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# SEX DETERMINATION IN BEES.
# III. CASTE DETERMINATION AND GENETIC CONTROL IN *MELIPONA* (1)

By WARWICK ESTEVAM KERR

*Departamento de Genética, Faculdade de Medicina, Universidade de São Paulo, 14.100 - Ribeirão Preto, S.P., Brazil.*

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## *SUMMARY*

Using the approach of Mahalanobis it was desclosed that workers of *Melipona quadrifasciata* are, as far as the external morphology is concerned, closer to males than to queens of this species. A preliminary interpretation of this is given based in a slight modification of the BRITTEN and DAVIDSON hypothesis (batteries of genes) applied to the sex determination system of these bees. There are two sets of sex genes : one set that acts in the embryo and determines ovary or testis and another that acts in the prepupal stage determining the transformation of the imaginal discs and tegument in adult female or adult male structures. A preliminary hypothesis for gene regulation is proposed for both sets. The mode of action of the second set implies that a substance produced by food above a given limit acts on the integrator genes $x^a$ and $x^b$ (with two alleles each) and these put a receptor gene ($R^{x^a} + x^b$) into action that by its turn, put into action the gene (or genes) for femaleness; *Melipona* workers are females inside (have ovaries) but are almost males in the outside, tegument, eyes, wings). The place of action of the juvenile hormone in this battery has not yet been experimentally determined, but this working hypothesis put it provisorily in the beginning of the battery.

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## ZUSAMMENFASSUNG

**Geschlechts Bestimmung bei Bienen.**
**III. Kastendetermination und genische Kontrolle bei Melipona.**

Mittels der von Mahalanobis vorgeschlagenen Annäherungsmethode wurde entdeckt, dass die Arbeitsbienen der *Melipona quadrifasciata*, soweit es sich auf die äussere Morphologie bezieht, den Drohnen ähnlicher sind, als den Königinnen dieser Art.

Eine vorläufige Deutung dieser Tatsache wird hier gegeben; sie beruht auf einer leicht geänderten Anwendung der Hypothese der Gen-batterien von Britten und Davidson auf das System der Geschlechtsbestimmung dieser Bienen.

Es gibt 2 Gruppen von Geschlechtsgenen : eine Gruppe wirkt im Embryo und bestimmt die Eierstöcke oder Hoden; die andere wirkt in der Vorpuppenstufe und bestimmt die Transformation der Imaginalscheiben und Tegumente in ausgewachsenen weiblichen oder männlichen Strukturen.

Eine vorläufige Hypothese für Genregulation wird für beide Gruppen vorgeschlagen. Die Wirkungsweise der zweiten Gruppe verlangt, dass eine durch Nahrungsmittel erzeugte Substanz, oberhalb einer gegebenen Grenze, auf die Integratorgene $x^a$ und $x^b$ (jede mit 2 Allelen) wirkt, und dass diese dann ein Empfangsgen $R(x^a + x^b)$ anregen, das dann seinerseits das Gen (oder die Gene) für Weiblichkeit aktiviert; dazu auch das Gen *N* der letzten 2 Ganglia des Ventralnervenstranges, das Verschmelzung bestimmt, oder *n*, das sie nicht bestimmt.

Die Angriffsstelle des jugendlichen Hormons in dieser Batterie wurde noch nicht experimentell bestimmt; aber diese Arbeitshypothese legt sie vorläufig in den Anfang der Batterie.

## INTRODUCTION

Unlike the situation in *Apis*, males and workers of *Melipona* are more noticeably similar to one another than either is to queens. This paper compares the similarity between the males, queens and workers of *Melipona quadrifasciata* and *Apis mellifera* using multivariate distance measures (Mahalanobis $D^2$). Using these results sex and caste determination in these bees are analysed.

## MATERIAL AND METHODS

Forteen males (sons of workers), twelve workers, and ten virgin queens (sisters of the workers), all from one same hive of *Melipona quadrifasciata* were measured for 14 characters. Twenty nine males, 30 queens and 29 workers of the same hive of *Apis mellifera* were measured for 21 characters. All measurements are given in arbitrary units read directly in the micrometer without transfering to metric system. The data processing for obtaing the Mahalanobis $D^2$ followed the steps described by Rao (1952) and two programs, for IBM 1130 and HP 9810-A, were developed by Mr. Luiz Antonio F. Bezerra under the instructions of Dr. Francisco A. Moura Duarte. In all figures, D (the square root of $D^2$) was used since it can be more easely put in a graph. The data in table II were collected by Prof. José Chaud Netto.

## *RESULTS*

The results are summarized in table I and figure 1 for *Melipona quadrifasciata* and in table II and figure 2 for *Apis mellifera.*

Workers in *M. quadrifasciata* are intermediate (fig. 1) between males and females (D equal to 52.02) while in *Apis mellifera* (fig. 2) workers are as different from males (109) as queens are to males (100). In order to more easily visualize this statement, figures 1 and 2 show in parenthesis all the data in uniform units, by making the distance between drones (perfect males) and queens (perfect females) equal to 100.

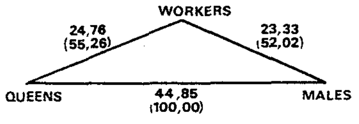


Fig. 1. — *Relative distances (D, of Mahalanobis) among 10 virgin queens, 12 workers and 14 males of the same hive of Melipona quadrifasciata.* Fourteen characters were used. The numbers in parenthesis are the distances transformed in relation to the distance between queens and males made equal to 100. See that workers are intermediate to males and queens.

Abb. 1. — *Relativabstände (D bei Mahalanobis) zwischen 10 unbefruchteten Königinnen, 12 Arbeiterbienen und 14 Drohnen desselben Stockes der Melipona quadrifasciata.* 14 Charakteristiken wurden gemessen. Die eingeklammerten Zahlen sind Relativabstände, bezogen auf 100 willkürliche Einheiten der zwischen Königinnen und Drohnen bestehenden Abstände. Man sehe, dass die Arbeiterbienen den Drohnen näher sind, als den Koniginnen.

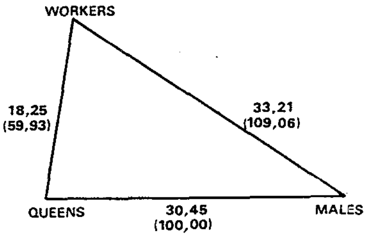


Fig. 2. — *Relative distances (D, of Mahalanobis) among 30 virgin queens, 29 workers and 29 males of the same hive of Apis mellifera adansonii.* Twenty-one charactes were used. The values in relation to the distance between queens and males made equal to 100. Workers and queens are practically equally distant from the males.

Abb. 2. — *Relativabstände (D bei Mahalanobis) zwischen 30 unbefruchteten Königinnen, 29 Arbeiterbienen und 29 Drohnen desselben Stokes der Apis mellifica adansonii.* 21 Charakteristiken wurden benutzt. Die Werte in Bezug auf die Abstände zwischen Königinnen und Drohnen wurden gleich 100 gesetzt. Arbeiterbienen und Königinnen sind praktisch äquidistant von den Drohnen.

TABLE I. — *Mean and variance for 14 characters of* Melipona quadrifasciata. *The measurements are in units of the micrometer ocular, not transferred to metric units.*

TABELLE I. — *Mittelwerte und Abweichungen von 14 Charakteristiken der* Melipona quadrifasciata. *Die Messeinheiten sind die des Okularmikrometers, nicht in Millimeter übertragen.*

| | Queen | | Worker | | Male | |
|---|---|---|---|---|---|---|
| Characters | Mean | Variance | Mean | Variance | Mean | Variance |
| Costal wing nerve | 51.4 | 1.1556 | 67.92 | 7.1742 | 66.21 | 1.2582 |
| Median wing nerve | 28.0 | 0.6667 | 37.58 | 4.0833 | 37.07 | 0.6868 |
| Anal-1 wing nerve | 29.1 | 0.7667 | 38.67 | 3.3333 | 37.93 | 1.4560 |
| Width of anterior wing | 23.6 | 0.4887 | 29.50 | 1.5455 | 28.14 | 0.4396 |
| Number of hamuli | 10.6 | 0.2667 | 11.83 | 0.1515 | 11.14 | 0.2851 |
| Width composite eye | 8.9 | 0.3222 | 14.08 | 0.2652 | 14.14 | 0.1319 |
| Width 3rd abd. segment | 65.7 | 4.0111 | 67.92 | 10.9924 | 60.93 | 4.9945 |
| Width of mesoscutum | 43.6 | 0.9333 | 50.92 | 4.0833 | 49.36 | 3.3242 |
| Length of scape | 38.3 | 0.2333 | 37.75 | 1.4773 | 28.14 | 0.7472 |
| Total width of head | 51.7 | 1.1222 | 61.42 | 7.3561 | 59.29 | 1.2967 |
| Total length of head | 40.9 | 0.3222 | 47.50 | 1.9091 | 45.57 | 1.6483 |
| Ocelorbital distance | 26.9 | 0.3222 | 29.25 | 1.1136 | 25.64 | 0.5549 |
| Width of clipeus | 25.6 | 0.2667 | 26.50 | 0.6364 | 25.29 | 0.9890 |
| Clipeorbital distance | 18.5 | 0.2778 | 15.08 | 0.8106 | 11.64 | 0.7088 |

TABLE II. — *Means and variances of 21 characters of queens, workers and males of* Apis mellifera. *Units are in millimeters.*

TABELLE II. — *Mittelwerte und Abweichungen von 21 Charakteristiken der Königinnen, Arbeiterbienen, und Drohnen der* Apis mellifica. *Messeinheiten in Millimetern.*

| | Queen | | Worker | | Male | |
|---|---|---|---|---|---|---|
| | Mean | Variance | Mean | Variance | Mean | Variance |
| Width anterior wing | 3.29 | 0.0058 | 2,96 | 0.0043 | 3.90 | 0.0153 |
| Length posterior wing | 6.92 | 0.0177 | 5.98 | 0.0100 | 8.01 | 0.0543 |
| Length radial cell | 3.25 | 0.0040 | 3.07 | 0.0041 | 4.40 | 0.0103 |
| Total length of thorax | 5.62 | 0.0402 | 4.35 | 0.0216 | 6.44 | 0.0760 |
| Length of head | 3.85 | 0.0157 | 3.49 | 0.0151 | 3.96 | 0.0204 |
| Length anterior wing | 9.52 | 0.0213 | 8.61 | 0.0149 | 11.77 | 0.0581 |
| Width of head | 3.92 | 0.0044 | 3.77 | 0.0113 | 4.47 | 0.0088 |
| Width posterior wing | 2.16 | 0.0059 | 1.70 | 0.0080 | 3.06 | 0.0074 |
| Width radial cell | 0.50 | 0.0017 | 0.45 | 0.0011 | 0.68 | 0.0019 |
| Length femur of median leg | 2.81 | 0.0052 | 2.13 | 0.0060 | 2.88 | 0.0079 |
| Length tibia of median leg | 2.17 | 0.0117 | 1.91 | 0.0027 | 2.34 | 0.0061 |
| Width tibia of median leg | 0.96 | 0.0027 | 0.47 | 0.0069 | 0.34 | 0.0013 |
| Width femur of median leg | 0.81 | 0.0038 | 0.55 | 0.0043 | 0.49 | 0.0012 |
| Length femur posterior leg | 3.26 | 0.0056 | 2.44 | 0.0033 | 3.29 | 0.0081 |
| Length tibia of posterior leg | 3.77 | 0.0113 | 2.96 | 0.0153 | 4.09 | 0.0098 |
| Width tibia of posterior leg | 1.26 | 0.0033 | 1.10 | 0.0029 | 1.18 | 0.0033 |
| Length tarsus of posterior leg | 4.07 | 0.0294 | 3.57 | 0.0217 | 4.13 | 0.0235 |
| Length of flagelum | 2.65 | 0.0035 | 2.61 | 0.0030 | 3.98 | 0.0128 |
| Length of scape | 1.39 | 0.0017 | 1.22 | 0.0015 | 0.74 | 0.1775 |
| Length pedicelum | 0.26 | 0.0006 | 0.23 | 0.0012 | 0.26 | 0.0019 |
| Width of basitarsus of posterior leg | 1.20 | 0.0040 | 1.05 | 0.0026 | 1.23 | 0.0025 |

## DISCUSSION

Why is the worker so similar to males in *Melipona* ? This is clearly a problem of gene regulation; many explanatory schemes were tried and the one which best fits the available data is the battery of genes proposed by Britten and Davidson (1969, 1971). The hypothesis of Britten and Davidson derives from the theory of gene control and corresponding experimental facts of Jacob Monod and Lwoff, and, it is our opinion that is would be better to call *regulator* (R) instead of *integrator* (I) genes, *operator* (o) instead of *receptors* (R) and *structural protein* gene instead of *producers* (P.). The hypothesis of Britten and Davidson (1969, 1971) defines the following elements : *sensor genes* (S) succeptible to the action of hormones, vitamins, and byproducts of other genes; *integrator genes* (I) that are put to work (in the production of activator RNA) by the sensor genes; *receptor genes* (R) that are put to work under the action of the activator RNA produced by the I genes; *producer genes* (P) that are put to work by the R genes and produce the enzymes or polypeptides needed for the metabolism or development of the individual. We should like to know to which of these 4 classes of genes (S, I, R, P) do the genes, $x^o$, $x^a$ and $x^b$ (that are part of the gene battery or batteries that confers femaleness) belong and how do they act. Trying all the four classes it becomes evident that they are integrator genes (see the model in fig. 4 to 10).

Sex in Hymenoptera is determined by a set of femaleness determining genes which are completely or almost completely additive, in balance with a set of maleness determining genes which are slightly additive (Cunha and Kerr, 1951; Kerr, 1975, 1974 *a*). The complementary multiple alleles $x^o$, that in total *(Apis, Bombus, Melipona)* or partial *(Tetragona)* heterozygosis determines the development of ovaries, are considered femaleness genes that have lost the additive property unless heterozygous (Kerr, 1975, 1974 *a*); their action is in the first days of embryo development. Demonstration of the existence of two sets of genes (one femaleness determining and one maleness determining) was provided by Garófalo and Kerr (1974), working with aneuploids of the bumblebee *Bombus atratus.* The slight additiveness of the maleness genes was shown by Chaud (1974) working with haploid and diploid males and with diploid and triploid workers of *Apis mellifera.*

Caste in *Melipona* is mainly determined by two major integrator genes $x^a$ and $x^b$, in another battery of femaleness genes, whose action is during the larval and, specially, in the prepupal stage (Kerr and Nielsen, 1966; Kerr, 1969, 1974 *a*). These two genes have two alleles each : $x^a_1$ and $x^a_2$, $x^b_1$ and $x^b_2$ (literature in Kerr, 1969). Homozygosity for any allele produces a worker (e.g. $x^a_1/x^a_1$, $x^b_1/x^b_2$; $x^a_1/x^a_2$, $x^b_1/x^b_1$; etc.) while the double heterozygotes are queens ($x^a_1/x^a_2$, $x^b_1/x^b_2$) if well fed but are workers if not well fed. Haploids ($x^a_1$, $x^b_1$; $x^a_1$, $x^b_2$; $x^a_2$, $x^b_1$; $x^a_2$, $x^b_2$) are males (references in Kerr, 1969, 1974 *a*). Diploid drones occur in *Melipona* if a hive is inbred (Camargo, 1974), due to homozygosis of the feminizing gene $x^o$.

The number of characters affected in caste determination is enormous (in the head, thorax, and abdomen, including development of ovaries and associated organs). These characters begun development in a given moment : all or none. This rules out the possibility of the $x^a$ and $x^b$ genes being either R or P. There is a sharp threshold for caste differentiation : If the *Melipona* larva receives sufficient food to become a pupa heavier than 80-85 mg, then heterozygotes will become queens; if the amount of food is insuficient, the imago will be a worker with masculinoid characters. This suggests that the amount of a hormone or other substance is important to bring into operation the S genes. All these facts suggests that the $x^a$ and $x^b$ loci and possibly $x^o$ are « integrator genes ».

The gene N affects the union (if the prepupa is double heterozygote) or lack of union (if the prepupa is homozygous for any of the 4 caste alleles) of the two last abdominal ganglia and in many lines is independent of the amount of food (Kerr and Nielsen, 1966). This suggests that until the prepupal phase, the sensor genes that control $x^a$, $x^b$ are acting and are putting them to work. The sensor genes could only stop acting at the end of the prepupal period. It is important to mention that at this moment the size of the *corpora allata* diminish (Wirtz, 1973) and can not furnish much juvenile hormone. Also, if the genes $x^a$ and $x^b$ (that are genetically independent) are not regulator genes, we would have the odd circumstance of N being strongly linked (no crossing-over so far detected) with two independent genes.

The gene $x^o$ (described in *Apis mellifera* by Mackensen, 1951, and recently discovered by Camargo, 1974, in *M. quadrifasciata*) is the first sex locus to enter into action (about 24 to 36 hours of embryonic development), since it determines ovary, and is not under the same control as $x^a$ and $x^b$; at this stage the food of the alveolus is not important. These two genes ($x^a$ and $x^b$) act during the larval stage and in the prepupal life.

The mechanisms found for male and female determination are put in figure 3 in the presumed evolutionary sequence. $F^o$ represent femaleness genes ($F^o$ is the ovary determiner); after, they become complementary through mutation, and are represented by $x^o$ and ${x^{oa}}_1$, ${x^{ob}}_1$. $F^a$ and $F^b$ are femaleness genes that act in larval and prepupal stages, $M^a$ and $M^b$ represent maleness genes. The following genotypes can be inferred as being involved in the sex determination (the black bar separates the genes that act in the embryonic stage from the ones that act in the prepupal stage) :

1. Primitive Hymenoptera, specially the endogamous microhymenoptera (Cunha and Kerr, 1951; Kerr, 1974 *a*) :

(n) $F^o F^o;\ M^a M^a \mid;\ F^a F^a;\ M^b M^b$ = females.
(2n) $F^o;\ M^a \mid;\ F^a;\ M^b$ = males.

2. *Apis mellifera* (Mackensen, 1951; Woyke, 1963; Chaud, 1974) :

(2n) ${x^o}_1\, {x^o}_n;\ M^a M^a \mid;\ F^a F^a;\ M^b M^b$ = females.
(2n) ${x^o}_n\, {x^o}_n;\ M^a M^a \mid;\ F^a F^a;\ M^b M^b$ = diploid males (most have underdeveloped testes, probably due to the presence of one more $F^o$ gene).
(n) ${x^o}_n;\ M^a \mid;\ F^a;\ M^b$ = haploid males.

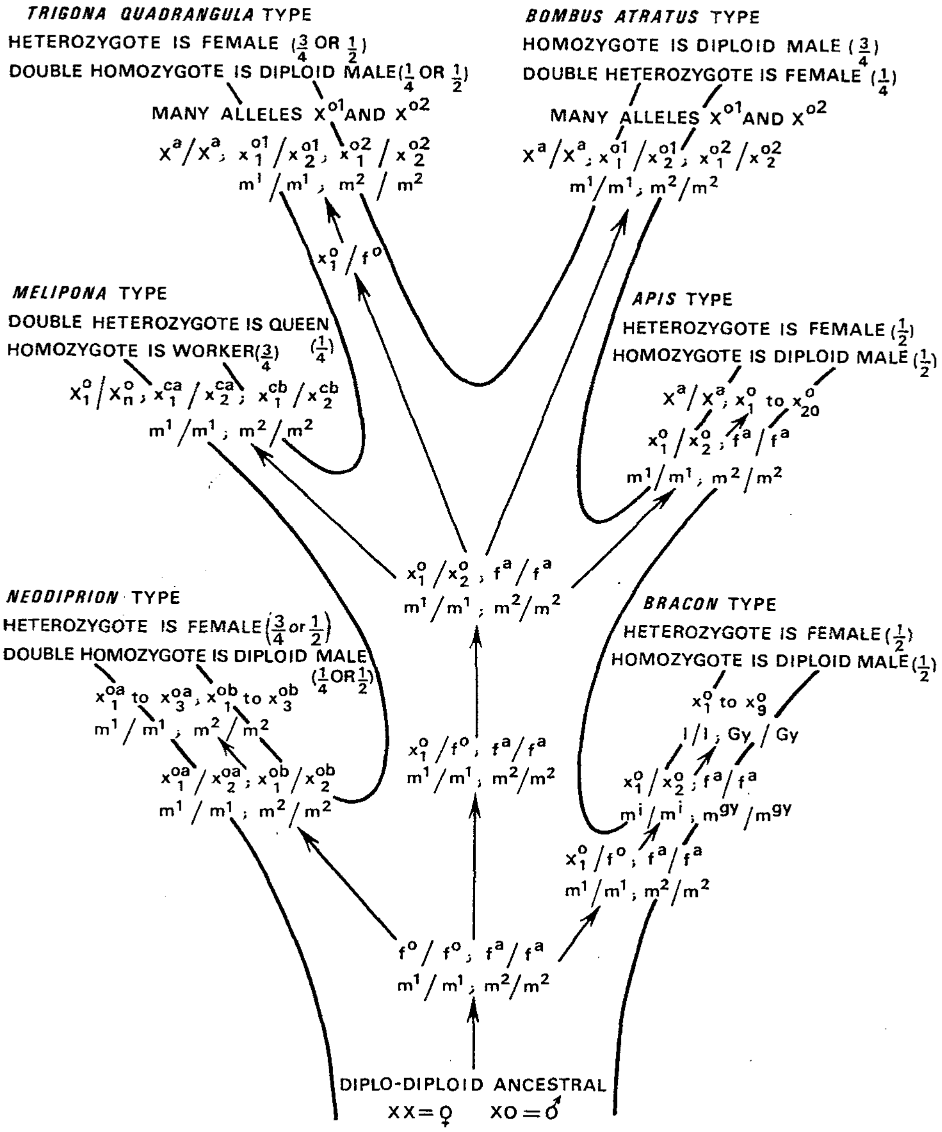


FIG. 3. — *Except for the unknown diplo-diploid ancestral stock, the mechanisms found in the sex determination of Hymenoptera are summarized in this figure, redrawn from* KERR *1974* b *and made up to date.* The genes $f^o$, $x^o$ and $m^1$ act in the embryo phase, and the genes $f^a$, $x^a$, $x^b$, $m^2$ act in the prepupal phase.

ABB. 3. — *Mit Ausnahme des unbekannten Diplo-Diploid Vorfahrenstockes sind die in der Geschlechtsbestimmung gefundenen Mechanismen in dieser Figur, nach* KERR *1974,* b. *zusammengefasst wiedergegeben und zeitgemäss vorwärtsgebracht.* Die Gene $f^o$, $x^o$ und $m^1$ wirken in der Embryophase; die Gene $f^a$, $x^a$, $x^b$ und $m^2$ wirken in der Vorpuppenphase.

3. *Bombus atratus* (bumble bees) (GARÓFALO, 1973; GARÓFALO and KERR, 1974) :

(2n) $x^{oa}_1\, x^{oa}_n;\; x^{ob}_1\, x^{ob}_n;\; M^a M^a\,|\,;\; F^a F^a;\; M^b M^b =$ females.

(2n) $x^{oa}_n\, x^{oa}_n;\; x^{ob}_1\, x^{ob}_n;\; M^a M^a\,|\,;\; F^a F^a;\; M^b M^b =$ diploid males (with underdeveloped testes).

(n) $x^{oa}_n;\; x^{ob}_n\; M^a\,|\,;\; F^a;\; M^b =$ haploid males.

In this case, it looks that a splitting of the original gene $f^o$ took place.

4. *Trigona (Tetragona) quadrangula* (TARELHO, 1973) :

(2n) $x^{oa}_1\, x^{oa}_n;\; x^{ob}_1\, x^{ob}_n;\; M^a M^a\,|\,;\; F^a F^a;\; M^b M^b =$ normal females.
(2n) $x^{oa}_n\, x^{oa}_n;\; x^{ob}_1\, x^{ob}_n;\; M^a M^a\,|\,;\; F^a F^a;\; M^b M^b =$ normal females.
(2n) $x^{oa}_1\, x^{oa}_n;\; x^{ob}_n\, x^{ob}_n;\; M^a M^a\,|\,;\; F^a F^a;\; M^b M^b =$ normal females.

Notice that in this case one heterozygous $x$ is enough for production of females. This sugests duplication of the original genes $x^o$ (and not splitting as in *Bombus atratus*).

(2n) $x^{oa}_n\, x^{oa}_n;\; x^{ob}_n\, x^{ob}_n;\; M^a M^a\,|\,;\; F^a F^a;\; M^b M^b =$ diploid males.
(n) $x^{oa}_n;\; x^{ob}_n;\; M^a\,|\,;\; F^a;\; M^b =$ haploid males.

5. *Melipona* species (KERR, 1948, 1969, 1975, 1974 *a*; KERR, STORT, MONTENEGRO, 1966; CAMARGO, 1974) :

(2n) $x^o_1\, x^o_n;\; M^a M^a\,|\,;\; x^a_1\, x^a_2;\; x^b_1\, x^b_2;\; M^b M^b =$ fertile females (queens).

(2n) $x^o_1\, x^o_n;\; M^a M^a\,|\,;\; x^a_1\, x^a_1;\; x^b_1\, x^b_2;\; M^b M^b =$ workers with underdeveloped ovaries and external male-like morphology.

(2n) $x^o_1\, x^o_n;\; M^a M^a\,|\,;\; x^a_1\, x^a_2;\; x^b_1\, x^b_1;\; M^b M^b =$ workers with underdeveloped ovaries and morphologically male like.

(2n) $x^o_1\, x^o_n;\; M^a M^a\,|\,;\; x^a_2\, x^a_2;\; x^b_1\, x^b_1;\; M^b M^b =$ workers with underdeveloped ovaries and male like appearence.

(n) $x^o_n;\; M^a\,|\,;\; M^b\, x^a_1$ or $x^a_2;\; x^b_1$ or $x^b_2;\; M^b =$ haploid males.
(2n) $x^o_n\, x^o_n;\; M^a M^a\,|\,;\; x^a\; x^a;\; x^b\, x^b_n;\; M^b M^b =$ diploid males.

In this case a splitting of an original gene in $x^a$ and $x^b$ is suspected. It is not known, at the present, if these two genes act under the action of juvenile hormone or if they act producing juvenile hormone at the end.

Taking all these facts in consideration, the genes $x^o$ of *Apis* and *Melipona*, $x^{oa}$ and $x^{ob}$ of *Bombus atratus*, and $x^a$ and $x^b$ *Melipona* can only be *integrator genes* and the following schemes can be suggested :

a) *For the control of the major femaleness gene or genes that determine the ovaries :* see figures 4, 5 and 6. The $x^o$ alleles are complementary and they only produce a complete, functional activator RNA if the alleles are different. $P^o$ represents the enzymes of polypeptides essential to trigger ovary development.

b) *For the control of the femaleness genes that determine the female morphological characters in the last stages of the prepupa :* see figures 7 to 10. The imaginal discs pass through complex development and the same basic genetic constitution produces queen, worker, or male. The figures 7, 8, 9 and 10 indicate

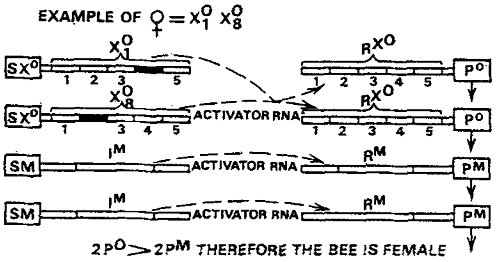


FIG. 4.

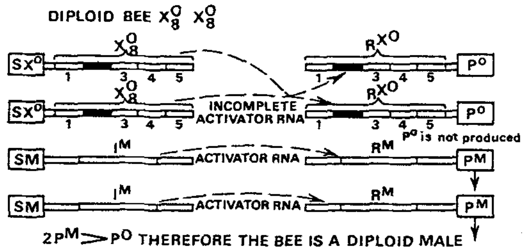


FIG. 5.

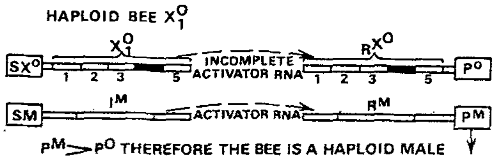
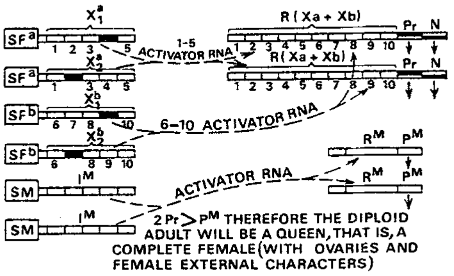


FIG. 6.

FIG. 4, 5 and 6. — *Genic control of sex determination genes which act during embryo development in Apis mellifera.* The model tries to fit known facts with the BRITTEN and DAVIDSON hypothesis. *Figure 4* indicates $x^{a}_{1}$ and $x^{o}_{8}$ as Integrator genes which are complementary, activating the Receptor $R^{xo}$, and in turn $P^{o}$ which produces the polypeptide (or enzyme) which determines cell proliferation in the ovary segment. *Figure 5* shows that whith incomplete activator RNA (since the bee is $x^{a}_{8}$, $x^{o}_{8}$), $R^{xo}$ will not act and $P^{o}$ will not produce ovary determiner protein. *Figure 6* shows the case for haploids. In both *figures 5* and *6* cell proliferation is activated in the positions which develop in testes.

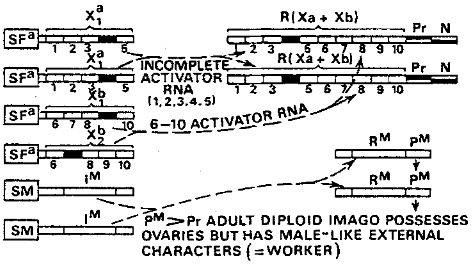
ABB. 4, 5 und 6. — *Genische Kontrolle der Geschlechtsbestimmungs-gene, die während der Embryoentwicklung in Apis mellifica wirken. Das Modell versucht die bekannten Tatsachen mit der Hypothese von* BRITTEN und DAVIDSON *in Einklang zu bringen. Abb 4* bezeichnet $x^{o}_{1}$ und $x^{o}_{8}$ als Integratorgene, die sich ergänzen, indem sie den Empfänger $R^{x}$, und darauf den Produktor $P^{o}$ aktivieren. Dieser produziert den Polypeptid (oder Enzym), welcher in dem Eierstocksegment die Vervielfältigung der Zellen bewirkt. *Abb. 5* zeigt, dass $R^{xo}$ mit unvollständigem Aktivator RNA nicht wirkt (das die Bienen $x^{o}_{8}$, $x^{o}_{8}$ sind), und dass das den Eierstock bestimmende $P^{o}$ kein Protein erzeugt. *Abb. 6* zeigt den Fall für Haploide. In Abb. 5 und 6 wird Zellenvervielfältigung an den Stellen aktiviert, die sich in den Hoden entwickeln.

the possible control of the genes $x^a$ and $x^b$. Since in the scheme it is obligatory to have a single receptor gene $R^{(x^a + x^b)}$, $x^a$ and $x^b$ may have resulted from a breakage in an original large *x* gene, very likely the same one still existing in *Apis, Bombus* and *Trigona.* Figure 9 indicates that, either the gene acts under the influence of the juvenile hormone or is the battery that produces it, its action follows a threshold : below a certain level something does not work and the male genes supplant the female effect. Caste determination appears to follow this same principle in the Trigonini; however, in *Bombus* and *Apis* the level of expression of the controller gene is dependent on the amount of hormone present. Intermediates between the castes are therefore easily found in these two genera. It must be emphasized that the present status of the research does not localize the action of the juvenile hormone in the *S* genes, or directly in the *I* genes; it may be that they are the final product of the first battery that would act in



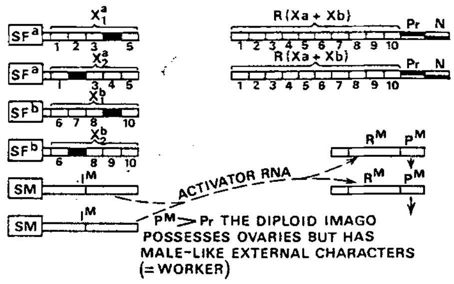
Case $x^a_1/x^a_2$; $x^b_1/x^b_2$ well fed prepura (heavier than 90 mg).

FIG. 7.



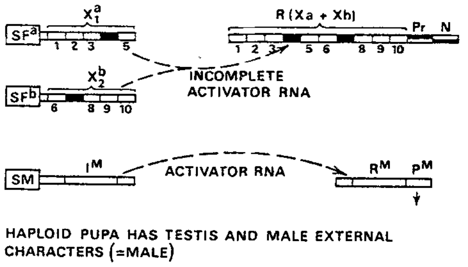
Case $x^a_1/x^a_2$; $x^b_1/x^b_2$ well fed.

FIG. 8.



Case $x^a_1/x^a_2$, $x^b_1/x^b_2$ and $x^a_1/x^a_1$, $x^b_1/x^b_1$. Undernourished. There is not enough amount of juvenile hormone; genes $x^a$ and $x^b$ do not work or work poorly.

FIG. 9.



Case of a haploid bee $x^a_1$; $x^b_2$.

FIG. 10.

FIG. 7 to 10. — *Genic control of sex determination genes which act during prepupal stage of the bee development, in Melipona quadrifasciata.* Five cases are presented : *figure 7* and *9* present the heterozygous bee, respectively well fed and undernourished; *figure 8* and *9* present the case of a homozygote, respectively, well fed and undernourished; *figure 10* shows the haploid condition.

ABB. 7 bis 10. — *Genische Kontrolle der Geschlechtsbestimmungs-gene, die während der Vorpuppenstufe der Bienenentwicklung in der Melipona quadrifasciata.* Fünf Fälle werden dargestellt. *Abb. 7 und 9* repräsentieren die heterozygotischen Bienen, wohlgenährt beziehungsweise unterernährten. *Abb. 8 und 9* zeigen den Fall einer wokl-genährten beziehungsweise unterernährten Homozygote. *Abb. 10* zeigt den Haploizustand.

a second battery that would be the actual femaleness genes. Only future research will clarify this point.

Both in cases of poorly fed *Melipona* female prepupae or when one $x^a$ or $x^b$ alleles is homozygous, the femaleness gene battery stops, the maleness genes take over and the insect, that already has ovaries, develops strong masculine external characters. Therefore, a worker is female inside but almost male outside. This explains why workers of *M. quadrifasciata* are more similar to males than to queens as shown by multivariate analysis (fig. 1).

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## REFERENCES

Britten (R. J.) and Davidson (E. H.), 1969. — Gene regulation for higher cells : a theory. *Science, 165,* 349-357. — 1971. Repetitive and non-repetitive DNA sequences and a speculation on the origins of evolutionary novelty. *Quart. Rev. Biol., 46,* 111-133.

Camargo (C. A.), 1974. — Produção de machos diplóides de *Melipona quadrifasciata* Lep. (Hymenoptera, Apidae). *Ciência e Cultura* (S.P.) *26* (Suplemento), 267.

Cunha (A. B.) and Kerr (W. E.), 1957. — A genetical theory to explain sex-determination by arrhenotokous parthenogenesis. *Forma et Functio, 1,* 33-36.

Chaud (J. Netto), 1974. — Sex-determination in bees. II. Additivity of maleness genes in *Apis mellifera. Genetics* (in press).

Garófalo (C. A.), 1973. — Occurrence of diploid drones in a neotropical bumblebee. *Experientia, 29,* 726-727.

Garófalo (C. A.) and Kerr (W. E.), 1974. — Sex determination in bees. I. Balance between femaleness genes and maleness genes in *Bombus atratus. Genetica* (Utrecht) (in press).

Kerr (W. E.), 1948. — Estudos sobre o gênero *Melipona. An. Esc. Sup. Agr.* « *Luiz de Queiroz* » (Piracicaba), *5,* 181-276. — 1969. Some aspects of the evolution of social bees (Apidae). *Evolutionary Biology, 3,* 119-175. — 1975. Geschlechts-und kastendetermination bei stachellosen Bienen. *In* « Sozialpolymorphismus bei Insekten », *C. H. Schimidt,* Verlag, Stuttgart, 964 p. — 1974 *a.* Evolution of the population structure in bees. Proceedings of the XIII Int. Congres. of Genetics (Berkeley). *Genetics* (in press).
— 1974 *b.* Advances in cytology and genetics of bees. *Ann. Rev. Ent., 19,* 253-268.

Kerr (W. E.) and Nielsen (R.), 1966. — Evidences that genetically determined *Melipona* queens can become workers. *Genetics, 54,* 859-866.

Kerr (W. E.), Stort (A. C.) and Montenegro (M.), 1966. — Importância de alguns fatores ambientais na determinação das castas do gênero *Melipona. An. Acad. Bras. Ciências, 38,* 149-168.

Mackensen (O.), 1951. — Viability and sex-determination in the honey bee (*Apis mellifera* L.). *Genetics, 36,* 500-509.

Rao (C. R.), 1952. — Advanced Statistical Method in Biometric Research. *John Wiley and Sons, Inc.* Publ., London, 390 p.

Tarelho (Z. V. S.), 1973. — Contribuição ao estudo citogenético dos Apoidea. *Ph. D. Thesis,* Dept. Genética, *Fac. Med. Univ. S. Paulo,* Brazil.

Wirtz (P.), 1973. — Differentiation in the honeybee larva. A histological, electron-microscopical and physiological study of caste induction in *Apis mellifera mellifera* L. VIII, Dept. Entomology, *Agric. Univ. Wageningen, Holland,* 155 p.

Woyke (J.), 1973. — Drone larvae from fertilized eggs of the honeybee. *J. Apic. Res., 2,* 19-24.