

The Number of Sex Alleles (CSD) in a Bee Population and its Practical Importance (Hymenoptera: Apidae)

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Abstract.—This work was carried out to monitor the number of *xo* sex alleles (CSD—Complementary Sex Determination) in a limited population of *Melipona scutellaris* Latreille (Apinae, Meliponini) and to verify if introduction of inseminated queens from distant populations is a good and practical method to avoid extinction of small ones. Twenty-two colonies of *Melipona scutellaris* were brought from Lençóis, Bahia (12°34'S; 41°23'W) to Uberlândia (18°52'56"S; 48°12'55"W), 500km south of the southern edge of its geographical distribution. Thirty foreign queens were introduced from 1992 to 1995, and the number of *xo* sex alleles oscillated from 7.0 to 36.0. The number of sex alleles was studied using a formula modified from the one of Laidlaw *et al.* (1956) $n = 2M(N+1)/(H+1)$ (where *n* is the number of *xo* sex alleles in the population, *N* is the total number of colonies sampled of this population, *H* is the number of colonies that produced diploid males and *M* is the number of males that mated with the queen). These results showed that the introduction of inseminated queens serves to maintain the variability of sexual alleles and, consequently, allow the existence of small populations of Meliponini. These data are being taught to *Melipona* beekeepers to improve their bee yards and help in protecting the species from extinction.

Brazilian stingless bees (Hymenoptera, Apidae) are among the main pollinators of the native Brazilian flora, varying from 30% to 90% of the native plants, according to the ecosystem they inhabit (Kerr *et al.* 1994). These bees belong to the subfamily Apinae, tribe Meliponini, and at least three species, *Melipona scutellaris* Latreille, *M. compressipes fasciculata* Smith and *M. beecheii*, were domesticated by native Pre-colombian populations, two in Brazil (Maranhão and Pernambuco States) and one in Mexico (Yucatan). Besides being good pollinators, two bees, the African honey bee and Uruçu (*Melipona scutellaris*) are the main producers of honey in north-eastern Brazil. *Melipona* honey reaches a price 4 to 10 times higher than honey bees because of lower production by individual colonies and the preference of local populations.

More than 350 species of Meliponini are distributed in the Tropical and Subtropical

Zones of South and Central Americas, Malaysia, India, Indonesia, Africa and Australia. Unfortunately about 100 species of stingless social bees are being seriously threatened with extinction as a consequence of the current forest destruction. In Brazil, annual deforestation increased 20% in the last 3 years due to the arrival of Chinese and Malaysian lumber companies.

Besides the forest destruction that is accompanied by both forest fires and plantations of grass, soybean, rubber trees, guarana, pepper, Brazil nuts and tropical fruits, there is the serious problem of CSD (Complementary Sex Determination) that in bee happens with one main gene (*xo*-sex alleles). According to Mackensen (1951) sex in *Apis mellifera* is determined by *xo* sexual alleles, where heterozygous larvae become females, hemizygous are males and homozygous are diploid males. This genetic system by *xo*-sex alleles de-

Table 1. Production of males in colonies of *Melipona scutellaris* and estimates xo alleles using $n = 2M(N + 1)/(H + 1)$ where n = number of xo , N is the total number of hives, H is the number of hives that produced 50% diploid drones and M is the number of males that inseminated the queen.

Year of sampling	Total number of colonies in <i>Melipona</i> apiary in each year	Number of colonies sampled	Number of colonies produced 100% of female	Number of colonies that produced 50% female:50% male (mated with one male)	Number of colonies that produced 75% female:25% male (mated with two males)	Estimated number of xo CSD alleles
1991	50	13	9	1	3	24.5
1992 (a)	80	27	19	7	1	7.86
1993 (b)	72	15	12	3	0	8.00
1994 (c)	79	16	13	1	2	28.34
1995 (d)	66	8	7	0	1	36.00
1997	65	7	5	1	1	12.00
1998	70	9	7	1	1	15.00
1999	60	29	23	5	1	11.67
Total	—	124	95	19	10	23.86

(a) introduction of 13 queens of Piatã, BA (14 June 1992)

(b) introduction of 3 queens of Catu, BA (19 July 1993)

(c) introduction of 11 queens of Lençóis, BA (24 May 1994)

(d) introduction of 3 queens of Lençóis, BA (26 March 1995)

termination is named "Complementary Sex Determination" (CSD) and was first described in the parasitoid wasp *Bracon hebetor* by Whiting (1943). He proposed that sex was regulated by a series of alleles segregating at a single locus. Camargo (1979) found the same CSD genetics for *Melipona quadrifasciata*, Kerr (1987) for *M. compressipes fasciculata*, and Carvalho *et al.* (1995) for *M. scutellaris*.

Kerr (1987) and Kerr *et al.* (1988) proposed that the xo^0 gene of primitive populations is still found in endogamous populations of Hymenoptera. It is mutated rarely, but constantly, to xo^1 , xo^2 , ... xo^{20} which were selected in panmitic populations giving origin to a series of multiple alleles that are involved in sex determination (CSD).

Yokoyama and Nei (1979) and Cornuet (1980) have shown that the number of CSD alleles maintained in a limited bee population depends directly on its size. Woyke (1980) demonstrated that in *Apis mellifera* the minimum number of CSD alleles that allows a population to survive is six. Our experience with three *Melipona* species indicates that six is also the minimum number of xo alleles required to

maintain restricted populations. Bellow six the population decreases rapidly due to the formation of diploid males. Stouthamer *et al.* (1992) and Heimpel *et al.* (1999) also observed the same in some hymenopteran parasitoids.

Unlike *Apis mellifera* queens that mate with 17 to 15 males (respectively Adams *et al.* 1977, Lobo and Kerr 1993), queens of stingless bees (Meliponini) mate with one or two males, rarely more (Kerr 1969, Contel and Kerr 1976, Paxton *et al.* 1999). Our data with *Melipona scutellaris* showed about 8% of crosses occurred with 2 males (Table 1). In small populations of *Melipona* with 6 xo alleles, $\frac{1}{3}$ of the new colonies will produce diploid males (Kerr and Vencovsky 1982). Many known panmitic Hymenoptera have xo^n sex alleles and have developed different methods to avoid the production of triploid females: a) in many Hymenoptera diploid males are semi-lethal or almost sterile (Inaba 1939, MacBride 1946, Hung *et al.* 1974, Naito and Suzuki 1991, Stouthamer *et al.* 1992, El Agoze *et al.* 1994); b) in *Apis mellifera* the workers eat the diploid male larvae (Woyke, 1980); c) in *Melipona* soon after they emerge from the brood cells, workers kill both diploid

males and the inseminated queen that is producing them (Camargo 1979, Kerr 1987, Kerr *et al.* 1996); d) increasing the number of *xo*-alleles and e) multiple mating diminishes the genetic load effect of 2n males.

Adams *et al.* (1977) and Lobo and Kerr (1993) respectively estimated the number of *xo*-alleles for open populations of *Apis mellifera* at 18.9 and 15.7, and Kerr (1987) in *Melipona compressipes* found it was 20.0. Using data based in the formula of Cornuet (1980), Kerr and Vencovsky (1982) estimated that in order to maintain 6 *xo*-alleles, the *Melipona scutellaris* population must contain 44 colonies or more. If the number of colonies is below 44 this whole mini-population is bound to be eliminated in a few generations. Carvalho *et al.* (1995) cited many examples of the extinction of small populations of stingless bees. This means that the decline of the number of *xo* sex alleles in stingless bees (Meliponini) is fatal and leads a population and even the species to elimination. Falk (1991) emphasized the fact that there is a worrying decline in the Aculeate population in Great Britain, with nearly half of the species described considered to be under threat. Of course, there is a genetic load associate with CSD sex determination in bees as it was demonstrated by Kerr (1975) and Werren (1993).

The objective of the research presented in this paper was to monitor the number of *xo* alleles year by year in a limited population of *Melipona scutellaris* and to study the effect of the introduction of inseminated queens in this population. Descendants of 22 colonies that came from the forests near Lençóis (Bahia) were maintained in Uberlândia (18°52'56"S; 48°12'55"W) about 500 km South of the southern border of its natural distribution, that is, there was no feral population of *Melipona scutellaris* within a radius of 500 kilometers of Uberlândia.

MATERIALS AND METHODS

The following material was used: 22 colonies of *Melipona scutellaris* collected randomly in the forest that surrounds Lençóis (12°34"S; 41°23"W), Chapada Diamantina, Bahia, Brazil—14 hives in 1988 and 8 hives in 1990. These colonies were divided and from their descendents 124 were sampled to be monitored. In order to count the number of *xo*-alleles the technique of Kerr (1987) was used, that is: take one or two brood combs with young bees of a given colony and put it in the place of the mother colony to receive the adult bees. In 1 to 10 days, one or several virgin queens emerge; four days after emerging one makes the nuptial flight and is inseminated. After 5 to 15 days the new queen begins egg laying. I marked this queen on her thorax. When the oldest brood comb of this new queen contains pupae, a small piece of it is taken (with about 10 to 30 pupae) and the number of workers, queens and males is counted. If the proportion fits 1:1 females: males, it indicates 50% production of diploid drones and is indication of insemination by one male only. Diploid males are confirmed by cytological analysis (diploid males have 18 chromosomes). If 25% males were produced it indicated that two males had inseminate this queen and if it was 12.5% three matings were indicated. Then the data are analyzed using the Laidlaw *et al.* (1956) formula $n = 2M(N+1)/(H+1)$ that is better than $n = 2MN/H$; in both equations *n* is the number of *xo* sex alleles in the population, *N* is the total number of colonies sampled in this population, *H* is the total number of colonies sampled that produced diploid males and *M* is the number of males that inseminated the queen. This formula assumes that the *xo* alleles have equal frequency, that there is random mating, and there is less bias in populations smaller than 10. Fisher's test was used to analyze the variation of *xo* alleles frequency by year. In order to mea-

sure the effect of the introduction of genetic material from outside the Uberlândia population as a search for a method of controlling the appearance of diploid males in small apiaries, 30 introductions of inseminated queens in orphaned colonies were carried out and these were: 13 queens of Piatã (Bahia) on 14 May 1992, 3 queens of Catu (Bahia) on 19 July 1993, 11 queens of Lençóis (Bahia) on 24 May 1994 and 3 queens of Lençóis (Bahia) on 26 March 1995. The mortality of egg and larvae of diploid males is near zero.

RESULTS

One hundred and twenty four samples of the new colonies made were taken from the *Melipona* apiary of which 95 had 100% females in the first series of eggs laid by the new queen, 19 presented about 50% diploid drones and 10 about 25%; none produced 12.5%. The results of these samples taken during 8 years are in Table 1.

This data demonstrated that 65.5% of mating occurred with one male and 34.5% with 2 males. Then, the number of *xo* alleles in this population is 8.18 (when queen mates with one male that has the same allele as she does) and 15.68 (when two males—one with the same allele and another with different allele) totaling 23.86 *xo* alleles.

Table 1 demonstrates the variation of *xo* alleles in this population as a consequence of sampling and matings with drones produced in this same population. As these colonies were divided (in order to increase our population) since their introduction (1990), the probability that one queen mates with a drone that has the same allele is high because the population is small. The population in Uberlândia was formed of colonies from a native population in Bahia, and the number of alleles in that original population was expected to be about 20 (Kerr and Vencovsky 1982). In our 67 colonies (average per year) the average number of alleles was 16.67. Looking at the data of 1991 to 1999, the number

of sex alleles does not differ statistically between these years (Fisher's Test, $P > 0.05$), which indicates the value of our method to avoid diminution of these alleles.

DISCUSSION

The behavior of stingless bee workers killing both diploid males and the queen is how they control the appearance of $2n$ males. It causes many beekeepers and bee scientists who collect and maintain less than 44 hives per species to lose all or almost all in short periods of time. Four examples are: a) W. E. Kerr collected 14 hives, in Parnaíba, SP, Brazil, of *Melipona marginata* in 1945. Six years later all had died; b) He collected 12 hives, in 1944, of *Melipona quadrifasciata* (also in Parnaíba, SP). In 1955 all had died; c) Eng. Agr. Rogério M. O. 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; d) Mr. Alvino Pianzolli collected 20 colonies of urucu-preto (*Melipona capixaba*) in Domingos Martins, Espírito Santo, Brazil in 1973. In June 1993, he still had 8, because the forest is not very far from the meliponary, so that his queens can mate with males from the forest. The same case was observed by Nascimento *et al.* (1996) at Archipelago of Fernando de Noronha (Pernambuco, Brazil) for three species of *Melipona*. They found, in 1996, only 18 colonies of *M. compressipes* out of 32 introduced, 11 of *Melipona subnitida* in 10 and none of *M. scutellaris* in 30 introduced in 1982 by Kerr and Cabeda (1985). These examples confirm the observations of Yokoyama and Nei (1979) about the extinction of small populations, and suggest the necessity of methods to avoid the decline of variability of sexual alleles.

The introduction of 13 foreign queens in 1992 and 3 in 1993 did not show its effect in the same year—the data show a decline in the number of alleles in 1992 and 1993. Due to the winter that followed the intro-

ductions of 1992 and 1993, the colonies usually became weaker than in the rest of the year and there was no regular production of males. Therefore, these introduced queens will be genetically active through their males only for the next queens naturally superseded.

When the queens were introduced in June or July (winter in the Southern Hemisphere) there were few or no males, but when they were introduced in May or March, the last season in which males are being produced, an apparent increase of the number of *xo* sex alleles was detected, reaching a value of $n = 17.92$, which is close to the values obtained by Kerr (1987) and Carvalho *et al.* (1995) for two *Melipona* species. Looking at the results of 1997 to 1999, it appears to indicate a small decline in the number of *xo* alleles. As the statistical analysis (Fisher's Test) showed, there was no divergence among the number of alleles by year, indicating that the introduction of these queens maintained the genetic variability in the population in Uberlândia, thus avoiding the appearance of diploid males and the extinction of their population.

Our data showed that the introduction of 3 to 4 inseminated queens per year in an apiary with about 65 colonies improved the genetic variability of the *xo* alleles becoming a good alternative to avoid the production of diploid males. It can save the production of hundreds of beekeepers who maintain small meliponaries because the production and sale of inseminated queens is much easier than selling a complete bee colony.

The main conclusion is: the method of introduction of 3 to 4 inseminated queens by year is good to avoid low sex allele diversity and consequent production of diploid males (the Yokoyama and Nei effect); it maintains the variability of *xo* sexual alleles and consequently allows the existence of restricted populations of *Meliponini ex-situ*, that is outside their area of geographic distribution.

ACKNOWLEDGMENTS

I thank Prof. Warwick Estevam Kerr for the advices provided during this seven years work; Prof. Gismar Silva Vieira for helping in statistical analysis, Prof. Rogério Marcos de Oliveira Alves for providing queens from Catu, BA; Mr. José Carlos Moraes for queens from Lençóis, BA. I thank Ms. Vania Alves Nascimento for her help in the cytological examinations. My acknowledgments are also to an anonymous referee that made important suggestions. CNPq (Conselho Nacional de Pesquisa e Desenvolvimento Tecnológico), FAPEMIG (Fundação de Amparo a Pesquisa de Minas Gerais) and Bank of Brazil Foundation provided the financial support.

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Carvalho-Zilse, Gislene Almeida. 2001. "The Number of Sex Alleles (CSD) in a Bee Population and its Practical Importance (Hymenoptera: Apidae)." *Journal of Hymenoptera research* 10, 10–15.

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