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Original article

Sex determination in bees. XXVII. Castes obtained from larvae fed homogenized food in *Melipona compressipes* (Hymenoptera, Apidae)

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Summary — One hundred and eighty-three larvae of *Melipona compressipes* were fed with mixed food collected from 391 cells containing eggs of various ages. Nine recently provisioned cells had an average of 238 mg of food; the maximum quantity of food a cell can receive if completely full averages 308 mg. Larvae were reared in wax cups receiving 350, 300 and 240 mg of food (control). Death rate was high due to the handling of larvae. In the 350 mg group, 17 workers and 4 queens were obtained and 34 died before the pupal stage. In the 300 mg group, 39 workers and 8 queens pupated and 38 died. In all there were 56 workers and 12 queens. If quality of food were decisive in queen determination, no segregation of queens and workers should occur, as the food was a homogenized mixture. Weight of last larval stage and genetics were the preponderant caste determinants. Below 184.7 mg of last larval weight no queens are produced; above it, a 3 workers to one queen segregation was observed; we therefore conclude that genetics is the ultimate determining factor of caste in *Melipona*.

***Melipona* / caste determination / genetic factor / food**

INTRODUCTION

Ihering (1903) first noted that in the horizontal brood combs of species of the genus *Melipona* the cells were about the same size, and that from them queens, workers and drones emerged. Kerr (1975; 1987a) proposed that sex in bees is determined by 2 sets of genes, one that acts in the first hours after the egg is laid and another set that acts during the last larval period. An important factor in the first set is the *xo* series of 20 heteroalleles (Kerr, 1987b) that determines ovaries when heterozygous and testes when hemizygous of homozygous.

In *Melipona* bees, the second set of genes has 2 main genes with 2 alleles each that are involved in the production of juvenile hormone (Kerr *et al,* 1975) and

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that activate the"femaleness" genes. This has been clearly demonstrated by Campos (1975, 1978) and Campos *et al* (1975) in *Melipona quadrifasciata*. *Melipona quadrifasciata* bees were studied (Kerr *et al*, 1966) in an observation hive for the following behavior: oophagy, number of workers that fill a brood cell with food, time spent by a queen eating food and bees that emerged from cells after the queen had eaten worker eggs, and quantity of food ingested. They found that queens were produced when > 150 mg of food was provided; above this amount a ratio of workers to queens of 3:1 emerged from the cells. Camargo *et al* (1976) raised larvae of 4 species (*Melipona quadrifasciata*, *Melipona rufiventris*, *Melipona scutellaris* and *Melipona marginata*) with different amounts of food and found that above a given amount specific for each species, a 3:1 worker/queen segregation was produced. However, Darchen and Delage-Darchen (1974, 1975) working with overfed *Melipona beechei* proposed that quantity, quality of food or both are the only factors involved in queen and worker determination. Wilde and Beetsma (1982) put forward the proposal that the bees could distribute food of different quality in different cells, thus determining queens. The present work was planned to contribute to our understanding of the problem of caste determination in *Melipona* by testing the effect of different quality and quantities of food on the determination of queens in *Melipona compressipes fasciculata*.

MATERIAL AND METHODS

Twenty-five colonies of *Melipona compressipes fasciculata* (Apidae) were used. This species of bee is large, larger than *Apis mellifera*, and is found in Maranhão State, in the northeastern part of Brazil. Its queens lay an average of 40 eggs per day, reaching a maximum rate of 150 eggs per day. Each cell has a total capacity of 0.277 ml. The food density is 1.11. If completely full, a cell can receive a maximum of 308 mg food. Nine cells from a natural comb, before egg eclosion, received an average of 238 mg of food (s d 13); 240 mg was therefore used as a control.

The first step consisted of making wax cups with hoods. Each cup was slightly smaller than *Apis mellifera* queen cell cups. Food was taken in batches of 20–150 cells from a total of 391 provisioned cells that contained eggs of various ages. The combined food was then placed in a large test tube and shaken. Food was distributed among 3 sets of cells as follows: group 1: 62 cups, provided with 350 mg of homogenized food; group 2: 97 cups provided with 300 mg of homogenized food; group 3: (control for this experimental condition) 24 cells provided with 240 mg of homogenized food; group 4: (natural control) a comb taken from the hive in June 1988.

The second step consisted of shaking the test tube each time a new cell was filled. After the artificial wax cell received the given amount of food a recently eclosed larva (< 20-h old) was deposited on the food and the cell was provided with a wax hood. The entire batch was then placed in a desiccator (240 mm inside diameter x 310 mm height) with a saturated solution of KCl which provided 84% relative humidity (ASTM, 1951). The desiccator (with the artificial cells) was placed in an incubator at a temperature of 30–33 °C. Castes were easily determined soon after the bee reached the white-eyed pupa, stage.

Since it is difficult to obtain *Melipona* material, each larva was weighed twice daily to determine whether the larvae continued eating after they began to spin the cocoon (Maciel-Silva, 1989). The weights were obtained when the larvae finished eating, prior to defecation. Due to unknown reasons many larvae did not eat all the food that was given, which is the reason why the weight of last-stage larvae was preferred to the weight of food deposited in the cups.

RESULTS AND DISCUSSION

The set of 62 cups with 350 mg of homogenized food produced: 17 workers and 4 queens (χ^2 (3:1) = 0.4) plus 34 larvae that

died before metamorphosis, 2 males, 4 malformed larvae, and 1 that reached a pupal stage but its caste could not be determined. The maximum capacity of a natural cell is 308 mg of food; thus, 350 mg is an amount which is not found in nature. The 3 heaviest larvae weighed 258.4 mg, 257.3 mg and 247.5 mg and became respectively: worker, worker, queen; *ie,* the heaviest larvae did not develop into queen bees.

The set of 97 cups that received 300 mg of homogenized food produced 39 workers and 8 queens (χ^2 3:1 = 1.6), 4 males, 3 malformed larvae, 38 dead larvae, and 5 unidentified larvae.

The lightest last-stage larva that developed into a queen weighed 184.7 mg. The 3 heaviest larvae of this group weighed 230.5, 230.5 and 228.2 mg and became queen, worker and worker bee, respectively.

The set of 24 larvae fed 240 mg produced 0 queens, 19 workers, 3 males, 1 malformed larva, and 1 dead larva. The heaviest larva of this batch weighed 178.6 mg and became a worker bee.

The brood comb collected in July 1988 produced 7 queens, 84 workers and 0 male bees.

Counting all bees with a larval weight of $\geq$ 184 mg in the first and second set resulted in a total number of workers and 12 queens (χ^2 3:1 = 0.4).

Splitting our sample into 2 groups: 184.7–216 and 216.1–247.5, 20 workers and 6 queens (χ^2 3:1 = 0.05) in the first group, 10 workers and 5 queens (χ^2 = 0.56) in the second group, were found: 216.1 mg was the average between the heaviest and the lighest queen larvae. Therefore, we concluded that overfeeding did not cause determination of castes.

Darchen and Delage-Darchen (1974, 1975) and Wilde and Beetsma (1982) advanced the idea that workers deposited a different quality of food in the cells from which queens emerged. However, the results of our experiments do not support the hypothesis that different food is deposited in different cell cups. Our results, however, support the hypothesis of Kerr (1975) that, in the presence of sufficient food in the cell, caste differentiation in *Melipona compressipes* is caused by genetic factors. Kerr proposes a model of gene action in which the queens are heterozygous ($x_a{}^1/x_a{}^1$; $x_b{}^1/x_b{}^2$) for 2 genes that control juvenile hormone production and, in the heterozygous condition, initiates femaleness. Males are hemizygous for 2 of the 4 alleles, while workers are homozygous for one or 2 alleles, producing JH deficiency. Therefore their eyes, head proportions, thorax and appendices, abdomen, and cuticle, are all masculine in form. Thus, the worker caste and males in meliponids are produced by a suppression of the action of femaleness genes.

CONCLUSION

Mortality was much higher in the 2 overfed groups (300 and 350 mg) than in the 240 g group for 2 reasons: a) the overfed group pupated later and was, therefore, manipulated more intensely; b) bees of this species provide cells with 150 to 270 mg of food; they do not reach 300 mg. Therefore higher mortality at those levels may also be due to unknown physiological effects of ingesting a large quantity of food.

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Résumé — Détermination du sexe chez les abeilles. XXVII. Castes obtenues à partir de larves de *Melipona compressipes* ayant reçu une nourriture homogénéisée. Cent quatre vingt trois larves de *Melipona compressipes* ont reçu une nourriture mélangée, prélevée dans 391 cellules d'ouvrières après la ponte de l'œuf et juste avant l'éclosion de la larve. Neuf cellules naturelles, qui venaient d'être approvisionnées, contenaient en moyenne 238 mg de nourriture. La quantité maximale qu'une cellule entièrement pleine peut contenir est d'environ 308 mg. Des cupules en cire ont été réparties en 3 séries : chaque cupule a reçu respectivement 350 mg, 300 mg et 240 mg de nourriture, la 3e série servant de témoin. Le taux de mortalité des larves a été élevé en raison des manipulations subies. La 1re série a fourni 17 ouvrières et 4 reines (mais 34 larves sont mortes avant le stade nymphe). Les 2 larves les plus lourdes au dernier stade pesaient 258,4 mg et 257,3 mg et donnèrent des ouvrières. La 2e série a donné 39 ouvrières et 8 reines (38 larves sont mortes). Cela fait au total 56 ouvrières et 12 reines. Si la qualité de la nourriture constituait le facteur décisif de la formation des reines, il ne devrait pas y avoir de différentiation entre reines et ouvrières, puisque toutes les larves avaient reçu la même nourriture homogénéisée. Le poids du dernier stade larvaire et la génétique sont les facteurs prédominants dans la détermination des castes. Lorsqu'au dernier stade la larve pèse moins de 184,7 mg, aucune reine n'est formée. Au-dessus de ce poids, on observe une différentiation entre ouvrières et reines dans le rapport de 3 à 1. Cela signifie que le facteur génétique est décisif dans la détermination des castes chez les mélipones.

***Melipona* / détermination des castes / facteur génétique / facteur trophique**

Zusammenfassung — Geschlechtbestimmung bei Bienen. XXVII. Kasten von *Melipona compressipes*, entstanden aus Larven, die mit homogenisiertem Futter ernährt worden waren. 183 Larven von *Melipona compressipes* wurden mit gemischtem Futter ernährt, das aus 391 Zellen nach der Eilage bis kurz vor dem Ausschlüpfem der Larven gesammelt worden war. Neun Zellen, die erst kürzlich mit Futter versorgt worden waren, enthielten im Durchschnitt 238 mg Futter. Die größte Menge, die eine vollständig gefüllte Zelle aufnehmen kann, betrug im Mittel 308 mg Futter. Ein Satz von Wachsbechern erhielt 350 mg, ein anderer 300 mg und die Kontrollgruppe 240 mg Futter. Die Sterblichkeitsrate war in Folge der Manipulation mit den Larven hoch.

Aus der Gruppe mit 350 mg entstanden 17 Arbeiterinnen und 4 Königinnen, aber 34 starben vor Erreichung des Puppenstadiums. Die zwei schwersten unter den letzten Larvenstadien wogen 258,4 mg und 257,3 mg und entwickelten sich zu Arbeiterinnen. Aus der Gruppe mit 300 mg verpuppten sich 39 Arbeiterinnen und 8 Königinnen, während 38 starben. Zusammen sind 56 Arbeiterinnen und 12 Königinnen entstanden. Wäre die Qualität des Futters der entscheidende Faktor bei der Königinnenentstehung, so dürfte es keine Aufspaltung in Königinnen und Arbeiterinnen geben, denn alle Larven erhielten dieselbe homogenisierte Futtermischung. Das Gewicht des letzten Larvenstadiums und Genetik waren die vorrangigen Faktoren bei der Kastenbestimmung:

Unterhalb eines Gewichtes des letzten Larvenstadiums von 184,7 mg wurden keine Königinnen erzeugt. Oberhalb dieses Gewichtes wurde eine Aufspaltung im Verhältnis von 3 Arbeiterinnen und einer Königin beobachtet. Das bedeutet, daß Genetik der entscheidende Faktor bei der Kastenbestimmung bei *Melipona* ist.

***Melipona* / Kastenbestimmung / Genetik / Futter**

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