

KARYOTYPIC EVOLUTION OF BEES AND CORRESPONDING TAXONOMIC IMPLICATIONS¹

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Karyotype evolution is one of the most important aspects of the whole evolutionary process (White, 1964). The orders which are closely related phylogenetically to Hymenoptera are: Strepsiptera, Coleoptera, Raphidioidea, Neuroptera, Megaloptera according to Wille (1960) and many earlier authors and Diptera, Mecoptera and Trichoptera according to Martynova (1959). The overwhelming majority of the species in these orders are diplo-diploid. The chromosomal catalog of Makino (1951) cites sex determination of the type XX females and XY or XO males for all these orders except for the Trichoptera and certain Coleoptera. In Coleoptera, mechanisms like XY, XO, parachute Y, X_1X_2-O , neo-X, three cases of arrhenotokous parthenogenesis, and one case of thelytokous parthenogenesis are found (White, 1954; Smith, 1971). In Trichoptera, males are XX and females XY, making Wille's ideas seem more likely. The great majority of hymenopteran species have arrhenotoky, that is, they are haplo-diploid organisms. However it is quite possible that, due to its origin from a diplo-diploid group, the genomes of many present day species still contain many genes that would provide better adaptive value in a diplo-tetraploid system than in a haplo-diploid one. In order to reach a diplo-tetraploid state for a gene or a group of genes, the species could

follow one of the following five paths: duplication, repeats, iso-chromosomes, polyploidy and polyploidy. Once polyploidy is attained, some genes which would be of greater adaptive value in a haploid system could come back through mutation, deletion, loss of a chromosome, or Robertsonian fusion (which produces a small deletion).

Muller (1925) proposed the hypothesis that complex sex determination mechanisms would prevent polyploidy in animals. However, when the gene number for sex determination is small, if polyploidy conferred better adaptation in some niches, sex genes would be rapidly reorganized in order to produce perfect males and females. The chromosome numbers published for all bees (Table 1) suggest that polyploidy originated independently at least 5 times: 1) From an ancestor of *Augochloropsis sparsilis* $n = 8$, to *Pseudoaugochloropsis graminea* $n = 16$; 2) from an ancestor of *Leurotrigona muelleri* $n = 8$ to *Frieseomelitta* (3 species) $n = 15$; 3) from an ancestral Trigonini $n = 9$ to *Plebeia* (6 species) $n = 18$; 4) from an ancestor of *Melipona quadrifasciata*, *Melipona marginata* and other species $n = 9$ to *Melipona quinquefasciata* $n = 18$; 5) from an ancestor of *Apis florea* $n = 8$, to *Apis cerana* and *Apis mellifera* $n = 16$. These facts indicate that some 65% of the total number of known species of bees (assuming our sample is random) are either polyploid or of polyploid origin; there were at least 5 or 6 independent events for establishing the doubling of chromosomes, implying that such a mechanism is very important in the evolution of bees (Kerr, 1952). Another possible conclusion is that the number of sex genes should be small, very likely a

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TABLE 1. Chromosome number in species of *Apoidea*.

Species	Spermatogenesis				Somatic Chromosomes		References
	Prophase I	Metaphase I	Metaphase II (in each pole)	Anaphase II	males	females	
<i>Lasio glossum rhytidophorum</i> (Halictidae)	12	6	6	6	-	12	Kerr, 1972
<i>Augochloropsis sparsilis</i> (Halictidae)	8	16	16	16	-	-	Kerr, 1972
<i>Pseudoaugochloropsis graminea</i> (Halictidae)	-	8	8	8	-	-	Kerr, 1972
<i>Augochlorella michaelsi</i> (Halictidae)	16	16	16	16	-	-	Kerr, 1972
<i>Osmia cornuta</i> (Megachilinae)	-	16	16	-	-	-	Armbruster, 1913
<i>Isepeolus viperinus</i> (Anthophoridae)	-	-	16	-	-	-	Kerr, 1972
<i>Exomalopsis (E.) aureopilosa</i> (Anthophoridae)	9	9	9	9	-	-	Kerr, 1972
<i>Xylocopa violacea</i> (Xylocopinae)	-	16	16	-	-	-	Granata, 1909
<i>Xylocopa fenestrata</i> (Xylocopinae)	-	16	16	16	16	32	Kumbkarni, 1965
<i>Bombus (F.) morio</i>	-	20	20	20	-	-	Silveira and Kerr, 1971
<i>Bombus (F.) atratus</i>	-	20	20	20	-	-	Silveira and Kerr, 1971
<i>Leurotrigona muelleri</i>	-	8	8	8	-	-	Silveira and Kerr, 1971
<i>Hypotrigona braunsi</i>	-	-	14 (or 15)	-	-	-	Kerr, 1972
<i>Hypotrigona gribodoi</i>	-	-	14 (or 15)	-	-	-	This paper
<i>Friesomelitta (Duckeola) ghiliani</i>	-	15	15	-	-	-	Kerr, 1972
<i>Friesomelitta (Friesomelitta) doederleini</i>	-	15	15	15	-	-	Silveira and Kerr, 1971
<i>Friesomelitta (Friesomelitta) varia</i>	-	15	15	15	-	-	Silveira and Kerr, 1971
<i>Celetrigona longicornis</i>	-	-	15	-	-	-	Silveira (pers. comm.)
<i>Meliponula bocandei</i>	9	9, 18	18	18	-	-	Kerr and Araujo, 1957
<i>Cleptotrigona cubiceps</i>	9	9?	18	18	-	-	Kerr and Araujo, 1957
<i>Azeotrigona ferruginea</i>	18	18	18	18	-	-	Kerr and Araujo, 1957
<i>Plebeia (Plebeia) droryana</i>	9	18	18	18	-	-	Kerr, 1952 published with n = 9
<i>Plebeia (Plebeia) emerina</i>	9	18	18	18	-	-	Kerr, 1972
<i>Plebeia (Plebeia) remota</i>	9	18	18	18	-	-	Kerr, 1972
<i>Plebeia (Friesella) schrottkyi</i>	9	18	18	18	-	-	Silveira and Kerr, 1971
<i>Plebeia (Plebeia) denotii</i>	9	18	18	18	-	-	Kerr, 1972

TABLE 1. (Continued).

Species	Spermatogenesis				Somatic Chromosomes		References
	Prophase I	Metaphase I	Metaphase II	Anaphase II (in each pole)	males	females	
					Chromosomes		
<i>Plebeia (Paratrigona) subnuda</i>	9	18	18	18	-	-	Kerr, 1972
<i>Tetragona (Tetragona) clavipes</i>	-	17	17	17	-	-	Silveira and Kerr, 1971
<i>Tetragona (Tetragonisca) angustula</i>	-	17	17	17	-	-	Silveira and Kerr, 1971
<i>Nannotrigona testaceicornis</i>	8, 9, 10	17	17	17	-	-	Silveira and Kerr, 1971
<i>Dactilurina staudingeri</i>	-	17	17	17	-	-	Kerr, 1972
<i>Meliplebia beccarii</i>	-	-	17	17	-	-	Kerr, 1972
<i>Trigona (Geotrigona) subterranea</i>	-	17	17	17	-	-	Silveira and Kerr, 1971
<i>Trigona (Trigona) fuscipennis</i>	-	17	17	17	-	-	Silveira and Kerr, 1971
<i>Trigona (Trigona) spinipes</i>	-	17	17	17	-	-	Silveira and Kerr, 1971
<i>Partamona (Partamona) cupira</i>	-	17	17	17	-	-	Silveira and Kerr, 1971
<i>Scaptotrigona postica</i>	-	17	17	17	-	-	Silveira and Kerr, 1971
<i>Scaptotrigona depilis</i>	-	17	17	17	-	-	Silveira and Kerr, 1971
<i>Oxytrigona tataira</i>	-	17	17	17	-	-	Kerr, 1972
<i>Melipona rufiventris</i>	-	-	-	-	-	18	Kerr, 1952
<i>Melipona nigra</i>	-	9	9	9	9	18	Kerr, 1952
<i>Melipona subnitida</i>	-	9	9	9	9	-	Silveira and Kerr, 1971
<i>Melipona interrupta fasciculata</i>	-	9	9	9	-	18	Kerr, 1952
<i>Melipona compressipes manausensis</i>	-	9	9	9	-	-	Kerr, 1972
<i>Melipona marginata marginata</i>	9	9	9	9	9	18	Kerr, 1948
<i>Melipona quadrijasciata</i>	9	9	9	9	9	18	Kerr, 1948
<i>Melipona quinquefasciata</i>	18	18	18	18	-	-	Kerr, 1972
<i>Apis (Megapis) dorsata</i>	-	-	8?	-	-	-	Deodikar and Thakar, 1966
<i>Apis (Mitrapis) florea</i>	8	8	8	8	-	16	Thakar and Deodikar, 1966
<i>Apis (Apis) cerana indica</i>	-	16	16	-	16	32	Deodikar, Thakar and Shah, 1959
<i>Apis (Apis) mellifera mellifera</i>	-	16	16	16	16	32	Meves, 1907
<i>Apis (Apis) mellifera adansonii</i>	-	16	16	16	-	-	Kerr, 1969

TABLE 2. Comparison of data for Morabinae and Bees.

	Morabinae	Bees
Fusions	21.3%	25%
Dissociations in the X	0	0
Polyploidy (ancient or recent)	0	67%

few major genes. There are two femaleness loci (x_a and x_b) with two alleles each known in *Melipona*, and in *Apis mellifera* only one femaleness locus with 12 alleles (x^1 to x^{12}) is known (Kerr, 1969; Mackensen, 1951).

Among 19 species of bees with $n = 18$ and $n = 17$ chromosomes, 10 have $n = 17$; in all these 10 species it is easy to detect a chromosome larger than the others and presumably the result of Robertsonian fusion. Since fusions only happened, in our sample, in diplo-tetraploid species, it is reasonably easy to know which two chromosomes fused (observing the pairing in prophase, and/or grouping the chromosomes by size and centromere position); among these 10 there are 4 independent fusions. One other group of Robertsonian fusions may have occurred in the *Hypotrigona* and *Frieseomelitta* group. *Leurotrigona muelleri* has $n = 8$ chromosomes, while *Frieseomelitta* (both subgenera) have $n = 15$; also *Hypotrigona braunsi* (from Angola) has $n = 14$ (*H. gribodoi*, from Moçambique also has $n = 14$, however they may be synonymous). These data suggest a possible polyploid intermediate $n = 16$ followed by one fusion (*Frieseomelitta* $n = 15$) or two fusions (*H. braunsi*, *H. gribodoi* $n = 14$). Therefore, 14 of 24 species have single fusions, and one species has two fusions; our data indicate 6 independent fusion origins. The frequency of independent fusions was 6/24 (= 25%); from this one would expect two fusions in 6.25% of the cases, a value which is not statistically different from what was found (1 in 24, or 4.2%). It is worth comparing our data with those obtained by White (1964) for 160 species of Australian grasshoppers of the subfamily Morabinae (Table 2).

White suggests that the lack of dissociations in the X has its root in the fact that a dissociation in the X would give the heterogametic sex two sex determining mechanisms segregating independently, leading to disturbances in sexual differentiation. Since in the Hymenoptera there is no heterogametic sex, and no dissociations were found, it is possible that some other reason, based on the haplo-diploidy itself, should be looked for.

The basic chromosome number in *Apis* and meliponids is $n = 8$; therefore, species of *Melipona* with $n = 9$ may be tentatively interpreted as having originated through polysomy. If this is the case, its rarity suggests that its establishment is difficult.

No case of tetravalents has been found in females of *Plebeia* species ($n = 18$) nor in females of any other diplo-tetraploid species. Actually, tetravalents would not be expected even in relatively recent polyploids, since diminishing of pairing is achieved rapidly by natural selection. Sears and Okamoto (1958) found a gene in chromosome V of wheat that reduces affinity between chromosomes, reestablishing order in the meiotic pairing in hexaploids.

However, in males there is strong pairing of chromosomes during meiosis of some presumed diplo-tetraploid species, in such a way that Kerr (1952) erroneously reported *Plebeia droryana* as having $n = 9$ chromosomes, since only diakinesis was observed at that time. In *Meliponula bocandei*, paired homologs are seen with greater clarity; in first and second metaphase, 18 chromosomes are counted. This suggests that natural selection has not acted to reduce the effects of the pairing protein (Stern and Hotta, 1968) as in females. It is likely that such a mechanism, that would cause the chromosomes to stick together, was very important in the possible origin of the Hymenoptera from a diplo-diploid stock with AAXX females and AAXO males. The subsequently originated AX haploid males would have a tendency to segregate the chromosomes with production of aneuploids (Hartl and Brown, 1970).

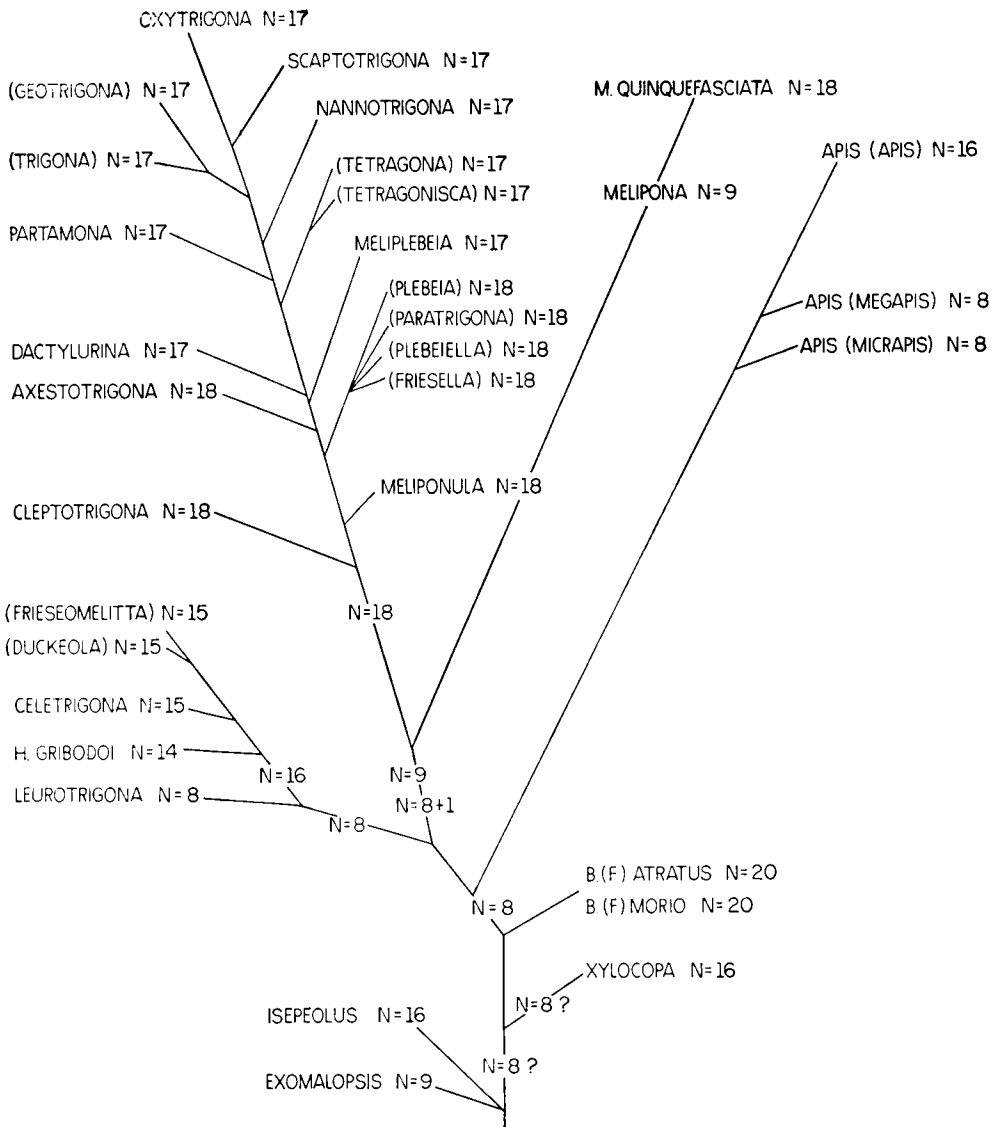


FIG. 1. Diagram of relationships among genera and subgenera of social Apidae and respective chromosome numbers. *Hypotrigona gribodoi*, from Moçambique (probably equal to *H. braunsi*, from Angola) *Bombus morio* and *B. atratus* have been cited under species names since it is expected that numbers different than those shown may be found. Three non-apid genera are mentioned (*Isepeolus*, *Exomalopsis* and *Xylocopa*) because they belong to the near family Anthophoridae. *M. quinquefasciata* is the only *Melipona* species with the number of chromosomes different than 9.

An excess of pairing protein in male meiotic cells would make those chromosomes stick together and they would then migrate all to one pole in the abortive first division. It is very likely that the persistent nuclear membrane (intra-nuclear spindle in the

first division) was selected for this same function.

The phylogenetic interrelationships among genera and subgenera of Apidae are being studied by taking into account morphology, ethology, caste determination, nest

type, communication, endocrine system, etc. Diagrams of relationships have been presented by several authors (Michener, 1944; Kerr and Esch, 1965; Cruz-Landim, 1967, Kerr, 1969) based on the available information. For the special case of the family Apidae all the previous data have been combined with the analyses of the chromosome numbers mentioned in Table 1 in order to produce Figure 1.

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