

Genetic variability of behavior in *Melipona quadrifasciata* (Hymenoptera: Meliponinae)

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ABSTRACT

Observation colonies containing only young workers from 10 matrix colonies were set up to investigate the genetic aspects involved in task division in *Melipona quadrifasciata*. Wide variation among origins was observed for all behaviors analyzed, but these differences were significant only for brood cell construction and propolis preparation.

INTRODUCTION

Among bees, the most complex social organization is found in the subfamilies Apinae (honey bees) and Meliponinae (indigenous bees) (Michener, 1974).

Each *Apis* queen mates with as many as 17 males (for a review, see Page, 1986), generating considerably high intracolony genetic variability. In these colonies, individuals belonging to the same subfamily (sharing the same parents) have a mean kinship coefficient of 0.75. In contrast, individuals produced by the same mother but by different fathers belong to different subfamilies and have an approximate kinship coefficient of 0.25 (Page and Laidlaw, 1988).

Genetic differences in the performance of activities such as guarding the nest (Robinson and Page, 1988), brood and queen care (Page *et al.*, 1992; Robinson *et al.*, 1994), ventilation (Kolmes *et al.*, 1989), body grooming (Frumhoff and Baker, 1988; Kolmes, 1989; Kolmes *et al.*, 1989) and swarming to a new site (Robinson and Page, 1988, 1989) have been demonstrated in *Apis mellifera*. Genetic differences have also been observed in the propensity of a given subfamily to forage for nectar or pollen (Calderone and Page, 1988; Calderone *et al.*, 1989; Robinson and Page, 1989; Oldroyd *et al.*, 1991a), to forage on different plant

species (Oldroyd *et al.*, 1992b), to opt for different sugar concentrations (Oldroyd *et al.*, 1991b) or to forage at different distances from their colonies (Oldroyd *et al.*, 1993). Genetic differences in trophallaxis, ovipositing, oophagia and care of male larvae have also been observed in queenless colonies (Hilleshein *et al.*, 1989; Moritz and Hilleshein, 1985; Robinson *et al.*, 1990).

The genetic differences existing among the workers of an *Apis* colony determined by multiple queen mating may be one of the factors responsible for the presence of individuals that perform some tasks earlier than others, i.e., individuals that present a lower sensitivity threshold for these tasks (Calderone and Page, 1988; Frumhoff and Baker, 1988; Robinson and Page, 1988).

Using molecular DNA fingerprinting, Oldroyd *et al.* (1994b) confirmed task specialization in *Apis florea*. They collected samples of workers that were performing a given task (foraging for nectar or pollen, guarding the nest, colony defense and ventilation). The DNA analysis of these samples showed differences between the behavioral classes, indicating the existence of genetic components in task division in this species.

The Meliponini species also show temporal polyethism and the first works on this subject were made by Bassindale (1955), Kerr and Santos-Neto (1956), Hebling *et al.* (1964) and Darchen (1969).

Since a Meliponine queen mates with a single male, an intracolony patrilineal effect on the tendency to present specific behavioral patterns is not expected to occur (Kerr *et al.*, 1962). All the individuals in the colony are produced by the same mother and father, and therefore share on average 75% of their genes (Hamilton, 1964). The genetic aspects related to the behavior of these bees are still little known and the objective of the present study was to determine whether there is genetic variability in some behavioral aspects between different colonies of *Melipona quadrifasciata*.

MATERIAL AND METHODS

The experiment was carried out at the Federal University of Viçosa (UFV) with *M. quadrifasciata* colonies obtained from the regions of Venda Nova, ES, Viçosa, MG, Brazil, and from the stock of the UFV apiary (colonies of various origins maintained at the apiary for several years). The first observation colony was set up on February 25, 1994, and the second and third on March 1 and March 6, 1995, respectively.

Brood combs were collected from the original colonies and placed in Petri dishes kept in an incubator at 29°C. As they emerged, the bees were marked with colored labels, numbered from 1 to 99 and placed in three observation colonies (I, II and III) located close to one another. When the bees were placed in the observation colony their age ranged from one to five days after emergence.

The colonies contained the marked bees, a small amount of wax, a physogastric queen that had no kinship with the introduced bees, and three artificial pots of *Apis* wax, two of them containing honey and one pollen. A 20-cm long plastic tube 1.5 cm in diameter permitted the bees to communicate with the external environment. Table I shows the composition of each colony. Despite the attempts made, it was not possible to place an identical number of individuals in the three replications.

The data were obtained by direct observation during a period of 90 min in the morning (9:00 to 10:30 a.m.) and 90 min in the afternoon (2:30 to 4:00 p.m.). Each colony was observed for a period not exceeding 10 min so that light would not interfere excessively with the behavior of the bees. A lamp with an incandescent bulb was used to aid observation. During each observation period we recorded the number of the bee, the time and day and the activity that was being performed, with care taken not to sample the same individual in a given activity during the same observation period. The observations were always started at the comb region

and continued towards the pots and waste disposal site. The entrance was always observed separately.

The population of each observation colony comprehended about 50% of the population of the normal colony of *M. quadrifasciata*, which has 300 to 400 bees (Nogueira-Neto, 1970).

Each colony replication will be mentioned in the text as colony I, II or III. The bees from the 10 original colonies will be referred to as bees of origin 1, origin 2, etc.

The following activities were observed as recommended by Sommeijer (1984) and Kolmes and Sommeijer (1992): brood cell construction (CC), food pot construction (PC), propolis preparation (PRO), waste disposal (WD), work at the entrance (WE), foraging for nectar (N), foraging for resin (RES), standing (S), and walking (W).

The observations were made for 35 days; for statistical analysis, this time was divided into four periods: IP (1-8 days), IIP (9-18 days), IIIP (19-27 days), and IVP (28-35 days). This division facilitates the data analysis and is also related to the beginning of some activities in each colony, such as building and foraging.

In view of the mortality and variation in number of bees observed during each sampling, statistical analysis was applied to the proportion of individuals performing a given activity. The proportions were calculated using the following formula:

$$P = \frac{nA_{ij}}{N_{ij}}$$

where: i = bee origin; j = colony; nA = number of individuals engaged in activity A; N = total number of individuals.

Table I - Number of labelled individuals of each origin in each colony replication.

Origin	Colonies		
	I	II	III
1	27	19	32
2	28	37	23
3	17	16	22
4	16	12	32
5	25	27	10
6	33	28	19
7	22	22	18
8	26	6	10
9	16	23	31
10	28	24	16
Total	238	214	213

To compare the performance of each origin in the colonies, the colonies were ordered according to the proportion of individuals involved in a given activity. The Friedman test (according to Snedecor and Cochran, 1980) was applied to test the null hypothesis of the absence of origins more involved in a given activity, considering the observation colonies as replications.

RESULTS AND DISCUSSION

Although the colonies consisted only of workers of the same age, the bees carried out all the activities usually performed by the workers in a normal colony consisting of individuals of varying ages. Because of the absence of individuals of other ages, the workers continued to perform practically all the activities up to the last day of observation (35th day), regardless of age.

The data presented in Table II show that the data referring to the behaviors analyzed are consistent with the hypothesis of genetic differences among the various origins only with respect to brood cell construction during periods II, III and IV and with respect to propolis preparation during period IV. In other words, some origins were outstanding in the performance of these activities during these periods. Since an *M. quadrifasciata* queen mates with only one male, intra-colony variation is very low, a fact that prevents the appearance of subfamilies specialized in certain tasks, as observed in *Apis* (Oldroyd *et al.*, 1994a).

The lack of differences during period I may have been due to the initial disorientation of the bees provoked by the absence of older individuals in the colony. The bees stood still or walked through the hive for long periods of time, and their first activity was the construction of the casing and pilasters.

Figure 1 shows that the engagement of workers of different origins in brood cell construction varied widely during the different periods. Origin 4 during period II and origin 1 during periods III and IV presented the highest proportions of work in the three colonies. Origin 1 reached approximately 43% activity in colony II during the third period (19 to 27 days) and approximately 37% in colony I during the last period (28 to 35 days).

Kolmes and Sommeijer (1992), working with *Melipona favosa*, found evidence that the construction of brood cells is a task performed by an "elite" group

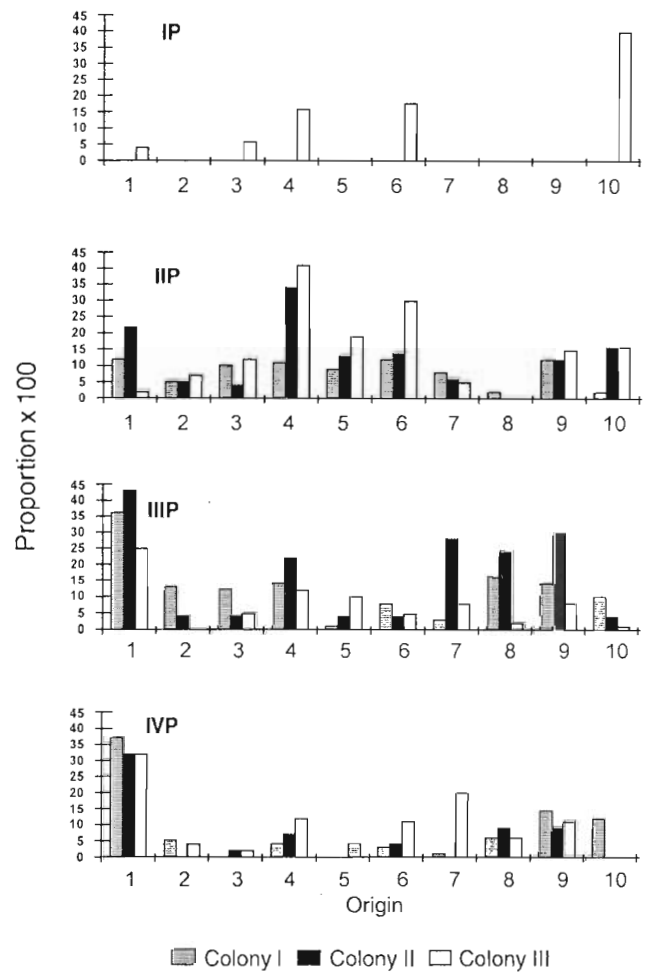


Figure 1 - Proportion of brood cell building activity presented by observation colonies of different origins initiated with only young bees (1-5 days of life after emergence from the cocoon) at different times: IP (1-8 days old), IIP (9-19 days old), IIIP (19-27 days old) and IVP (28-35 days old). All observations were made during a 35-day sample period.

Table II - Results of the Friedman test of behavioral variability in colonies containing only young bees (1 to 5 days of life after emergence from the cocoon). (FRIED is the statistical parameter of the Friedman test, and P is the associated probability).

Activities	Periods							
	I		II		III		IV	
	FRIED	P	FRIED	P	FRIED	P	FRIED	P
CC	2.6	0.977	21.2	0.012*	16.7	0.054*	16.8	0.052*
PC	4.9	0.845	14.4	0.109	7.6	0.569	4.9	0.844
WD	2.4	0.772	14.3	0.111	9.7	0.383	9.6	0.381
W	7.8	0.242	11.9	0.216	15.6	0.074	11.5	0.242
S	4.7	0.866	9.8	0.365	11.9	0.220	10.9	0.281
PRO	-	-	5.6	0.777	13.9	0.126	17.7	0.039*
WE	-	-	2.0	0.992	9.6	0.385	8.7	0.461
RES	-	-	-	-	5.5	0.790	8.8	0.459
N	-	-	-	-	9.5	0.392	12.2	0.204

CC = Brood cell construction, PC = pot construction, WD = waste disposal, W = walk, S = stand, PRO = propolis preparation, WE = work at the entrance, RES = foraging for resin, N = foraging for nectar.

*Significant and marginally significant values at P = 0.05.

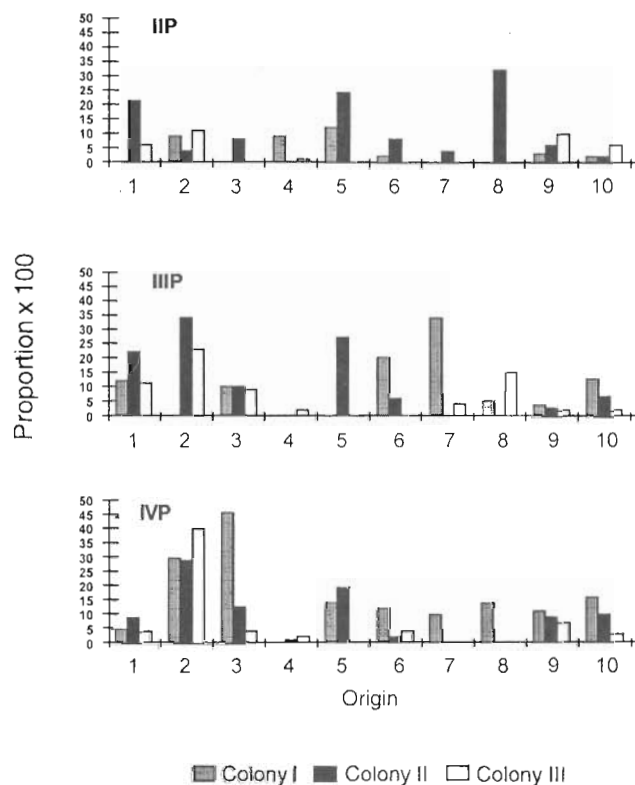


Figure 2 - Proportion of propolis production activity presented by observation colonies of different origins containing only young bees (1-5 days of life after emergence from the cocoon) at different times: IIP (9-18 days old), IIIP (19-27 days old) and IVP (28-35 days old). All observations were made during a 35-day sample period.

of workers. According to Page and Robinson (1991), genotypic differences in the response threshold are one of the factors that might explain the occurrence of "elite" workers. Thus, workers of certain subfamilies in *Apis* colonies may present a lower sensitivity threshold for some tasks compared to others. In this experiment the bees of origins 1 and 4 may have a genotypic constitution that causes them to present more sensitivity, responding more promptly to the stimuli for brood cell construction.

Although the activity of propolis preparation started during the second period, the bees started to forage and consequently to collect resin only after 20 days of life. During period IV, this behavior agreed with the hypothesis of genetic differences among the different origins (Table II). Possibly, the origins presenting genetic predisposition for this activity were outstanding during this period. The bees of origin 2 presented a high proportion of work with propolis in the three colonies during this period (Figure 2). No studies are available about honeybees to determine if this behavior suffers a genetic influence, and further studies should be performed in *Meliponines*.

Although differences in the proportion of bees of each origin were observed in the execution of tasks

such as construction of food pots, waste disposal, work at the entrance, foraging for nectar and resin, standing still and walking, these differences were nonsignificant at the 5% level of probability by the Friedman test. Studies on *Apis* have demonstrated that cleaning behavior involving the removal of dead bees from the colony (these bees are directly transported outside the colony and not placed in the garbage container as is the case for *Meliponines*) (Robinson and Page, 1988, 1989; Rothenbuhler, 1964), and foraging for nectar are behaviors that suffer genetic influence (Calderone and Page, 1988; Robinson and Page, 1988, 1989; Calderone et al., 1989; Oldroyd et al., 1991a).

The differences between colonies in the day when the workers started their activities were probably related to general colony conditions, such as the number of individuals present. Mortality was higher in colony 2, followed in decreasing order by colony 3 and colony 1.

Considering the papers on temporal polyethism in *Meliponids*, as well as the results obtained in this work, we can infer that in this group of bees aspects as the age together with the necessities of the colony are factors that critically influence the behavior of these bees.

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RESUMO

Para o estudo dos aspectos genéticos envolvidos na divisão de trabalho em *Melipona quadrifasciata* foram construídas colônias de observação contendo apenas operárias jovens originadas de 10 colônias. Em todos comportamentos analisados houve uma grande variação entre as origens, mas somente os comportamentos de construção de células de cria e propolição apresentaram diferenças significativas entre as origens.

REFERENCES

- Bassindale, R. (1955). The biology of the stingless bee *Trigona* (*Hypotrigona*) *gribodoi* Magretti (*Meliponidae*). *Proc. Zool. Soc. Lond.* 125: 49-62.
- Calderone, N.W. and Page, R.E. (1988). Genotypic variability in age polyethism and task specialization in the honey bee, *Apis mellifera* (Hymenoptera: Apidae). *Behav. Ecol. Sociobiol.* 22: 17-25.

- Calderone, N.W., Robinson, G.E. and Page, R.E. (1989). Genetic structure and division of labor in honey bee societies. *Experientia* 45: 765-767.
- Darchen, R. (1969). Sur la biologie de *Trigona (Apotrigona) nebulata*. *Biol. Gabonica* 3: 151-183.
- Frumhoff, P.C. and Baker, J. (1988). A genetic component to division of labour within honey bee colonies. *Nature* 333: 358-361.
- Hamilton, W.D. (1964). The genetical evolution of social behaviour. *J. Theor. Biol.* 7: 1-52.
- Hebling, N.J., Kerr, W.E. and Kerr, F. (1964). Divisão de trabalho entre operárias de *Trigona xanthotricha* Moure. *Pap. Avulsos Zool.* 16: 115-127.
- Hillesheim, E., Koeninger, N. and Moritz, R.F.A. (1989). Colony performance in honeybees (*Apis mellifera capensis* Esch.) depends on the proportion of subordinate and dominant workers. *Behav. Ecol. Sociobiol.* 24: 291-296.
- Kerr, W.E. and Santos-Neto, G.R. (1956). Contribuição para o conhecimento da bionomia dos Meliponini V: Divisão de trabalho entre operárias de *Melipona quadrifasciata* Lep. *Insectes Soc.* 3: 423-430.
- Kerr, W.E., Zucchi, R., Nakadaira, J.T. and Bertolo, J.E. (1962). Reproduction in social bees (Hymenoptera: Apidae). *J. New York Entomol. Soc.* 70: 256-276.
- Kolmes, S.A. (1989). Grooming specialists among worker honey bees *Apis mellifera*. *Anim. Behav.* 37: 1048-1049.
- Kolmes, S.A. and Sommeijer, M.J. (1992). Ergonomics in stingless bees; changes in intranidal behavior after partial removal of storage pots and honey in *Melipona quadrifasciata* (Hym. Apidae, Meliponinae). *Insectes Soc.* 39: 215-232.
- Kolmes, S.A., Winston, M.L. and Fergusson, L.A. (1989). The division of labour among worker honey bees (Hymenoptera: Apidae): the effects of multiple patrillines. *J. Kans. Entomol. Soc.* 62: 80-95.
- Michener, C.D. (1974). *The Social Behavior of the Bees; a Comparative Study*. Belknap/Harvard Univ. Press, Cambridge, pp. 404.
- Moritz, R.F.A. and Hillesheim, E. (1985). Inheritance of dominance in honeybees (*Apis mellifera capensis* Esch.). *Behav. Ecol. Sociobiol.* 17: 87-89.
- Nogueira-Neto, P. (1970). *A criação de Abelhas Indígenas Sem Ferrão*. Ed. Chácaras e Quintais, São Paulo, pp. 365.
- Oldroyd, B.P., Rinderer, T.E. and Buco, S.M. (1991a). Honey bees dance with their super sisters. *Anim. Behav.* 42: 121-129.
- Oldroyd, B.P., Rinderer, T.E. and Buco, S.M. (1991b). Intra-colonial variance in honey bee foraging behaviour: the effects of sucrose concentration. *J. Apic. Res.* 30: 137-145.
- Oldroyd, B.P., Rinderer, T.E., Harbo, J.R. and Buco, S.M. (1992b). Effects of intracolony genetic diversity on honey bee (Hymenoptera: Apidae) colony performance. *Ann. Entomol. Soc. Am.* 85: 335-343.
- Oldroyd, B.P., Rinderer, T.E., Buco, S.M. and Beaman, L.D. (1993). Genetic variance in foraging bees for preferred foraging distance. *Anim. Behav.* 45: 323-332.
- Oldroyd, B.P., Rinderer, T.E., Schewenke, J.R. and Buco, S.M. (1994a). Subfamily recognition and task specialization in honey bees (*Apis mellifera* L.) (Hymenoptera: Apidae). *Behav. Ecol. Sociobiol.* 34: 169-173.
- Oldroyd, B.P., Sylvester, H.A., Wongsiri, S. and Rinderer, T.E. (1994b). Task specialization in a wild bee, *Apis florea* (Hymenoptera: Apidae), revealed by RFLP banding. *Behav. Ecol. Sociobiol.* 34: 25-30.
- Page, R.E. (1986). Sperm utilization in social insects. *Annu. Rev. Entomol.* 31: 297-320.
- Page, R.E. and Laidlaw, H.H. (1988). Full sisters and super sisters: a terminological paradigm. *Anim. Behav.* 36: 944-945.
- Page, R.E. and Robinson, G.E. (1991). The genetics of division of labour in honey bee colonies. *Adv. Insect Physiol.* 23: 118-168.
- Page, R.E., Robinson, G.E. and Fondrk, M.K. (1992). Genotypic variability for rates of behavioral development in worker honey bee (*Apis mellifera* L.). *Behav. Ecol.* 3: 173-180.
- Robinson, G.E. and Page, R.E. (1988). Genetic determination of guarding and undertaking in honey bee colonies. *Nature* 333: 356-358.
- Robinson, G.E. and Page, R.E. (1989). Genetic determination of nectar foraging, pollen foraging, and nest-site scouting in honey bee colonies. *Behav. Ecol. Sociobiol.* 24: 317-323.
- Robinson, G.E., Page, R.E. and Fondrk, M.K. (1990). Intracolony behavioral variation in worker oviposition, oophagy, and larval care in queenless honey bee colonies. *Behav. Ecol. Sociobiol.* 26: 315-323.
- Robinson, G.E., Page, R.E. and Arensen, N. (1994). Genotypic differences in brood rearing in honey bee colonies: context-specific? *Behav. Ecol. Sociobiol.* 34: 125-137.
- Rothenbuhler, W.C. (1964). Behavioral genetics of nest cleaning in honey bees. IV. Responses of F1 and backcross generation to disease-killed brood. *Am. Zool.* 4: 111-123.
- Snedecor, G.W. and Cochran, W.G. (1980). *Statistical Methods*. The Iowa State University Press, Iowa, pp. 507.
- Sommeijer, M.J. (1984). Distribution of labor among workers of *Melipona favosa* f.; age polyethism and worker oviposition. *Insectes Soc.* 31: 171-184.

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