

## SEX DETERMINATION IN BEES. XVII. SYSTEMS OF CASTE DETERMINATION IN THE APINAE, MELIPONINAE AND BOMBINAE AND THEIR PHYLOGENETICAL IMPLICATIONS\*

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### ABSTRACT

The generalized distances of Mahalanobis between queens, males and workers have been estimated in the literature for 5 Apidae species. Queen-to-worker distance is shorter than male-to-worker distance in *Apis mellifera* (0.6), but is longer, i.e., workers resemble more the males than the true females (queens) in *Melipona compressipes* (1.8), *M. marginata* (1.4), *M. quadrifasciata* (1.1) and *Melipona scutellaris* (1.7). This paper shows that the ratio of queen-to-worker distance divided by male-to-worker distance in *Bombus atratus* (2.7) is similar to that found in *Melipona*. The proposed hypothesis is that juvenile hormone levels above some threshold turn on the femaleness determining genes in Bombinae and Meliponinae and this determines queen development; its production below a given level does not turn on the femaleness determining genes and thus the female pupae acquire masculinoid characters. A phylogenetic diagram of relationships is proposed that tentatively puts both Bombinae and Meliponinae closer to each other than any of them to Apinae.

### INTRODUCTION

Bee species have two peculiarities in their sex determination. First, like the majority of Hymenoptera, they are haplo-diploid organisms with two sets of genes, one acting during the first hours of embryo development and the other after the larvae

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finish eating. The first set comprises one out of 20 *xo* heteroalleles plus some minor genes that primarily determine ovaries, genitalia and antennae. According to the genic balance hypothesis (Cunha and Kerr, 1957; Kerr and Nielsen, 1966; Kerr, 1969; Kerr *et al.*, 1975) the fact that haploids are males and diploids (for this same set of genes) are females can be explained by assuming that male determining genes are nonadditive (compensated) or only slightly additive, and female determining genes are fully additive (dose-dependent). This hypothesis explains all the cases of endogamous microhymenoptera studied thus far.

In many Hymenoptera groups, one of the female determining genes (responsible for ovary determination) mutated from *xo* to  $xo^1$ , an event that probably occurred several times independently. Likely, in a hymenopterous group in which  $xo^1$  appears, it was a mutation that was selected for. The assumption is that  $xo^1/xo^1$  were diploid drones which acted as diploid-limited lethals, and this would only be compensated for by the higher adaptive value of the  $xo/xo^1$  heterozygote or of the male  $xo^1$ ; each new mutation  $xo^2$ ,  $xo^3$ , ...  $xo^{20}$  reduced the probability of a diploid drone appearing ( $xo^1/xo^1 : xo^2/xo^2, \dots, xo^{18}/xo^{18}$ ) and, therefore, had higher probability to become established. These *xo* heteroalleles are responsible for the determination of ovaries (when heterozygous) in the first hours of embryonic development and in later larval stages, for the female-type antennae and genitalia.

In the following hymenopterous species diploid males have been found: *Bracon hebetor* (Whiting, 1943); *Neodiprion nigroscutum* (Smith and Wallace, 1971); *Nasonia vitripennis* (not biparental) (Whiting, 1960); *Apis mellifera* (Mackensen, 1951); *Melipona quinquefasciata* (Kerr and Silveira, 1972; only interpreted as diploid males when Tarelho, p.c., found haploid males of this species with 9 and 10 chromosomes); *Tetragona quadrangula* (Tarelho, 1973); *Bombus atratus* (Garofalo, 1973); *Melipona quadrifasciata* (Camargo, 1974, 1977, 1979); *Apis cerana* (Woyke, 1979; Hoshiba *et al.*, 1981); *Melipona compressipes* (Kerr, 1987); *Scaptotrigona postica* (Kerr, 1986) and the ants (apud Ross and Fletcher, 1985): *Pseudolasius* sp (Hung *et al.*, 1972), *Rhytidoponera* spp. (Ward, 1980), *Lasius alienus* and *L. niger* (Pearson, 1983), *Formica pressilabris* (Pamilo and Rosengren, 1984), *Solenopsis invicta* (Hung *et al.*, 1974; Glancey *et al.*, 1976; Ross and Fletcher, 1985). Diploid drones were found only in panmictic species; no diploid drones were normally found in species with autogamic reproduction. In dozens of microhymenoptera no diploid males ever appeared in controlled inbred lines (*Telenomus*, *Melittobia*, etc.) and in a great number of species males inseminate sisters soon after they emerge (e.g. Agaonidae). In order to promote the differentiation of the adult male and female characters another set of sex genes acts (Kerr, 1975). In the highly social bees, the small larva develops until the end of the last instar with non-differentiated integument and imaginal discs. Right after its last feeding there is production of Juvenile Hormone (JH), proportional both to the amount of food received and to the size of the *corpora allata* (Beig, 1968, 1972;

Kerr *et al.*, 1975). In *Apis*, even with insufficient JH, some femaleness genes act. By means of the generalized distance of Mahalanobis, one can see that *Apis* workers are much closer to queens than to males. In Meliponids food quantity increases the size and number of cells of the *corpora allata*; high production of J.H. puts femaleness genes to work, which predominate over male determining genes, and the bee will become a queen; but if the JH does not exceed a certain threshold then the female determining genes do not come into action and the bee will be a female inside, but masculinoid outside, i.e. a worker. At first sight it is very difficult to distinguish a meliponid worker from a male of the same species (Kerr, 1975).

Therefore, there are two distinct ways to produce caste in bees; the *Apis* system and the meliponid system. The objective of this paper is to find which system *Bombus* belongs to.

The method of estimating the generalized distance ( $D^2$ ) of Mahalanobis has been used by Pisani and collaborators (literature in Pisani *et al.*, 1968) to estimate genetical distances between different species of Apidae. The generalized distances between males, queens and workers of *Melipona quadrifasciata* (Kerr, 1974; Campos, 1975), *M. scutellaris* (Almeida, 1985); *M. compressipes* and *M. marginata* (Bonetti and Kerr, 1985) and *Apis mellifera* (Kerr, 1974) have been estimated using the same technique. In the present study this technique was applied to *Bombus atratus*.

## MATERIAL AND METHODS

Thirty three males, 27 workers and 22 queens of *Bombus atratus* were collected from three nests in Ribeirão Preto, São Paulo, Brazil. The nests were strong ones at the stage when males and queens begin to emerge and each provided about the same numbers of queens, males and large workers. The workers selected were the heaviest bees, to avoid size bias in comparison with queens.

Forty-three morphological characters listed in Table I were measured on each bee using an ocular micrometer and stereomicroscope. The data were analyzed by the method of Pisani *et al.* (1968) and the final distances were standardized by multiplying by 100 and dividing by the distance between males and queens.

## RESULTS

Tables with data concerning individual measurements and containing the Rao coefficient vectors are available at this Journal office upon request.

The  $D^2$  values obtained are given in Table II. The D values obtained after correcting queen-to-drone distance to 100 are given in parentheses in the same Table.

Table I - List of the measurements of *Bombus atratus* used in this paper.

Items marked by arrows are the ones used for estimating $D^2$	
C01 - Costal vein	C23 - Length of hind basistarsus
C02 - First discoidal cell	C24 - Length of hind femur
C03 - Anal lobe	C25 - Length of flagellum
C04 - Second discoidal cell	→ C26 - Length of scape
→ C05 - Sub-median cell	C27 - Width of scape
C06 - Median cell	C28 - Length of pedicel
C07 - First sub-marginal cell	C29 - Width of pedicel
C08 - Length of anterior wing	C30 - Total width of head
C09 - Width of anterior wing	→ C31 - Total length of head
C10 - Width of posterior wing	C32 - Maximum interocular distance
→ C11 - Length of posterior wing	C33 - Superior interocular distance
C12 - Number of hamuli	C34 - Inferior interocular distance
C13 - Width of eye	C35 - Interocellar distance
C14 - Length of eye	→ C36 - Ocellorbital distance
C15 - Width of third abdominal segment	C37 - Alveolorbital distance
→ C16 - Width of mesoscutum	C38 - Alveolocellar distance
C17 - Length of mesoscutum	C39 - Diameter of antennal alveolus
C18 - Length of mesonotum	C40 - Length of clypeus
C19 - Width of scutellum	→ C41 - Width of clypeus
C20 - Length of scutellum	C42 - Clipeocellar distance
→ C21 - Length of hind tibia	C43 - Clipeorbital distance
C22 - Width of hind basistarsus	

Table II - Mahalanobis generalized distances between queens, workers and drones of *Bombus atratus* given as  $D^2$ , D and percentages.

Intercaste distance	$D^2$	D	Standard value
Queens to males	8651.47	93.01	(100.00)
Queens to workers	6039.99	77.72	( 83.56)
Workers to males	849.64	29.15	( 31.34)

## DISCUSSION

After standardization to units of 100, these results may be compared with others in the literature. See Table III, in which  $D^2$  was reduced to D and to percentage for comparison among six species of Apidae bees.

Table III - Generalized distances (D) of Mahalanobis between queens, workers and males in six species of Apidae.

Species	Generalized distance (D)				References
	Queen to	Queen to	Male to	B/C	
	male	worker	worker		
	A	B	C		
<i>Bombus atratus</i>	100	83.56	31.34	2.7	This paper
<i>Melipona compressipes</i>	100	76.58	41.99	1.8	Bonetti and Kerr, 1985
<i>Melipona marginata</i>	100	56.27	41.07	1.4	Bonetti and Kerr, 1985
<i>Melipona quadrifasciata</i>	100	55.26	52.02	1.1	Kerr, 1974
<i>Melipona scutellaris</i>	100	134.1	86.1	1.6	Almeida, 1985
<i>Apis mellifera</i>	100	59.93	109.06	0.6	Kerr, 1974

Two different groups are formed: *Bombus* and *Melipona* on one side, in which workers are closer to males than to queens, and *Apis* on the other, in which workers are closer to queens.

Kerr (1974, 1975), Kerr *et al.* (1975), Campos (1975, 1978, 1979) and Campos *et al.* (1975) have gradually demonstrated that the worker caste of *Melipona* evolved as a byproduct of sex determination by a mechanism in which female determining genes do not work unless sufficient Juvenile Hormone is present. When there is a lack or deficient production of JH in diploid prepupae, female determining genes are not put into action and the final development of both integument and imaginal discs is toward male characteristics.

According to the above results, this caste determination mechanism also exists in *Bombus atratus* and by visual comparison it exists also in all stingless bees I know of, but not in *Apis*. Since caste determination is basic to the evolution of social life, our findings suggest that Meliponinae and Bombinae may be closer to each other than either is to Apinae, and very likely this method of repression of female determining genes is the most evolved system of caste determination among social Apidae. Alternatively, of course, the common features of Meliponinae and Bombinae could be a result of parallel evolution.

The phylogenetical relationships between Bombinae and Meliponinae are strengthened by other characteristics, e.g., eyes with no hairs, the young queen leaving the nest to found a new colony, brood in clusters, pots for honey and pollen reserves, tubes for pollen in some species, sheets of wax for covering the brood (these are quite similar between *Bombus* and many stingless bees), ability to get pollen of *Cassia*,

*Solanum*, etc, by vibration of anthers with their mandibles and thorax, type of ovary and testis (with few and long ovarioles and testioles in both *Meliponinae* and *Bombinae* and many and short ones in *Apis*), and, according to this paper, the system of caste determination.

Winston and Michener (1977), based on some morphological characters and on pollen collection behavior, propose a dual origin for highly social behavior among bees with which the present data agree.

They propose the dendrogram shown in Figure 1, but it appears that the dendrogram of Figure 2 is equally possible. More research is necessary to decide upon these hypotheses.

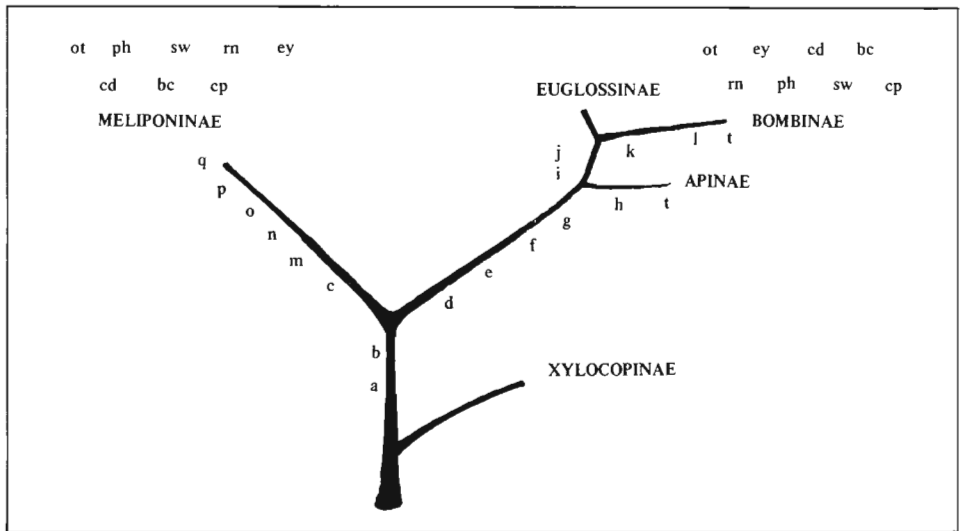


Figure 1 - Dendrogram of Winston and Michener (1977) where *a* = presence of corbicula; *b* = rastellum; *c* = penicillum; *d* = auricle; *e* = maxilla process lost; *f* = subgaleal sclerite lightly sclerotized and straight; *g* = reduced stigma of the forewing; *h* = elongated and submarginal cells; *i* = large size; *j* = papillate distal wing membranes; *k* = loss of jugal lobes of the hind wings; *l* = large number of grooves on the outer mandibular surfaces; *m* = reduced sting; *n* = reduced tergal and external apodemes; *o* = reduced wing venation; *p* = pollen gathered by fore basitarse is brushed off onto hairs of the thoracic venter, picked up by the middle legs and transferred to the hind legs; *q* = lack of tibial spurs; *t* = progressive feeding. Characters that are similar, at least in some genera, between *Bombinae* and *Meliponinae* have two letters and are: *bc* = brood in cluster; *cd* = caste determination by masculinization of workers; *cp* = ability to collect pollen by vibrating anthers holding them with mandibles; *ey* = eyes without hairs; *ot* = ovaries and testes with 4 long ovarioles and 4 testioles, respectively; *ph* = pots for honey; *m* = colony founded by young queens (young queens leave the nest); *sw* = sheets of wax to protect against cold weather.

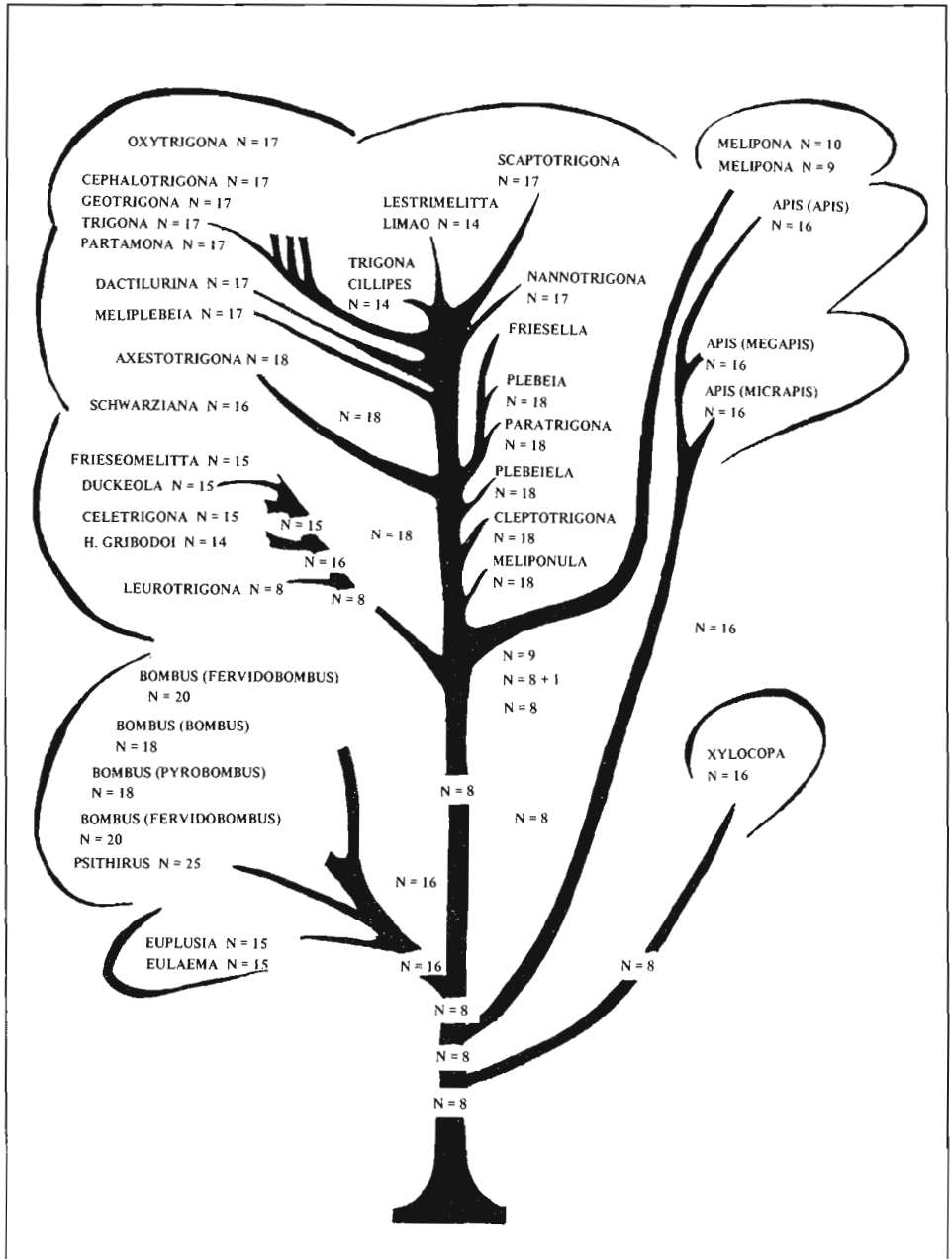


Figure 2 - Dendrogram redrawn and enlarged from the one proposed by Mello and Kerr (1984). The chromosome numbers in *Bombus* are according to Tarelho (1973) and Owen (1983). The chromosome number of *Eulaema* is described by Pompolo *et al.* (1986). N= Number of chromosomes.

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## RESUMO

As distâncias generalizadas de Mahalanobis já foram estimadas para 5 espécies de abelhas. A distância relativa entre rainha-e-operária é menor que entre macho-e-operária em *Apis mellifera* (0,6), porém é maior (isto é, as operárias parecem-se mais com os machos que com as fêmeas férteis) em: *Melipona compressipes* (1,8), *M. marginata* (1,4), *M. quadrifasciata* (1,1) e *M. scutellaris* (1,6). Esta publicação demonstra que o mesmo que ocorre com as *Melipona* acontece também com *Bombus atratus* (2,7). A hipótese proposta aqui é que o Hormônio Juvenil (HJ) após um dado limiar aciona os genes determinadores de feminidade nos Bombinae e Meliponinae e isso determina rainhas nas larvas bem nutridas; a produção de HJ abaixo de um certo nível não põe a funcionar os genes determinadores de feminidade e, portanto, a pupa fêmea adquire caracteres masculinoides. Um diagrama de interrelações filogenéticas é proposto que, tentativamente, põe Bombinae e Meliponinae mais próximos entre si que qualquer deles aos Apinae.

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