EVIDENCES THAT GENETICALLY DETERMINED MELIPONA QUEENS CAN BECOME WORKERS[1]

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IHERING (1903) suggested that caste determination in Melipona (Hymenoptera Apidae) was not phenotypic but was possibly determined by some mechanism before the egg was laid. On the basis of observations by many authors, we can say that in all genera of social Apidae, except Melipona, caste determination is controlled by quantity or quality of food or both.

KERR (1946) found in four colonies of *Melipona quadrifasciata* Lepeletier and one of *Melipona marginata* Lepeletier that workers and queens emerged from the same type of cells. Queens were randomly scattered and emerged in a fixed proportion. This proportion appeared to be 12.5% in *M. quadrifasciata* and 25% in *M. marginata.* It was postulated that *M. quadrifasciata* queens are heterozygous for three genes and *M. marginata* queens for two. By this hypothesis, homozygosis for 1, 2, or 3 genes would cause the insect to be a worker. During the winter the percentages of queens produced dropped to 3.45 in *M. quadrifasciata* and 6.50 in *M. marginata.* Four other papers have since been published on the subject of caste determination in Melipona. KERR (1948) gave data on five additional colonies of *M. quadrifasciata,* two of *M. marginata* and one each of *Melipona nigra* Lepeletier, *M. melanoventer* Schwarz and *M. rufiventris* Lepeletier. *M. rufiventris, M. nigra,* and one colony of *M. quadrifasciata* gave proportions of queens closer to 25% than 12%. KERR (1950a) gave more information on two colonies each of *M. quadrifasciata* and *M. interrupta* Latreille and one colony each of *M. melanoventer* and *M. favosa* (Fabricius). KERR (1950b) proposed tentatively that caste had been determined phenotypically in the ancestors of Melipona and that a succession of gene mutations resulted in the genotypic mechanism. It remained to be explained why queens are not produced in winter, or in poorly fed colonies. KERR, STORT, and MONTENEGRO (1966) examined this problem to find whether caste determination was purely environmental, or if genetic factors contributed as well. They concluded that the following factors have no bearing on caste determination: (1) temperature; (2) eggs eaten by the queen; (3) time spent by the queen feeding herself from the brood cell; (4) number of eggs laid by the queen in the same brood cell; (5) increase of protein ratio within the nest. They found that each alveolus is normally filled by four to 11 workers and the entire operation takes from 15 to 64 seconds. Queens emerged from cells provisioned by four to eight

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TABLE 1

Weight of the ovaries of white-eyed pupae of workers and queen of Melipona quadrifasciata

Number of the bee	Weight of bee (mg)	Weight of ovary (mg)	Capacity of cell(ml)	Caste or sex
1	89.9	1.9	0.162	queen
2	93.7	. .	0.165	male
3	80.1	0.1	0.154	worker
4	87.0	0.1	0.162	worker
5	83.5	0.1	0.156	worker
6	85.6	0.1	0.164	worker
7	89.3	0.3	0.166	worker
8	81.8	0.28	0.150	worker
9	88.9	0.23	0.164	worker
10	85.6	0.4	0.156	worker
11	88.0	0.3	0.158	worker
12	85.4	0.1	0.150	worker
13	99.2	0.23	0.160	worker
14	89.8	0.24	0.144	worker
15	81.1	0.2	0.156	worker
16	80.1	0.1	0.148	worker
17	95.7	0.1	0.172	worker
18	95.9	0.1	0.162	worker
19	90.1	0.1	0.162	worker
20	84.7	0.34	0.158	worker
21	93.0	0.3	0.165	worker
22	87.5	0.18	0.162	worker
23	86.5	0.2	0.162	worker
24	96.4	0.2	0.158	worker
25	95.2	0.3	0.154	worker
26	90.3	0.4	0.158	worker
27	83.2	0.4	0.155	worker

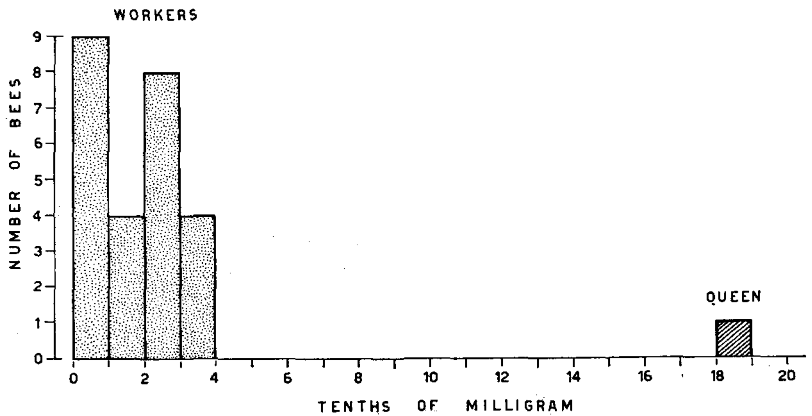

FIGURE 1.—Weight of the ovary of workers and queen of *Melipona quadrifasciata.*

workers, but no queen emerged when the alveolus was provisioned by more than nine workers. The quantity of food put into the brood cells was negatively correlated with the number of bees provisioning the cells ($r = -0.43$). The quantity of food eaten by the larvae was indirectly estimated by weighing very young pupae. Among *M. quadrifasciata* pupae weighing less than 72.0 mg, no queens were observed. However, a 3:1 segregation between workers and queens was found among those weighing more than 72.0 mg. They concluded that all queens of Melipona are double heterozygous (*AaBb*), but before a 3:1 segregation could be expressed pupae must be heavier than 72 mg.

The apparent genetic segregation of seven workers to one queen in *M. quadrifasciata* was considered by KERR to result from a study of combs containing pupae not fed well enough to obtain the full genetic capability of 25% queens. However, there was no direct evidence concerning the final disposition of larvae which should be genetically queens. This paper reports the results of our studies on the latter problem.

MATERIALS AND METHODS

Colonies of the following species of Melipona from Brazil were used: five colonies of *M. quadrifasciata*, three from Rio Claro, one from Santa Rita do Passa Quatro (two counties of the State of São Paulo), one from Pocinhos do Rio Verde, Minas Gerais; two of *M. quinquefasciata* Lepeletier from Cristalina, Goiás; one of *M. marginata* from Pocinhos do Rio Verde, Minas Gerais; and one of *M. seminigra merrillae* Cockrell from Manaus, Amazonas. All colonies were collected in their natural habitats, brought to the laboratory, and kept either in regular nest boxes or observation hives.

During the exploratory phase of the work we looked for characteristics by which virgin queens and workers could be distinguished. The main distinctive structure is the ovary, which is enormous in queens and rudimentary in workers. KERR, STORT and MONTENEGRO (1966) had found that the first stage in which a worker could be differentiated from a queen was the white-eyed pupae. We, therefore, compared ovary weights of 27 white-eyed pupae (Table 1, Figure 1). Ovaries of the one queen weighed 1.9 mg and those of the 25 workers ranged from 0.1 to 0.4 mg. There is a slightly bimodal distribution among the workers. It was apparent, however, that ovary weight alone was not a sufficient measure of ovarian development.

A careful study then was made of the anatomy of the worker ovaries. Great variability was found among the different parts of the reproductive system, including large, medium and small main oviducts, the sizes of the spermathecae, the sizes of the ovaries, and the shapes of the ovaries and meristematic cells of the ovaries. One striking difference was found in *Melipona quadrifasciata:* the abdominal nerve cord had at the white-eyed pupa stage, four nerve ganglia in most of the workers, and five in the queen and in some workers. Consequently, our subsequent studies were concentrated on this character. No differences between the abdominal nerve cord were detected among workers and queens of *M. quinquefasciata.* A slight difference was observed in bees of *M. seminigra merrillae*, but not distinct enough to work with it easily. Since the differences observed for *M. quadrifasciata* were found in only one other species (*M. marginata*) counts were confined to these two species.

Although the number of abdominal ganglia varied from four to five in the early pupae, it was invariably four by the late pupal stage as a result of fusion of the terminal two. Consequently, the number of ganglia was useful as a genetically determined character only in pink- and white-eyed pupae.

The pupae were dissected under a stereoscopic microscope (up to 40×) with the bees immersed in Ringer, saline, or water. Water was used successfully with pupae, but in the adults it caused the ganglia to expand into large vesicles. Methyl Blue was often used to improve observation. Picroformol was used for fixing when necessary.

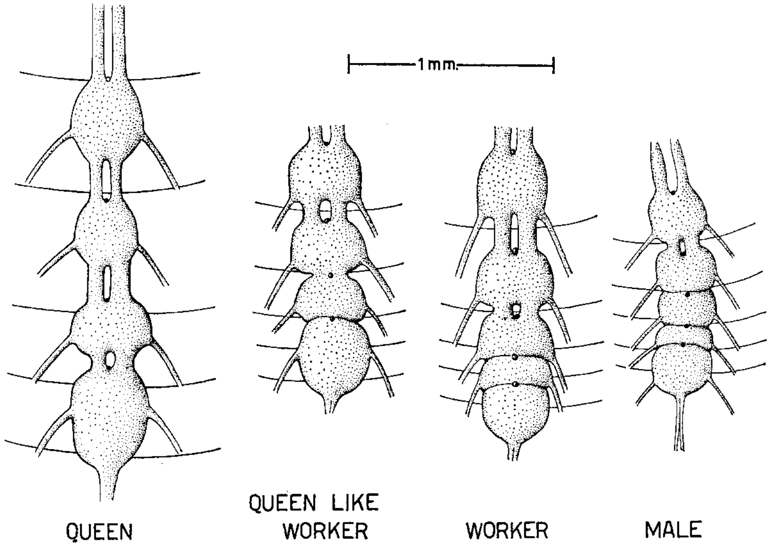

FIGURE 2.—Abdominal section of the ventral nerve cord of *Melipona marginata* for male and different female castes.

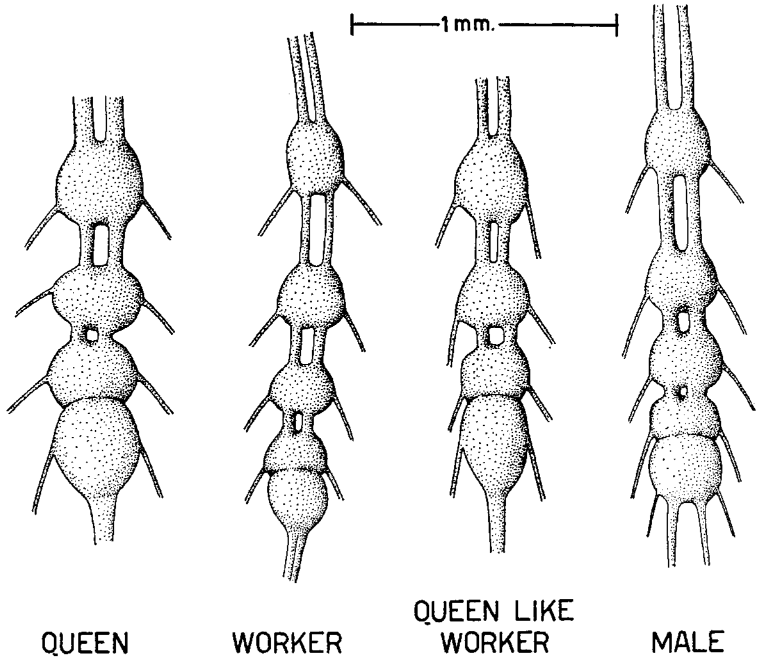

FIGURE 3.—Abdominal section of the ventral nerve cord of *Melipona quadrifasciata* for male and different female castes.

RESULTS

In *M. marginata* the white- and pink-eyed queens and some workers had four ganglia while most of the workers had five ganglia in the abdominal nerve cord (Figure 2). Of a total of 47 bees examined, 35 workers had five ganglia, six queens had four, and six workers had four. These six were considered to be phenotypically workers but genotypically queens.

White-eyed pupae from five colonies of *M. quadrifasciata* were examined. The first colony observed, from Rio Claro, had no queens. Out of 42 workers examined, 29 had four ganglia and 13 had five. Several days later three white-eyed queen pupae were found, all of which had five ganglia. To avoid weakening the colony, no workers were taken at this time. We interpret the 13 workers with five ganglia as being genetically *AaBb* (like the queens) but phenocopies of workers. Agreement with a 3:1 segregation is thus good ($P > 0.36$). Data from the remaining colonies are also in agreement with a 3:1 or 1:1 ratio. (See KERR 1950a for evidence that queens are *AaBb*.)

DISCUSSION

It is evident that the ratios of queens and workers observed in Melipona, if genetically controlled, can be explained on a rational basis only if queens mate but once. If multiple mating occurred (as it does with honey bees) there would be no consistent ratios on a genetic basis. However, two papers (KERR and KRAUSE 1950; KERR, ZUCCHI, NAKADAIRA, BUTULO 1962) gave information on the mating of *Melipona quadrifasciata*, showing by sperm count and the presence of the chitinous male genitalia in the young queen genital tract that such queens mate only once. Other unpublished data of this Department show that single mating is the case in other Melipona and Trigona species.

In combs of Melipona brood, when the larvae spin their silk cocoons inside the wax alveoli, the old workers remove the wax. This permits more intense respiration within the cocoon. Any failure to spin a cocoon causes the adult workers to remove the entire alveolus and its larva. Since no holes were observed in combs producing zero percent queens, we concluded that lack of queens in such cases was not caused by death.

KERR, STORT and MONTENEGRO (1966) demonstrated that not only was genetic constitution (*AaBb*) necessary for queen production but also interaction with quantity (and/or quality) of food. No one had demonstrated, however, that genetically determined Melipona queens sometimes becomes workers. We decided to look in the prepupal stage for evidence of such an occurrence. This was a complete failure. We now suspect that the separation of queen from workers becomes irrevocable in the late feeding stage but that the two forms cannot be distinguished morphologically until the pupae are formed.

Since the nervous system is determined quite early in the life of insects, we were not surprised to find evidence for the transformation of queen to worker phenocopies in the ventral nerve cord. Also, DIAS (1957) describes the ventral nerve cord of several meliponids, indicating queen and worker differences in *M. quadrifasciata.*

If the "queen conditions" of genetic constitution and food ingested are satisfied, the head and thorax will be relatively underdeveloped while the abdomen becomes unusually large. Only after the abdomen develops, do the ovaries start to grow. One-hour old pupae have very small ovaries, but two-day old pupae can have enormous ones.

It is interesting to note that hemizygous males of *M. marginata* and *M. quadrifasciata* have the same number of ganglia as the homozygous workers.

When *M. marginata* white-eyed and pink-eyed pupae were examined, we thought queens having four ganglia and workers having five could result from a pleiotropic effect of either or both of the caste determining genes *A* or *B*. In *M. quadrifasciata* a possible interpretation would be that a third dominant gene *N* conditions the homozygous constitutions (*AABB*, *AABb*, *AAbb*, *AaBB*, etc.) to become 5-ganglia workers whereas recessive *nn* individuals have five ganglia if *AaBb*, but four ganglia if homozygous at *A* or *B locus*. Segregation would be ¾ five ganglia : ¼ four ganglia in the following crosses: *NN* queen × *N* male, *NN* queen × *n* male, *Nn* queen × *N* male and *nn* queen × *N* male. Examples of such cases are colonies 2, 3, and 5 (Table 3).

The cross *Nn* queen × *n* male should produce ½ *Nn* plus ½ *nn*. The *Nn* bees should segregate ¾ five ganglia in all "homozygous" pupae to ¼ four ganglia in all heterozygous pupae. The *nn* bees should segregate ¼ five ganglia in all heterozygous to ¾ four ganglia in all homozygous bees. This should give a final segregation of ½ five ganglia to ½ four ganglia in both workers and queens, as in colony 4 (Table 3).

In the cross *nn* queen × *n* male the heterozygous (*AaBb*) bees should have five ganglia in the abdominal nerve cord and the homozygous bees for any of these four genes (*A*, *a*, *B*, *b*) should have four ganglia, as in colony 1 (Table 3).

We have observed many colonies of Trigona which die out owing to lack of queens. This we have never observed in Melipona, probably because queen production does not depend on the direct "wish" of the workers to make a queen cell.

TABLE 2

Segregation of castes (queens and workers) and of ganglia in the abdominal section of the ventral nerve cord in Melipona marginata

	Phenotype			
	Abdominal nerve cord	Caste	Observed	Expected
A.	White eyed pupae			
	5 ganglia	workers	22	21.75
	4 ganglia	"workers"	4 } (7)	7.25
	4 ganglia	queen	3 }	
B.	Pink and red eyed pupae			
	5 ganglia	worker	13	13.5
	4 ganglia	worker	2 } (5)	4.5
	4 ganglia	queen	3 }	

TABLE 3

Segregations for caste and abdominal nerve cord in Melipona quadrifasciata

Colony No. and source	Caste	Number of ganglia in abdominal nerve cord	Number of bees counted	Expected values
1. Rio Claro	worker	4	29	31.5*
	worker	5	13 } (13)	10.5
	queen	5	0 }	
2. Pocinhos do Rio Verde	worker	5	58	58.5†
	worker	4	14 } (20)	19.5
	queen	4	6 }	
3. Observation hive, Rio Claro	worker	5	21	22.5†
	worker	4	8 } (9)	7.5
	queen	4	1 }	
4. Santa Rita do Passa Quatro	worker	4	31	27.0‡
	worker	5	23	27.0
	queen	4	5	4.5
	queen	5	4	4.5
5. Rio Claro	worker	5	1	. .
	worker	4	1	. .
	queen	4	1	. .

None of the deviations from expected values are statistically significant.
* Based on postulated parent genotypes: *AaBb nn* ♀ × *n* ♂ (see DISCUSSION).
† Based on postulated parent genotypes: *AaBb* (*NN*, *Nn*, or *nn*) ♀ × *N* ♂.
‡ Based on postulated parent genotypes: *AaBb Nn*♀ × *n* ♂.

This advantage may have been responsible for establishing among the Melipona species a system by which an *AaBb* female larva needs less food to become a queen; also a system by which in time of food scarcity there is less waste by production of large numbers of unneeded queens, which would be continuously killed a few days after emergence.

SUMMARY

Castes in Melipona species (Hymenoptera, Apidae) are determined through an interaction between food and genetic factors. Only a doubly heterozygous *AaBb* bee can become a queen. Food acts as an effectuator and the four genes (*A*, *a*, *B*, *b*) may act as a set of regulator genes. In the examined colony of *M. marginata*, ¾ of the young pupae were workers with five ganglia and ¼ (all queens and some workers) had four ganglia in the abdominal section of the ventral nerve cord. In *M. quadrifasciata* the segregation ratios are as would be expected if a pair of alleles (*N*,*n*) determines the number of ganglia; if colonies in which all individuals are *NN* or *Nn* segregate 3:1 for five ganglia (workers) : four ganglia (queens or underfed queen-workers); and if *nn* colonies, on the contrary, segregate 3:1 for four ganglia (workers) : five ganglia (queens or queen-workers). Colonies from *Nn* queen × *n* drone would segregate 1:1 for five ganglia : four ganglia in both workers and queens; one example of such a colony was observed.

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