

Sex determination in bees. XXXIII. Decrease of xo heteroalleles in a finite population of *Melipona scutellaris* (Apidae, Meliponini)

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ABSTRACT

The descendants of a sample of 22 colonies of *Melipona scutellaris* (Meliponini, Apidae) from the forest of Lençois, Bahia (Brazil), and kept in Uberlândia, Minas Gerais, where they do not naturally occur, were monitored yearly for the number of xo alleles remaining in this finite population. Altogether, 32 daughter colonies were artificially produced: 15 colonies in 1991 and 17 colonies in 1992. Five of these colonies produced diploid drones. The estimated number of xo alleles for 1991 is 15 and for 1992 it is 7.5. Usually, wild populations of Hymenoptera have around 20 xo alleles. Therefore, there was a decline in the number of xo sex-alleles resulting from the reduction in the size of the population. Recommendations to avoid the loss of sex alleles in bee populations are: maintain meliponaries with more than 44 hives, maintain larger contiguous areas of forest, teach honey hunters to transfer the colonies to wooden boxes, instead of destroying them, and exchange queens with other beekeepers.

INTRODUCTION

The number of xo heteroalleles in a Brazilian population of *Apis mellifera*, near Rio Claro, SP, Brazil, was estimated to be 18.9 by determining the percentage of diploid drones in samples from each of 90 colonies (Adams *et al.*, 1977) among a population of 500 colonies. The average number of drones that inseminated each queen in that population was 17.3. Fourteen years later, using a different methodology, Lobo and Kerr (1993) found that the average number of drones inseminating queens in five different populations was 15.8, not statistically different from 17.3. These results demonstrate that the two estimates obtained by Adams *et al.* (1977) were robust. Page *et al.* (1983) studied the

distribution of xo alleles in closed populations of *Apis mellifera*. Stingless bee queens (Meliponini, Apidae) are mated by only one male.

Yokoyama and Nei (1979) pointed out the danger of maintaining small populations of bee colonies - the number of xo heteroalleles surely will diminish and the frequency of diploid male producing colonies will increase. The loss of alleles is responsible for the elimination of stingless bees in many places where the fragment of the forest left after harvesting is too small to support a sufficient number of colonies of each species. Workers of *Melipona quadrifasciata* (Camargo, 1982), *M. compressipes* (Kerr, 1987), and *M. scutellaris* kill their queen when the first diploid males emerge. Diploid males are also killed. Our experience shows that six xo alleles is about the minimum a population can stand; below that the whole population is rapidly eliminated. Kerr and Vencovsky (1982) showed that a popu-

lation of 44 colonies of stingless bees can maintain six *xo* alleles. In a reasonably large and old forest of São Paulo State, Pedro and Camargo (1991) found 192 species of bees of which 25 were stingless bees, representing 49% of the 4086 bees collected during one year. Since these bees are in equilibrium for these *xo* alleles, the data of Pedro and Camargo mean that every one of these species has a population greater than 44 colonies, in their respective reproductive area (Laidlaw *et al.*, 1956).

Kerr (1987) found that 49 colonies of *M. compressipes* sampled from a native population of a forest at the margins of the Mearim river (Maranhão State, Brazil) had a total of 20 (± 1.36) *xo* heteroalleles. This estimate is similar to that found for *A. mellifera* (Adams *et al.*, 1977). Colonies with 50% males result from queens mating with a male that has the same *xo* allele. For example, if a xo^1/xo^2 queen is inseminated with an xo^1 or xo^2 male, it will produce 50% diploid drones. Diploid-male producing queens (those that mated with a male having a matching *xo* allele) are replaced with a daughter who then mates with a male drawn from the original parental gene pool. This paper presents data from a population of stingless bees monitored for diploid drone production from April 1991 to April 1993.

MATERIAL AND METHODS

A collection of *M. scutellaris* was begun in 1987 at the Universidade Federal de Uberlândia with 14 colonies from the province of Lençóis, Bahia, Brazil. Eight more hives were added to this collection in November, 1990, totaling 22 colonies in this original sample, this species does not normally occur in Minas Gerais. *M. scutellaris* is one of the three species of stingless bees that were domesticated before the "discovery" of the Americas.

We used the method developed by Kerr (1987) to determine the number of *xo* alleles in our *M. scutellaris* population. This method is as follows: a new colony (A) is initiated with two or three large brood combs containing older pupae and young bees collected from a strong hive (B). The new hive (A) exchanged place with (B), or with another strong one (C), in order to collect some of its foragers to constitute a work force. Within five to 20 days a virgin queen will emerge in the new hive (A), it is inseminated in a nuptial flight with only one male, and begins laying (this always occurs because queens are constantly being reared in all species of *Melipona*). Forty days after the beginning of egg-laying, a piece of brood comb containing about

15 to 20 cells is taken from the new colony and examined. New brood combs commonly contain either 100% females (workers and queens), and rarely 100% males (from eggs of workers or, very rarely, of a queen that has not been inseminated) or 50% males and 50% females. In this last case, three or four males from each such colony were dissected, and their chromosomes counted in order to find out if the males had nine (haploid) or 18 (diploid) chromosomes. Then an additional brood sample was taken from the same new hive and counted. Diploid-male producing queens are killed by the workers. This continues until a suitable mating occurs within that maternal lineage, one that does not produce diploid drones. If this does not happen the colony dies out.

RESULTS

Among 32 colonies studied, five showed 50% diploid drones (Table I).

The formula of Laidlaw *et al.* (1956) was applied, $n=2(N+1)/(H+1)$, where *n* = number of *xo* heteroalleles, *N* = total number of colonies (32), *H* = number of colonies that produced diploid drones (5), therefore $n = 2(33)/6 = 11.0$ *xo* heteroalleles.

However, if only colonies made in 1991 are considered, *n* is 15 and for colonies in 1992 *n* is 7.5; which suggests that the number of *xo* alleles is diminishing each year.

A complete record of all colonies has been maintained from the original 22, including 18 hives that left descendents to the current 62 hives. Going back from the present 62 hives to the founders, the following was obtained: original hives (that left descendents) = 18, number of generations elapsed between hives tested (F1, F2 and F3) and the founders (P) of our population = 4.0 generations.

Further data and conclusions

The behavior of stingless-bee workers killing both diploid males and the queen causes many beekeepers and bee scientists who collect and maintain less than 44 hives per species to lose all or almost all of them in short periods of time. Four examples are:

- 1) W.E. Kerr collected 14 hives, in Parnaíba, S.P., Brazil, of *M. marginata* in 1945. Six years later all had died.
- 2) He collected 12 hives in 1944, of *M. quadrifasciata* (also in Parnaíba, S.P.). In 1955 all had died.

Table I - Number of worker, queen and male pupae produced in the brood combs of the first laying queen before the emergence of the first offspring.

No. of the queen	Day the new colony was assembled	Day the queen begin laying	Date the counts were made	Female	Male	2n Male
330-4-91	21/09/91	28/09/91	04/11/91	17	7	-
338-4-91	change	06/11/91	11/03/91	19	8	-
130-6-91	change	28/11/92	11/01/92	11	1	-
306-2-91	21/09/91	28/09/91	11/10/91	17	0	-
244-3-91	28/09/91	11/12/91	17/12/91	20	0	-
423-1-91	31/08/91	14/09/91	07/01/92	52	1	-
407-3-91	change	06/11/91	28/11/91	22	0	-
416-1-91	16/08/91	31/08/91	28/11/91	13	0	-
305-3-91	28/09/91	19/10/91	17/12/91	19	0	-
324-2-91	change	28/11/91	30/12/91	36	0	-
300-4-91	19/10/91	17/12/91	11/01/92	43	0	-
309-3-91	04/11/91	17/12/91	30/12/91	12	0	-
424-2-91	04/11/91	30/11/91	11/01/92	9	-	8
414-3-91	25/01/92	31/01/92	11/03/92	24	0	-
425-3-91	29/12/91	17/01/92	31/03/92	32	0	-
433-1-92	07/02/92	06/03/92	18/03/92	16	0	-
430-1-92	07/02/92	09/03/92	02/04/92	11	-	11
301-4-92	29/01/92	18/02/92	06/04/92	25	0	-
347-2-92	24/04/92	13/05/92	13/06/92	28	0	-
445-2-92	02/07/92	09/07/92	22/08/92	55	0	-
442-2-92	13/06/92	25/06/92	04/08/92	24	14	-
444-3-92	13/06/92	25/06/92	22/08/92	20	0	-
428-2-92	13/05/92	01/07/92	04/08/92	57	0	-
303-5-92	13/06/92	13/06/92	04/08/92	38	0	-
448-1-92	14/08/92	29/08/92	10/10/92	18	0	-
450-1-92	14/08/92	20/08/92	10/10/92	15	0	-
345-2-92	22/08/92	26/08/92	14/10/92	6	-	7
446-2-92	03/09/92	08/09/92	10/10/92	10	-	6
130-7-92	24/08/92	20/09/92	19/10/92	19	0	-
334-3-92	change	14/08/92	21/09/92	23	-	22
447-1-92	orphan	19/05/92	19/08/92	18	5	-
454-1-92	24/08/92	10/09/92	14/10/92	9	0	-

change = means that the mother queen was naturally superseded.

orphan = means that the colony had the queen removed.

3) Mr. Rogério Alves collected 11 colonies of *M. quadrifasciata* near Catu, Bahia, Brazil, in May, 1990; by December, 1991, he had eight colonies and in June, 1993, only one was left.

4) Mr. Alvino Pianzoli collected 20 colonies of urucu-preto (*Melipona capixaba*, Moure and Camargo, in press) in Domingos Martins, Espírito Santo, Brazil in 1973. In June, 1993, he still has 8, probably because the forest is not very far from the apiary, so that his queens can occasionally mate with males from the forest.

The gradual loss of alleles and respective disappearance of species in both restricted and finite populations appears to be more important to the conservation of panmitic hymenopteran species than presently considered. Miss Maria C. Lorenzon, Professor of Apiculture in the Universidade Federal da Paraíba, Campus Areias informed us recently that many species of meliponids of Northeastern Brazil are not found anymore, since the remaining forest fragments are not large enough in order to maintain the necessary number

of colonies. Falk (1991) emphasizes the fact that there is a decline in the aculeate population in Great Britain, with nearly half of the species described considered to be under threat.

These data could be due to the gradual loss of the xo sex multiple-alleles in restricted finite populations of Hymenoptera.

As result of this and Kerr and Vencovsky's (1982) paper we are carrying out a campaign among *Melipona* beekeepers of Brazil asking them to keep more than 44 colonies, if possible more than 100 and to engage in queen exchange with other beekeepers. Also the Brazilian Institute of Environment (IBAMA) is being alerted about this problem, so that forest fragments can be of an adequate size to avoid the extinction of the species; and finally, the honey gatherers should be taught how to harbor colonies in wooden boxes instead of destroying them.

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RESUMO

Foi retirada da floresta de Lençóis, Bahia, uma amostra de 22 colônias de *Melipona scutellaris*. Esta amostra estudada está sendo monitorada quanto ao número de alelos xo, determinadores de sexo. Usualmente uma população grande de abelhas tem 20 heteroalelos xo. A amostra deu origem a 32 filhas sendo 15 colônias em 1991 e 17 em 1992. Cinco colônias produziram machos diplóides, sendo estimado que a população de 1991 tinha 15 alelos xo e a de 1992 apenas 7,5 o que indica um declínio no número desses alelos, declínio esse que é devido a população ser pequena e limitada. Apresenta-se cinco sugestões para evitar essa diminuição que pode levar a extinção da espécie: 1 - cada meliponicultor deve ter no mínimo 44 colônias de cada espécie que crie; 2 - os fragmentos florestais devem ser maiores, 3 - os "meleiros" devem ser instruídos a guardarem suas colônias em colméias de madeira, 4 - os meliponicultores devem proceder a troca de rainhas com outros criadores e, 5 - ao dividir a colméia, levar a colônia com rainha virgem para fecundar na mata ou em outro meliponário.

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