

Sex determination in honey bees (Apinae and Meliponinae) and its consequences

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ABSTRACT

The first experiments on sex determination in bees began with Dzierzon, Meves, Nachtsheim, Paulcke, Petrunkevitch, Manning. Whiting, (1943) found multiple alleles in *Bracon xo* that are the Rosetta stone of sex determination in Hymenoptera. Whiting also discovered that some species of microhymenoptera do not possess *xo* sex alleles. Therefore, Hymenoptera apparently presents two types of sex determination superimposed on haplodiploidy. In the panmictic groups hemizygous (xo^1, xo^2, \dots, xo^n) and homozygous ($xo^1xo^1, xo^2xo^2, \dots, xo^nxo^n$) are males while heterozygous ($xo^1xo^2, \dots, xo^{n-1}xo^n$) are females. There is no such series of xo^n in endogamous Hymenoptera, since the constant elimination of diploid males would be damaging to the population and the mutation of xo to xo^n would be quickly eliminated. Besides the Whiting hypothesis, four others are discussed. The new hypothesis of genomic imprinting, of Beukeboom, is eliminated since: a) spermatozoa that develop within the egg produce male tissue; b) telitokous parthenogenesis due to the fusion of two haploid cells develop into females; c) last instar larvae treated with juvenile hormone become queens. The Cunha and Kerr hypothesis (female determining genes are totally or partially additive and male determination is totally or partially nonadditive) explains all known cases. The *xo* is a female determining gene. Sex determination in social bees led to the gradual evolution of two systems of caste determination: one in which queens and workers are similar and males are very different (Apinae), and another in which workers and males are very similar and both very different from the queens (Meliponinae). This second system in stingless bees implies that many of the mutations that improve worker capacities also affect the males that will carry out some activities that in *Apis* are clearly female ones. Ten of these activities are described.

INTRODUCTION

The first scientific studies of sex determination in the Hymenoptera were those of Johannes Dzierzon (1845, 1854, 1898, 1899). He found that a virgin queen only laid eggs that developed into drones and he concluded correctly that males originate from unfertilized eggs and females from fertilized ones. Siebold (1856a,b) found spermatozoa in 30 eggs (out of 52 analyzed) collected in worker cells and none in 27 taken from drone cells, which confirmed the assertion of Dzierzon. Cytological evidence was provided by

Meves (1907), who found 16 chromosomes in spermatogonia, and Nachtsheim (1913) who found 32 chromosomes in fertilized eggs. Makino (1951) and Imai *et al.* (1977) have shown that the presence in females of $2n$ chromosomes and of n in males is general for all Hymenoptera, except for the thelytokous ones. The first cytological evidence for this in bees was provided by Paulcke (1899), Petrunkevitch (1901), Nachtsheim (1913) and Manning (1950), who showed that each egg of *Apis mellifera* receives from 8 to 20 spermatozoa, of which only one joins with one of the four haploid nuclei of the egg and later on becomes either a worker or a queen; unfertilized eggs have no spermatozoa, and develop into males that are true representatives of a

queen's gametes. Bridges (1925), working with *Drosophila melanogaster*, demonstrated that its sex is determined by a balance between genes determining maleness, found on the autosomes (especially in chromosome II; Kerr, 1962), and genes determining femaleness, found on sex chromosome X. Dobzhansky (1930) demonstrated the importance of environment in the sex determination of *Drosophila melanogaster*.

HYPOTHESES FOR SEX DETERMINATION

The statement that Hymenoptera females are diploid and males haploid is a cytological description that deserves a detailed genetic explanation. Five genetic hypotheses of sex determination have been presented. Briefly summarized they are:

1) Involvement of a single series of multiple alleles (Whiting, 1943, 1945; Mackensen, 1951). Whiting based this on the studies of diploid males of *Bracon hebetor* (published as *Habrobracon juglandis*). From his crosses he concluded that sex in this Braconid is determined by a series of at least 8 heteroalleles that in hemizygous ($xo^1, xo^2 \dots xo^8$) or homozygous ($xo^1xo^1, xo^2xo^2 \dots xo^8xo^8$) condition are males and in heterozygosis ($xo^1xo^2, \dots, xo^7xo^8$) are females. This was the Rosetta stone of the studies of the sex determination in the Hymenoptera, but Whiting himself found that some species of microhymenoptera do not produce diploid males. Mackensen (1951), using inbred lines of *Apis mellifera*, suggested that the same multiple alleles occurred in *Apis mellifera*. Woyke (1963, 1979, 1986) actually demonstrated that in *Apis mellifera* and *Apis cerana* the homozygous diploids for the sex alleles were males (Hoshiba *et al.*, 1981). All endogamous Hymenoptera that have been studied cytologically and genetically have not such series of multiple alleles (Kerr *et al.*, 1988).

Among the Hymenoptera there are two systems of sex determination: one is found in endogamic species where the xo allele is male and xo/xo is female. The mutation rate of xo to xo^1 , or to xo^2 , or ... xo^n occurs at an average rate of 1.6×10^{-6} (Kerr *et al.* 1980; Chaud-Neto *et al.*, 1983; Kerr, 1986b). This means that on average for every 1,600,000 gametes of a hymenopterous species about one xo mutates to xo^n . However, in endogamous populations no new xo^n mutations (that are constantly being produced) will be established because of the severe conditions for its persistence (Kerr *et al.*, 1988), namely, xo/xo^n needs to have an adaptive value 1/3 greater than that of xo/xo or the haploid xo must have an adaptive value of 0.50 or smaller (the new xo^n and xo/xo^n being equal to 1). In a panmictic

population, however, the new xo^n mutation (the homozygote produces diploid xo^n/xo^n drones) will become automatically established provided those xo/xo^n females have a slightly better adaptive value than xo/xo . The large advantage of 1/3 is not required, as is necessary in an endogamous population.

Since this mutation will occur randomly in all panmictic species, and diploid males (that produce spermatozoa with 2n chromosomes) cause genetic problems if they mate, different species selected different methods to avoid the effects of having them, for example: sterility of the diploid males, higher mortality of them, diploid male larvae being eaten by the workers (Woyke, 1986) or adult diploid males and their queen being killed (Camargo 1977a, 1979, 1984; Kerr 1987c,d); Carvalho *et al.*, 1995). The sooner the bees get rid of the diploid males, the greater will be the adaptive value of the species.

2) Goldschmidt (1958), being aware of the work of Whiting (1945) but not knowing the work of Cunha and Kerr (1957), hypothesized that sex in Hymenoptera may be reached by an equilibrium between male determining genes (M) and female determining genes (F) in the following way (page 475): "The M and F determiners are not located in different chromosomes, but both within the X-chromosome; further, they are completely linked here, and the F/M balance is such that F has a high potency which is additive in compounds but not in homozygotes; the M potency is such that it has no action in the heterozygous condition (recessiveness of m) but an additive one in hemizygotes and homozygotes, so that MM (= mo, or mm, or $m^a m^a$ $m^b m^b$) is epistatic over F or FF". Goldschmidt tried to explain and generalize the *Bracon hebetor* case. But there is no evidence of an X-chromosome in bees and Camargo (1977a,b) demonstrated that the feminizing genes x_a, x_b, x_o are not linked.

3) Crozier (1977) suggested the hypothesis of many series of multiple alleles. In a certain way the *Melipona* case fits in Crozier's idea since the triple heterozygotes are females ($x^{n-1}/x^n; x_a^1/x_a^2; x_b^1/x_b^2$). However three facts negate such series in the sex determination of endogamous populations: a) The lack of diploid males in species that naturally reproduce by obligatory endogamy (e.g., Agaonidae, *Telenomus*, *Melittobia*, *Goniozus*, etc.). b) Lack of diploid males in experimentally inbred populations. No diploid males were detected in 100 controlled endogamic populations (crossing brother x sister or mother x son) of *Melittobia hawaiiensis* (Varanda, E.A., personal communication) and in 22 lines with three three to 18 generations of mother x son and 110 generations of brother x sister of *Muscidifurax raptor* and *M. zaraptor* (Legner, 1979). c)

Cook (1993) carried out two inbred experiments with *Goniozus nephantidis* and obtained diploid males in none of them.

4) Genomic imprinting model. Beukeboom (1995) developed a model based on genomic imprinting, that assumes: 1) sex-specific imprinting of a sex-determining locus (S) that binds a product (P) which is present in the egg. 2) Females imprint the S locus during oogenesis in such a way that it cannot bind a product P and therefore eggs carry it in the inactive state (Si). 3) Males do not imprint the S locus and transmit it in the active state Sa, in which it does bind P. 4) The model assumes that the imprint is erased during zygotic development. 5) Binding of P to S activates the genes that turn on the female pathway, where non-binding leads to male development. Unfertilized haploid eggs are always male (non-active S locus) and fertilized diploid eggs are always female (one Sa and one Si).

Three facts eliminate this model: 1) The experiments of Walter Rothenbuler *et al.* (1952) and Rothenbuler (1957) have shown that male sperm can develop into male tissues (n) and some (that join the female nucleus) into female tissues (2n) in the same fertilization event (same egg). 2) The telytokous parthenogenesis of *Apis mellifera* shows females being produced without males (Tucker, 1958) by fusion of two post meiosis haploid nuclei. 3) Sex determination in bees is accomplished in two steps: a) few hours after fertilization in which ovary or testis are determined and b) in the prepupa when the adult characters (head, thorax, wings, abdomen, and integument) are fixed; artificial addition in Meliponinae larvae of juvenile hormone determines queens (Bonetti *et al.*, 1995), which shows that female-determining genes of the second step are put into action by juvenile hormone and not by imprinting genes.

5) Cunha and Kerr (1957) suggested that the genes involved in the determination of male characters in endogamous Hymenoptera are not additive (are compensated) while the genes involved in the determination of femaleness are additive (or non-compensated). Subsequent to the data of Chaud-Neto (1972, 1975) and Chaud-Neto and Duarte (1975) the expression "not additive" was replaced by "not additive or slightly additive" for maleness genes; for genes determining femaleness it became "additive or almost additive". The xo^1 to xo^n are derived from mutations in the xo gene or from themselves (xo^3 to xo^8 , for instance)

The following experiments favor this genic balance hypothesis.

a) Devlin *et al.* (1985) studied trisomics for the autosomes 3L and 2R in *Drosophila melanogaster* and found that four out of six enzymes determining loci

produce disomic levels of the genic product, while two have levels that are dosage dependent (trisomic). However, in the X-chromosome (that acts in a haplodiploid fashion) all five loci studied compensate the enzymatic level in trisomics.

b) Oliveira (1992) and Oliveira *et al.* (1992) estimated the number of protein molecules in young male and female (white eyed) pupae and in adults of both *Melittobia hawaiiensis* and *Scaptotrigona postica* (Apidae, Meliponinae). They found that 9.2% of protein products are compensated, and 8.2% partially compensated. In adults, in a sample of 101 genic products, 5.9% were compensated, and 5.9% were partially compensated. This is the first biochemical evidence for the existence of genes with properties equal to those we have hypothesized to characterize sex determining genes in the Hymenoptera. The adjustment of the genes producing these different proportions of the same proteins in males and females may be done by specific genes, like the *sdc* and *dpy* genes of *Caenorhabditis elegans* (revision in Kelley and Kuroda, 1995). Gergen (1987) found that in *Drosophila* sex determination precedes the establishment of a segmental pattern in the embryo because expression of the X-linked pair-rule gene *runt* is already dosage compensated. Parkhurst *et al.* (1990) found that *Sxl-n1* males are lethal because their inappropriate *Sxl* expression leads to a failure of dosage compensation (*Sxl* = sex lethal gene); *Sxl* is transcribed in both sexes but translation is truncated in males, therefore *Sxl* protein is not detectable in male embryos.

c) Campos (1975, 1978) and Campos *et al.* (1975) working with *Melipona quadrifasciata*, and Bonetti and Kerr (1985) with *Melipona compressipes*, treated about 200 last stage larvae with juvenile hormone; the majority of these treated larvae were females, but several male larvae were also treated. Campos *et al.* (1983) demonstrated the action of juvenile hormone in Trigonini. Juvenile hormone is very effective in activating femaleness genes. Using techniques of numerical taxonomy it was demonstrated that Meliponinae workers are nearer to males than to functional females (queens), and that juvenile hormone-treated males are closer to queens than to the untreated ones.

d) Fascinating was the work of Conceição Camargo (1977a,b): in the offspring of a brother x sister mating of *Melipona quadrifasciata* a 1:1 female to diploid males was obtained. In a group of 83 pupae raised from treated larvae with 10^{-6} mg of juvenile hormone III, both female and diploid male adults emerged. Camargo obtained 83 adults of which 38 were female queens and 45 diploid males (1:1). But among the diploid males a 3:1 segregation of phenotypes was obtained, that is, 3/4 (38) were males maintaining their beautiful four yellow

bands while 1/4 (12) were brown and had a queen like shape. This indicates that besides being $2n$ those males were also double heterozygous for the caste factors (xa and xb).

e) Chaud-Neto (1975) inseminated *Apis mellifera* queens with sperm of several diploid drones and obtained triploid workers and queens; estimating the generalized distance between them, Chaud-Neto and Duarte (1975) demonstrated that female-determining genes are not completely additive and male-determining genes are not entirely compensated, which would allow other genes and the environment (temperature, pollen, nectar, humidity, for instance) to have a role.

f) In some species, where the genic balance is very near to equilibrium, intersexes may appear frequently. This is the case for several species of *Megachile* (such as *vidua*, *parallela*, *gemula*, *intergradus*, etc.) (Mitchell, 1929, 1941) of *Ooencyrtus* (Wilson and Woolcock, 1960) and of Mutillidae (Quintero and Cambra, 1994, mentioned four cases of intersexed mutillids). This balance can also be broken by aneuploidy as in *Bombus atratus* (Garofalo, 1973 and Garofalo and Kerr, 1975).

g) The balance theory also predicts the existence of specific sexual genes, different from xo ; for instance, the mutation i (allele of the normal gene I) induces intersexuality in the haploids of *Bracon hebetor* (Borstel and Smith 1960).

Velthuis and Sommeijer (1991) proposed a new model for the genetic determination of caste. It is a physiological model and deals with phenotypes. However, genetically it is equal to the one here proposed (Kerr *et al.*, 1966; Kerr, 1975; Campos *et al.*, 1979, Maciel-Silva and Kerr, 1991). Using the own words of Velthuis and Sommeijer (1991, pages 366 to 368) we have: "This alternative model includes the interaction of two independent genetic factors with food conditions (Figure 1). In this model, the first factor concerns the genetic system that regulates development in the young larva. This development could be related to the rate of food intake, which is either high or low, for instance, as the result of differences in the quality or amount of an enzyme. The enzyme system is less efficient if alleles are homozygous than if they are heterozygous. The heterozygous condition promotes queen development; the homozygous condition, worker development. However, both queen and worker disposition are not irreversibly fixed. They can still be modified later in life. A second gene, being fully independent of the first, operates at a later larval phase. It regulates the production of juvenile hormone at either a high or a low level. This gene is again, in the same way, more effective in its

heterozygous form than in its homozygous constellation. Two alleles are envisaged. The physiological translation of this genetic system can have various forms; one of them could be that the genes lead to the production of an enzyme such as a juvenile hormone synthetase. A set of two genes operating in this way allows for the existence of four classes of bees: A^+/B^+ , A^+/B^- , A^-/B^- , and A^-/B^+ . In connection with the further development of these four classes, a sensor mechanism is assumed that measures the amount of food ingested in the course of larval development. For the purpose of our discussion we distinguish between large and small amounts, but probably a continuous distribution exists in the amount of food ingested.

The combination of two sets of genes and two classes of food amount leads to eight categories. An ideal development program for a *Melipona* queen is $A^+/B^+/H$, for a worker bee $A^-/B^-/L$. In the latter case the lower level of juvenile hormone is considered to be an adaptation to inferior food conditions. If the hormonal activity were higher, a higher metabolic activity would result, which would in turn lead to reduced body weight in the adult; this could be disadvantageous, if not fatal ($A^-/B^+/L$). However, if food amounts were higher ($A^-/B^-/H$), a larger worker or a worker that had some reserves could develop. In this part of Figure 1 the category $A^-/B^+/H$ represents the larvae that are the nearest to queen development, although they will still develop into a firm worker only.

In the upper part of Figure 1, A^+ animals are predestined to become queens but B^- levels preclude this in half of these larvae. By consequence, all A^+/B^- animals become workers. The $A^+/B^+/L$ will become workers too, so only the $A^+/B^+/H$ type will develop into queens.

The action of B^+ is designed to be the most influential, because the artificial application of juvenile hormone has stronger effects than those of increasing

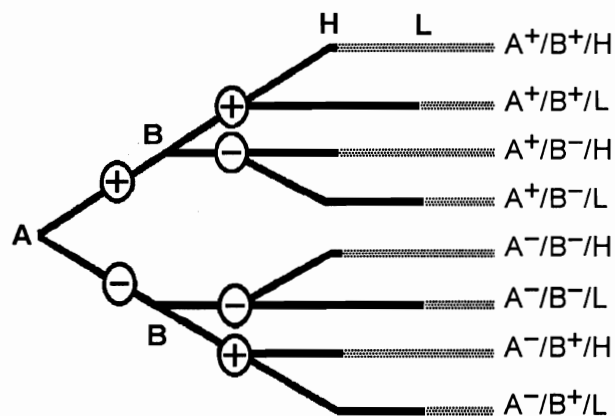


Figure 1 - Phenotypic representation of the model for caste determination in the genus *Melipona* according to Velthuis and Sommeijer (1991).

the amount of food. It is to be expected, however, that the various categories respond differently to such experimental treatments, and in addition to inducing queen development the effect of juvenile hormone application on mortality should be considered.

The model is consistent with the observations that under natural conditions the maximum production of queens is 25%. This occurs when food conditions are excellent and, as a consequence, no *L* categories are present. More frequently the percentages of queens under natural conditions are lower and correspond to the ratio *H* to *L* in the A^+/B^+ category".

The demonstration that this model is equal to the genetic model is:

**Velthuis and
Sommeijer
model**

Kerr model

A^+	=	xa^1/xa^2
A^-	=	xa^1/xa^1 or xa^2/xa^2
B^+	=	xb^1/xb^2
B^-	=	xb^1/xb^1 or xb^2/xb^2

therefore, in genetic terms we have:

$1 = A^+/B^+ =$	$xa^1/xa^2; xb^1/xb^2$
$2 = A^+/B^- =$	$xa^1/xa^2; xb^1/xb^1$ or $xa^1/xa^2; xb^2/xb^2$
$3 = A^-/B^- =$	$xa^1/xa^1; xb^1/xb^1$ or $xa^1/xa^1; xb^2/xb^2$; or $xa^2/xa^2; xb^1/xb^1$ or $xa^2/xa^2; xb^2/xb^2$
$4 = A^-/B^+ =$	$xa^1/xa^1; xb^1/xb^2$ or $xa^2/xa^2; xb^1/xb^2$

TWO SYSTEMS OF CASTE DETERMINATION

Sex determination in bees occurs in two distinct phases: i) the first takes place in the early hours of embryo development (three to eight hours, according to the species).

In endogamic populations of Hymenoptera, sex is controlled by an interacting set of maleness- and femaleness-determining genes. In panmictic populations one of the *xo* femaleness-determining gene mutated to a series of xo^n , which in bees contains eight to about 34 hetero-alleles (Mackensen, 1955; Laidlaw *et al.*, 1956; Adams *et al.*, 1977; Kerr, 1987a,b) depending on the population size and species. This locus xo^n determines ovaries when heterozygous, diploid males when homozygous and normal haploid males when hemizygous. The xo^1 to xo^{34} hetero-alleles originated by mutation of the primitive gene *xo* (Kerr *et al.*, 1988). ii) The second phase occurs under the control of genes

that act in the last larval stage, that is, they determine the future of the integument and of the majority of the imaginal discs, except those whose fate had been determined in an anterior larval stage, like the antennae and genitalia (Kerr, 1987a). In the stingless bees femaleness-determining genes are made functional mainly by juvenile hormone (Campos, 1975; Campos *et al.*, 1975; Kerr *et al.*, 1975; Campos *et al.*, 1983).

A direct consequence of this system of sex determination is the evolution of two types of caste determination in superior bees (Kerr *et al.*, 1978; Kerr, 1987a,b,c; Kerr and Cunha, 1990).

a) One, in which specialization of the queen and workers has not drastically affected the workers morphology in their female characters, that is, the workers are more similar to queens than both are to males (as in *Apis*).

b) Another in which juvenile hormone became, in many evolutionary steps, gradually more important in putting femaleness genes into action determining modifications in the workers and in the queens and, consequently workers become more similar to males at each step (as in Meliponinae and some *Bombus*) and queens become gradually more different from workers.

WORKERS SIMILAR TO MALES; MALES DOING FEMALE WORK

As a result of the Meliponinae system of caste determination, workers *are similar to males* (Kerr and Cunha, 1990) and many mutations that are selected for improving worker capacities (and consequently the colony survival) may also affect the males.

The demonstration of this is found in males of Meliponinae and Bombinae (and also in some Halictidae and in some wasps) that perform activities that in *Apis* are reserved to the workers, like:

1) Incubation. Males of all stingless bees so far studied remain in the comb of young brood in an incubating position as do the workers (Kerr, 1987a; Cortopasi-Laurino, 1979). The incubation behavior of males of *Bombus griseocollis* and *Bombus pennsylvanicus* has also been described (Cameron, 1985).

2) Trophallaxis. Males of *Plebeia droryana* perform trophallaxis with males and with workers for most of their life within the hive (about 15 days) (Cortopasi-Laurino, 1979). Trophallaxis between males was also seen in *Melipona quadrifasciata* (Silva, 1977), *Scaptotrigona postica* (Engels and Engels, 1988) and I observed it in *Melipona compressipes*.

3) Feeding the queen. Cortopasi-Laurino (1979) saw young males feeding the queen of *Plebeia droryana*. Imperatriz-Fonseca (1970) found frequent trophallaxis

between males and a virgin queen of *Paratrigona subnuda*. I saw a male of *M. quadrifasciata* feeding the queen before mating, in a cage.

4) Self-cleaning. Males of *Plebeia droryana* (Cortopasi-Laurino, 1979) and of *Melipona*, *Scaptotrigona* and *Bombus* do self-grooming.

5) Secretion of wax. Drory (1874, 1877, 1883) was the first bee researcher to observe wax scales in males of *Melipona marginata* and *Melipona scutellaris*. Kerr (1951, p. 313) observed in colonies of *Melipona marginata* and *Plebeia droryana* hundreds of males producing wax abundantly. Cruz-Landim (1967) studied the histology of wax glands in males. Nogueira Neto (1963) observed wax being produced by males of *Nannotrigona testaceicornis*. Males of *M. rufiventris* (Kerr, 1950), and *M. compressipes* (Kerr, 1987a) were seen producing wax. I saw males of *M. compressipes* behaving like workers, taking the wax scales from their tergites and putting the wax in the wax deposits around the brood nests.

6) Working with the wax. The most general task that every worker carries out is to work with the wax. I saw males of *Melipona rufiventris* and *Melipona compressipes* working with wax, building small wax columns, pots and involucrum sheets. I never saw them building the cells in which queens are going to deposit eggs. This task appears to be done exclusively by the selected group of female workers that provides cells for oviposition.

7) Receivers and dehydrators. Males normally receive nectar directly from field workers and dehydrate it within the hive in the same way as do workers. Imperatriz-Fonseca (1973) saw males of *Schwarziana quadripunctata* and Cortopasi-Laurino (1979) saw males of *Plebeia droryana* dehydrating nectar; Ferreira, H. (personal communication) saw males of *Melipona marginata* doing this task within the hive. No males were seen depositing concentrated nectar in the honey pots. Recently, I observed in my meliponid apiary males of *Melipona rufiventris* and *M. scutellaris* dehydrating nectar outside the hive (standing on the wall of the building) one to five meters far from the nearest hive, or right on the front hive board 20 cm far from the entrance. Males of these meliponid species permanently leave the colony after they reach about one third of their adult life and search for food in flowers by themselves. During the time a virgin queen normally goes on her nuptial flight (each species has its preferred hours) many males stop foraging and stay close to the hive; dehydration of a nectar with 30 to 70% sugar would make a 90-mg male 33% lighter, what could allow it to be "the" only male that inseminates a meliponid virgin queen.

8) Foraging. Many researchers found either through direct observation, or by occasional collection in insect nets, males of many species of meliponids on flowers. Males of *Scaptotrigona postica* were seen collecting nectar and pollen in *Senecio brasiliensis* and *Dombeya acutangula* (Kerr *et al.*, 1962) and males of *Plebeia droryana* and *P. mosquito* collected nectar in flowers of *Croton floribundus* (Nogueira-Neto, 1964). I observed males of *Geotrigona mombuca* visiting daisies (*Chrysanthemum leucanthemum*). Brenha (1986) found males of *Melipona compressipes* in flowers of *Cordia multispicata* and Gondin (1984) saw males of *M. seminigra* in flowers of guaraná (*Paullinia cupana sorbilis*).

Males of Euglossinae and Bombinae are, in many cases, considered good pollinators. Therefore, the case of *Apis mellifera* in which males are worker dependent for their feeding may be a recent evolution.

9) Communication. Workers of *Melipona rufiventris*, *Melipona quadrifasciata*, *Melipona bicolor*, *Melipona compressipes* and *Melipona scutellaris* (Kerr and Rocha 1988; Kerr, 1994) were found to perform communication by a smell odor track made near to the food source and by sounds produced inside the hive whose frequency and sound duration, like in *Apis*, have a high correlation with the distance between the hive and the food source (Kerr and Esch, 1965; Von Frisch, 1967; Kerr and Rocha, 1988). In January 1989, I saw males of *Melipona rufiventris*, and on November 16, 1989 males of *M. scutellaris* on an artificial food source 12 m from the hives. This was observed again many times. These facts indicate that those males are also able to understand and follow the same communication signs used among their sisters or mothers (0 to 95% of males, according to the species, are sons of the workers; Beig, 1968).

10) Guards. In most meliponid species males leave the colony after migration of spermatozoa from the testes to the seminal vesicles is completed. Then, gradually they increase the distance from the mother hive and have a tendency to congregate near a queenless colony (Kerr *et al.*, 1962; Kerr, 1973). Three times I saw a male of *Melipona scutellaris* that was watching the hive entrance when a common spider (Salticidae) was approaching the entrance in order to catch one of the leaving or entering workers. Suddenly, the male flew toward the spider that jumped and ran away, interrupting the hunting of the day. Therefore, these males can occasionally act as external guards of the colony. Drummond (in press) observed that males of the wasp species *Zethus miniatus* (Eumenidae) have an important role in nest defense during periods when females are absent from the nests. Sphecid males are

also commonly involved in nest guarding (reviewed in Drummond, in press).

All these "worker" tasks carried out by males of stingless bees indicate that many more observations on males of different meliponine and other Hymenoptera species should be made. The observations cited here support the idea that, since the method of worker determination is a lack of activating femaleness-determining genes (due to a small amount of juvenile hormone), workers gradually become very similar to males. Consequently, each mutation that would improve the fitness of the workers would also induce the males to carry out those or part of those same tasks. The main selective forces that would keep males from becoming true workers would be the need to be fit and to be in the right time and in the right place for reproduction (Kerr, 1990).

The Brazilian Indians are very good observers of nature, but they cannot distinguish workers from males in Meliponinae bees; they use the word "fathers" for both adult males and workers of stingless bees.

The first mention in the scientific literature that the males "are very similar in appearance to the workers, at a point that it is not known whether they were among the swarms" ... is from Salt (1929, p. 435).

Workers and males of stingless bees have an external morphology very similar to each other as one can see by the figure painted by Camargo of *Melipona quadrifasciata* (front cover of both the journal "Ciência e Cultura", Kerr, 1973, and the book by Schmidt, 1974). Distinction of males and workers of *Tetragonisca angustula* by one of the greatest experts in bee taxonomy can only be precisely made with the help of a magnifying glass.

Kerr and Cunha (1990) studied, using various methods, the morphological differences and similarities between workers, males and queens. They made 3096 measurements in *Bombus atratus*, 1935 in *Scaptotrigona postica* using 43 characters for each bee; generalized distances of Mahalanobis were estimated for six species; in four species of Meliponinae and in *Bombus atratus* their final morphology indicates that workers of these species are much more similar to males than to queens, contrary to what happens in *Apis* species. The physiological explanation rests in the action of juvenile hormone that, as demonstrated by Campos, Velthuis, Bonetti, Camargo, Cruz-Landim and Kerr, activates the genes that determine femaleness if applied near the prepupal phase; lack of juvenile hormone, due to a small size of the worker *corpora allata* or a small number of cells in the *corpora allata*, caused by haploidy or by a smaller amount of food (Kerr *et al.*, 1966), makes both the integument and the organs derived from the imagi-

nal discs acquire male forms. Larger titers of juvenile hormone activate the battery of genes that determine female characteristics.

Morphological variations are the product of two evolutionary forces: selective (Darwinian) or casual, neutral (Kimuran). For this case of morphological convergence between males and females, it is very difficult to justify a Kimuran process, especially since it happens in a great number of species; therefore, it may be assured with a good degree of certainty that they were submitted to strong and permanent selective pressures. Secondary consequences for the workers were: a) being masculine on the outside, they do not attract males; b) their glandular system is more similar to males than to queens (Mota, 1988; Kerr, 1987a,c,d); c) in many species the workers do not lay eggs even when a colony is queenless for many days (Kerr, 1987a,b).

Hartfelder and Engels (1992) found that among four characters (their Figure 4) when analyzed by double logarithmic plots, in two of them (eye width and tergite 2 length) workers are nearer to males than to queens, in one (head length) the data are overlapping, and the only one that makes the workers closer to queens than to males is the scape. It is not surprising that scape difference between males and females is one of the few to persist since, like distinctive genitalia, it is a very fundamental and evidently ancient sex-specific character. In all Sphecoidea, Apoidea, Vespoidea and Scolioidea there are almost always 13 segments in the male antennae and 12 in the female (Riek, 1970). Figure 5 of Hartfelder and Engels (1992) is very similar to the ones found for four species of *Melipona*, but all of them are very different from *Apis mellifera* (Kerr and Cunha, 1990). Hartfelder and Engels (1992) may have discovered that the development of males and workers follows an allometric growth pattern in order to attain a similar adult appearance, where selection acts.

In all social insects caste is determined by genes and environment. A very elegant work in *Apis mellifera* was carried out by Severson *et al.* (1989). They provided clear evidence that the worker and queen castes show differences in patterns of transcriptional activity during the larval and prepupal stages of development. Gel electrophoresis shows that queens and workers exhibit caste-specific differences in levels of translatable RNAs by the 83rd h after hatching. These differences become more striking by 156 h, with an increase in 70, 68, 47, 36 and 27-kDa translation products in queen prepupae, while worker exhibit an obvious increase in only the 54-kDa translation product. Since queen and worker pupae exhibit less defined caste-specific differences in levels of translatable RNAs the conclusion is that the genic mechanisms of caste determination occur mainly

in the last larval stages and prepupae. These stages are the only ones found in female larvae of *Melipona* species by Campos, Velthuis, Bonetti and Kerr to react with juvenile hormone, transforming 100% of them into queens, when good techniques are used (Bonetti *et al.*, 1995).

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RESUMO

São relatados os primeiros experimentos sobre determinação do sexo em abelhas, iniciando com os de Dzierzon, Meves, Nachtsheim, Paulcke, Petrunkevitch e Manning. Whiting descobriu em *Bracon* alelos múltiplos xo que são considerados a pedra Rosetta da determinação do sexo em himenópteros. O próprio Whiting constatou que algumas espécies de micro-himenópteros não têm alelos múltiplos xo^1 a xo^n . Logo, nos himenópteros há aparentemente dois tipos de determinação do sexo superpostos a haplo-diploidia. Nos panmíticos há uma série de genes $xo^1, xo^2 \dots xo^n$ para os quais os hemizigotos xo^1 a xo^n e homozigotos, xo^1xo^1 até xo^nxo^n são machos e os heterozigotos xo^1xo^2 , até $xo^{n-1}xo^n$ são fêmeas. Nos endogâmicos não há essa série de alelos múltiplos, pois a mutação de x^o para x^n é rapidamente eliminada, a não ser quando o heterozigoto for muito mais viável. Além da hipótese básica de Whiting, discutem-se outras 4 hipóteses que tentam explicar a genética da determinação do sexo nos Hymenoptera. A nova hipótese, da impressão genômica, de Beukeboom, é eliminada pelos seguintes fatos: a) espermatozoides que se desenvolvem em ovos dão origem a tecidos masculinos; b) partenogênese telítoca produz fêmeas e c) as larvas em último estágio, tratadas adequadamente com hormônio juvenil, desenvolvem-se em rainhas. A hipótese que explica todos os casos conhecidos ainda é a do balanço gênico, de Cunha e Kerr, com pequenas modificações. A determinação do sexo nas abelhas sociais implicou a gradual evolução de dois sistemas de determinação das castas. Um em que as rainhas e as operárias se parecem e o macho é diferente (Apíneos), e outro em que as operárias e os machos se parecem e são diferentes da rainha. Neste segundo sistema (Meliponíneos), os machos passam a, gradualmente, assumir

atividades que em *Apis* são claramente de fêmeas. São apresentadas 10 dessas atividades.

REFERENCES

- Adams, J., Rothman, E.D., Kerr, W.E. and Paulino, Z.L. (1977). Estimation of the number of sex alleles and queen matings from diploid male frequencies in a population of *Apis mellifera*. *Genetics* 86: 583-593.
- Beig, D. (1968). Produção de machos e desenvolvimento embrionário em *Trigona (Scaptotrigona) postica*. PhD thesis, UNESP, Rio Claro, SP.
- Beukeboom, L.W. (1995). Sex determination in Hymenoptera: a need for genetic and molecular studies. *BioEssays* 17: 813-816.
- Bonetti, A.M. and Kerr, W.E. (1985). Sex determination in bees. XX. Estudo da ação gênica em *Melipona marginata* e *Melipona compressipes* a partir de análise morfométrica. *Braz. J. Genet.* 8: 629-638.
- Bonetti, A.M., Kerr, W.E. and Matusita, S.H. (1995). Effects of juvenile hormones I, II and III, in single and fractionated dosage in *Melipona* bees. *Braz. J. Genet.* 55 (Suppl. I): 113-120.
- Borstel, R.C. Von and Smith, P.A. (1960). Haploid intersexes in the wasp *Habrobracon*. *Heredity* 15: 29-34.
- Brenha, S.L.A. (1986). Abelhas sociais (Apidae, Apoidea) e seus hospedeiros alimentares em São Luís, MA, Brasil. Monography for obtaining the degree of BSc of Biology. Fed. Univ. Maranhão, São Luís, MA, Brasil.
- Bridges, C.B. (1925). Sex in relation to chromosomes and genes. *Am. Nat.* 59: 127-137.
- Camargo, C.A. (1977a). Properties of the xo gene, sex determination in *Melipona quadrifasciata* Lep. (Hymenoptera, Apidae). VIII Congress Int. Union for the Study of Soc. Insects, pp. 191-192.
- Camargo, C.A. (1977b). Effect of juvenile hormone on diploid drones of *Melipona quadrifasciata* Lep. (Hymenoptera, Apidae). VIIIth Int. Congress IUSSI, pp. 193-194.
- Camargo, C.A. (1979). Sex determination in bees. XI. Production of diploid males and sex determination in *Melipona quadrifasciata*. *J. Apic. Res.* 18: 77-84.
- Camargo, C.A. (1984). Spermatozoa numbers and migration to the seminal vesicles in haploid and diploid males of *Melipona quadrifasciata* Lep. *J. Apic. Res.* 23: 15-17.
- Cameron, S.A. (1985). Brood care by male bumble bees. *Proc. Nat. Acad. Sci. USA* 82: 6371-6373.
- Campos, L.A.O. (1975). Determinação de castas do gênero *Melipona* (Hymenoptera, Apidae). Papel do hormônio juvenil. Master's thesis, Dept. Genética, Fac. Medicina de Ribeirão Preto, USP, Ribeirão Preto, SP, Brasil.
- Campos, L.A.O. (1978). Sex determination in bees. VI. Effect of a juvenile hormone analogue in males and females of *Melipona quadrifasciata* (Apidae). *J. Kans. Entomol. Soc.* 51: 228-234.
- Campos, L.A., Velthuis, K.F. and Velthuis, H.H. (1975). Sex determination in bees. VII. Effect of juvenile hormone

- analogues in caste determination of *Melipona*. *Naturwissenschaften* 62: 98-99.
- Campos, L.A.O., Drummond, M.S. and Lacerda, L.M.** (1983). Determinação do sexo em abelhas. XVIII. Papel do hormônio juvenil I, II, III, na diferenciação das castas de *Scaptotrigona xanthotricha* (Hymenoptera, Apidae). *Ciênc. Cult.* 35: 209-211.
- Carvalho, G.A., Kerr, W.E. and Nascimento, V.A.** (1995). Sex determination in bees. XXXIII. Decrease of xo heteroalleles in a finite population of *Melipona scutellaris* (Apidae, Meliponinae). *Braz. J. Genet.* 18: 13-16.
- Chaud-Neto, J.** (1972). Operárias triplóides em *Apis mellifera*. In: *Em homenagem a Warwick Estevam Kerr*, Rio Claro, Brasil, pp. 63-70.
- Chaud-Neto, J.** (1975). Sex determination in bees. II. Additivity of maleness genes in *Apis mellifera*. *Genetics* 79: 213-217.
- Chaud-Neto, J. and Duarte, F.M.** (1975). Sex determination in bees. V. The action of sexual genes in *Apis mellifera*. *Ciênc. Cult.* 27: 125-129.
- Chaud-Neto, J., Kerr, W.E. and Bezerra, M.A.F.** (1983). Mutation in honeybees. 2. Average rate of mutation based on seven genes for eye colour. *J. Apic. Res.* 22: 17-21.
- Cook, J.M.** (1993). Experimental tests of sex determination in *Goniozus nephantidis* (Hymenoptera: Bethyridae). *Heredity* 71: 130-137.
- Cortopasi-Laurino, M.** (1979). Observações sobre a atividade de machos de *Plebeia droryana* Friese (Apidae, Meliponinae). *Rev. Bras. Ent.* 2: 177-191.
- Crozier, R.H.** (1977). Evolutionary genetics of the Hymenoptera. *Ann. Rev. Entomol.* 22: 263-288.
- Cruz-Landim, C.** (1967). Estudo comparativo de algumas glândulas de abelhas e respectivas implicações evolutivas. *Arq. Zool. São Paulo*, 15: 177-290.
- Cunha, A.B. and Kerr, W.E.** (1957). A genetical theory to explain sex determination by arrhenotokous parthenogenesis. *Forma Functio* (4): 33-36.
- Devlin, R.H., Grigliatti, T.A. and Holm, D.G.** (1985). Gene dosage compensation in trisomics of *Drosophila melanogaster*. *Dev. Genet.* 64: 39-58.
- Dobzhansky, T.** (1930). Genetical and environmental factors influencing the type of intersexes in *Drosophila melanogaster*. *Am. Nat.* 64: 261-271.
- Drory, E.** (1874). Arbeit über Meliponen. *Bienenzeitung* 30: 281-286.
- Drory, E.** (1877). Les mélipones et les trigones. *Rucher J. d'Apic. Rationnelle* 5: 144-149.
- Drory, E.** (1883). Nouvelles observations sur les mélipone. *Le Rucher du Sud-Duest* 1: 59-61.
- Drummond, M.S.** (1997). A system of social interaction in *Zethus miniatus* based on the analysis of descendant viability: consideration on the evolution of eusociality in Hymenoptera. *Braz. J. Genetics* (in press).
- Dzierzon, J.** (1845). Gutachten über die von Herrn Direktor Stohr im ersten und zweiten Kapitel des General-Gutachtens aufgestellten Fragen. *Bienenzeitung* 1: 109-113, 119-121.
- Dzierzon, J.** (1854). Fernere Beobachtungen über Vermehrung und Reinerhaltung der italienischen Bienen. *Bienenzeitung* 10: 251-254.
- Dzierzon, J.** (1898). Widerlegung der jüngst gegen meine Theorie der Fortpflanzung der Bienen erhobene Einwände. 43 Wanderversammlung. Salzburg. *Bienenzeitung* 54: 299-302.
- Dzierzon, J.** (1899). Grundlosigkeit und Haltlosigkeit der neuen Lehre. Erste gemeinsame Wanderversammlung... in Köln a. Rh. *Bienenzeitung* 55: 318-320.
- Engels, E. and Engels, W.** (1988). Age-dependent queen attractiveness for drones and mating in the stingless bees, *Scaptotrigona postica*. *J. Apic. Res.* 27: 3-8.
- Garofalo, C.A.** (1973). Occurrence of diploid drones in a neotropical bumble bee. *Experientia* 29: 726-727.
- Garofalo, C. and Kerr, W.E.** (1975). Sex determination in bees. I. Balance between femaleness and maleness genes in *Bombus atratus*. *Genetica* 45: 203-209.
- Gergen, J.P.** (1987). Dosage compensation in *Drosophila*: evidence that daughterless and sex-lethal control X-chromosome activity at the blastoderm stage of embryogenesis. *Genetics* 117: 477-485.
- Goldschmidt, R.B.** (1958). *Theoretical Genetics*. X. University of California Press, Berkeley, USA, pp. 56.
- Gondim, C.G.E.** (1984). Alguns aspectos da biologia reprodutiva do guaraná (*Paullinia cupana* var. *sorbilis* (Mart., Ducke). Master's thesis, Univ. Amazonas, Manaus, AM, Brasil.
- Hartfelder, K. and Engels, W.** (1992). Allometric and multivariate analysis of sex and caste polymorphism in the neotropical stingless bee *Scaptotrigona postica*. *Insectes Soc.* 39: 1-16.
- Hoshiba, H., Ichiji, O. and Kusanagi, A.** (1981). The diploid drone of *Apis cerana japonica* and its chromosomes. *J. Apic. Res.* 20: 143-147.
- Imai, H.T., Crozier, R.H. and Taylor, R.W.** (1977). Karyotype evolution in ants. *Chromosoma* 59: 341-393.
- Imperatriz-Fonseca, V.L.** (1970). Aspectos da biologia de *Paratrigona subnuda*. Master's thesis, Dept. Zool., Univ. de São Paulo, Brasil.
- Imperatriz-Fonseca, V.L.** (1973). Miscellaneous observations on the behavior of *Schwarziana quadripunctata*. *Biol. Zool. Biol. Mar.* 30: 633-640.
- Kelley, R.L. and Kuroda, M.I.** (1995). Equality of X chromosomes. *Science* 270: 1607-1610.
- Kerr, W.E.** (1950). Estudos sobre a genética de populações de himenópteros em geral e dos apídeos sociais em particular. "Livro Docência" thesis, ESALQ, USP.
- Kerr, W.E.** (1951). Bases para estudo da genética de populações dos Hymenoptera em geral e dos Apinae sociais em particular. *Anais da ESALQ, USP*, Piracicaba, SP, Brasil, 8: 219-354.
- Kerr, W.E.** (1962). Genetics of sex determination. *An. Rev. Entomol.* 7: 157-176.
- Kerr, W.E.** (1973). Genética e Biologia de abelhas. *Ciênc. Cult.* 25: 927-934.

- Kerr, W.E.** (1986a). Determinação do sexo nas abelhas. XVI. Informações adicionais sobre alelos *xo*, *xa* e *xb*. *Rev. Bras. Biol.* 47: 111-112.
- Kerr, W.E.** (1986b). Mutation in bees. 3. Application in bee populations of a mutation rate of $\mu = 1.6 \times 10^{-6}$. *Braz. J. Genet.* 9: 1-10.
- Kerr, W.E.** (1987a). Biologia, manejo e genética de *Melipona compressipes fasciculata* Smith (Hymenoptera, Apidae). Thesis for Full Professor of Genetics, Univ. Federal do Maranhão, S. Luis, Brasil.
- Kerr, W.E.** (1987b). Sex determination in bees. XVII. Systems of caste determination in the Apinae, Meliponinae and Bombinae and their phylogenetical implications. *Braz. J. Genet.* 10: 685-694.
- Kerr, W.E.** (1987c). Sex determination in bees. XXIV. Critical appraisal of sex determination in bees (in Japanese). *Honeybee Sci.* 8: 176-182.
- Kerr, W.E.** (1987d). Sex determination in bees. XXI. Number of *xo*-heteroalleles in a natural population of *Melipona compressipes fasciculata* (Apidae). *Insectes Soc.* 34: 274-279.
- Kerr, W.E.** (1990). Why are workers in social Hymenoptera not males? *Braz. J. Genet.* 13: 133-136.
- Kerr, W.E.** (1994). Communication among *Melipona* workers (Hymenoptera, Apidae). *J. Insect Behav.* 7: 123-128.
- Kerr, W.E. and Cunha, R.** (1990). Sex determination in bees. XXVI. Masculinism of workers in the Apidae. *Braz. J. Genet.* 13: 479-489.
- Kerr, W.E. and Esch, H.** (1965). Comunicação entre as abelhas sociais brasileiras e sua contribuição para o entendimento de sua evolução. *Ciênc. Cult.* 17: 527-538.
- Kerr, W.E. and Rocha, R.** (1988). Comunicação em *Melipona rufiventris* e *M. compressipes*. *Ciênc. Cult.* 40: 1200-1202.
- Kerr, W.E., Zucchi, R., Nakadaira, I.T. and Butolo, J.E.** (1962). Reproduction in the social bees. *J. N. Y. Entomol. Soc.* 70: 265-276.
- Kerr, W.E., Stort, C.G. and Montenegro, M.J.** (1966). Importância de alguns fatores ambientais na determinação de castas do gênero *Melipona*. *An. Acad. Bras. Ciênc.* 32: 149-168.
- Kerr, W.E., Akahira, Y. and Camargo M.A.** (1975). Sex determination in bees. IV. Genetic control of juvenile hormone production in *Melipona quadrifasciata*. *Genetics* 81: 749-759.
- Kerr, W.E., Cunha, R. and Pisani, I.F.** (1978). Genética da determinação do sexo. XII. Aplicação de métodos numéricos para agrupar sexos e castas de *Melipona quadrifasciata anthidioides* Lep. (Apidae). *Rev. Bras. Biol.* 38: 319-324.
- Kerr, W.E., Chaud-Neto, J. and Silva, A.T.** (1980). Mutação em abelhas. I. Taxa de mutação reversa para genes que afetam a cor dos olhos nas abelhas. *Braz. J. Genet.* 3: 275-284.
- Kerr, W.E., Monteiro, S.G. and Kerr, H.A.S.** (1988). Sex determination in bees. XXV. Adaptive value of the *xo*' gene in its origin. *Braz. J. Genet.* 11: 469-473.
- Laidlaw, H.H., Gomes, F.P. and Kerr, W.E.** (1956). Estimation of the number of lethal alleles in a panmitic population of *Apis mellifera*. *Genetics* 41: 179-188.
- Legner, E.F.** (1979). Prolonged culture and inbreeding effects on reproductive rates of two Pteromalid parasites of muscoid flies. *Ann. Soc. Am.* 72: 114-118.
- Maciel-Silva, V. and Kerr, W.E.** (1991). Sex determination in bees. XXVII. Castes obtained from larvae fed homogenized food in *Melipona compressipes* (Hymenoptera, Apidae). *Apidologie* 22: 15-19.
- Mackensen, O.** (1951). Viability and sex determination in the honey bee (*Apis mellifera*). *Genetics* 36: 500-509.
- Mackensen, O.** (1955). Further studies on a lethal series in the honey bee. *J. Hered.* 46: 72-74.
- Makino, S.** (1951). *An Atlas of the Chromosome Numbers in Animals*. 2nd edn. (First American Edition). XXVIII. 290. The Iowa State College Press, Ames, Iowa, USA.
- Manning, F.J.** (1950). Sex determination in the honey bee. V. The sperm. *Microscope* 7: 303-305.
- Meves, F.** (1907). Die Spermatocytenteilung bei der Honigbiene (*Apis mellifica* L.), nebst Bemerkungen über Chromatinreduktion. *Arch. Mikr. Anat. Entw.* 70: 414-491 + 5 tables.
- Mitchell, T.B.** (1929). Sex anomalies in the genus *Megachile*, with descriptions of new species (Hymenoptera, Megachilidae). *Trans. Am. Entomol. Soc.* 54: 321-383.
- Mitchell, T.B.** (1941). Some additional intersexes in *Megachile* (Hymenoptera, Megachilidae). *Pan-Pac. Entomol.* 17: 165-168.
- Mota, M.H.V.B.** (1988). Estudo comparativo das glândulas epidérmicas do abdômen dos meliponíneos (Hymenoptera, Apidae, Meliponinae). Doctoral thesis. IBRC/UNESP, Rio Claro, SP, Brasil.
- Nachtsheim, H.** (1913). Cytologische Studien über die Geschlechtsbestimmung bei der Honigbiene (*Apis mellifica* L.). *Arch. Zellforsch.* 11: 169-241.
- Nogueira-Neto, P.** (1963). Novas pesquisas sobre Meliponíneos. *Chácaras Quintaes* 108: 2690-2691.
- Nogueira-Neto, P.** (1964). Abelhas indígenas sem ferrão - algumas observações. *Chácaras Quintaes* 110: 691-692.
- Oliveira, D.A.G.** (1992). Compensação de dose em *Scaptotrigona postica* (Hymenoptera, Apidae) e em *Melittobia hawaiiensis*. Master's thesis, UNESP, Rio Claro, SP.
- Oliveira, D.A.G., Kerr, W.E. and Palma, M.S.** (1992). Compensação de dose em *Scaptotrigona postica* (Hymenoptera, Apidae). Proceedings of the X Congresso Latino Americano e XXXVIII Congresso Nacional de Genética. *Braz. J. Genet.* 15 (Suppl. 1): 256-260.
- Parkhurst, S.M., Bopp, D. and Horowicz, I.S.H.** (1990). X:A, The primary sex-determining signal in *Drosophila* is transduced by Helix-loop proteins. *Cell* 63: 1179-1191.
- Paulcke, W.** (1899). Zur Frage der parthenogenetischen Entstehung der Drohnen (*Apis mellifica*). *Anat. Anz.* 16: 185-187.
- Petrunkewitsch, A.** (1901). Die Richtungskörper und ihr Schicksal im befruchteten und unbefruchteten Bienen. *Zool. Jahrb. Anat. u. Ont.* 14: 573-608.
- Quintero, D.A. and Cambra, R.A.T.** (1994). Systematics of *Pseudomethoca areta* (Cameron): sex association, description of the male and a gynandromorph and a new

- synonymy (Hymenoptera, Mutillidae). *J. Hym. Res.* 3: 303-308.
- Riek, E.F.** (1970). Hymenoptera (wasps, bees, ants). In: *The Insects of Australia* (Michener, C.D., ed.). Division of Entomology, C.S.I.R. Org., Canberra, Australia, pp. 867-983 (pages 943-951, on bees).
- Rothenbuler, W.** (1957). Diploid male tissue as new evidence on sex determination in honey bees. *J. Hered.* 48: 160-168.
- Rothenbuler, W.C., Gowen, J.W. and Park, W.** (1952). Androgenesis and zygogenesis in gynandromorphic honeybees (*Apis mellifera* L.). *Science* 115: 637-638.
- Salt, G.H.** (1929). A contribution to the ethology of the Meliponinae. *Trans. Ent. Soc.* 77: 431-470.
- Schmidt, G.H.** (1974). Sozialpolymorphismus bei Insekten. Wissenschaftliche Verlag, MBH Stuttgart, Germany.
- Severson, D.W., Williamson, J.L. and Aiken, J.M.** (1989). Caste-specific transcription in the female honey bee. *Insect Biochem.* 19: 215-220.
- Siebold, C.TH.** (1856a). Wahre Parthenogenesis bei Schmetterlingen und Bienen. Engelmann, Leipzig.
- Siebold, C.TH.** (1856b). Die Drohneneier sind nicht befruchtet. *Bienenzeitung* 12: 181-184.
- Silva, D.L.N.** (1977). Estudos bionômicos em colônias mistas de Meliponinae (Hymenoptera, Apoidea). *Bol. Zool. Univ. S. Paulo* 2: 7-106.
- Tucker, K.W.** (1958). Automitotic parthenogenesis in the honey bee. *Genetics* 43: 299-316.
- Velthuis, H.H.W. and Sommeijer, M.J.** (1991). Roles of morphogenetic hormones in caste polymorphism in stingless bees. In: *Morphogenetic Hormones in Arthropods: Roles in Histogenesis, Organogenesis and Morphogenesis* (Gupta, A.P., ed.). Rutgers University Press, New Brunswick, New Jersey, USA, pp. 346-383.
- Von Frisch, K.** (1967). *The Dance Language and Orientation of Bees*. Belknap Press of Harvard Univ. Press, Cambridge, MA, USA.
- Whiting, P.W.** (1943). Multiple alleles in complementary sex determination of *Habrobracon*. *Genetics* 28: 365-382.
- Whiting, P.W.** (1945). The evolution of male haploidy. *Quart. Rev. Biol.* 20: 231-260.
- Wilson, F. and Woolcock, L.T.** (1960). Temperature determination of sex in a parthenogenetic parasite, *Ooencyrtus submetallicus* (Howard) (Hymenoptera: Ooencyrtidae). *Aust. J. Zool.* 8: 153-169.
- Woyke, J.** (1963). Drone larvae from fertilized eggs of the honeybee. *J. Apic. Res.* 2: 73-76.
- Woyke, J.** (1979). Sex determination in *Apis cerana indica*. *J. Apic. Res.* 118: 122-127.
- Woyke, J.** (1986). Sex determination. In: *Bee Genetics and Breeding* (Rinderer, T.E., ed.). Academic Press Inc., New York, pp. 91-119.

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