

GENETIC BACKGROUND OF SEXUALITY IN  
THE DIPLOID DRONE HONEYBEE

J. WOYKE

Agricultural University, Bee Division, Warszawa 13, Ursynów, Poland

Manuscript received for publication 12 February 1979

Summary

According to the latest version of the genic balance hypothesis, diploid drones should show super-male characters. Almost 38 thousand measurements or counts on haploid and diploid drones, and queens and workers, showed that (except the testes and structures on the wings) all characters were larger in diploid than in haploid drones. Many body parts of the haploid drone were larger than those of females, but some (such as mouthparts and abdomen parts) were smaller. Diploid drone body parts, being larger than those of the haploids, showed (in relation to the females) super-male character in half the dimensions investigated and, in the other half, inclination toward female characters ranging from intersex through caste and female to super-female characters. Of 10 body parts larger in females than in haploid drones, only one was smaller in diploid than in haploid drones, exhibiting a super-male character. The others tended toward female characters. In size, diploid drone body parts were closer to the queen than to the worker. Thus the main difference between the haploid and diploid drone was the larger size of the diploid and not a shift in one direction of sexuality; this disagrees with the genic balance hypothesis. The sexuality of diploid drone body parts depends upon their size and upon the relation to the haploid drone and to the females. However, the size of different body parts is not the result of genic balance, but of higher polyploidization of body tissues. This hypothesis enables a forecast to be made of sexuality of body parts not yet investigated. Higher polyploidization will cause larger body parts in diploid than in haploid drones. Diploid drone body parts will show super-male character if the parts are larger in the haploid drone than in females, and an inclination toward the female character if the parts are smaller in the haploid drone than in the females.

Introduction

Whiting (1940), working with *Bracon*, discovered that homozygosity at the sex locus  $X$  resulted in diploid males, but he did not try to explain their degree of maleness. According to Cunha and Kerr (1957) sex would be determined by a balance  $2F > M > F$  between maleness genes  $M (= m_1 + m_2)$  having non-cumulative effects and femaleness genes  $F (= f + f)$  having cumulative effects. In the haploids  $M$  dominates  $F$ , producing males, whereas in the diploids  $2F$  dominates  $M$ , producing females. The  $X$  alleles were considered as major  $f$  genes which, unless heterozygous, lose their additivity. Therefore diploid males develop from fertilized eggs homozygous at the sex locus. Woyke (1963) proved that in *Apis mellifera* diploid drones develop from fertilized eggs homozygous at the sex locus  $X$ . Kerr (1974a, 1974b) and Chaud-Netto (1975) explained the sexual genetical constitution and genic balance between the maleness genes  $m_2 + m_1$  and the femaleness genes  $X + f$  as follows;

haploid drone  $X_1 + f + m_1 + m_2$ , where  $m_1 + m_2 > X_1 + f$

diploid drone  $X_1X_1 + 2f + 2m_1 + 2m_2$ , where  $2m_1 + 2m_2 > X_1X_1 + 2f$

diploid female  $X_1X_1 + 2f + 2m_1 + 2m_2$ , where  $2m_1 + 2m_2 < X_1X_2 + 2f$ .

However, the femaleness genes were considered to be somewhat cumulative in the homozygote  $X_1X_1 + 2f$ , since *Habrobracon* diploid males were not normal. Woyke (1965a, 1965b, 1973) showed that diploid *A. mellifera* drones had much smaller testes than the haploids, and Kerr and Nielsen (1967) presented data according to which the rudiments of external parts of reproductive organs in the youngest *A. mellifera* larvae were less distinctive in diploids than in haploids. Kerr and Nielsen (1967) concluded.

\*This investigation was supported in part by a research grant from the USDA authorized by PL 480.

that honeybee males homozygous at the sex locus  $X$  are more female-like than the haploids. Both these facts support the hypothesis of additivity of femaleness genes.

Later Woyke (1971a, 1971b) showed in his detailed biometrical investigation that diploid drones have many super-male characters. This led Kerr (1974a, 1974b) to conclude that the male-determining genes must also be additive, resulting in the super-male characters of diploid drones.

Woyke (1974) showed that the size of testes of diploid drones, and thus their potential degree of maleness, did not depend either on the composition of different sex alleles  $X$  or on the level of homozygosity.

Chaud-Netto (1973, 1975) and Chaud-Netto and Duarte (1975) repeated biometrical measurement of 22 morphological characters on the head and thorax of 16 diploid drones, 29 haploids and 29 workers. The estimated generalized Mahalanobis distance between the different groups of bees showed that the diploid drones were almost 10% more masculinized than the haploids. So they concluded that  $2m_1 + 2m_2$  genes have a maleness determining effect greater than the femaleness effect produced by  $ff$  genes, and that this can happen if the maleness genes  $2m_1 + 2m_2$  are additive. However, they had already stated that the maleness additivity could be detected for certain characters, only thus suggesting different action in different characters.

Therefore the question arises as to whether the sexuality of the diploid drone is the result of genic balance between femaleness  $X + f$  and maleness  $m_1 + m_2$  genes, or of another mechanism. If the genic balance hypothesis is correct in the recently modified version, then all or almost all body parts of diploid drones should show super-male characters.

The present paper also concludes the author's work on the biometry of diploid drones (Woyke, 1977, 1978a, 1978b).

## Materials and Methods

The studies were conducted on the size of testes of haploid and diploid drones already published by the author (Woyke, 1973, 1974), and on the number or size of different body parts of the four honeybee castes, *Apis mellifera* (Woyke, 1977, 1978a, 1978b).

Altogether 37.9 thousand measurements or counts were made, of 37 characters; 7.5 thousand on 2.5 thousand testes, about 1.5 thousand being from diploid drones. Altogether 518 drones (of which 272 were diploids) 0-1 day old were weighed. External body parts were measured or counted on 35 characters of 269 haploid drones, 295 diploid drones, 109 queens and 248 workers. All the individuals originated from 12 queens. Almost 29.9 thousand measurements or counts of the external body characters were made, 9.8 thousand on the head, 10.8 thousand on the thorax and 9.3 thousand on the abdomen.

Methods of rearing or measuring are given in the papers cited above.

## Results

### Comparison of haploid and diploid drone body sizes

Table 1 shows averages of all the measurements made, and statistical differences between haploid and diploid drones. Table 2 shows that out of 37 characters investigated, 33 were larger in diploid than in haploid drones, one was similar (index of forewing) and two were smaller (number of bristles on the forewing and number of hooks on the hind wing). With the exception of the testes and structures on the wings, all the other body parts were larger in diploid than in haploid drones. Thus the first conclusion is that diploid drones are larger than haploids.

TABLE 1. Size (mm unless indicated) or number of body parts in male and female honeybees (L = length, W = width)

Body parts	Males		Females	
	Diploid	Haploid	Queen	Worker
<i>Whole body</i>				
Weight (mg)	262.1+	226.0	200.0	100.0
<i>Testes</i>				
Volume (mm <sup>3</sup> )	1.27+	11.30	—	—
<i>Head</i>				
Length	3.976+	3.790	3.293	3.296
Width	4.501+	4.336	3.833	3.747
Middle ocellus diameter	0.332+	0.328	0.265	0.261
Ten facets L	0.466+	0.416	0.251	0.245
Antennal flagellum L	4.361+	4.337	2.919	3.036
Labrum L	1.003+	0.955	1.202	1.102
Labrum W	0.311+	0.302	0.343	0.333
Mandibles L	1.058+	1.024	1.257	1.357
Mandibles W	0.216+	0.208	0.410	0.304
Labium L	4.063+	3.748	3.725	6.075
Labial palpus L	1.805+	1.710	1.523	2.434
<i>Thorax</i>				
Length	4.598+	4.289	3.582	2.983
Width	4.786+	4.528	3.651	3.055
Thickness	6.098+	5.850	5.053	3.950
<i>Forewing</i>				
Length	12.337+	12.008	9.820	9.070
Width	3.994+	3.929	3.221	3.114
Cubital index	1.77 o	1.79	2.55	2.28
No. bristles	26.90+	30.30	124.50	66.10
<i>Hind wing</i>				
No. hooks	19.00+	21.40	18.20	20.70
<i>Hind leg</i>				
Tibia L	4.162+	4.038	3.697	3.240
Tibia W	1.175+	1.125	1.194	1.112
Basitarsus L	2.669+	2.606	2.370	2.027
Basitarsus W	1.326+	1.281	1.166	1.172
<i>Abdomen</i>				
Tergite 4 L	13.525+	13.249	10.986	9.233
Tergite 4 W	2.923+	2.893	3.199	2.231
Sternite 4 L with lateral apodemes	7.690+	7.448	6.920	6.067
Sternite 4 L without apodemes	5.123+	4.973	5.482	5.052
Sternite 4 W with front apodeme	2.962+	2.847	3.691	2.829
Sternite 4 W without apodeme	1.278+	1.264	2.468	1.699
Tergite 5 L	13.174+	12.899	10.635	8.665
Tergite 5 W	2.888+	2.857	3.131	2.173
Sternite 5 L with lateral apodemes	7.862+	7.723	6.291	5.945
Sternite 5 L without apodemes	5.235+	4.966	5.181	4.979
Sternite 5 W with front apodeme	2.987+	2.875+	3.849	2.818
Sternite 5 W without apodeme	1.249 o	1.240	2.662	1.759

+ Difference between haploid and diploid drones statistically significant.

o Difference not statistically significant.

TABLE 2. Body size relationships of diploid drones (D) to haploid drones (H) and females (F): queen (Q), worker (W).

*Analysis of D in relation to H*

<i>Body part</i>	<i>No. characters</i>	<i>D &gt; H</i>	<i>D = H</i>	<i>D &lt; H</i>
Weight	1	1	—	—
Testes	1	—	—	1
Head	11	11	—	—
Thorax	12	9	1	2
Abdomen	12	12	—	—
Total	37	33	1	3

*Analysis of D in relation to F and H*

<i>Body part</i>	<i>Super male</i>	<i>Male</i>	<i>Intersex</i>	<i>Caste</i>	<i>Female</i>	<i>Super female</i>
	<i>D &gt; H &gt; F</i> <i>D &lt; H &lt; F</i>	<i>D = H</i>	<i>F &gt; D &gt; H</i> <i>F &lt; D &lt; H</i>	<i>Q &gt; D &gt; H &gt; W</i> <i>Q &lt; D &lt; H &lt; W</i>	<i>Q &gt; D &gt; W &gt; H</i> <i>Q &lt; D &lt; W &lt; H</i>	<i>D &gt; F &gt; H</i>
Weight	1	—	—	—	—	—
Testes	—	—	1	—	—	—
Head	5	—	4	2	—	—
Thorax	9	1	—	1	1	—
Abdomen	4	—	2	4	1	1
Total	19	1	7	7	2	1

### Relation of diploid drone body parts to those of haploid drones and females

In neither type of drone are all body parts larger than those of the females. Many parts of both the mouth and abdomen are larger in females than in drones. Those body parts which are larger in diploid than in haploid drones, but smaller than in the females, do not show super-male character, but tend towards the female character. Table 2 shows that, in relation to both haploids and females, diploid drone body parts had 19 super-male characters, 1 male character, and 17 characters ranging from intersex through caste and female to super-female characters. So little more than half the characters measured showed a super-male character in diploid drones, the other half tending towards the female; the various body parts which were larger in diploid drones than in haploids were not a sign of super-male character. The sexuality of the characters studied showed equal tendencies towards super-maleness and femaleness.

### Test of genic balance hypothesis

The genic balance hypothesis may be tested by the size of those body parts which are larger in females than in drones. If the hypothesis is correct, and additivity of maleness genes results in super-male characters, then the super-male character in diploid drones would be verified by smaller sizes of those body parts. Table 3 shows that out of 10 characters studied only the number of bristles was significantly lower in diploid than in haploid drones, thus truly showing the super-male character. This is in accord with the genic balance hypothesis.

All the other 9 characters were larger in diploid than in haploid drones. Two did not differ significantly, but the other 7 did, weighing against the genic balance hypothesis.

TABLE 3. Test of super-male character of those diploid drone (D) body parts which are larger in the females (worker W, queen Q) than in the haploid (H) drone (mm or no.) (L = length, W = width).

<i>Body part</i>	<i>Smallest</i>		<i>Intermediate</i>		<i>Largest</i>		<i>D character</i>
Labrum L	H 0.96	+D 1.00	W 1.10	Q 1.20			intersex
Labrum W	H 0.30	+D 0.31	W 0.33	Q 0.34			intersex
Mandibles L	H 1.02	+D 1.06	Q 1.26	W 1.36			intersex
Mandibles W	H 0.21	+D 0.22	W 0.30	Q 0.41			intersex
Cubital index	D 1.77	<i>o</i> H 1.79	W 2.28	Q 2.55			male
No. bristles	D 26.9	+H 30.3	W 66.1	Q 124.5			super-male
Sternite 4 L without apodemes	H 4.97	W 5.05	+D 5.12	Q 5.48			female
Sternite 4 W without apodemes	H 1.26	+D 1.28	W 1.70	Q 2.47			intersex
Sternite 5 L without apodemes	H 4.97	W 4.98	Q 5.18	+D 5.24			super-female
Sternite 5 W without apodemes	H 1.24	<i>o</i> D 1.25	W 1.76	Q 2.66			intersex

+ Difference between haploid and diploid drone statistically significant; *o* difference not found statistically significant.

### Caste characters of diploid drones

Unless the size of the queen's body parts are considered, caste characters would not be detected in diploid drones. These body parts would be considered as super-male or intersex characters in diploid drones. Table 4 shows that of 7 characters studied, all dimensions were larger in diploid than in haploid drones, 2 being closer to workers and 5 closer to queens.

Excluding the testes, of all 36 characters of the diploid drone studied in relation to the females, 12 were closer to the worker and 23 to the queen. One was ambiguous: the width of the queen hind leg basitarsus was equal to that of the worker. So the diploid drone was more often closer to the queen than to the worker, and its sexuality should probably be discussed in relation to the queen rather than the worker. Nevertheless, no consistent inclination was found towards one or other of the female castes in diploid drones.

TABLE 4. Size (mm) of those body parts in haploid drone (H), worker (W) and queen (Q) which show caste character in diploid drone (D) (L = length, W = width).

<i>Body part</i>	<i>Smallest</i>		<i>Intermediate</i>		<i>Largest</i>	
Labium L	Q 3.73	H 3.75	+D 4.06	W 6.08		
Labial palpus L	Q 1.52	H 1.71	+D 1.81	W 2.43		
Hind leg tibia W	W 1.11	H 1.12	+D 1.17	Q 1.19		
Tergite 4 W	W 2.23	H 2.89	+D 2.92	Q 3.20		
Sternite 4 W with apodeme	W 2.83	H 2.85	+D 2.96	Q 3.69		
Tergites W	W 2.17	H 2.86	+D 2.89	Q 3.13		
Sternites W with apodeme	W 2.82	H 2.88	+D 2.99	Q 3.85		

+ Difference between haploid and diploid drone statistically significant.

### Discussion and Conclusion

The main difference between the diploid and haploid drone is the larger size of the diploid, rather than a shift in one direction of sexuality.

A similar conclusion may be derived from results presented by Chaud-Netto (1975) and Chaud-Netto and Duarte (1975). Here out of 22 dimensions investigated the diploid drone showed 10 super-male and 12 intersex characters.

Depending upon the body parts chosen, the generalized effect may show super-male character if the head (without mouthparts) or thorax is considered, or an inclination towards the female character if mouthparts and abdomen are taken into account. How-

ever, it is difficult to accept that the dimensions of different body parts of a diploid drone are the result of a new genic balance between maleness and femaleness genes, as almost the same number of characters in its sexuality react in opposite directions. Thus the above results do not support the hypothesis of genic balance between maleness and femaleness genes.

The results obtained may be better explained by the hypothesis presented below. The male sex is determined by the hemi- or homozygosity at the sex locus  $X$ . The female sex is determined by heterozygosity at the sex locus  $X$ . The sexuality of different body parts of the diploid drone depends on the size and the relation to the haploid drone and to the females. The size of diploid drone body parts is not the result of genic balance but of higher polyploidization and/or of higher gene dosage, as a result of which almost all characters are larger in diploid than in haploid drones, and present different sexuality.

According to the genic balance hypothesis, the sexuality of different body parts should be almost consistently super-male, whereas the size relation of diploid to haploid drone body parts should differ in the opposite direction to the female sizes (if  $m_1 + m_2$  are additive). Diploid drone dimensions should be larger in those body parts which are larger in haploids than in females ( $D > H > F$ ), and smaller in those which are smaller in haploids than in females ( $D < H < F$ ). However, this was not the case.

According to the hypothesis presented here the size relation of different body parts in diploid and haploid drones should be more or less constant. Depending upon polyploidization, and the reaction to it, diploid drone body parts should be mostly larger than those of haploids. However, the sexuality (relation to both haploid drones and females) should shift in the opposite direction. This is what actually happened.

Using the genic balance hypothesis suggesting intersexuality of all body parts in the first version, or super-male character in the last version, it was not possible to forecast the sexuality of those parts not yet investigated.

The hypothesis presented forecast the sexuality of those body parts. Higher polyploidization usually leads to larger body size (but fewer bristles and hooks on the wings), and—depending upon the size in females and haploids—the sexuality of diploid drone body parts may be forecast quite accurately. It will show super-male character in parts which are larger in the haploid drone than in the females, and a tendency towards femaleness in parts which are smaller in the haploid than in females. Thus, according to this hypothesis the size of cell nuclei in different tissues should be larger, and the DNA content in the nuclei should be higher, in diploid than in haploid drones. However, the relation of these characters to the females should vary. Furthermore, triploid workers should be larger than diploid ones, but the sexuality of different body parts should differ.

The body size is larger in the queen than in the worker and is also related to higher polyploidization in body tissues of the queen. In this instance, however, the polyploidization is caused by environmental conditions, whereas in the diploid drone it is caused by cytogenetical factors.

All the data presented above allow us to make the following conclusions. Although many body parts of the haploid drone are larger than those of females, some are smaller. Since the diploid drone is larger than the haploid, it shows many super-male characters, but also some which range from intersex to super-female. Thus the sexuality of different body parts of the diploid drone is not the result of a genic balance between femaleness and maleness genes, but is the result of polyploidization of body tissues.

## References

- CHAUD-NETTO, J. (1973) Machos diplóides de *Apis mellifera*: Sua bionomia e contribuição ao problema da determinação do sexo. *Faculdade de Medicina de Ribeirão Preto: Tese de Mestrado*
- (1975) Sex determination in bees. II. Additivity of maleness genes in *Apis mellifera*. *Genetics* 79(2): 213-217
- CHAUD-NETTO, J.; DUARTE, F. A. M. (1975) Sex determination in bees. V. The action of sexual genes in *Apis mellifera*. *Ciênc. Cult., S Paulo* 27(2) : 125-129
- CUNHA, A. B. DA; KERR, W. E. (1957) A genetical theory to explain sex-determination by arrhenotokous parthenogenesis. *Forma et Functio* 1(4) : 33-36
- KERR, W. E. (1974a) Advances in cytology and genetics of bees. *A. Rev. Ent.* 19 : 253-268
- (1974b) Genética de determinação do sexo em abelhas X. Programação da atividade dos genes determinadores de sexo e casta. *III Congr. bras. Apic.* : 179-190
- KERR, W. E.; NIELSEN, R. A. (1967) Sex determination in bees (Apinae). *J. apic. Res.* 6(1) : 3-9
- WHITING, P. W. (1940) Multiple alleles in sex determination of *Habrobracon*. *J. Morph.* 66 : 323-355
- WOYKE, J. (1963) Drone larvae from fertilized eggs of the honeybees *J. apic. Res.* 2(1) : 19-24
- (1965a) Study on diploid drone honeybees. *C. r. V Congr. UIEIS* : 257-262
- (1965b) The diploid drones. *XX Int. Beekeep. Congr.* : 152-154
- (1971a) New experimental data in the honeybee genetics. *XXIII Int. Beekeep. Congr.* : 365-367
- (1971b) Biology of reproduction as a basis for production of new varieties of honeybees. Final Technical Report (PL 480). *Warsaw: Agricultural University, Bee Culture Laboratory*
- (1973) Reproductive organs of haploid and diploid drones. *J. apic. Res.* 12(1) : 35-51
- (1974) Genic balance, heterozygosity and inheritance of size of testes in diploid drone honeybee. *J. apic. Res.* 13(2) : 77-91
- (1977) Comparative biometrical investigation on diploid drones of the honeybee I. The head. *J. apic. Res.* 16(3) : 131-142
- (1978a) Comparative biometrical investigation on diploid drones of the honeybee II. The thorax. *J. apic. Res.* 17(4) : 195-205
- (1978b) Comparative biometrical investigation on diploid drones of the honeybee. III. The abdomen and weight. *J. apic. Res.* 17(4) : 206-217