

EVOLUTION OF THE MECHANISM OF CASTE DETERMINATION IN THE GENUS *MELIPONA*

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INTRODUCTION

Social bees (*Apidae*) belong to three tribes and five genera: *Bombus* (*Bombini*), *Apis* (*Apini*) and *Trigona*, *Lestrimelitta* and *Melipona* (*Meliponini*). These three tribes are phylogenetically related to one another possessing certain characters in common, and thus are united in the same subfamily (Apinae). Their morphological relationships were studied by Michener (1944), who constructed from these studies, a phylogenetic tree, suggesting a common ancestor for the tribes of social bees. Recent work (Kerr, 1946) has disclosed the existence of two very distinct methods of determination of castes (queens or functional females on one hand, and workers or females with under-developed ovaries on the other). It was known for some time that in the honeybee (*Apis mellifera*) a larva develops either into a worker or into a queen depending upon the food on which it is fed. The difference between a worker and a queen is, thus, phenotypic or trophogenic. A situation similar in principle seems to obtain also in *Bombus* and in *Trigona* and *Lestrimelitta*. In *Melipona*, no difference is observed between the feeding of future queens and of future workers. *Melipona* queens are genetically different from *Melipona* workers, the former being heterozygous for two or for three (depending upon species) genes, homozygosis for any one of which makes a diploid individual a worker. Some consideration on the probable course of evolution of the caste-determining mechanisms are reported in the present article.

EVOLUTIONARY RELATIONSHIPS AMONG THE SOCIAL BEES

Paleontological evidence, as well as morphology and bionomy, indicate that the tribe Bombini is the most primitive among the three tribes of social bees. Six genera of *Bombini*: *Protobombus*, *Electrapis*, *Chalcobombus*, *Sophrobombus*, *Ctenoplectrella*, *Glyptapis* are known from Oligocene (Baltic) amber (Wheeler, 1928). No fossil *Apini* are known, but the genera *Electrapis* and *Protobombus* are regarded morphologically intermediate between *Bombus* and *Apis*. Three Miocene *Meliponini* are known: *Meliponorytes succini*, and *M. sicula* from Sicilian amber, and *M. devictus* from Burmese amber (Tosi 1896, Cockerell 1921).

The anatomical traits of the modern *Apini* and *Meliponini* may be derived from those of *Bombini*. Thus, *Bombus* has dorsal as well as ventral wax glands, and both females and males possess them. The *Meliponini* have only dorsal wax glands, and even these are present only in young workers and in drones. The *Apini* have only ventral wax glands, and these are restricted to the workers.

Bombus has 3 to 6 egg-strings in the ovaries and an equal number of seminal tubes in the testes (Bordas, 1895). *Apis* has about 180 egg strings and seminal tubes, which represents a modification induced by strong selection for high fecundity. The *Meliponini* have 4 egg-strings and seminal tubes. *Bombus* and *Apis* have an accessory gland in the male reproductive system which is absent in

the *Meliponini*. The sting is well developed in *Bombus* and *Apis* but it has suffered atrophy in the *Meliponini*.

External morphology also suggests that the *Bombini* are close to the ancestral stock of social bees (Michener, 1944). Thus, *Bombus* has bare eyes and cleft claws; *Apis* has pilose eyes and cleft claws, and the *Meliponini* have bare eyes, simple claws in females, and cleft ones in males.

Bombus stores honey and pollen in oval pots of cerumen (wax mixed with gum, resin, latex, etc.) and in old cocoons. Some European species, such as *B. pomorum*, keep pollen stocks in cylindrical tubes. *Apis* builds no pots and keeps honey and pollen in new or old wax cells. In the *Meliponini* honey and pollen are stored in cerumen pots but a group of *Trigona* species (such as *T. silvestrii*) uses for this purpose cylindrical tubes, like *Bombus pomorum* (Wheeler, 1928). The writer has observed two weak colonies of *Melipona marginata* and *Melipona schencki* which were exceptional in that brood cells were used for honey storage, instead of pots, as is normal. This is a link between the food storage systems used by the *Meliponini* and the *Apini*. The ancestors of *Apis* may have stored food in pots, as the *Bombini* and *Meliponini* do now.

The *Apini* and *Meliponini* have, however, some traits suggesting a common origin. Both have the same type of corbicula, no hind tibial spurs, and have well developed jugal lobes in their hind wings and labrum three to four times as broad as long (Michener, 1944). Both construct individual cells for the progeny, special queen cells (in *Apis*, *Trigona* and *Lestrimelitta*), perennial colonies (instead of annual ones as in *Bombus*), and practice individual feeding of larvae.

CASTE DETERMINATION IN THE BOMBINI AND APINI

In *Bombus*, no queens develop in young colonies. The first larvae raised by the founding female receive little food and de-

velop into diminutive workers. Only when a colony reaches the stage of having numerous workers, does the food given to the brood become more abundant, and some fertile females begin to appear.

In *Apis dorsata*, the most primitive *Apis* species, drones and workers develop in similar cells (Singh, 1943). The domestic bee, *Apis mellifera*, raises workers in small and drones in larger hexagonal cells, while queens develop in peanut-shaped cells placed preferentially at the bottom of the comb. Larvae of queens are fed on a so-called "royal jelly," while worker and drone larvae receive pollen and honey from the third day of their life on (Snodgrass 1925). The well-known evidence of phenotypic caste determination in *Apis mellifera* is that when eggs originally placed by the bees in worker cells are transferred to queen cells, the larvae arising from these eggs develop into queens.

CASTE DETERMINATION IN THE MELIPONINI

In the *Meliponini* larvae are raised in individual cells, as in *Apis*. Each cell is filled with honey, pollen and a glandular secretion. In most species, as soon as the queen lays an egg in a cell, the latter is sealed, to be opened again only by the emerging imago. In some species (such as *Trigona carbonaria*, Rayment 1932, 1935) the cells are however kept open for several days, new food being added even after the hatching of a larva. In either case, feeding is "progressive," i.e., a young larva eats first the glandular secretion, then the honey, and lastly pollen. The system of larval feeding used by *Trigona carbonaria* resembles, therefore, that practiced for queen larvae of *Apis mellifera* (Kerr, 1948).

Trigona and *Lestrimelitta* have two kinds of cells: small ones for workers and drones and large ones for queens. The queen cells may be twice, or even more than twice, as large as the worker ones, according to species. This writer has established by statistical tests that

queen cells are placed mostly at the margins of horizontal combs. Although one can not be absolutely certain about this, all the available evidence indicates that the food placed in queen and in worker cells seems to differ only in quantity.

There can not be any doubt that caste determination in *Melipona* is not trophogenic, but genotypic. The evidence, given in more detail elsewhere (Kerr, 1946, 1950), can be summarized as follows:

a) There are no differences in the quantity of food provided for the larva, as can be concluded from the following facts: all cells are of the same size and contain the same amount of food, with an average weight of 148 mg. per cell. The food absorbed by both queen and worker larvae is the same, as shown by the identity of the mean weight of young queen and worker pupae. The only statistical difference noticeable is the higher variability of worker pupae, as measured by the standard error.

b) It seems impossible to assume that there exist any differences in quality of food, since queens' cells are distributed at random in the horizontal combs and are found in normal hives in fixed ratios.

c) Under unfavorable conditions, or in diseased hives there is a considerable reduction in the frequency of emerging queens, to one fourth or less of the expected ratio, which needs a special explanation. No elimination of eggs, larvae or pupae occurs, with individuals emerging from every one of the closed cells. There are no intermediates and queens are always of normal appearance and quite distinct from workers as verified in more than 50,000 individuals studied to date. If one wanted to refer to some trophogenic hypothesis to explain this situation, one would have to make the rather improbable assumptions: 1) that workers provide or omit completely a certain foodstuff necessary for queen formation, 2) that they deposit this stuff completely at random, 3) that they apply it to less and less cells under unfavorable

conditions, never exceeding however a fixed maximum under favorable ones. This maximum is about 25 per cent in one species and about 12.5 per cent in seven other species.

There can be little doubt that the process which reduces the number of queens is a general cytogenetical phenomenon since it is not limited to the ratios of queens to workers but also applies to the segregation of the monofactorial difference: black against yellow first abdominal segments.

d) Thus, limiting the discussion to normal hives it was found that in *Melipona marginata* and its subspecies workers are about three times as numerous as queens (ratio 3:1). In other species so far studied in this respect, namely *Melipona quadrifasciata*, *Melipona schencki*, *Melipona favosa orbigny*, *Melipona interrupta fasciculata*, *Melipona rufiventris paraensis*, *Melipona flavipennis*, and *Melipona fuscata melanoventer*, workers are seven times more numerous than queens (ratio 7:1).

The occurrence of the 3:1 ratio suggests that the queens are heterozygous for two pairs of genes (AaBb), homozygosis for either or both of which makes a female a worker (thus, workers are AABb, AaBB, aabb, aaBb, AABB, etc.) and males are AB, Ab, aB, or ab). In species from which the 7:1 ratio is characteristic, the queens are heterozygous for three pairs of genes (AaBbCc), while workers are homozygous for one, two, or three of these genes (AaBBCC, AaBbCC, AABbCC, etc.). It is easy to show that, with such a system, any mating of a queen with a haploid male (drone) results in production of a single genetic type of queens, several genetic types of workers, and several types of males (Kerr, 1946, 1950).

THE ORIGIN OF THE GENOTYPIC MECHANISM OF CASTE DETERMINATION

The phenotypic, or trophogenic, caste determination is presumed to be phylogenetically more ancient than the geno-

typic caste determination mechanism. The antiquity of the trophogenic mechanism is indicated by its occurrence in the genus *Bombus*, which, as shown above, must be regarded as primitive for a variety of reasons. Furthermore, this mechanism occurs in all three tribes of social bees, while the genotypic one exists only in a part of the tribe *Meliponini*.

The ancestors of *Melipona* probably had their castes determined trophogenically, as is the case at present in *Trigona* and *Lestrimelitta*. In such an ancestor, with a genetic constitution AABBCDD . . . , a mutation took place from A to a, the heterozygote Aa being heterotic, thus possessing a higher adaptive value than the original genotype. Assuming that the drone which fertilized this Aa queen was A, AA and Aa queens and AA and Aa workers would be produced, depending upon the feeding of the larvae. The heterosis might, however, make Aa workers fertile, and not sterile as their AA sisters. The heterosis of the Aa gene combination would, then, lead to increase of the frequency of the new allele a in the population, up to the point when the disadvantages of homozygosis for A (AA) would be counterbalanced by the disadvantages of homozygosis for a (aa). Modern genetics demonstrates many situations in which both homozygotes for a gene or a chromosome structure have adaptative values inferior to the corresponding heterozygote (Wright and Dobzhansky, 1946).

A new mutation, B to b, has then happened at another locus, having the same heterotic properties as the A locus, females Bb will, then, have an advantage over BB and bb. Heterozygosis for both genes, AaBb, might confer so great an increase of vigor on the double heterozygote that the latter might develop into a queen regardless of kind and quantity of food which it received in the larval stage. This would make the trophogenic mechanism superfluous, and all the homozygotes would develop automatically into workers. Owing to the existence of

fertile heterozygous females in every colony, mutations that cause loss of the instincts connected with the construction of distinct royal and worker cells would now be tolerated instead of being eliminated by natural selection, as would occur in a species with trophogenic caste determination. *Melipona marginata* and its subspecies may be regarded as representing the most primitive now existent type of genotypic caste determination among bees.

The transition from the bifactorial (AaBb) to the trifactorial (AaBbCc) type of caste determination could occur by addition of a third mutation, C to c, with heterotic properties. The triple heterozygotes, having selective advantages thus became established as the exclusive genotype in queens, while the AaBbCC and AaBbcc type became a worker. With a trifactorial mechanism a decrease in the proportion of queens in colonies would occur (1:7 instead of 1:3 ratio). Indeed, with the bifactorial mechanism the queens constitute 25 per cent of all diploid females, while with the trifactorial one only 12.5 per cent of the females are queens, the remaining 87.5 per cent being workers. This may have an important adaptative advantage. Most virgin queens appearing in a *Melipona* colony are killed off by the workers within 15 days after emergence (Kerr, 1950). This means that with the bifactorial mechanism, twice as many queens will be eliminated as with the trifactorial mechanism. The lesser "cost" of the trifactorial, compared to the bifactorial mechanism may explain why a majority of species of *Melipona* studied in this respect have the former, and only one species, *M. marginata*, still preserves the latter type of mechanism. The genes involved in bifactorial as well as in the trifactorial mechanism have a sex-limited action. Males, despite being homogametic, remain fertile. The abundance of genes with sex-limited effects in *Hymenoptera* is quite understandable. As expressed by White (1945, pp. 279): "Recessive mutants that are sex-limited in their expression so that they produce

no effect in the male, will be in special position in organisms with male haploidy. Such mutations may exist in wild populations for a long while, even though deleterious, since in the males they will not affect the phenotype. This may possibly explain why in the social *Hymenoptera* the males are all of one type while the females are differentiated into several castes. We are not suggesting here that the various female castes are genotypically different—merely that a large number of mutants whose effects are limited to the female sex may have accumulated in the species, gradually building up a genotype that is very plastic in the female sex (producing several entirely different phenotypes according to the environment and nutrition of the larvae)."

Genotypic mechanisms of caste determination have, however, certain adaptive disadvantages. The reproductive wastage involved in killing off a part of the queens emerging in the colony has already been mentioned. Anyway, a rigid mechanism which produces a fixed proportion of queens and workers may be less desirable than a mechanism which allows a greater plasticity in this respect. It appears that, superimposed on the genotypic caste determination, the genus *Melipona* has evolved another mechanism which permits variation in the proportion of queens to workers among the developing larvae. During winter, or in colonies with few nurse bees, or in hives infected with parasites or diseases, the percentages of queens among the hatching brood may be very small or even zero. The precise nature of this additional mechanism has not been completely clarified. We have mentioned in a general way the reasons why we do not think that any phenotypic or trophogenic mechanism can be responsible. Thus we have arrived by exclusion of other possibilities to the following hypothesis which fits all data so far on hand. If there were a parthenogenetical development of eggs with a following fusion of two nuclei of identical constitution, either derived from an equational

meiotic division or from an early cleavage division, we would obtain diploid homozygous individuals. In accordance with our theory of caste determination, these must be all workers. A somewhat analogous reduction is known in the fish *Lebistes reticulatus* in which Winge (1934) found the sex-ratio to vary greatly with season. In spring a normal sex-ratio of 1 ♀: 1 ♂ is produced, while at other times most of the young are females.

DISCUSSION

According to the hypothesis presented above, the transition from the trophogenic to the genotypic mechanism of caste determination entails production of mutations (A to a, B to b, C to c) with heterotic effects in heterozygotes (Aa, Bb, Cc) and deleterious ones in homozygotes (AA, aa, BB, bb, CC, cc). Such heterotic effects are indeed possible in organisms with the haplo-diploid sex-determination, characteristic for *Hymenoptera*. Mackensen (in Farrar, 1943–1947, and Farrar, 1948) obtained offspring from honeybee queens fertilized by their own sons. Such an inbreeding process results in a rapid loss of vigor of the colony and, particularly, in a loss of ability of the eggs to hatch. Intercrossing of inbred lines gives, on the contrary, a pronounced hybrid vigor. Some double-hybrid colonies were highly productive, but their worker bees were "intolerably vicious." This latter quality may perhaps be regarded as an additional manifestation of heterosis. The genetic mechanism involved in production of heterosis evidently must involve genes that are advantageous when heterozygous in diploid individuals (Aa superior to both AA and aa) but which do not affect adversely the haploid individuals, A and a. Both, increase and loss of vigor in *Apis* have not yet been studied genetically, but it seems to be of the same nature as this phenomenon in maize. In this species recent studies by Crow (1948) and Brieger (1948, 1949) have shown that the heterotic mechanism can be attributed only to a small extent

to the combination of dominant factors, being mainly due to the interaction of heterotic genes in the heterozygous conditions. Our Mendelian formula applies the same genetical principle of a heterotic gene interaction for caste determination. Individuals completely heterozygous are fully fertile (queens), while partially or fully homozygous individuals are sterile (workers).

The type of sex-determination discovered by Whiting (1940) in *Habrobracon juglandis* may be regarded as involving a special type of heterosis. Here, a diploid individual must, in order to be a female, be heterozygous for different alleles (or chromosome sections). Homozygosity for either allele gives a poorly viable diploid male, but the haploid males carrying either allele are fully vigorous. In *Melipona*, the heterosis is exploited for purposes of caste rather than sex-determination. And furthermore, since the caste-determining genes (A, B, C) are localized in different chromosomes, any queen is automatically heterozygous for three chromosome segments, in which further heterotic genes may accumulate.

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SUMMARY

Two types of mechanism of caste determination exist in social bees. In the tribes *Bombini*, *Apini*, and in a part of the *Meliponini* (genera *Trigona* and *Leptotrimelitta*), a diploid individual develops into a fertile female (queen) or into sterile female (worker) depending upon the quality and quantity of food which it receives in the larval stage. This is the phenotypic, or trophogenic, caste determination. But in the genus *Melipona*, the caste determination is genotypic. Queens are heterozygous for two (*Melipona marginata*) or three (all other spe-

cies of *Melipona* so far studied) genes, each of which, when homozygous, makes the individual a worker.

Among the social bees, the trophogenic mechanism of caste-determination is phylogenetically primitive and the genotypic mechanism secondary. The former mechanism exists in every one of the three tribes of social bees, and is the only one in the tribe *Bombini*, which must be regarded as primitive on several grounds. The genotypic mechanism is known only in the specialized genus *Melipona*.

The emergence of the genotypic mechanism in the ancestors of *Melipona* which are presumed to have had their castes determined phenotypically, is visualized as a succession of mutations in genes with sex-limited heterotic effects. The transition from the genotypic mechanism involving two genes to one involving three genes had an adaptive advantage because of the decrease of the proportion of the obligatorily produced queens. An additional mechanism, which permits variations in the proportions of queens born in a colony at different seasons, has become superimposed on the genotypic caste determination in *Melipona*.

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