

Proportion of males sons-of-the-queen and sons-of-workers in *Plebeia droryana* (Hymenoptera, Apidae) estimated from data of an MDH isozymic polymorphic system

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Abstract

Stingless bees from 14 hives of *Plebeia droryana* were analysed for the MDH isozymic polymorphic system, which is controlled by four alleles, *MDH1-1*, *MDH1-2*, *MDH1-3* and *MDH1-4*. The hives came from four different localities in Brazil and at least 15 drones were tested from each one, to estimate the proportion of them that are sons of the queen or of workers; the obtained values were 83.8% (range 100% to 43%) and 16.2% (range 0% to 57%), respectively. It is suggested that male-producing workers evolved from the need to preserve xo -heteroalleles.

Introduction

In the genus *Plebeia* the brood cells are of equal size for workers and males, those producing queens are much larger. *Apis* queens are fertilized by several drones during their mating flight (Taber, 1954; Woyke, 1955, 1960; Kerr & Bueno, 1970; Roberts, 1974; Adams *et al.*, 1977) while *Melipona* queens are fertilized by a single drone (Kerr & Krause, 1950; Kerr, 1969; Silva *et al.*, 1972; Contel & Kerr, 1976). In *Apis* all males are sons of the queen when one is present, i.e., workers lay eggs that give rise to males only in queenless hives, whereas in *Melipona* and *Scaptotrigona* male production is shared by queens and workers (Beig, 1969; Tambasco, 1971; Silva, 1974). These conclusions were based on direct observation. Mechanisms for the transmission of some kind of inhibitory substance possibly secreted by the queen are still unknown in Meliponini, whereas queen interference with workers' egg-laying has been demonstrated in the genus *Apis* (Butler, 1967).

Sakagami *et al.* (1963) elaborated an hypothesis for the explanation of the various patterns of ovarian development in 16 Meliponini species and an

intermediate percentage of workers with developed ovaries was found in *Plebeia* (*Plebeia*) *droryana*; Terada (1980) elucidated some aspects of the regulation of ovarian development in adult workers in this species. According to Terada (1980), when the two types of ovules are considered (nutrient and functional), the majority of ovules found in workers in a hive when the queen is present are of the nutrient type since the queen acts as an inhibitor of oocyte growth; in queenless colonies, ovarian development becomes activated in workers. Even in cases where a certain number of workers' ovules (generally of the nutrient type) are present in hives with queens, a significant increase in ovule number occurs when the queen is removed from the hive, with an initial increase in the frequency of nutrient ovules, followed by the development of functional ovules that will give rise to males. Terada (1980) reported that, in queenless hives, 37.63% of 5 400 nurse workers had ovaries of the type described above, and the presence of males was influenced by the percentage of functional ovules. This indicates that *droryana* workers contribute maximally 37.6% to the eggs that give rise to males.

The present study is an attempt to check the

proportion of males produced by workers in *P. (P) droryana* using the genetical method previously utilized by Contel and Kerr (1976), and to compare the results with the data obtained by Terada (1980) on the basis of direct observation and of the patterns of development of workers ovaries.

Material and methods

Populations studied

Four populations of *Plebeia droryana* were investigated. The samples came from 4 different locations in Brazil: 28 hives from Pocinhos do Rio Verde (State of Minas Gerais), 10 from Prudentópolis (State of Paraná), 6 from Viçosa (State of Minas Gerais), and 5 from Ribeirão Preto (State of São Paulo). At least 15 males per hive were studied, including pupae and adult insects.

Electrophoretic analysis

Pupae and adult *droryana* samples were homogenized in 0.25 and 0.35 ml of twice-distilled water, respectively, using a glass stick and a test tube, and centrifuged at room temperature in an International centrifuge (Model K, size 2) with a no. 250 rotor, for 10 min at 3000 rpm. The clear supernatant thus obtained was used as sample. Samples were immediately absorbed with Whatman No. 3 paper (5 × 6 mm) and inserted in vertical slots in the gel.

An 11% starch gel (Sigma) in 0.02 M Tris-HCl buffer, pH 8.6, and 0.3 M Tris-HCl buffer, pH 8.6 in the cuvettes with the electrodes were used. Electrophoresis was carried out for 6 h, at 110 V measured in the gel, and at low temperature (approximately 10 °C). Common filter paper folded four times was used for the bridges between the gel and the electrode compartments. The gel was then cut lengthwise into two homologous parts, one of which was stained for NAD-dependent MDH. The isoenzymes were developed by incubating the gel at 37 °C in the dark with another gel prepared with the following reaction mixture: 210 mg sodium L-malate; 6 mg NAD; 15 ml 0.1 M Tris-HCl buffer, pH 8.6; 0.6 ml MTT solution (5 mg/ml of twice-distilled water); 0.6 ml PMS solution (5 mg/ml twice-distilled water) and 15 ml 2% agar in twice-distilled water. After 20 min incubation, dark blue bands

were visible, indicating malate-dehydrogenase activity.

Genetic system

The individual variations observed in the 4 *Plebeia droryana* populations investigated suggested the presence of 4 alleles located in a single locus (*MDH1-1*, *MDH1-2*, *MDH1-3* and *MDH1-4*). The polymorphism of this system has been described in detail by Machado (1982).

Determination of the origin of males

The proportion of males produced by the queen and by the workers was estimated according to Contel & Kerr (1976), that is, only crosses in which the genotypes of the queen and of the male that fertilized her are known to be different, and that produced 12 or more male descendants, were considered.

Samples of males with *MDH1-1*, *MDH1-2* and *MDH1-3* genotypes were obtained and three types of crosses were made:

- (1) $AA \times B$ – the queen is homozygous for one of these three alleles, and crossed with a male differing from her in genotype: in this case the proportion r of males produced by the queen equals $(A-B)/N$ and the proportion $op = 1-r$ of males produced by workers descending from this cross equals $2B/N$. (A and B are the numbers of males observed to have A and B genotype, respectively, and N is the total number of males investigated).
- (2) $AB \times A$ – the queen is heterozygous and crossed with a male whose genotype has one of the alleles of the queen. In this case $r = (3B-A)/N$ and $op = (2A-B)/N$.
- (3) $AB \times C$ – the queen is heterozygous and crossed with a male that has no allele in common with her. In this case $r = (A + B - C)/N$ and $op = 2C/N$.

Results

The genotypes of the queens and of the males that inseminated them, mentioned in Tables 1 and 3, were inferred from the worker segregation (Table 2) and drone segregation (Table 1) obtained.

Table 1. Crosses analyzed to demonstrate the inheritance of the MDH-NAD dependent variants in drone pupae descendant from 4 populations of *Plebeia droryana*.

Locality	Number of the hive	Type of cross ♀	Drones obtained				Total
			♂	MDHI-1	MDHI-2	MDHI-3	
Pocinhos do Rio Verde (MG)	236	MDHI-3/MDHI-3 × MDHI-2				24	24
	06				34		34
	137	MDHI-2/MDHI-3 × MDHI-2		13	8		21
	50			9	6		15
	01	MDHI-3/MDHI-3 × MDHI-4				16	16
Prudentópolis (PR)	5P	MDHI-3/MDHI-1 × MDHI-3	15			10	25
	9P					17	17
	1P	MDHI-3/MDHI-3 × MDHI-1				19	19
	6P					24	24
	13P	MDHI-1/MDHI-1 × MDHI-3	17				17
Viçosa (MG)	26M	MDHI-2/MDHI-3 × MDHI-3		7	8		15
	2M			12	11		23
Ribeirão Preto (SP)	Z	MDHI-3/MDHI-3 × MDHI-2				16	16
	CM	MDHI-2/MDHI-3 × MDHI-2		14	11		25
Total			32	55	204	0	291

Table 2. Segregation among workers of the 14 crosses studied in order to infer the queen and mating male genotypes.

Number of hive	Workers obtained					Total
	MDHI-2/MDHI-2	MDHI-3/MDHI-3	MDHI-1/MDHI-3	MDHI-2/MDHI-3	MDHI-3/MDHI-4	
236				89		89
06				48		48
137	41			47		88
50	8			3		11
01					11	11
5P		7	10			17
9P		3	5			8
1P			9			9
6P			19			19
13P			8			8
26M		6		4		10
2M		12		7		19
Z				11		11
CM	13			16		29
Total	62	28	51	225	11	377

Figure 1 shows the three phenotypes of the male pupae sampled, and 4 of the 10 theoretically expected phenotypes for female pupae in terms of this system. The data presented in Table 3 were obtained by applying the formulas described and show that in *P. droryana* both queens and workers lay eggs that develop into males.

Of the five hives from Pocinhos do Rio Verde, three showed the type-1 cross, two the type-2 cross, and 88.2% of the males in this population were produced by the queen, 17.8% by the workers. The same proportion of crosses was observed in the Prudentópolis population, with 77.2% of the males produced by the queen and 22.8% by the workers.

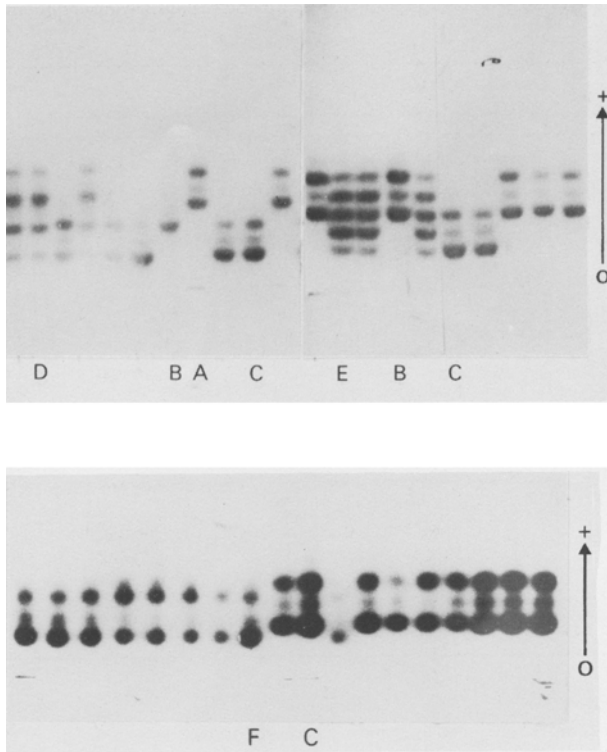


Fig. 1. Starch gels showing variation in the MDH-NAD dependent system in worker pupae of *Plebeia (Plebeia) droryana*. The phenotypes correspond to the following genotypes: (A): *MDH1-1/MDH1-1*; (B): *MDH1-2/MDH1-2*; (C): *MDH1-3/MDH1-3*; (D): *MDH1-1/MDH1-3*; (E): *MDH1-2/MDH1-3* and (F): *MDH1-3/MDH1-4*. The arrows indicate the direction of migration.

Only 2 hives each were studied in the populations from Ribeirão Preto and Viçosa. Male offspring of type 1 and 2 crosses were observed in the Ribeirão Preto population, with 88% of the males produced by the queen and 12% by the workers, whereas only the type-2 cross was observed in the Viçosa population, with 100% of the males being produced by the queen. When all 14 *droryana* hives are considered, 7 hives showed a type-1 cross and 7, type 2.

The results taken together indicate that in *droryana* 83.8% of the males are produced by the queen, and 16.2% are produced by the workers (Table 3).

In all crosses in which a homozygous queen was crossed with a male of a different genotype (type-1), in our sample 100% of male offspring was produced by the queen.

Discussion and conclusions

The results indicate that in the *P. droryana* species 83.8% of the males are produced by the queen and only 16.2% by the workers. These data agree with those reported by Terada (1980) on the basis of direct observation and of the developmental patterns of workers ovaries, which indicate that workers have a much lower rate of production of eggs that give rise to males than the queen has. According to Terada (1980), the production of males in *P. droryana* is associated with hive conditions and time of the year, occurring when the hive has a high populational density and the queen's egg-laying process is activated.

Four out of fourteen colonies had workers which produced males. Therefore, the probability of having, at this moment, a colony with male-producing workers was 4/14 and the probability of being in a period of no male-producing workers was 10/14. The probability of having six hives with homozygous queens but workers not in the phase of producing males is $(10/14)^6$ or 0.13, which means that this is not an altogether improbable event.

The production of sexual individuals (males and queens) occurs exactly at the time when the hive is heavily populated and when the queen is actively laying eggs, and our samples were collected at this phase, that is, the estimate of the proportion of males produced by the queen and by workers was made in the presence of the queen.

Contel and Kerr (1976) using esterase alleles to estimate the proportion of males produced by the queen and by the workers in *Melipona subnitida* showed that workers contribute with an average of 38.8% to male production.

According to the data reported here, the production of males by workers is small, a fact that places *P. droryana* between species where workers have highly developed ovaries, such as *Scaptotrigona postica* (Sakagami *et al.*, 1963; Bego, 1977), and species whose workers do not develop ovaries whether the queen is present or absent, such as *Frieseomelitta varia* (Terada, 1974) and *Duckeola ghiliani* (Zucchi, 1977) with the reproductive caste limited to the queen of the hive.

These findings in *P. droryana* cannot be extended to other species of the genus *Plebeia*. According to Bego (1977) and Camillo-Atique (1977), male production in *Plebeia (Friesella) shrottkyi* is

Table 3. Estimated proportion of drones sons of the queen (r) and of the workers (op) in 4 populations of *Plebeia* (*Plebeia droryana*).

Locality	Type of cross	Type of cross		Number of hives	Type of descendants r		op	
		♀	♂		A	B		
Pocinhos do Rio Verde (MG)	Type 1	AA	× B	3	74	0	1.000	0.000
	Type 2	AB	× A	2	22	14	0.556	0.444
Average							0.822	0.178
Prudentópolis (PR)	Type 1	AA	× B	3	60	0	1.000	0.000
	Type 2	AB	× A	2	27	15	0.429	0.571
Average							0.772	0.228
Viçosa (MG)	Type 2	AB	× B	2	19	19	1.000	0.000
Average							1.000	0.000
Ribeirão Preto (SP)	Type 1	AA	× B	1	16	0	1.000	0.000
	Type 2	AB	× A	1	14	11	0.760	0.240
Average							0.880	0.120
Total	Type 1	AA	× B	7	160	0	1.000	0.000
	Type 2	AB	× A	5	63(1)	40(1)	0.553	0.447
	Type 2	AB	× B	2	19	19	1.000	0.000
Average							0.838	0.162

Type 1: $r = (A-B)/N$ $op = 2B/N$

Type 2: $r = (3B-A)/N$ $op = (A-B)/N$

(1) χ^2 for 1:1 = 5.14 (P = 0.02)

mostly accounted for by workers. The statement by Zucchi that egg-laying by workers in Meliponini varies between species is supported by the data obtained by Terada (1980), Bego (1977), Camillo-Atique (1977) and the present paper.

Similarly to the data obtained in *Melipona* (Kerr & Krause, 1950; Kerr, 1969; Silva *et al.*, 1972; Conzel & Kerr 1976), all the segregations obtained with *Plebeia droryana* allow us to agree with Kerr (1969) that queens are fertilized by a single male during their wedding flight. All species of stingless bees studied so far mate with only one drone. This has been checked by sperm counts (Kerr, pers. comm.) in three species. Also, the male hooks, sticking firmly to the female genitalia, take the queen one to five days to get rid of. Segregations of polymorphic genes studied in 20 species of meliponids never demonstrated mating by more than one male.

Crozier (1977) indicates that male production by workers is in fact somewhat maladaptive (from the population point of view); also, that it induces loss of variation and decreases effective population size. We think that the great selective agent favoring populations with male-producing workers is the

xo-heteroalleles. When a queen xo^1/xo^2 crossed to an xo^3 male is the only producer of males, the segregation for these sex hetero-alleles is $1/2 xo^1:1/2 xo^2$. In case workers also lay eggs the ideal proportion for greater diversity would be for $1/3$ of the males to be produced by the queen and $2/3$ by the workers since, then, the proportion of *xo*-heteroalleles would be: $1/3 xo^1 + 1/3 xo^2 + 1/3 xo^3$.

Different selective agents may act in different species since we found 0% sons of workers in the genera *Apis*, *Frieseomelitta* and *Duckeola*; 16% in *Plebeia*, 39% in *Melipona subnitida*, and 95% in *Scaptotrigona postica*. *Apis mellifera* found a way to control the defects of the *xo* heteroalleles by having up to 18 mates (Adams *et al.*, 1977; Page, 1980).

Possibly, an other factor favoring male-producing workers in meliponids would be the fact that the size of worker and drone cells is equal, which makes it more difficult for the queen to produce males. Since in meliponids the queen lays the egg a few seconds after the food poured into a brood cell reaches a level equal to or above a threshold, and immediately after this the cell is closed by a worker

pulling the collar and making the cap, there is no recognition by the workers of either male or worker larvae. Also, in large populations the selective advantage of laying workers diminishes.

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