

SEX DETERMINATION IN BEES. XXVI. MASCULINISM OF WORKERS IN THE APIDAE

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ABSTRACT

The methodology of Sokal and the computational system of Rohlf were used on 3096 measurements of 72 *Bombus atratus* and 1935 measurements of 45 *Scaptotrigona postica*. The analysis revealed that workers of both species are more similar externally to their respective males than to their queens. It is suggested that evolution of the worker and queen caste in the social Apidae followed a series of steps: 1) workers and queens morphologically similar to one another and very different from males; 2) some females lay more eggs than other, but are morphologically very different from males; 3A) specialization and more efficient use of food by queens (as in *Apis*) and specialization of workers; 3B) increasing role of Juvenile Hormone in queens and diminution of it in workers, making workers (due to gene regulation) more and more similar to haploid males (as in *Bombus atratus* and in all meliponids).

INTRODUCTION

A comprehensive review and study of the development and physiological determinants of castes in bees, ants and wasps was made by Wheeler (1986), considering the many levels of evolution of hymenopteran societies. The present paper refers to a specific point not discussed in Wheeler's paper, namely, the evolution of the worker caste towards a masculine external morphology, that is, the female determining genes become more and more dependent on Juvenile Hormone for the regulation of female determining genes.

We dedicate this publication to Dr. Harry H. Laidlaw and to Dr. Walter C. Rothenbuhler in honor of their invaluable contribution to the science of Genetics.

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MATERIAL AND METHODS

In order to augment the information available from the species already studied (Kerr, 1987a) two species, namely *Bombus atratus* (Bombinae) and *Scaptotrigona postica* (Meliponinae), were measured and analysed. Seventy-two specimens (OTU's = Operational Taxonomic Units) of *Bombus atratus*, 23 males, 27 workers, and 22 queens, from 3 different nests maintained by Dr. C. Garófalo, Ribeirão Preto, SP, Brazil, and 20 females, 20 workers and 5 queens from 3 nests of *Scaptotrigona postica* were used in this study. For each bee, 43 characters (listed in Table I of Kerr, 1987a) were measured. The 5031 measurements are in tables deposited in our laboratories and in the office of this journal, and will be sent to readers upon request.

Table I - Generalized distances of Mahalanobis (D, in percentages) in six Apidae species (after Kerr 1987a page 689).

Species	Distances (1) in %			B/C
	Male to queen (A)	Worker to queen (B)	Worker to male (C)	
<i>Apis mellifera</i>	100	59.93	109.06	0.6
<i>Melipona quadrifasciata</i>	100	55.26	52.02	1.1
<i>Melipona compressipes</i>	100	76.58	41.49	1.8
<i>Melipona scutellaris</i>	100	134.10	86.10	1.6
<i>Melipona marginata</i>	100	56.27	41.07	1.4
<i>Bombus atratus</i>	100	83.56	31.34	2.7

(1) These distances have relative values, since for each species different character measurements are used for estimating D2.

The 3096 measurements of *Bombus atratus* and the 1935 of *Scaptotrigona postica* were analysed using phenetic techniques of clustering (UPGMA = Unweighted Pair-Group Method - Arithmetic Averages) - and of ordination, principal coordinate analysis - as discussed in Sneath and Sokal (1973), with help of the NTSYS-PC computational system developed by Rohlf (1986).

RESULTS

Using 43 characters, two matrices of similarities among the OTU's, consisting of taxonomic distance coefficients were constructed from a matrix of standardized

data using the 72 OTU's of *Bombus atratus* and the 45 OTU's from *Scaptotrigona postica*. Phenograms in Figure 1 (for *Bombus atratus*) and Figure 2 (for *Scaptotrigona postica*) were obtained with the use of the UPGMA clustering method. Using the same taxonomic matrices, Figure 3 (for *Bombus atratus*) shows a three-dimensional plot of the scores for the first three principal coordinates, each one accounting for 58.7%, 31.9% and 1.6% of the total variation. Figure 4 (for *Scaptotrigona postica*) shows a projection of the scores for the two principal coordinates, accounting for 76.0% and 19.4% of the total variation.

These results demonstrate the existence of a greater external morphological similarity between males and workers than between workers and queens both in *Bombus atratus* and in *Scaptotrigona postica*.

DISCUSSION AND CONCLUSIONS

The bumblebees (Bombinae) are reasonably primitive eusocial Apidae, because their colonies are founded by only one queen and, therefore, still have a solitary phase. Kerr (1987a) compared generalized Mahalanobis distances among workers and queens, and workers and males (transformed to make males to queens equal to 100) for six species of Apidae (Table I). The present analysis made by quite a different method yields a result that demonstrates the same phenomenon even more dramatically (Figures 1 to 4), that is, there is greater similarity between workers and males than between workers and queens, the exception being *Apis mellifera*. Since many wasps and social Halictidae are similar to *Apis* in this respect we suggest the existence of two systems of caste determination among bees: one in which both workers and queens remain similar but specialize in their respective functions and another in which queens get specialized but workers become more and more similar to males (Figure 5). The fact that workers are more similar to males than to queens is interpreted by Kerr (1975) as being due to gene regulation. That is, female-determining genes are not activated in the last larval phase because of a lack or deficiency of Juvenile Hormone. That would cause the development of workers that are females internally (since in the egg and larval phases they developed ovaries, female genitalia and female antennae) but with male-like external morphology (head, thorax, abdomen, epidermis). This phenomena was first demonstrated in *Melipona quadricincta* using similar technique (Kerr, 1974).

Looking at Table I, one sees that *Apis mellifera* workers show more resemblance to the queen than to males. Meliponids and *Bombus atratus* (Figures 1 to 4) workers have an external morphology closer to males than to queens. This indicates the existence of two systems of caste determination: a) workers resemble the queens to different degrees in the Halictidae, Xylocopidae, Euglossinae, Apinae and b) workers resemble males, to different degrees in *Bombus atratus* and all meliponid species. Mota (1988) studied the presence or absence of different epidermic glands

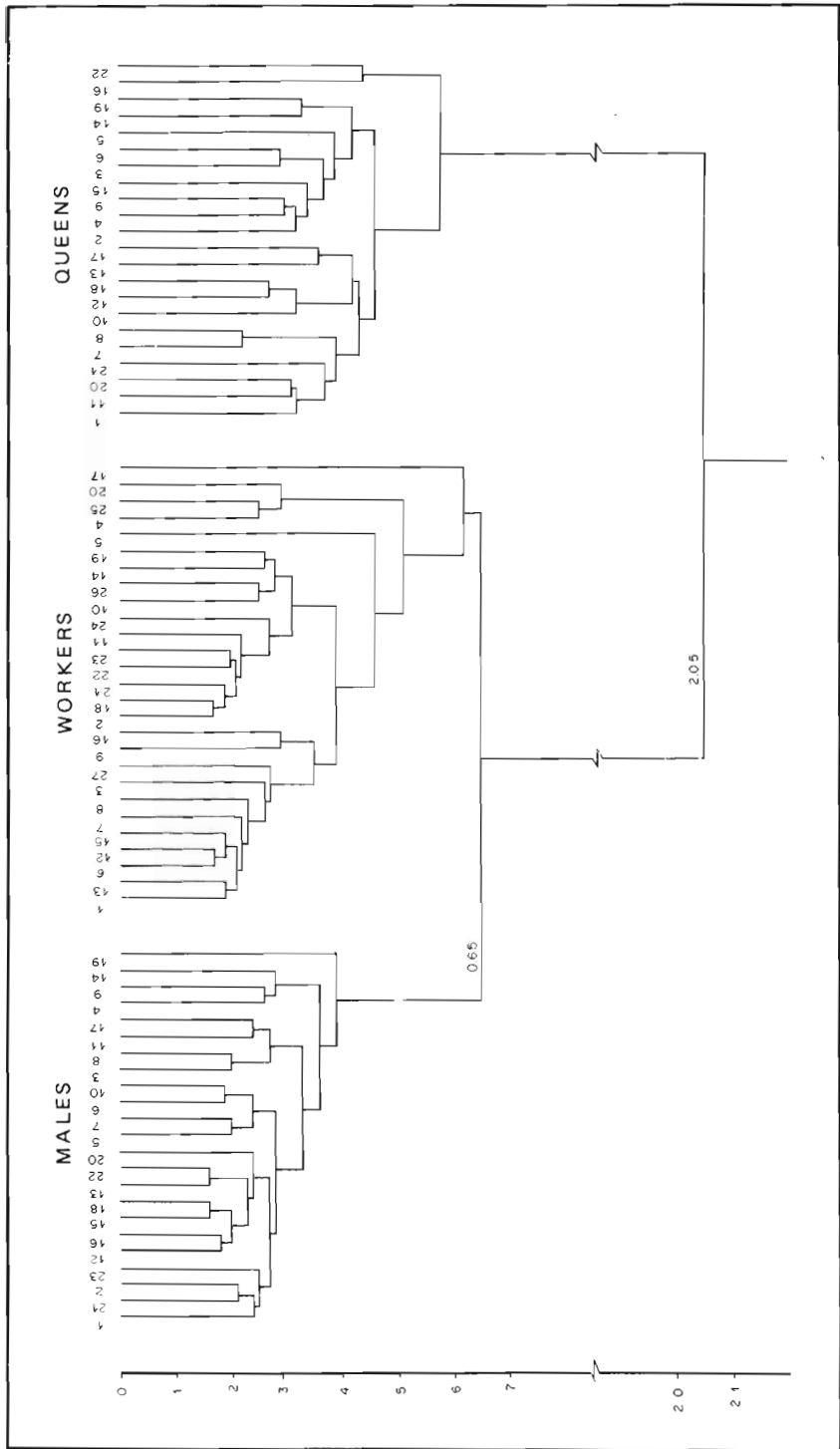


Figure 1 - UPGMA phenogram for males, females and queens of *Bombus atratus*, based on taxonomic distances. Cophenetic correlation is $r = 0.97$.

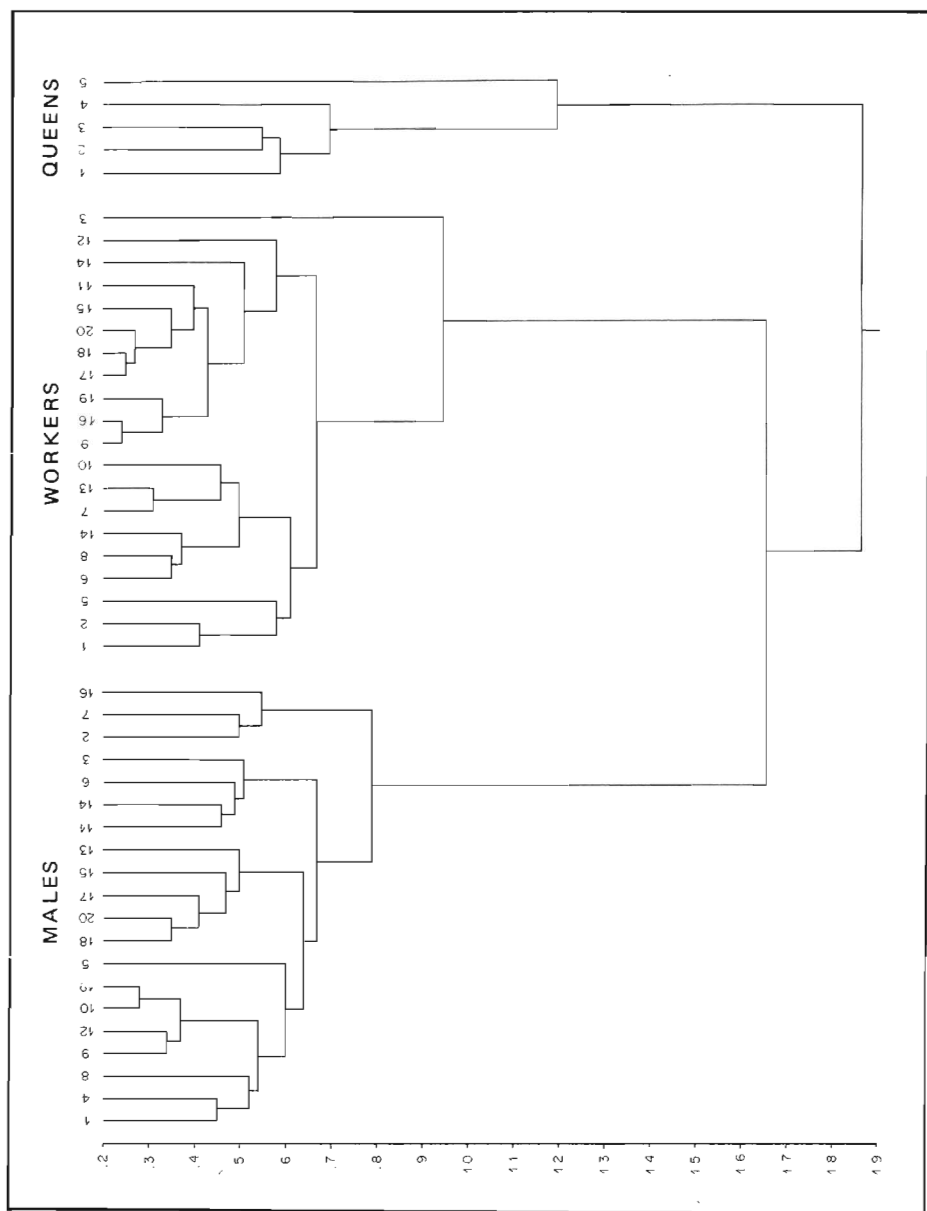


Figure 2 - UPGMA phenogram for males, females and queens of *Scaptotrigona postica*, based on taxonomic distances. Cophenetic correlation is $r = 0.98$.

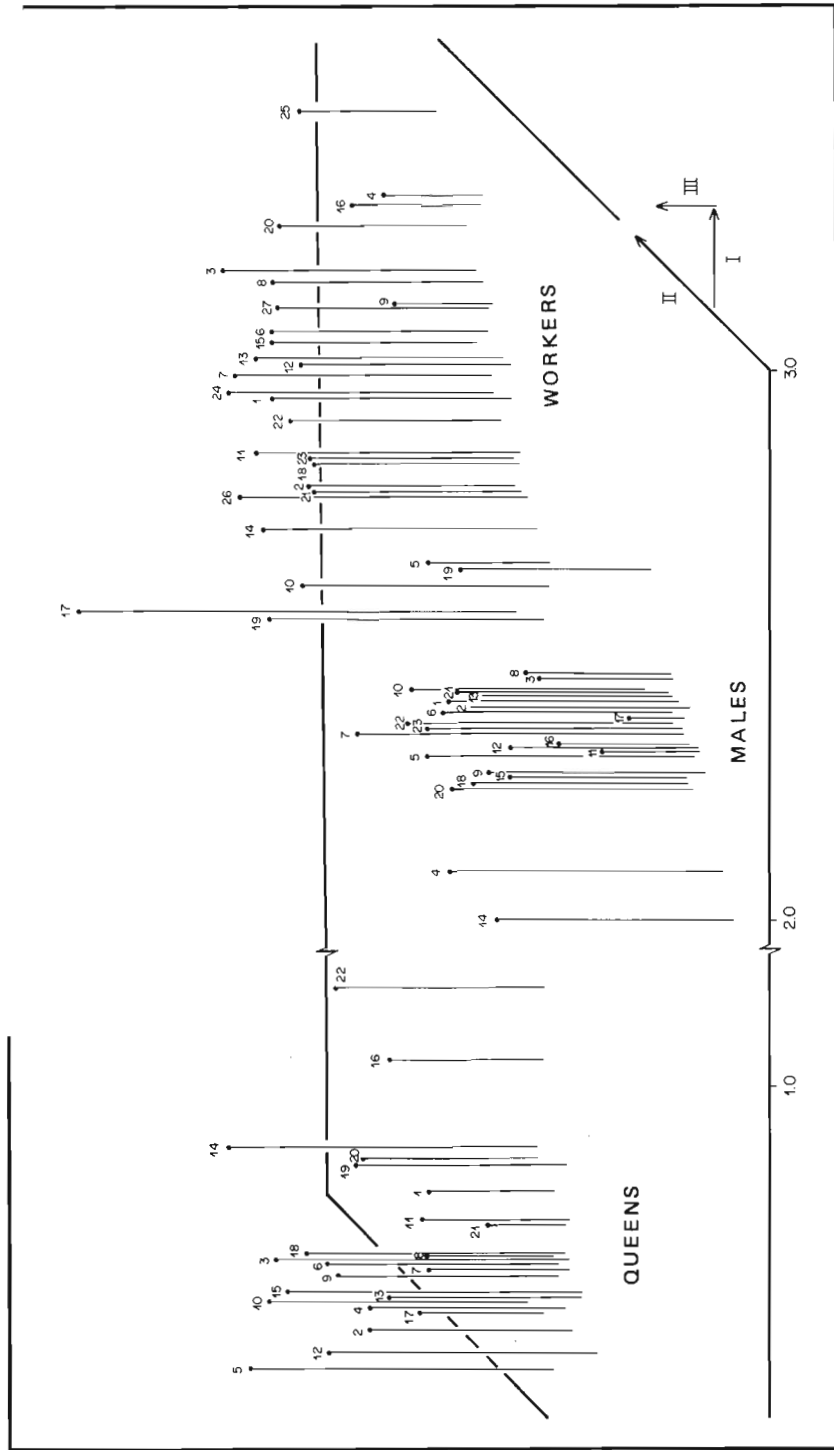


Figure 3 - Projection of the scores for males, females and queens of *Bombus atratus* onto the three principal coordinates.

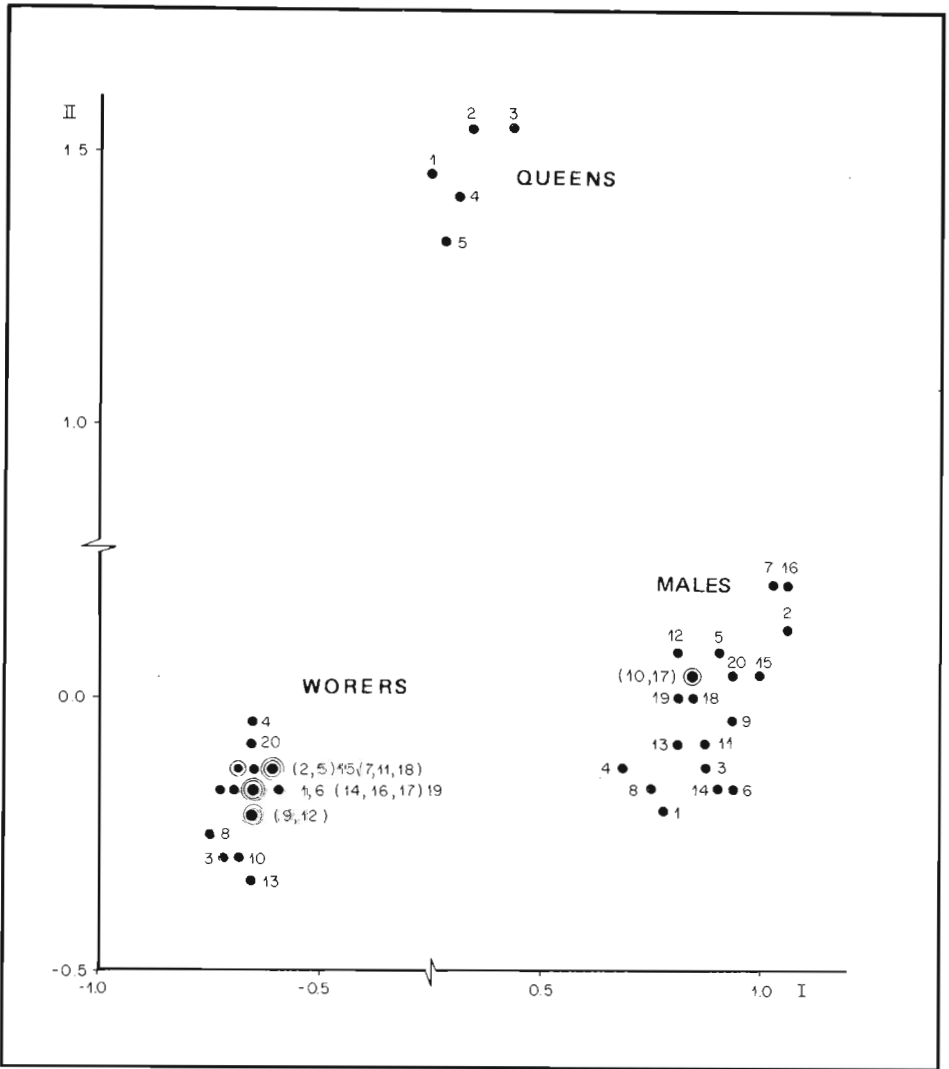
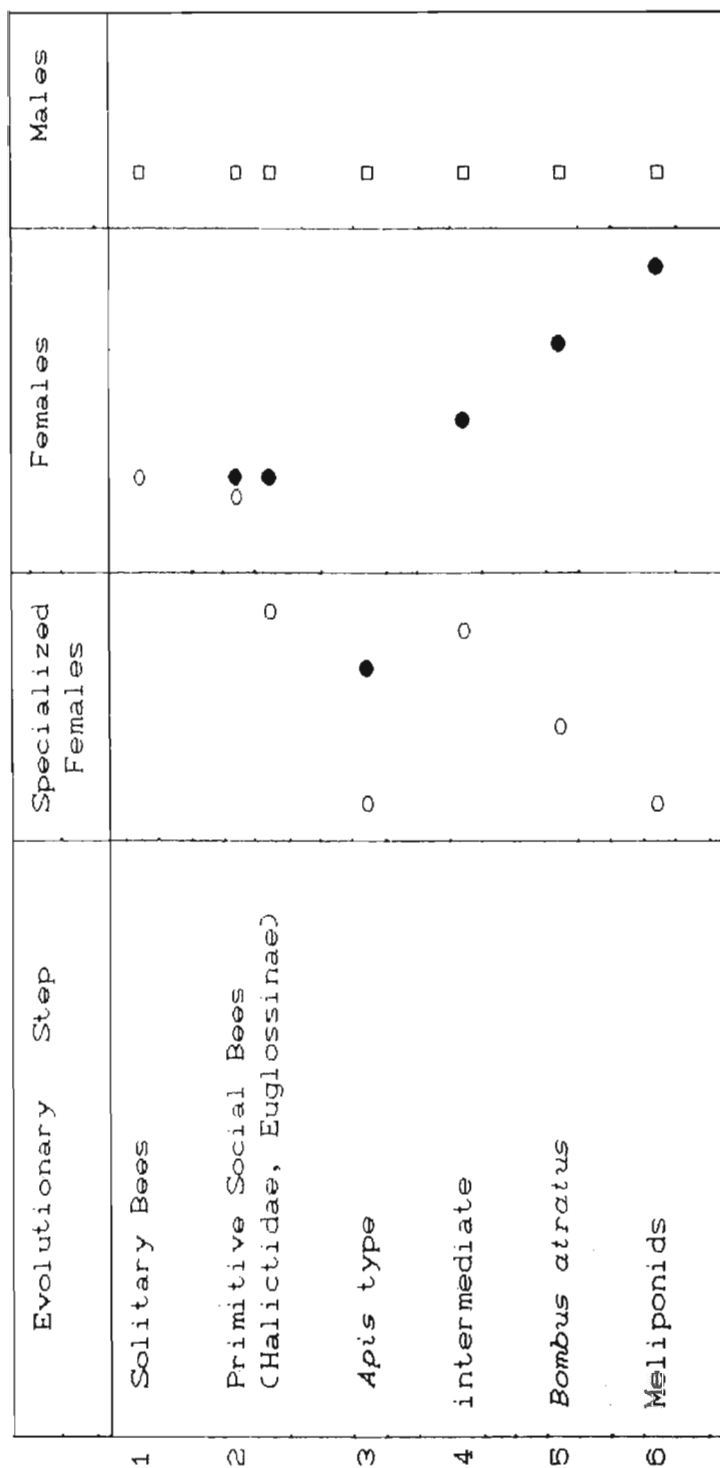


Figure 4 - Projection of the scores for males, females and queens of *Scaptotrigona postica* onto the two principal coordinates.

of the abdomen of males, workers and queens of seven species of meliponids (*Friesella schrottkyi*, *Melipona bicolor*, *M. marginata*, *M. quadrifasciata*, *Plebeia droryana*, *Scaptotrigona depilis*, *Trigona crassipes*). In her Table VI there are 37 coincidences between workers and males and only 12 between workers and queens. Since these glands develop in the second phase of sex determination, they are more similar in the workers to males than to queens. *Bombus atratus* is the only known *Bombus* with permanent nests and may not represent the general case in this genus because primitive-



○ = queen ● = worker □ = male
 ← specialization of females and workers → specialization of workers toward males

Figure 5 - Sequence of evolutionary steps in determination of the worker caste in bees. Females became more and more specialized and became physogastric queens; workers with less and less juvenile hormone became more and more similar to males.

ly social bees have workers and queens which are very similar to one another. Primitive Apidae (Euglossinae) and Xylocopinae have females morphologically very different from males.

These data suggest that the steps followed in the evolution of this character (see Figure 5) are:

1) Solitary bees. No queens present, only males and females. Males of some species are macroscopically different from females, like many *Xylocopa* in which males are brown and females black.

2) Some females lay more eggs than others, e.g., in the parasocial euglossine *Eulaema nigrita*, a range of zero to 11 eggs per female was found (Pereira and Kerr, in press). In some aspects such as *Lasioglossum zephyrum* and *Ceratina japonica* castes are determined in the adult stage (Brother and Michener, 1974; Sakagami and Maeta, 1984). According to Michener (personal communication) all social Halictidae have workers with external morphology very similar to queens and males are very different from females.

3A) In many species food received by the larva is decisive in determining caste as in *Apis mellifera*. This implies a selection for genes that allow a more efficient reaction to environmental conditions. Both queens and workers in their evolution get specialized in their functions, but workers do not become similar to males.

3B) Gradually, caste determination is more and more dependent on gene regulation in which Juvenile Hormone activates female determining genes. There is an increase in Juvenile Hormone production in queens and diminution of its production in workers. The masculinism of workers is effective only in characters determined after the last larval stage; therefore antenna, genitalia and internal organs are not affected.

Many functional characters have developed, but are not directly related to the problem of masculinism. It is probable that when workers are male-like, mutations that make workers more fit also will affect males, because they are morphologically similar. This may explain why males of meliponids produce and work with wax in the beginning of their adult life (Kerr, 1987b), males of *Schwarziana* dehydrate nectar (Fonseca, 1973), all males leave the hive by the end of the first third of their lives, foraging by themselves, and, recently I found that males of *Melipona rufiventris* can follow the communication system of the workers.

Males, workers and queens can be on their own evolutionary trajectory as a consequence of natural selection and of sexual selection. Workers develop corbiculae, stings, and glands; males can have larger eyes and a yellow or white clypeus; queen have a larger abdomen, a white or yellow clypeus, and usually, bright brown tergites.

Presence of corbicula constitutes an important morphological difference between workers and males. This must be a result from one or a series of worker limited genes independent of the sex genes, since after a species becomes cleptobiotic

(*Lestrimelitta*, *Cleptotrigona*) or is not a pollen collector (*Trigona hypogea*) the corbicula is selected against and disappears.

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RESUMO

A metodologia de Sokal e o sistema de computação de Rohlf foram aplicados a 3096 medidas feitas em 72 exemplares de rainhas, machos e operárias de *Bombus atratus* e a 1935 medidas feitas em 45 exemplares de rainhas, operárias e machos de *Scaptotrigona postica*. A análise mostrou que as operárias de ambas as espécies são, externamente, mais semelhantes aos seus machos do que às suas rainhas. Sugere-se que a evolução das castas, operária e rainha, nos Apídeos sociais seguiu a seguinte série de passos: 1) operárias e rainhas são morfologicamente muito parecidas entre si e diferentes dos machos; 2) algumas fêmeas põe mais ovos que outras, porém as que não o fazem continuam diferentes dos machos; 3A) as rainhas desenvolvem uma especialização e uso mais eficiente do alimento (como em *Apis*) e há uma especialização no trabalho pelas operárias; 3B) as rainhas se especializam e desenvolve-se nelas um papel muito importante do Hormônio Juvenil e concomitante diminuição desse papel nas operárias, fazendo com que as operárias sejam cada vez mais semelhantes aos machos haplóides, como em *Bombus atratus* e em todos os meliponíneos.

REFERENCES

- Brother, d.J. and Michener, C.D. (1974). Interaction in colonies of primitively social bees. III. Ethometry of division of labor in *Lasioglossum zephyrum* (Hymenoptera: Halictidae). *J. Comp. Physiol.* 90: 129-168.
- Fonseca, V.L.I. (1973). Miscellaneous observations on the behavior of *Schwarziana quadripunctata* (Hym., Apidae, Meliponinae). *Biol. Zool. e Biol. Mar. N.S.* 30: 633-640.
- Kerr, W.E. (1974). Sex determination in bees. III. Caste determination and genetic control in *Melipona*. *Insectes Sociaux* 21: 357-368.
- Kerr, W.E. (1975). Evolution of population structure in bees. *Genetics* 79: 73-84.

- Kerr, W.E. (1987a). Sex determination in bees. XVII. Systems of caste determination in the Apinae, Meliponinae and Bombinae and their phylogenetical implications. *Rev. Bras. Genet.* 10: 685-694.
- Kerr, W.E. (1987b). Biologia, manejo e genética de *Melipona compressipes fasciculata* Smith (Hymenoptera, Apidae). Full Professor Thesis, Universidade Federal do Maranhão, São Luis, MA.
- Mota, M.H.V.B. (1988). Estudo comparativo das glândulas epidérmicas do abdômen dos meliponíneos (Hym. Apidae, Meliponinae). PhD Thesis, UNESP, Rio Claro, SP.
- Pereira, S.R.F. and Kerr, W.E. Biologia de *Eulaema nigrita*. 3. Inferências evolutivas. *Papeis Avulsos do Museu de Zoologia da USP.* (in press).
- Rohlf, F.J. (1986). *NTSYS-pc: Numerical taxonomy system for the IBM-PC microcomputer and compatibles.* Dept. of Ecology and Evolution, SUNY at Stony Brook, N.Y.
- Sakagami, S.F. and Maeta, Y. (1984). Multifemale nests and rudimentary castes in the normally solitary bee *Ceratina japonica* (Hymenoptera, Xylocopinae). *J. Kansas Entomol. Soc.* 57: 639-656.
- Sneath, P.H.A. and Sokal, R.R. (1973). *Numerical Taxonomy.* W.H. Freeman & Co., San Francisco.
- Wheeler, D.E. (1986). Development and physiological determination of caste in social Hymenoptera: Evolutionary implications. *American Naturalist* 128: 13-34.

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