

GENETIC DETERMINATION OF CASTES IN THE GENUS MELIPONA

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DIFFERENT mechanisms of caste determination are encountered in different genera of stingless bees (KERR 1946, 1947, 1948). In *Trigona* and in *Lestrimelitta* a phenotypic caste determination, long known in *Apis*, is observed: a larva develops into a queen or into a worker depending upon the food it receives. In *Melipona*, on the other hand, caste determination is genotypic. Fertile females (queens) are heterozygous in some species for two, and in other species for three, pairs of genes, homozygosity for any one of which makes the individual develop into a worker. A review of the previously published evidence, as well as new data, relevant to the problem of caste determination in *Melipona* are reported in the present article.

It should be noted that males of stingless bees, like those of other Hymenoptera, differ from females in having a haploid, instead of diploid, chromosome complement. Females of several species of *Melipona* are known to have 18, while drones have only 9 chromosomes. Spermatogenesis in *Melipona* follows the well-known *Apis* scheme. The first meiotic division is abortive, and results in expulsion of a cytoplasmic bud; the second division is equational, and a bud of cytoplasm containing a nucleus is produced which subsequently degenerates, while the remaining cell develops into a spermatid and a spermatozoon. Cytological abnormalities in spermatogenesis occur in *Melipona*, as they do in *Apis*, in testes of pupae exposed to temperature variations. The oogenesis in *Melipona* has not been studied owing to technical difficulties. The chromosome counts were made in cells of nerve tissue and in those of the ovarial wall.

RESULTS

Form and Position of Royal Cells in the Combs

In *Apis*, the queen cells are much larger than those of workers and drones, and are localized principally at the bottom of the comb. In *Trigona*, the difference in size between queen cells and worker cells varies from species to species. Royal cells are localized peripherally in the horizontal combs.

A statistical study was made of the distribution and localization of different cells in combs of various species of *Melipona* and a random distribution was found with regard to queen and worker cells, while the drone cells were found chiefly at the center. All cells are of the same size in all three castes of *Melipona*.

Morphological Differences of the Castes in Melipona

Queens: Virgin queens are smaller than the workers, with a narrower head, eyes and thorax. The abdomen swells considerably after fertilization. Body color, in most species, is darker in the queen than in the workers and drones, and without marks. The queens are somewhat more precocious in their development than the workers (data for *M. quadrifasciata*):

egg	4.5 to 6.5	days
larva	7 to 8	days
prepupa	4.5 to 5	days
pupa	11.5 to 15	days
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Total	30 to 34	days

Virgin queens, after emerging in the presence of the mother queen, are killed by the workers, or die within 15 days. If no fecundated queen is present, then one, or under exceptional conditions more young queens, after their flight and fecundation, are given a special diet for 6–10 days in strong hives and for 20 to 30 days in weak colonies. During this time the sexual organs complete their development and the egg laying may begin.

Workers: Workers have undeveloped ovaries, but in rare instances they may lay unfertilized eggs which produce only drones. The morphological characters of this caste are mainly used by taxonomists for the identification of species.

Generally the workers have more brilliant colors than the queens: For instance, in *M. quadrifasciata*, *M. favosa*, *M. scutellaris*, *M. interrupta* and others, the queens are all brownish and the workers are black banded with yellow, brown banded with yellow, or black banded with white.

The development follows approximately the following time table (data for *M. quadrifasciata*):

egg	4.5 to 6.5	days
larva	7 to 8	days
prepupa	5 to 5.5	days
pupa	15.5 to 18	days
<hr/>		
Total	34 to 37	days

Drones: The main differences between males and females are the forms of the antennae and the genitalia. The antenna has 13 segments in males and 12 in females.

Frequency and Determination of the Castes

Combs with a variable number of cells were taken and the caste of each bee was registered either in the pupal stage or a short time after emergence in the laboratory.

These counts show that the proportion of males (drones) in a colony is not fixed but varies in accordance with the age of the colony and with the season. At certain times most males are killed off by the workers; but in the period preceding the nuptial flight even males from other colonies are admitted and are permitted to move around in the hive without being molested.

In contrast to this, the proportion of queens and workers in colonies is constant, except under certain abnormal conditions to be discussed later. As shown in table 1, in *Melipona marginata* and subspecies a ratio of 3 workers:1 queen is observed. But in *Melipona quadrifasciata*, *Melipona schencki*, *Melipona fuscata melanoventer*,¹ *Melipona favosa orbigny*, *Melipona interrupta fasciculata*, *Melipona flavipennis*, *Melipona rufiventris paraensis*,¹ the ratio is 7 workers:1 queen (tables 2-8). The occurrence of these ratios suggests at once the following hypothesis of caste determination.

TABLE 1

Melipona marginata.(χ² computed on the basis of a 3:1 expectation for the ratio workers:queens)

NO. OF COLONY	PROBABLE DATE OF EGG LAYING	CASTES ♀		χ ²	DRONES	NOTES*
		WORKERS	QUEENS			
1946						
5	3-24 to 4- 1	34	14	0.44	0	(1)
5	4- 1 to 4-14	83	27	0.01	0	(1)
5	6- 2 to 6-18	118	11	18.67	0	(3)
9	6- 8 to 6-18	73	2	19.95	0	(3)
9	7-23 to 8- 4	158	11	30.82	0	(3)
5	8-18 to 8-25	14	0	4.67	0	(3)
5	8-21 to 9- 3	9	1	1.20	45	(3)
9	9-18 to 9-24	62	12	3.05	0	(1)
15	11- 2 to 11-10	32	6	1.72	4	(1)
15	11- 2 to 11-10	39	7	2.34	34	(1)
15	11-13 to 11-18	1	0	—	17	(1)
15	12-28, 1946 to 1-11, 1947	60	21	0.04	3	(1)
1948						
22	1- 1 to 1- 2	20	1	4.59	5	(2)
22	1- 4 to 1- 6	89	2	25.23	0	(2)
76	4- 1 to 4- 5	27	7	0.35	0	(1)
Total	(1)	338	94	2.42		
Total χ ² (1); nf=7				7.96		

* (1)—strong hives; (2)—weak hives; (3)—winter.

Since *Melipona* males are haploid, all gametes which they produce are, barring mutation, genotypically alike. Females are, on the contrary, diploid, and may accordingly produce several kinds of gametes. We assume, then, that the queens of *Melipona marginata* are always heterozygous for two pairs of genes, *AaBb*, homozygosis for any one of which makes a diploid individual a worker. Males would, then, be of four kinds, any one of which would produce, when mated to a queen, a ratio of 1 queen:3 workers among the female offspring, as follows:

¹ These names are given in accordance with the new classification of the subspecies of *M. fasciata*, by MOURE and KERR (1949).

$$\begin{aligned}
 \text{Queen} \times \text{Drone} &= 1 \text{ Queen} + 3 \text{ workers} \\
 AaBb \times AB &= AaBb + (AABB + AABb + AaBB) \\
 AaBb \times Ab &= AaBb + (AABb + AAbb + Aabb) \\
 AaBb \times aB &= AaBb + (AaBB + aaBB + aaBb) \\
 AaBb \times ab &= AaBb + (Aabb + aaBb + aabb)
 \end{aligned}$$

For other species of *Melipona*, in which the ratio 1 queen: 7 workers has been found, we assume that a queen is always heterozygous for three pairs of genes, *AaBbCc*, homozygosis for any one of which makes a diploid individual a worker. The possible types of matings are, then, as follows:

$$\begin{aligned}
 \text{Queen} \times \text{Drone} &= 1 \text{ Queen} + 7 \text{ workers} \\
 AaBbCc \times ABC &= AaBbCc + (AABBCC + AABBCc + AABbCC + AAbBCC + AaBBCC \\
 &\quad + AaBBcC + AaBbCC) \\
 AaBbCc \times ABc &= AaBbCc + (AABBCc + AABbCc + AAbBcc + AaBBCC + AaBBcc \\
 &\quad + AaBbcc) \\
 AaBbCc \times AbC &= AaBbCc + (AABbCC + AAbBcC + AaBbCC + AaBbCC + AaBbCC + AabbCC \\
 &\quad + AabbCc) \\
 AaBbCc \times Abc &= AaBbCc + (AABbCc + AAbbcc + AaBbCc + AaBbcc + AaBbcc + AabbCc \\
 &\quad + Aabbcc) \\
 AaBbCc \times aBC &= AaBbCc + (AaBBCC + AaBBcC + AaBbCC + aaBBCC + aaBBcC + aaBbCC \\
 &\quad + aaBbCc) \\
 AaBbCc \times aBc &= AaBbCc + (AaBBcC + AaBbcc + AaBbcc + aaBBcC + aaBBcc + aaBbCc \\
 &\quad + aaBbcc) \\
 AaBbCc \times aBC &= AaBbCc + (AaBbCC + AabbCC + AabbCc + aaBbCC + aaBbCc + aabbCC \\
 &\quad + aabbCc) \\
 AaBbCc \times abc &= AaBbCc + (AaBbcc + AabbCc + Aabbcc + aaBbCc + aaBbcc + aabbCc + aabbcc)
 \end{aligned}$$

Data

The actual data to which these hypotheses are to be applied are, according to species, as follows:

a) *Melipona marginata* (table 1)—We studied first the 3:1 type, found only in *M. marginata* and its subspecies. The colonies studied were collected in the States of Santa Catarina, São Paulo and Minas Gerais, in Brazil.

b) *Melipona quadrifasciata* (table 2) with subspecies: *quadrifasciata*, living in southern Brazil (southern part of São Paulo State, and the States of Paraná, Santa Catarina, and Rio Grande do Sul) and adjacent countries (Argentina and Paraguay); and *anthidioides*, living in the northern part of São Paulo State and all States to the north as far as Pernambuco. This species has been studied in most detail owing to its high frequency in our region. The colony number 4 is hybrid of the subspecies *quadrifasciata* \times *anthidioides*.

c) *Melipona schencki*, (table 3) was collected in the State of São Paulo. Its domestication is harder than that of the former species.

d) *Melipona fuscata* subspecies *melanoventer* (table 4)—This subspecies is found only in the lower Amazon region, and colony 90 was examined by us during our trip to that zone.

e) *Melipona favosa* (subspecies *orbigny*) (table 5)—Only one colony from State of Mato Grosso between the basin of Paraguay and Paraná rivers.

f) *Melipona interrupta* (subspecies *fasciculata*) (table 6) collected in the State of Maranhão.

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TABLE 2

Melipona quadrifasciata.(χ^2 computed on the basis of a 7:1 expectation)

NO. OF COLONY	PROBABLE DATE OF EGG LAYING	CASTES ♀		χ^2	DRONES	NOTES*
		WORKERS	QUEENS			
1946						
1	2-16 to 2-27	69	8	0.31	0	(1)
1	2-20 to 3- 5	144	18	0.29	0	(1)
2	2-27 to 3- 7	52	8	0.04	0	(1)
3	3- 6 to 3-17	45	10	1.67	0	(1)
4	3-14 to 3-26	56	4	1.87	0	(1)
4	3-26 to 3-28	29	4	—	0	(1)
3	5- 9 to 5-16	52	2	3.82	0	(6)
2	5-14 to 5-24	73	2	6.63	0	(6)
3	5-28 to 6- 6	56	2	4.34	0	(6)
8	6-20 to 7- 1	43	2	2.67	0	(6)
1	8- 9 to 8-16	79	3	5.86	1	(6)
8	8-12 to 8-22	77	4	4.23	57	(6)
12	8-12 to 8-17	31	2	—	0	(6)
3	9-13 to 9-17	85	1	10.64	0	(6)
1	9-16 to 9-22	40	0	5.71	0	(6)
2	9-29 to 10- 6	78	8	0.80	2	(1)
1	10-16 to 10-24	111	4	8.56	0	(2)
14	10-26 to 11-10	57	9	0.08	15	(1)
2	11-11 to 11-19	91	8	1.14	0	(1)
1	11-28 to 12- 2	57	3	3.09	0	(2)
12	12-13 to 12-23	156	23	0.02	0	(1)
14	12-15 to 12-28	121	9	3.70	4	(3)
1947						
12	1-17 to 1-25	97	12	0.22	7	(1)
12	2-19 to 2-28	83	2	8.22	0	(4)
12	2-22 to 3-11	177	17	2.48	106	(1)
12	4-10 to 4-23	100	20	1.90	28	(1)
12	5-24 to 6- 4	104	5	6.24	0	(6)
23	7- 1 to 7- 3	34	4	0.14	0	(5 and 6)
12	8- 4 to 8-23	93	9	1.26	0	(5 and 6)
23	9-20 to 9-21	25	1	—	0	(6)
35	11-10 to 11-25	61	13	1.74	20	(1)
1948						
35	3- 1 to 3- 3	72	14	1.12	0	(1)
12	3-10 to 3-10	43	1	4.21	0	(1)
12	3-20 to 3-22	41	5	1.12	0	(1)
23	3-30 to 4- 7	57	2	4.48	0	(3)
8	4-10 to 4-14	83	9	0.62	0	(1)
35	5- 4 to 5- 8	54	10	0.57	0	(1)
35	5- 9 to 5-19	53	0	7.57	0	(6)
Total (1) and (5)		1632	214	1.39		
Total χ^2 of (1) and (5); $nf=20$				21.60		

* (1)—strong hives; (2)—parasitized by phorides (Dipt.); (3)—weak colonies; (4)—experimental weak colonies; (5)—colonies with temperature control and artificially fed; (6)—winter.

TABLE 3

Melipona schencki. $(\chi^2$ computed on the basis of a 7:1 expectation)

NO. OF COLONY	PROBABLE DATE OF EGG LAYING	CASTES ♀		χ^2	DRONES	NOTES*
		WORKERS	QUEENS			
1946						
13	9-20 to 9-28	11	4	—	75	(1)
13	10-28 to 11- 2	71	8	0.41	20	(1)
13	11- 6 to 11-13	41	2	2.42	5	(1)
1947						
13	1-26 to 2- 1	119	5	8.13	0	(3)
13	2-25 to 3- 2	147	9	6.46	2	(3)
13	3- 6 to 3-10	80	3	5.99	0	(3)
13	3-19 to 3-26	174	9	9.62	1	(2)
Total (1)		123	14	0.65		
Total χ^2 (1); $nf=2$				2.83		

* (1)—strong hives; (2)—experimental weak colony; (3)—bacterial infection.

g) *Melipona flavipennis* (table 7) was collected in the State of Pará (Amazon basin) but it occurs throughout northern and northeastern South America.

h) *Melipona rufiventris paraensis* (table 8) is a species living in the north of Brazil. Strong colonies have been found on the frontier of French Guiana, but attempts to maintain hives of this species in Piracicaba were unsuccessful, owing to its complete inadaptability to this climate.

TABLE 4

M. fuscata melanoventer. $(\chi^2$ computed on the basis of a 7:1 expectation)

NO. OF COLONY	PROBABLE DATE OF EGG LAYING	CASTES ♀		χ^2	DRONES	NOTES*
		WORKERS	QUEENS			
1946						
16	11-18 to 11-20	28	2	—	0	(3)
1948						
90	9-23 to 10- 2	90	7	2.48	30	(1) and (2)
90	10- 1 to 10- 7	106	9	2.30	83	(1)
90	10- 7 to 10-11	111	15	0.04	43	(1)
Total (1)		307	31	3.42		
Total χ^2 (1); $nf=3$				4.81		

* (1)—strong hive; (2)—In the Amazon valley in the period between December 15th and July 15th the rains are strong enough to interfere with the normal work of the bees. But in the "dry" season it often rains so much as to interfere somewhat with their work; (3)—weak hive.

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TABLE 5

Melipona favosa orbigny.

(χ^2 computed on the basis of a 7:1 expectation)

NO. OF COLONY	PROBABLE DATE OF LAYING OF EGGS	CASTES ♀		χ^2	DRONES	NOTES*
		WORKERS	QUEENS			
40	5-23 to 6-1, 1948	105	4	7.77	0	(2)
40	9- 1 to 9-5, 1948	49	8	0.12	0	(1)

* (1)—strong hive; (2)winter.

TABLE 6

Melipona interrupta fasciculata.

(χ^2 computed on the basis of a 7:1 expectation)

NO. OF COLONY	PROBABLE DATE OF EGG LAYING	CASTES ♀		χ^2	DRONES	NOTES*
		WORKERS	QUEENS			
82	11-29 to 12- 8, 1948	55	4	1.77	0	(1)
82	12- 8 to 12-13, 1948	27	7	2.03	0	(1)
89	3- 3 to 3- 9, 1949	44	9	0.97	0	(1)
Total (1)		126	20	0.19		
Total χ^2 (1); nf=3				4.77		

* (1)—strong hive.

TABLE 7

Melipona flavipennis.

(χ^2 computed on the basis of a 7:1 expectation)

NO. OF COLONY	PROBABLE DATE OF EGG LAYING	CASTES ♀		χ^2	DRONES	NOTES*
		WORKERS	QUEENS			
79-A	9-12 to 9-21, 1948	110	7	4.54	35	(1)
79-A	9-22 to 9-27, 1948	38	3	1.01	3	(1)
Total (1)		148	10	5.50		
Total χ^2 (1); nf=2				5.55		

* (1)—strong hive (see note 2, table 4).

TABLE 8

Melipona rufiventris paraensis.
(χ^2 computed on the basis of a 7:1 expectation)

NO. OF COLONY	PROBABLE DATE OF EGG LAYING	CASTES ♀		χ^2	DRONES	NOTES*
		WORKERS	QUEENS			
81	10-15 to 10-20, 1948	51	3	2.38	0	(1)

* (1)—strong hive.

DISCUSSION AND CONCLUSIONS

Table 1 contains data for 15 samples taken in 5 colonies of *Melipona marginata*. Among these, 8 samples (marked (1) in the column "Notes") were collected while the colonies were in good health and weather conditions were favorable; seven of these eight samples are large enough to be analyzed statistically. The χ^2 tests have been made taking 25 percent queens and 75 percent workers as the expected ratio (1:3 segregation).

Tables 2-8 contain data for 57 samples taken in colonies of seven species of *Melipona*. Among these, 34 samples (marked (1) or (5) in the column "Notes") were collected while the respective colonies were in good health and while weather conditions were favorable; 32 of these 34 samples are large enough to be analyzed statistically. The χ^2 tests have been made taking 12.5 percent queens and 87.5 percent workers as the expected ratio (1:7 segregation). It can be seen that the ratios observed fit the expected one as well as could be expected. The remainder of the samples (marked (2), (3), (4) or (6) in the tables) have been taken either during unfavorable season (May to early September, i.e., winter) or in weak or parasitized colonies. In such samples the percentages of queens fall below the expected 25 percent in *Melipona marginata*, and below 12.5 percent in the other species.

The reduction of the percentage of queens during winter is due to the lowering of the temperature of the hives rather than to any general effect of winter conditions. This has been shown experimentally by raising artificially the temperature of the colonies in winter; in such colonies the proportion of queens returns to normal (colonies 12 and 23 in table 2). Colonies with too few nurse bees, or those attacked by parasites or diseases, give low frequencies of queens even if the honey and pollen storage is sufficient. It can be shown experimentally that the loss of queens is also produced by deficient nourishment of the queen.

Several hypotheses that might account for these deviations have been considered in previous publications (KERR 1948):

a) Elimination of queens by the workers during the larval or pupal stage. This does not occur, since all cells are sealed after egg laying and are not opened again before emergence of the imago.

b) Preferential oösortion of the eggs which should produce queens. This hypothesis was rejected since there is not sufficient time after fecundation for a preferential degeneration of eggs.

c) Loss of chromosomes. Cytological aberrations were observed in two male pupae after they had been kept from 3 to 5 days at a low temperature (20°C) and it was supposed that the same could occur in the fertile female and, preferentially a female with one chromosome carrying a caste determinative gene. This cytological hypothesis was rejected because on counting the chromosomes of nearly 50 larvae during the winter it was found that all of them had 18 chromosomes.

d) From a poorly nourished egg (which has suffered little oöSORPTION) a queen develops into a worker. This hypothesis is plausible but suggests that one could find intermediates; these were not found to date.

e) Abnormalities during meiosis and fertilization. 1—Preferential fertilization. 2—Parthenogenetic development with preferential fusion of two haploid nuclei or fusion of cleavage nuclei; if the egg be fertilized the male nucleus is eliminated. All these hypotheses are plausible, but at present the only hypothesis that fits the available data is that, under unfavorable conditions of temperature or of feeding, the male nucleus in fertilized eggs is absorbed or eliminated, the female pronucleus divides, and the division products fuse to give rise to diploid cleavage nuclei. Diploid females of such origin will evidently be homozygous for the caste-determining genes, and will therefore develop into workers instead of into queens. Owing to the technical difficulties of cytological investigation of bee eggs, no direct proof of this hypothesis has thus far been secured. The hypothesis is made plausible, however, by the fact that cases of female parthenogenesis have been reported in Hymenoptera by several authors. HASKINS and ENZMANN (1945) described such a process in ants, MACKENSEN (1943) in the honey bee, SMITH (1941) in *Dyprion*, etc. The effect of low temperature causing fusion of nuclei in eggs of *Triturus* has been observed by FRANKHAUSER (1942). Recently a promising new method for solution of this problem was found. Some genes affecting body color have been found in colonies of *Melipona marginata*. By following the segregations of these genes at different seasons, it should be possible to adduce conclusive evidence for or against diploid parthenogenesis as the cause of the reduction of normal proportions of queens in colonies of this species. Thus far the data are small but they are consistent with the hypothesis. This topic will be discussed elsewhere.

SUMMARY

The development of a diploid larva into a queen or into a worker bee depends in several species of *Melipona* upon the genetic constitution of that larva. In *Melipona marginata* the queens are always heterozygous for two pairs of genes, *AaBb*. Homozygosis for either of these genes produces a worker. Males are either *AB*, or *Ab*, or *aB*, or *ab*. The mating of any of these males with the *AaBb* females gives segregation in a ratio of 1 queen: 3 workers among the diploid zygotes. In *Melipona quadrifasciata*, *Melipona schencki*, *Melipona fuscata melanoventer*, *M. fava orbigny*, *Melipona interrupta fasciculata*, *Melipona flavipennis* and *Melipona rufiventris paraensis* females are heterozygous for three pairs of genes (*AaBbCc*), homozygosis for any one of which makes a diploid zygote develop into a worker. In these species the normal ratios of

queens and workers in colonies approach 1:7. Deviations from these ratios are, however, observed in winter and in colonies that are attacked by parasites or diseases. These deviations may be accounted for by supposing that under certain conditions the male pronucleus is eliminated from the egg, and the division products of the female pronucleus fuse to give rise to diploid cleavage nuclei.

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