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SEX DETERMINATION IN BEES. XXI. NUMBER OF XO-HETEROALLELES IN A NATURAL POPULATION OF *MELIPONA COMPRESSIPES FASCICULATA* (APIDAE)

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SUMMARY

Data are produced that allows estimating the number of xo-heteroalleles of *Melipona compressipes fasciculata* as being equal to 20.0 (s = 1.36) which does not differ significantly from *Apis mellifera* (n = 18.9). Emphasis is given to the fact that the lethal equivalents estimate for *Apis mellifera* (1.29 to 1.36) also does not differ from the one found for meliponids (1.08 to 1.14). These equalities are supposed to be due to similar evolutionary pressures that sex determination and eusocial life exercise upon the genetic parameters of the populations of these bee species with large *Ne*.

RESUME

Détermination du sexe chez les abeilles. XXI. Nombre de xo-hétéroallèles dans une population naturelle de Melipona compressipes fasciculata **(Apidae).**

Les résultats permettent d'estimer à 20.0 (s = 1.36) le nombre de xo-hétéroallèles de *Melipona compressipes fasciculata*, ce qui ne diffère pas significativement d'*Apis mellifera* (18.9). On doit remarquer le fait que les équivalents létaux estimés pour *Apis mellifera* (1.29 à 1.36) ne diffèrent pas de ceux trouvés pour les Meliponides (1.08 à 1.14). Ces données semblent démontrer que cette ressemblance est due aux pressions évolutives similaires provenant de la détermination du sexe et de la vie eusociale s'exerçant sur les paramètres génétiques des populations de ces abeilles avec un grand *Ne*.

INTRODUCTION

In *Apis mellifera* the number of xo-heteroalleles in a population has been studied by many authors (MACKENSEN, 1955 ; LAIDLAW *et al.*, 1956 ; KERR, 1975 ; WOYKE, 1976 ; ADAMS *et al.*, 1977). These estimates vary from 6 to 19 in this species.

Since 1981, work has been done with the bee *Melipona compressipes fasciculata* in order to evaluate how many xo-heteroalleles exist in a natural population of these bees.

MATERIAL

Colonies of *Melipona compressipes fasciculata* were obtained in the forest near Arari, Maranhão State, Brazil (around 3° 30' S and 44° 40' W).

METHOD

Instrumental insemination has not yet been developed for *Melipona compressipes*. But it was found that only one male mates with each queen (KERR, unpubl. data). Therefore the following methodology was applied. A new colony was made as follows (KERR, 1986) : 1) Provided a 30 × 30 × 30 cm hive with an entrance 2 cm in diameter and three 5 × 5 cm openings in the back for ventilation and, consequently, nectar dehydration ; this back openings remained closed and was opened only after the colony was well established (± 3 months) and in days of rain when the bees closed it with resin and mud in less than 24 hours. 2) One comb of emerging brood (150 to 300 cells) from a very strong colony was put on the floor of the new hive ; there was no need to look for queen cells since 3 % to 25 % of the emerging bees are queens. 3) A glass (± 200 cc) full of sugar syrup (40-50 %) was put in the hive upside down upon a toothpick and a small plate. 4) Two to three pots of pollen were added near the brood comb (*Melipona* bees keep their honey and pollen in pots of about 25 cc capacity). 5) All craks in the hive were closed with tape or melted wax to avoid ant attack and temperature variation. 6) A strong colony was moved one to four meters away from its old place and slightly disturbed. The new hive (with brood comb, syrup, pollen, and with the cover on) was put on the site of this strong hive in order to receive all its field workers (and the ones that flew due to the disturbance). 7) Each 5 days this new hive was opened, more syrup added in glass, and was examined for an accepted queen or, after 15 days, a laying queen. Forty days after the new queen began egg laying, the oldest comb (containing 30 to 40 day old pupae) was taken out, brought to the laboratory and all the pupae were removed from their cells. Then each pupa examined to find out if it was a female (queen or worker) or a male. Usually, there are very few or no males, but when the ratio between females and males was about 1.1, chromosome counts were carried out in a random sample of about 10 males in order to see whether they were diploids (18 chromosomes) or haploids (9 chromosomes).

RESULTS AND ANALYSES

The results of all counts are in *table I.* All males tested cytologically showed 18 chromosomes.

In 49 colonies studied from 1982 to 1987, 4 hives showed approximately 50 % diploid drones and the remaining 45 had only workers. Applying the formula of LAIDLAW *et al.* (1956) $n = \frac{2(N+1)}{(H+1)}$ and making a jackknife estimate of n and s, were H is the number of hives that produced 50 % diploid males (4) ; *N* is the total number of experimental colonies (49) ; *s* is the standard deviation and *n* is the number of xo-heteroalleles, the following parameters were obtained : N = 49 (or 48 in the jackknife estimate), n = 20.0, s = 1.36.

DISCUSSION AND CONCLUSIONS

The similiarities in two important parameters of populations of *Apis mellifera* and meliponids are remarkable.

The number of xo-heteroalleles estimated in one population af Apis is 18.9 (ADAMS *et al.*, 1977) and 20.0 in *Melipona compressipes fasciculata.* Also the number of lethal equivalents in *Apis mellifera* is 1.29 to 1.36 (KERR, 1975) and in meliponids is 1.08 to 1.14 (KERR and ALMEIDA, 1981). These values are not significantly different of each other.

The average rate of mutation found for six genes in *Apis mellifera* ($\mu = 1.6 \times 10^{-6}$, CHAUD *et al.*, 1983) also applies to the populations of meliponids.

The main reasons for these convergences are the great similarities in the system of sex determination (haplodiploidy and xo-heteroalleles), and a social life with a similar high degree of complexity (eusociality) that puts pressure on the genetic constitution of the populations of these species. Of course large Ne is also important. A large population of *Apis mellifera* of Central Brazil has Ne = 4524 (KERR, 1974) what can maintain 36 xo-hetoeralleles according to YOKOYAMA and NEI (1979). Why a greater number of xo-heteroalleles was not found ? One possibility is that a number around 20 complementary alleles is the upper limit allowed by the length of this type of gene. Of course, small populations should not share the same alleles, since fixation is an alleatory process.

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Table I. — Number of worker, queen and male pupae produced in the brood comb of 49 young queens, before the emergence of their first offspring.

Tableau I. — Nombre de pupes d'ouvrières, de reines et de mâles produites dans la descendance de 49 jeunes reines, avant les premières éclosions.

Number of the queen	Day the new colony was assembled	Day the queen began laying	Date counts were made	Pupal counts			% diploid males	
				Workers	Queens	Males		
L73-2-83	12-12-82	16-1-83	12-5-83	30	5	0	0	1
C79-1-83	25-2-83	16-3-83	28-4-83	24	4	0	0	2
C81-1-83	25-2-83	12-3-83	28-4-83	26	9	0	0	3
C80-2-83	4-3-83	25-4-83	12-5-83	10	5	4	0	4
C84-1-83	7-3-83	19-3-83	6-5-83	38	2	38	50 %	5
C83-1-83	7-3-83	18-3-83	12-5-83	25	7	0	0	6
C82-1-83	4-3-83	15-3-83	12-5-83 3-5-83	25 24	1 8	0 0	0 0	7
L72-1-83	25-5-83	4-6-82	13-5-82	30	3	0	0	8
L75-1-82	28-10-82	14-11-82	13-5-83	22	5	0	0	9
L78-1-83	31-1-83	14-2-83	13-5-83	18	2	0	0	10
O81-2-83	9-7-83	14-7-83	9-9-83	22	3	0	0	11
A16-3-83	16-4-83	4-5-83	4-7-83	21	0	0	0	12
C84-2-83	(1)	±25-4-83	30-6-83	21	1	0	0	13
O86-1-83	24-8-83	9-9-83	27-10-83	21	0	0	0	14
A18-3-83	21-1-84	6-2-84	20-3-84	29	3	0	0	15
O87-1-83	24-8-83	22-9-83	27-10-83	19	4	0	0	16
C85-1-83	2-8-83	19-9-83	30-10-83	31	7	0	0	17
O88-2-83	26-10-83	3-11-83	20-1-84	23	5	0	0	18
N89-1-83	25-10-83	15-11-83	20-1-84	38	4	0	0	19
C79-2-83	28-10-83	12-11-83	21-1-84	34	2	0	0	20
V32-2-83	±20-10-83	5-11-83	19-1-84	40	2	0	0	21
R99-1-84	(1)	3-8-85	2-11-85	21	3	26	50 %	22

O94-1-84	5-2-84	17-2-84	26-3-84	18	2	0	0	23
C90-1-84	24-1-84	25-2-84	2-4-84	25	5	0	0	24
C82-2-84	8-2-84	21-2-84	4-17-84	18	0	0	0	25
A5-3-84	5-3-84	20-3-84	23-4-84	11	8	0	0	26
C83-2-84	6-2-84	29-2-84	9-4-84	22	4	0	0	27
F200-1-84	24-3-84	8-4-84	20-5-84	25	5	0	0	28
Q96-1-84	20-4-84	3-5-84	21-6-84	19	5	0	0	29
Q93-3-85	5-1-85	13-3-85	6-8-85	22	0	26	50 %	30
R110-1-85	16-6-85	—	11-8-85	25 (W + q)	—	0	0	31
R111-1-85	16-6-85	—	11-8-85	25 (W + q)	—	0	0	32
R112-1-85	16-6-85	—	11-8-85	25 (W + q)	—	0	0	33
R113-1-83	16-6-85	—	11-8-85	25 (W + q)	—	0	0	34
K45-5-86	25-12-86	—	7-2-87	19	2	0	0	35
K146-1-86	21-10-86	1-11-86	7-2-87	7	1	0	0	36
Q96-2-87	2-1-87	1-2-87	19-4-87	28	1	0	0	37
Z10-1-87	4-4-87	16-5-87	3-7-87	19	2	0	0	38
PN89-2-87	2-1-87	10-1-87	14-3-87	13	4	0	0	39
Q150-1-87	2-1-87	17-1-87	14-3-87	18	1	0	0	40
A20-3-87	12-1-87	7-2-87	14-3-87	14	3	0	0	41
A132-2-87	27-12-86	15-1-87	14-3-87	17	3	0	0	42
A11-3-87	12-1-87	7-2-87	14-3-87	19	2	0	0	43
Q95-2-87	2-1-87	7-2-87	4-4-87	23	2	0	0	44
Q155-1-87	2-1-87	7-2-87	16-5-87	13	3	0	0	45
Q128-2-87	(1)	7-2-87	4-4-87	14	0	12	50 %	46
Q129-2-87	22-1-87	7-2-87	16-5-87	19	4	0	0	47
Q135-2-87	2-2-87	7-2-87	3-4-87	22	5	0	0	48
Q122-2-87	2-1-87	7-2-87	3-4-87	10	0	0	0	49

(W + q) = queens and workers were counted together.
(1) = natural superseding.

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