Rev. Brasil. Genet. III, 2, 127-138 (1980)
(Brazil. J. Genetics)

GENETIC MECHANISMS FOR THE DEVELOPMENT OF REPRODUCTIVE ORGANS OF *Apis mellifera* WORKERS AND DIPLOID DRONES: A COMPLEMENTARY HYPOTHESIS *

José Chaud-Netto[1] and Warwick E. Kerr[2]

ABSTRACT

A study of the normal variation of ovariole number in *Apis mellifera* workers was carried out in groups of 30 female pupae from each of 11 *Apis mellifera adansonii* hives and 11 *Apis mellifera ligustica* hives. The number of ovarioles for *adansonii* pupae varied between 1 and 11 per ovary, and between 1 and 24 for *ligustica* pupae. A parallel study of the normal variation in size of testes in haploid drones was also carried out utilizing 20 *adansonii* and 20 *ligustica* drone pupae. The weight of testes varied between 46 and 56.5 mg for the *adansonii* drones, and between 56 and 77 mg for the *ligustica* drones. Twenty-seven pupae of diploid *Apis mellifera* males were also studied for weight of testes. Of these, 21 (78%) had almost normal testes while 6(22%) had very small testes. In contrast, 26 haploid drones from the same queen had normal testes. Workers from

---

* This paper is part of the M.Sc. thesis of J.C.N. and was supported by Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP) and by the Brazilian National Research Council (CNPq)

[1] Departamento de Biologia do Instituto de Biociências (UNESP), 13.500 Rio Claro, S.P., Brasil.

[2] Departamento de Genética, Faculdade de Medicina de Ribeirão Preto, (USP), 14.100 – Ribeirão Preto, S.P., Brasil.

this hive were studied for ovariole number, with 75% showing a normal number (1 to 20 per ovary) and 25% a larger number (21 to 95). These data suggest that the number of ovarioles in workers and the size of testes in diploid drones are governed by two major genes.

INTRODUCTION

Queen and worker honeybees have the same number of ovarioles up to the fifth larval day. After this the worker's ovaries degenerate with a drastic reduction in ovariole number to between 2 and 20 per ovary at birth, while newborn queens have 160 to 180 ovarioles per ovary (Meier, 1916; Zander *et al.*, 1916, 1925). This is due in part to the "alimentary castration" of worker honeybees after 60 hours of larval life, in contrast to the superalimentation of the queens with royal jelly throughout the larval stage (Von Rhein, 1933; Weaver, 1957). *Apis dorsata* workers have 11 to 53 ovarioles per ovary, while their queens have 124 to 137 (Velthuis *et al.*, 1971). *Apis cerana* workers commonly have 1 to 12 ovarioles per ovary (Kapil, 1962) but in some cases this number may reach 30 per ovary (Sakagami and Akahira, 1958).

Homozygosis for *x* alleles causes retarded development and decreased size of testes in diploid *A. mellifera* drones. The size of testes does not depend on the different *x* alleles, but is governed by polygenes (Woyke, 1969, 1974). Fundamental differences in reproductive organs have been observed between haploid and diploid drones (Woyke, 1973; Chaud-Netto, 1973): 1) mean testes volume for haploid drones from a given queen is generally 10 times that for diploid males; 2) diploid drones have approximately half the number of testicle tubules found in haploids; 3) the seminal vesicles and mucus glands of diploid drones are 85 to 95% the size of the haploid; 4) diploid drones mature 10–15 days later than haploids.

The objective of this investigation was to study the genetic mechanisms involved in the development of the reproductive organs of *Apis mellifera* workers and diploid drones by determining the normal variation in ovariole number and in size of testes in workers and haploid drone pupae, respectively, and by comparing the results with data obtained for workers and diploid drones from an experimental hive of *Apis mellifera ligustica* and *Apis mellifera adansonii* hybrids.

MATERIAL AND METHODS

The study was divided into two parts. The first was concerned with the normal variation in ovariole number and in size of testes for *Apis mellifera* workers and haploid drones, respectively. Eleven hives containing Italian honeybees (*Apis mellifera ligustica*) and 11 hives containing Africanized honeybees (*Apis mellifera adansonii*) were used for the initial part of this research. Each Italian and each African queen had been previously mated to a single drone of the respective strain by instrumental insemination. Thirty worker pupae from each of the 22 hives were dissected in water on the 18th day of development. Their ovaries were excised and lightly dehydrated with 96% alcohol, and the number of ovarioles was recorded for each female. For the study of the normal variation in size of testes, 20 *Apis mellifera adansonii* drone pupae and 20 *Apis mellifera ligustica* drone pupae were dissected in saline (0,75% NaCL), on the 18th day of development, and their testes were excised and weighed.

The second part of this research was carried out in order to obtain information on a specific experimental group of hybrids. Twenty-six haploid drones, 27 diploid drones and 30 workers, all sons and daughters of the same queen (129) (a hybrid of *Apis mellifera ligustica* and *Apis mellifera adansonii)* were utilized in this experiment. The male pupae were dissected in saline (0.75% NaCl) on the 18th day of development. The workers, of approximately the same age as the males (black-eyed pupae with pigmented bodies), were dissected in water, their ovaries were excised and lightly dehydrated with 96% alcohol, and the number of ovarioles was recorded for each female.

RESULTS

Experiment n ♀ 1 – The number of ovarioles per ovary in workers of *Apis mellifera adansonii* varied between 1 and 11. The *Apis mellifera ligustica* workers had 1 to 24 ovarioles per ovary (see Table I), with only two of the 660 females having more than 20 ovarioles per ovary. The haploid drones of *Apis mellifera adansonii* had testes weighing 46 to 56.5 mg ($\overline{X}$ = 51.55 ± 2.36 mg), while the testes of haploid *Apis mellifera ligustica* drones weighed 56 to 77 mg ($\overline{X}$ = 63.90 ± 5.27 mg). The coefficient of variation was about 4.57% for the sample of *adansonii* drones, and 8.23% for the *ligustica* drones.

Table I - Characteristics of frequency distributions of ovarioles in workers from 22 hives of *Apis mellifera.*

Subspecies	Hive	Mean	Mode	Maximum
A. m. ligustica	3	3.85	4	8
	97	4.77	5	9
	225	5.45	3	12
	206	5.48	4	14
	136	5.72	3	15
	227	6.52	4	16
	140	6.78	7	15
	210	6.87	6	15
	122	7.03	8	16
	38	7.05	7	16
	91	9.38	8	24
A. m. adansonii	226	2.47	2	6
	20	2.63	3	6
	22	2.75	2	8
	28	3.23	3	6
	56	3.62	2	9
	64	4.15	3	10
	137	4.47	3	9
	23	4.68	3	10
	117	5.30	4	11
	21	5.53	4	10
	49	6.02	4	11

Experiment nº 2 – The testes weights obtained for 27 diploid drones could be divided into two distinct groups: 6 to 18 mg, and 30 to 66 mg (see Fig. 1). The coefficient of variation obtained was 41.55%. Genetically, the data suggested a simple Mendelian segregation (3:1), i.e. 3/4 of diploid drones with normal testes and 1/4 of diploid drones with small testes. This hypothesis was tested by applying the χ^2 test to the data and the result ($\chi^2 = 0.11$, $P = 0.74$)

was consistent with 3:1 segregation. This implies that the mother of the diploid drones had an *A/a:B/b* genotypic constitution, and that the father that supplied the semen for the instrumental insemination was *a;b*. The diploid drones which had inherited at least one of the dominant genes (*A* or *B*) would have normal testes, while those with double recessive genotypes (*a/a : b/b*) would have small testes.

A group of 26 haploid drones from the same queen (129) was used as control in this experiment. One of the objectives of this study was to make a comparative analysis of testes weight for haploid and diploid drones originating from the same queen to find out whether the hypothesis postulated for the genetic control of the diploid drones testes was valid also for the haploid drones. The 26 haploid drones had testes weighing between 44 and 74 mg (coefficient of variation: 9.66%). A frequency diagram for the values obtained for haploid drones shows that the weight variation in this sample was relatively small when compared to that for their diploid brothers (their coefficient of variation was 41.55%, i.e. 4.3 times that for the haploid drones) (Fig. 2). The data provided a unimodal distribution suggesting that the two gene hypothesis formulated for the development of the diploid drones testes cannot be applied to these haploid drones. Thus it appears that the haploid drones in this hive have a genetic mechanism for testes development which is not ruled by the mechanism observed in their diploid brothers.

The variation in number of ovarioles was next studied in workers of the same hive. The results of these observations are shown in the frequency diagram in Fig. 3. It can be easily seen that the distribution is bimodal and that about 25% of the workers (small group) have 21 to 95 ovarioles per ovary (*Apis mellifera* workers commonly have 1 to 20 ovarioles per ovary). Genetically, the data suggest a simple Mendelian segregation, i.e. 3/4 workers with a normal number of ovarioles (1 to 20) and 1/4 workers with a larger number of ovarioles (21 to 95). Once more, this hypothesis was found to be acceptable (the χ^2 test applied to the data gave a nonsignificant result: $\chi^2 = 0.08$). It is important to point out that these results suggest a specific genetic mechanism responsible for the expression of the "number of ovarioles" character in these worker honeybees.

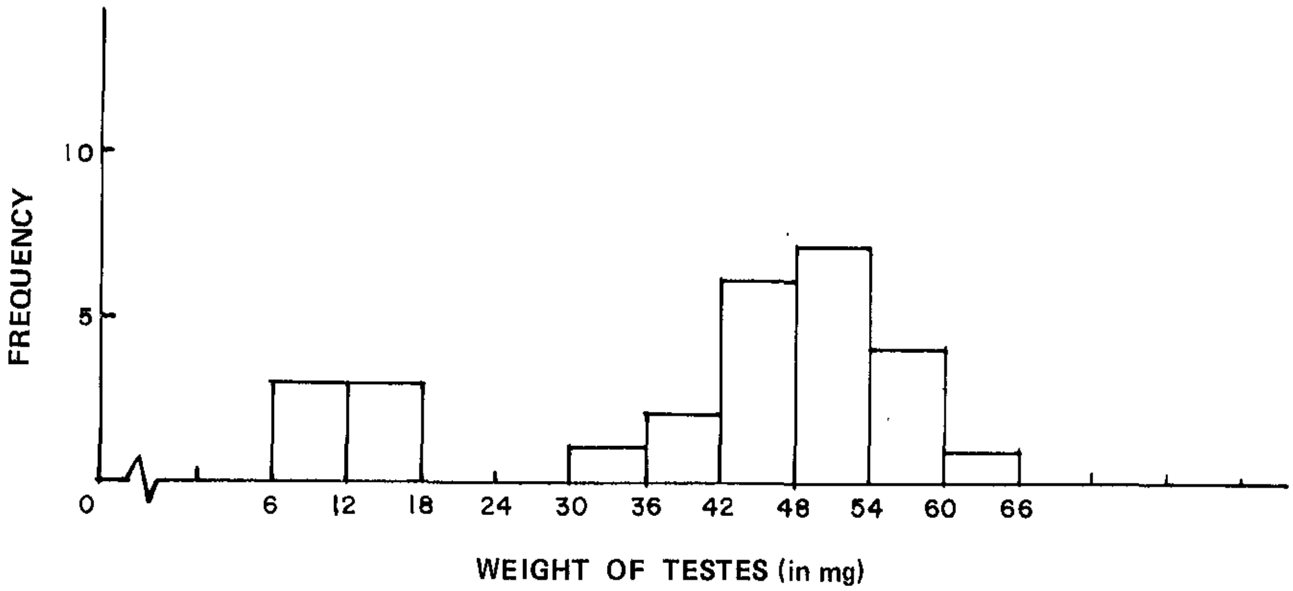

Figure 1 - Frequency-distribution of the weight of testes in diploid drones (queen 129).

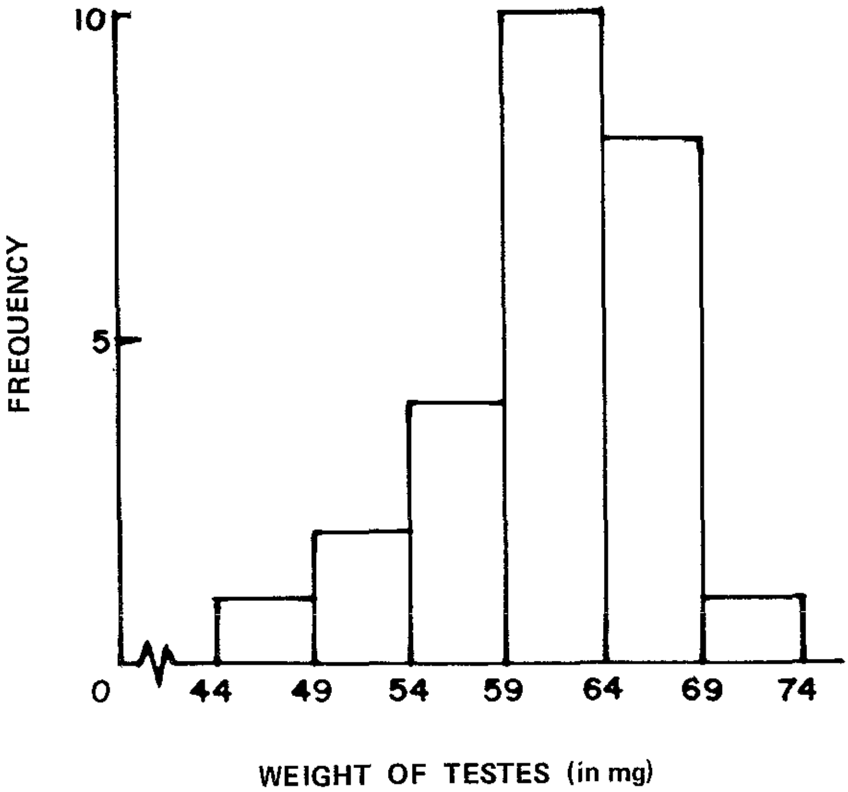

Figure 2 - Frequency-distribution of the weight of testes in haploid drones (queen 129).

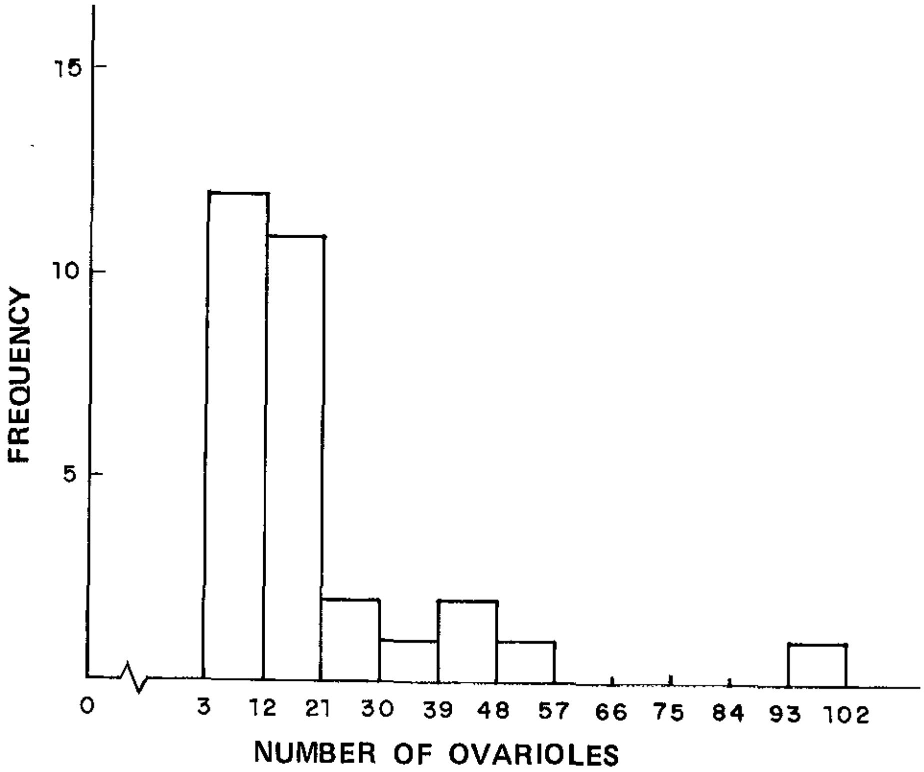

Figure 3 - Frequency-distribution of the number of ovarioles per ovary in workers (queen 129).

DISCUSSION

The normal variation in number of ovarioles for workers from the 22 hives studied in our first experiment can be considered to be 1 to 20 ovarioles per ovary, since only 0.3% of the dissected workers showed more than 20 ovarioles per ovary. Normal variation in testes weight for haploid drones was 46 to 77 mg.

Woyke (1971, 1974) interpreted the variation in testes size of diploid drones as being caused by polygenes that would act differently in *Apis mellifera ligustica* and *Apis mellifera adansonii*. However, a character determined by polygenes would give values which, if plotted on a frequency diagram, should show a unimodal distribution. The data obtained for weight of diploid drone testes yielded a bimodal distribution characteristic of bifactorial segregation (in bees). In contrast, the haploid drones from the same queen had a unimodal distribution and thus no evidence was found for a 3:1 segregation for testes weight. Again, a bimodal distribution characteristic of bifactorial segregation was obtained for ovarioles in workers.Approximately 75% of the workers had a practically normal number of ovarioles (3 to 20 ovarioles per ovary) while approximately 25% showed more than 20 ovarioles per ovary.

Using these data and the basic idea elaborated by Woyke (1969, 1974), we can formulate a complementary hypothesis for the development of testes in diploid drones and number of ovarioles in worker honeybees. These two characters would be determined by polygenes but among these there may be two major genes, each having two alleles. One of their combinations could originate a large number of ovarioles in workers and small testes in diploid drones of *Apis mellifera*. These two genes would act only in diploid individuals and, since there is no normal occurrence of diploid drones in nature, they would act only on the females. This hypothesis is very probable if we calculate that in *Apis mellifera* 15% of the genes that contribute to the genetic load (Kerr, 1975) and 40% of the quantitative effect genes (Gonçalves, 1970) are limited to females. These genes responsible for the development of reproductive organs of workers and diploid drones of *Apis mellifera* are indicated here with the letters *M* and *Ov*. Each one of these genes would have two alleles (*M, m* and *Ov, ov*, respectively). Crossing of an *M/m;Ov/ov* female and of an *m;ov* male would produce the following combinations:

Genotype	Diploid drones	Workers
M/m; Ov/ov	Normal testes	Normal number of ovarioles
M/m; ov/ov	Normal testes	Normal number of ovarioles
m/m; Ov/ov	Normal testes	Normal number of ovarioles
m/m; ov/ov	Small testes	Large number of ovarioles

The recessive combination (*m/m; ov/ov*) would lead to small testes in the diploid drones and a large number of ovarioles in the workers. The presence of a single dominant gene (*M or Ov*) would originate diploid drones with normal testes and workers with a normal number of ovarioles. The recessive combination is rare and perhaps the genes that govern these extraordinary characteristics are primitive ones, since large numbers of ovarioles in workers are normally found in the primitive *Apis dorsata.*

ACKNOWLEDGMENT

The authors are grateful to Dr. H.H.W. Velthuis for calling their attention to the case of *Apis dorsata.*

RESUMO

Foi estudada a variação normal do número de ovaríolos de operárias de *Apis mellifera* em grupos de 30 pupas provenientes de 11 colmeias de *Apis mellifera adansonii* e 11 de *Apis mellifera ligustica.* O número de ovaríolos das pupas de *adansonii* variou de 1 a 11 por ovário, e de 1 a 24 nas pupas de

ligustica. Estudou-se também, em paralelo, a variação normal do tamanho testicular em zangões haplóides, utilizando 20 pupas de zangões *adansonii* e 20 de *ligustica.* O peso testicular variou de 46 a 56,5 mg nos zangões *adansonii* e de 56 a 77 mg nos de *ligustica.* Estudou-se também o peso testicular de 27 pupas de machos diplóides de *Apis mellifera.* Vinte e uma destas (78%) tinham testículos quase normais, enquanto que 6 (22%) os tinham muito pequenos. Em contraste, 26 zangões haplóides provenientes da mesma rainha tinham testículos normais. As operárias desta mesma colmeia foram estudadas quanto ao número de ovaríolos, sendo que 76% apresentaram um número normal (de 1 a 20 por ovário) e 25% um número grande (de 21 a 95). Estes resultados sugerem que o número de ovaríolos das operárias e o tamanho dos testículos dos zangões diplóides são regulados por dois genes principais, além dos demais genes quantitativos.

REFERENCES

Chaud-Netto, J. (1973). Machos diplóides de *Apis mellifera:* Sua bionomia e contribuição ao problema da determinação do sexo. Masters Thesis, 140 pp. Universidade de São Paulo.

Gonçalves, L.S. (1970). Análise genética do cruzamento entre *Apis mellifera ligustica* e *Apis mellifera adansonii.* Doctoral Thesis. Universidade de São Paulo.

Kapil, R.P. (1962). Anatomy and histology of the female reproductive system of *Apis indica* F. (Hymenoptera, Apidae). *Ins. Soc. 9* (2): 145-163.

Kerr, W.E. (1975). Population Genetic Studies in bees (Apidae, Hymenoptera). 1. Genetic Load. *An. Acad. Brasil. Ciên. 47* (2): 319-334.

Sakagami, SH. F. and Akahira, Y. (1958). Comparison of the ovarian size and of ovarioles between the worker bees of Japanese and European honeybees. Studies of the Japanese honeybee. *I, Kontyu* 26: 103-109.

Velthuis, H.H.W., Clement, J., Morse, R.A., and Laigo, F.M. (1971). The ovaries of *Apis dorsata* from the Philippines. *J. Apic. Res.* 10 (2): 63-66.

Von Rhein, W. (1933). Uber die Entstehung des Weiblichen Dimorphismus in Bienenstaate. *Wilhelm Roux'Archiv. F. Entwickl. Organ 129* (4): 601-665.

Weaver, N. (1956). Ovarian development of worker honeybees. *Econ. Entomol. 49* (6): 854-857.

Weaver, N. (1957). Effects of larval age on dimorphic differentiation of the female honeybees. *Ann. Entomol. Soc. Amer. 50* (3): 283-294.

Woyke, J. (1969). The role of sex alleles in the development of reproductive organs of diploid drones. *XXII Int. Apic. Congr. Summ. Paper*: 187-188.

Woyke, J. (1971). New experimental data on honeybee genetics. *XXIII Int. Beekeep. Congr.*: 365-367.

Woyke, J. (1973). Reproductive organs of haploid and diploid drone honeybees. *J. Apic. Res.* 12 (1): 35-51.

Woyke, J. (1974). Genic balance, heterozygosity and inheritance of size of testis in diploid drone honeybees. *J. Apic. Res.* 13 (2): 77-91.

(Received May 18, 1979)