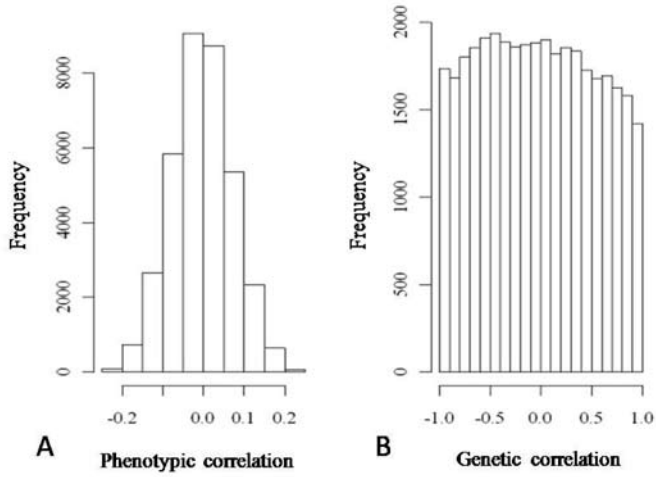

Fig. 2. Phenotypic (A) and genetic (B) correlation of hygienic behavior with infestation rate of the mite *Varroa destructor*

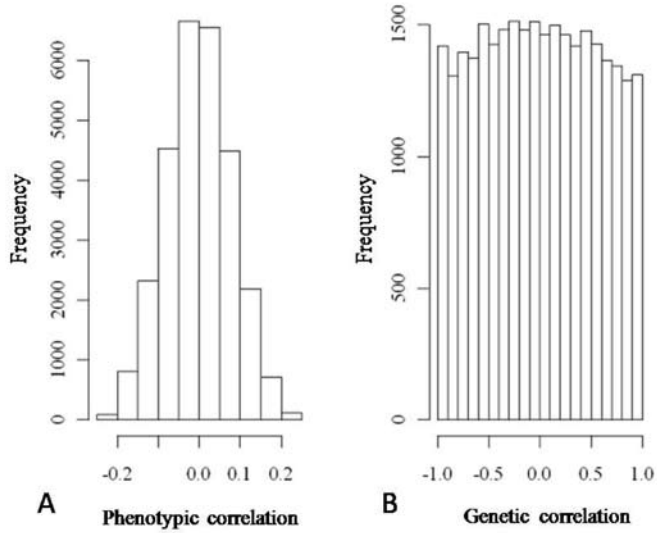

Fig. 3. Phenotypic (A) and genetic (B) correlation of hygienic behavior with invasion rate of the mite *Varroa destructor*.

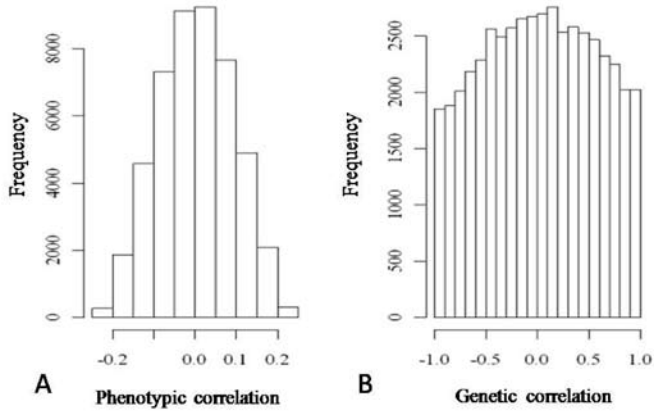

Fig. 4. Phenotypic (A) and genetic (B) correlation of hygienic behavior with effective reproduction rate of the mite *Varroa destructor.*

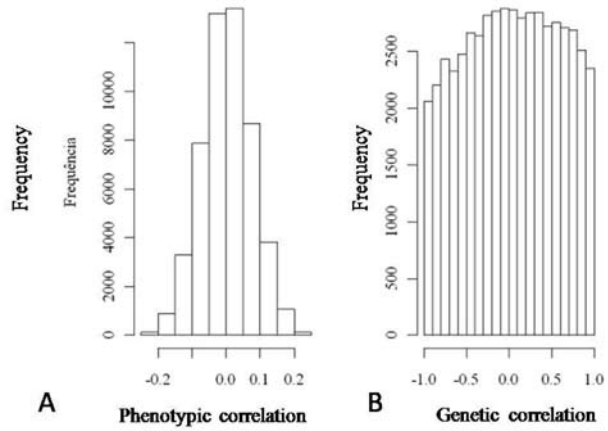

Fig. 5. Phenotypic (A) and genetic (B) correlation of infestation rate and invasion rate of the mite *Varroa destructor.*

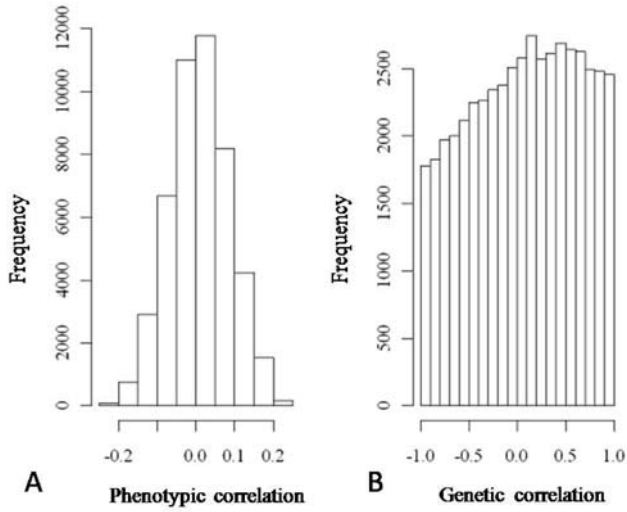

Fig. 6. Phenotypic (A) and genetic (B) correlation of invasion rate and effective reproduction rate of the mite *Varroa destructor*.

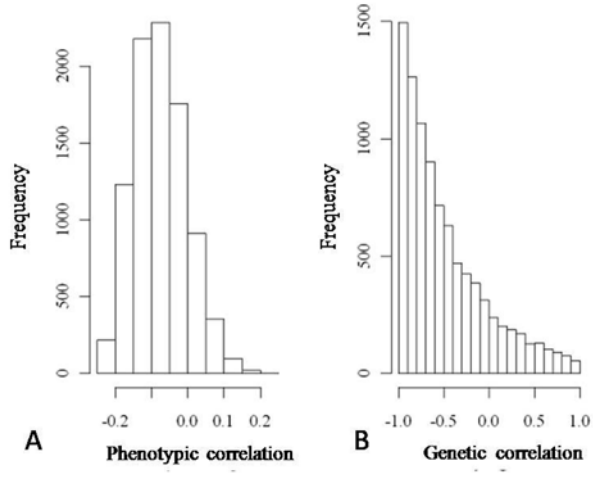

Fig. 7. Phenotypic (A) and genetic (B) correlation of hygienic behavior with total reproduction rate of the mite *Varroa destructor*.

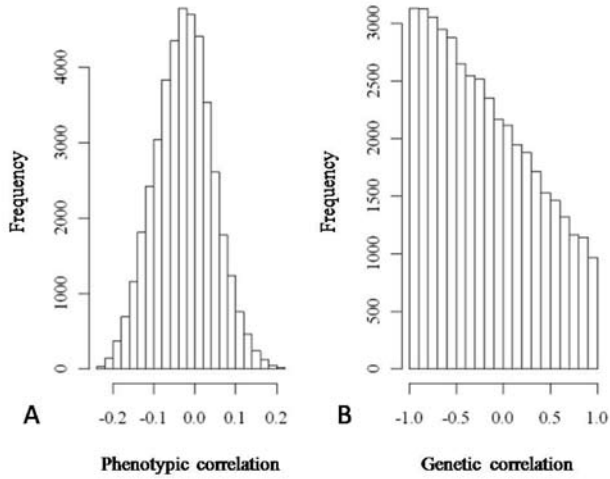

Fig. 8. Phenotypic (A) and genetic (B) correlation of infestation rate with total reproduction rate of mite *Varroa destructor*.

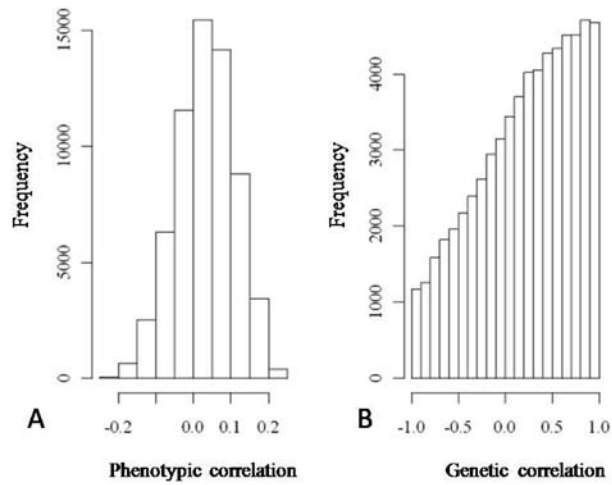

Fig. 9. Phenotypic (A) and genetic (B) correlation of infestation rate with effective reproduction rate of the mite *Varroa destructor*.

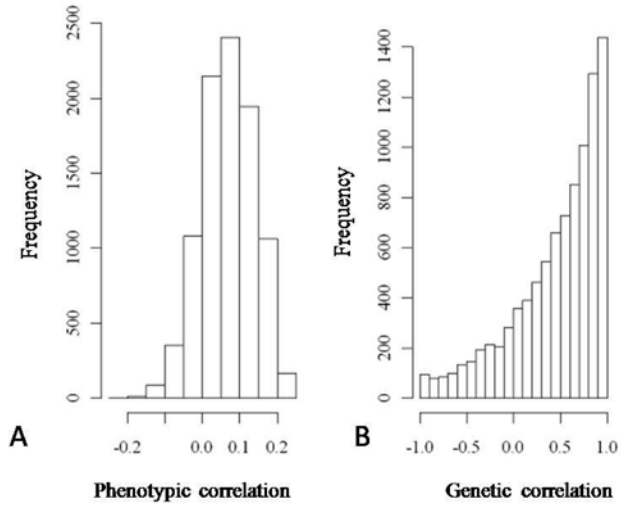

Fig. 10. Phenotypic (A) and genetic (B) correlation of invasion rate with total reproduction rate of the mite *Varroa destructor*.

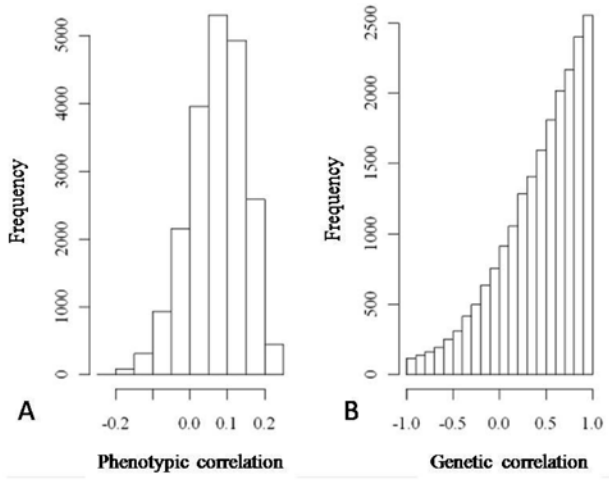

Fig. 11. Phenotypic (A) and genetic (B) correlation of total reproduction rate with effective reproduction rate of the mite *Varroa destructor*.