Journal of Apicultural Research 13(3) : 183–190 (1974)

SPERMATOGENESIS IN DIPLOID DRONES OF THE HONEYBEE*

J. WOYKE AND W. SKOWRONEK

Bee Division, Agricultural University, Warszawa-Ursynów, Poland

Manuscript received for publication 20th June 1973

Summary

The process of spermatogenesis was investigated in 35 diploid honeybee drone pupae and compared with that in 15 haploid pupae. Altogether 25 013 chromosomes were counted, and 1200 measurements were made of cells in different stages of spermatogenesis. The spermatocytes of the diploids contained twice as many chromosomes and were twice as large as those of the haploids. The chromosomes did not pair or separate during metaphase I (prometaphase), when only a cytoplasmic bud was formed. The chromatids separated during metaphase II, and in anaphase a diploid set of chromosomes was visible. Only one spermatid and one polar body with chromatin were formed from one spermatocyte. Both the nucleus of the spermatid and the polar body were twice as large as in the haploids. The process of spermatogenesis in diploids was very similar to that in the haploids, with no reduction of the number of chromosomes, and thus the diploid drones produced diploid spermatozoa. This was probably caused by the homozygosity of the sex locus.

Introduction

After diploid drone honeybees (*Apis mellifera*) were identified (Woyke, 1963) and the method of rearing them was worked out (Woyke, 1969), the most important question was whether they produced haploid or diploid spermatozoa: if the latter, then it would be possible to rear triploid bees.

The following is a summary of relevant work on the honeybee. Spermatogenesis in haploid drones was described by Meves (1903, 1907). He found 16 chromosomes in the spermatocytes, in which two divisions occurred, although only a cytoplasmatic bud without chromatin was formed during the first division. The second division was of a mitotic character, resulting in one spermatid and one polar body (Mark & Copeland, 1906). Doncaster (1906, 1907) described pairing of chromosomes during spermatogenesis, and this was confirmed by Nachtscheim (1913) in the anaphase stage only. Sanderson and Hall (1948) found one hooked chromosome, and Manning (1949) called this an X-chromosome and described how it was extruded during the maturation of the spermatocyte. This loss of one chromosome meant that all spermatozoa had only 15 chromosomes, as confirmed by Kerr (1951). Re-investigation of spermatogenesis by Sanderson and Hall (1951) and by Ris and Kerr (1952) led to rejection of these later findings and confirmed the earlier one, according to which there were 16 chromosomes in the spermatids. Wolf (1960) interpreted the divisions of spermatocytes as different steps of one division only.

The synchronized divisions of gonocytes in the same cyst were thought by MacKinnon and Basrur (1970) to be the result of interconnection by intercellular bridges. The ultrastructure of spermatocytes was described by Hoage and Kessel (1968).

---

* This investigation was supported in part by a research grant from the United States Department of Agriculture, authorized by Public Law 480.

According to Hachinoe and Onishi (1952) meiosis occurred within 24 hours of the 8th day in the pupal stage, when the eyes of the drone were white-coloured.

Spermiogenesis in haploid drones was described in detail by Orska (1939).

The present investigation sought to clarify the process of spermatogenesis in diploid drones. Preliminary results were reported by Woyke and Skowronek (1967).

Materials and Methods

Spermatogenesis was investigated in 35 pupae of diploid drones originating from 7 sibling-mated queens and, for comparison, in 15 haploid pupae originating from 3 queens. The chromosomes were counted in 1146 spermatocytes, of which 662 were from diploids. Altogether 25 013 chromosomes were counted. In addition, 1200 measurements of the diameter of cells in different stages of spermatogenesis were made.

Diploid drones were reared by the method described by Woyke (1969). Pupae 1 or 2 days old, i.e. with white eyes, were dissected.

Smears of testes were fixed and stained in aceto-carmine or aceto-orcein. The results were good, but difficulties were encountered in obtaining permanent mounts. Some testes were fixed in Flemming's fixative. When iron haematoxylin was used, many cytoplasmic structures were stained, and it was rather more difficult to count the chromosomes. The best results for counting chromosomes were obtained after the testes were fixed in Gilson, embedded in paraffin wax, and sectioned at 5 μm. The sections were stained with iron haematoxylin and mounted in Canada balsam. A few sections were stained for DNA using the Feulgen reaction. The chromosomes of one spermatocyte had to be counted on one, two or even three sections. Whole spermatocytes as well as nuclei were measured with a micrometer eyepiece. The volume was calculated on the assumption that the cells and nuclei were elipsoids.

Results

Divisions of spermatocytes were found in haploid or diploid pupae 13 or 14 days, respectively, after the larva hatched from the egg. Spermatogenesis occurred in the diploids as follows. All spermatocytes in one cyst were at almost the same stage of development, but those in cysts near the seminal duct were further developed than those at the distal end of the tubules.

Before divisions of spermatocytes occurred, they enlarged. Two centrioles were located on opposite sides of the cell: one was on the tip of a cytoplasmic process (called distal), and the other on a small cone (called proximal). But very soon the spermatocyte became rounded at this point and the proximal centriole moved to the surface of the cell, or looked as if it was inside it. The whole process was very similar to that described in haploids by Meves (1907, Fig. 24 – Fig. 28). Spindle fibres extended from both centrioles, passing outside the nucleus. Sometimes three centrioles were visible, with fibres extending from each of them.

The chromatin was visible at first in the form of a reticulum and granules in the clear caryolymph of the nucleus. Next the chromosomes appeared as rod-shaped structures located immediately beneath the nuclear membrane. This stage of spermatogenesis was diakinesis (Fig. 1, Fig. 2). It was very suitable for counting the chromosome number, but the microscope had to be focused on all the chromosomes at different levels, and successive sections searched. Table 1 shows that the mean number of chromosomes found in the diploids was double that in the haploids. The most frequent number found in the diploids was 32 and in the haploids 16. No

TABLE 1. Chromosome numbers in various stages of spermatogenesis in diploid and haploid drones.

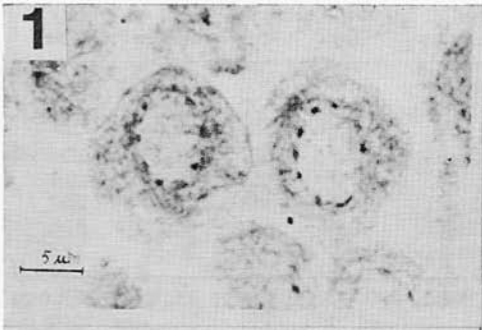
Queen no.	*Diakinesis*			*Metaphase II plate*			*Anaphase*		
	No. cells	*Range*	*Mean*	*No. cells*	*Range*	*Mean*	*No. cells*	*Range*	*Mean*
				Diploids					
883	60	23–36	29·7	7	26–30	28·4	72	15–30	21·3
885	29	25–38	31·1	—	—	—	—	—	—
914	78	26–36	30·7	32	27–33	29·5	57	13–28	22·4
63	42	27–32	30·3	19	28–32	30·0	37	17–29	22·1
89	29	28–35	31·8	36	27–32	29·9	58	18–30	24·7
173	36	28–33	30·8	8	29–32	30·4	3	23–25	23·6
187	23	28–34	30·5	10	26–34	30·1	26	20–26	22·6
Total ± SE	297	23–38	30·7 ± 0·12	112	26–34	29·7 ± 0·16	253	13–30	22·6 ± 0·20
SD			2·00			1·73			3·14
				Haploids					
1	42	12–18	15·1	11	12–17	14·9	37	8–17	12·1
2	72	13–17	15·5	30	13–17	15·2	89	9–15	12·4
3	98	13–18	15·6	42	13–17	15·3	63	8–15	12·1
Total ± SE	212	12–18	15·5 ± 0·07	83	12–17	15·2 ± 0·11	188	8–17	12·2 ± 0·11
SD			1·05			0·97			1·57

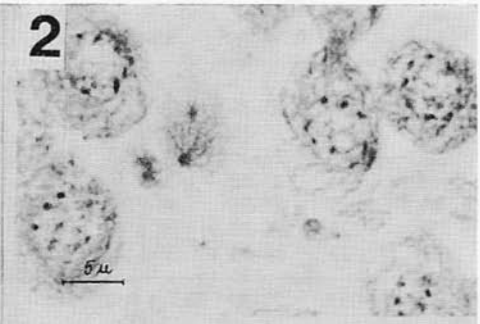
conjugation or pairing of chromosomes into bivalents was found. This is contrary to normal spermatogenesis in most other diploid individuals, where pairing always occurs. Table 2 shows that in diakinesis the volume of the spermatocytes and of their nuclei was twice as great in the diploids as in the haploids.

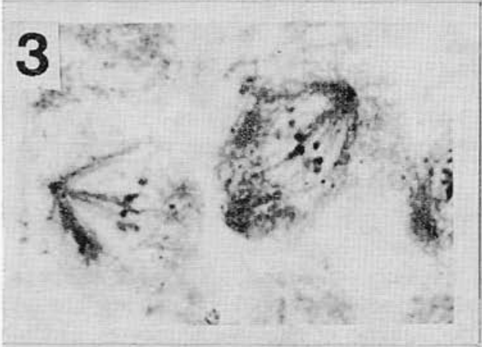
The chromosomes now moved together inside the nucleus, which became spindle-shaped. The nuclear membrane did not dissolve. Spindle fibres appeared inside the nucleus, and the chromosomes made a rather irregular metaphase plate (Fig. 3). This stage is called first metaphase by Meves (1907) and prometaphase by Wolf (1960). The irregular metaphase plate made it difficult to count the exact number of chromosomes in a sufficient number of diploid spermatocytes, but it was ascertained that no pairing into bivalents occurred. Next the chromosomes dispersed at intervals along the longitudinal axis of the spindle inside the nucleus, but there was no division into two groups (Fig. 4). Next the chromosomes concentrated more along the axis, before moving as an almost homogeneous mass towards one pole (Fig. 5).

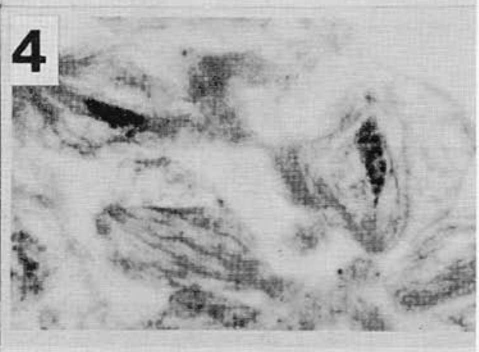
Meanwhile, a cytoplasmic bud was formed on the distal part of the cell. Although no chromosomes were visible in this bud, at times some granules which stained black with iron haematoxylin appeared there (Fig. 5). But these granules were Feulgen negative (Fig. 6), which showed that the chromosomes did not enter the cytoplasmic bud. The buds became connected by intercellular bridges.

The chromosomes then migrated again into the centre of the cell. The nuclear membrane dissolved, and a very distinct spindle and a typical metaphase plate were formed (Fig. 7). Metaphase II could easily be distinguished from metaphase I by the regularity of the plate, distinct spindle, and presence of cytoplasmic polar bodies. There were more chromosomes lying close to one another in the metaphase II plate of the diploids than of the haploids (Fig. 8). It was sometimes difficult to count their exact number. Table 1 shows that the average number of chromosomes found in the metaphase II plate of the diploids was approximately twice that in the haploids.









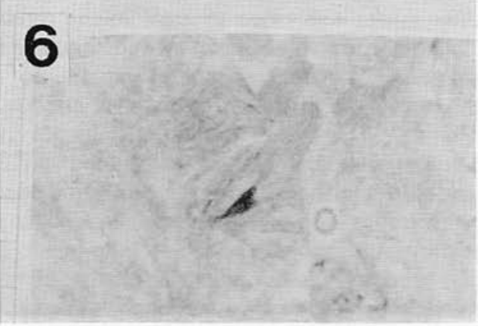


FIG. 1. Diakinesis in diploid drone.

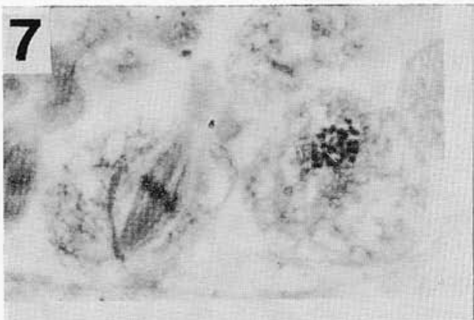
FIG. 2. Diakinesis in haploid drone.

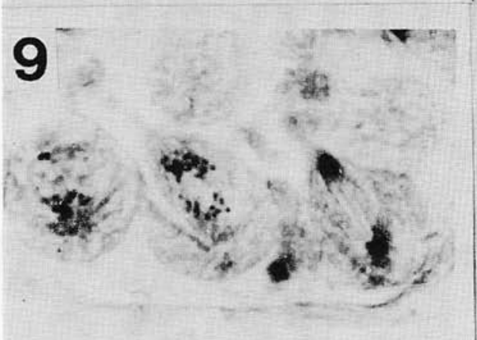
FIG. 3. Metaphase I in diploid drone: chromosomes on metaphase plate.

FIG. 4. Metaphase I in diploid drone: chromosomes along the axis.

FIG. 5. Anaphase I in diploid drone: chromatin aggregated at one pole, and cytoplasmic bud containing granules stained black with iron haematoxylin.

FIG. 6. Anaphase I in diploid drone showing cytoplasmic bud after Feulgen reaction.





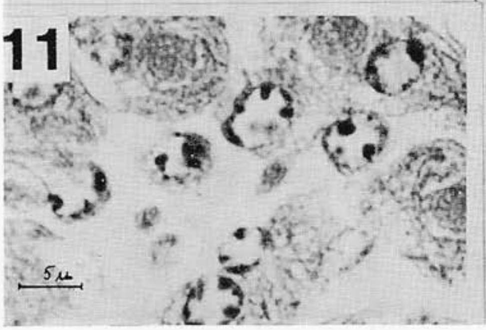


FIG. 7. Metaphase II in diploid drone.

FIG. 8. Metaphase II in haploid drone.

FIG. 9. Anaphase in diploid drone.

FIG. 10. Anaphase in haploid drone.

FIG. 11. Spermatids and polar bodies in diploid drone.

FIG. 12. Spermatids and polar bodies in haploid drone.

TABLE 2. Volume and area of different parts of reproductive cells in diploid and haploid drones.

Queen no.	Vol. spermatocytes in diakinesis (μm^3)		Vol. nuclei of spermatocytes in diakinesis (μm^3)		Area of metaphase II plate (μm^2)		Vol. nuclei in spermatids (μm^3)		Vol. 2nd polar bodies (μm^3)	
	No. cells	Mean	No. cells	Mean	No. cells	Mean	No. cells	Mean	No. cells	Mean
					Diploids					
883	32	2522	20	279	30	24·53	40	73·8	24	100·7
885	32	2088	20	305	12	18·01	40	71·6	24	97·7
914	64	2360	40	189	24	20·75	60	68·3	36	101·3
63	24	2338	10	222	12	19·62	50	65·4	24	105·0
89	24	2348	15	256	12	18·03	60	75·5	36	112·3
173	16	2006	10	244	12	16·25	30	74·3	18	100·3
187	16	2611	10	241	6	17·05	30	66·4	18	92·3
Total ± SE	208	2182 ± 43·7	125	248 ± 6·6	108	20·34 ± 0·359	310	70·8 ± 0·89	180	101·4 ± 1·79
SD		628·8		73·7		4·27		15·8		18·1
					Haploids					
1	24	1133	15	133	18	11·14	30	38·1	18	48·3
2	32	1103	20	117	18	10·63	60	34·7	36	47·6
3	40	984	30	93·5	24	9·65	40	29·2	24	43·0
Total ± SE	96	1073 ± 21·8	65	115 ± 3·4	60	10·34 ± 0·306	130	34·0 ± 0·72	78	46·3 ± 0·74
SD		222·4		27·4		2·81		8·8		7·6

Table 2 shows that the area of the diploid metaphase plate was also twice as large as that in the haploid. Both these results indicate that no reduction in the number of chromosomes in diploids occurred up to this stage of spermatogenesis.

The chromatids in the spermatocytes of diploid drones then separated, and the two groups of chromosomes migrated in opposite directions, to the poles of the spindle (Fig. 9). Fig. 10 shows a similar stage in haploid drones. The number of chromosomes in anaphase was that characteristic of spermatids and spermatozoa. The chromosomes were more aggregated now than in metaphase, and each group was located on several levels, which allowed counts of chromosomes in cells with spindles lying perpendicular to the observer.

It was difficult at this stage to see all the chromosomes, Table 1 shows that the mean number found for each group of sister chromosomes in the anaphase was 22·6 in the diploids and 12·2 in the haploids—lower than the expected diploid (32) or haploid (16) set, but by a similar percentage (71% and 76%). This fact, as well as the range of the number of chromosomes counted in the diploids (13–30) and the haploids (8–17), suggests that aggregation or pairing of some chromosomes occurred during the anaphase, as was found by Doncaster (1907) and Nachtscheim (1913) for haploids. Both the mean number and the minumum and maximum numbers of chromosomes found in the anaphase in diploid drones were approximately double those found in the haploids. This suggests strongly that there was a diploid set in both groups of sister chromosomes in the anaphase of the spermatocytes of diploid drones.

When the chromosomes arrived at the poles, both groups aggregated into a mass of chromatin (Fig. 9). The mass was surrounded by a nuclear membrane, and two small nuclei with reticular chromatin were formed on opposite sides of the cell. One of the nuclei remained in the cell, but the other entered a cytoplasmic process. Both nuclei increased in volume. The nucleus in the process became surrounded by a thin layer of cytoplasm, and was detached from the cell as the so-called polar body. The other nucleus was that of the spermatid (Fig. 11). So only one spermatid was formed from each spermatocyte during spermatogenesis in a diploid drone. Fig. 12 shows a similar stage in a haploid drone.

Table 2 shows that in diploids the volumes of the nucleus of the spermatid and the polar body itself were twice those in haploids. This is an additional indication that there was twice as much chromatin (chromosomes) in the spermatids of diploid drones as in haploids.

Conclusions

The above description shows that the process of spermatogenesis in diploid drones is very similar to that in haploids (Meves, 1907; Mark & Copeland, 1906). No reduction in the number of chromosomes occurred in the diploid drones, but the number of chromosomes (including the last stages of spermatogenesis), and the size of the reproductive cells and of their nuclei, were twice as great in the diploids as in the haploids at the corresponding stage. Thus the diploid drones produced diploid spermatozoa. The hypothesis is proposed that homozygosity of the sex locus results in the lack of pairing of chromosomes in gametogenesis in the honeybee. Both hemi- and homozygosis of the sex locus result in the production, after metaphase I, of a cytoplasmic bud without chromosomes.

References

DONCASTER, L. (1906) Spermatogenesis of the hive bee (*Apis mellifica*). *Anat. Anz.* 29(18) : 490–491

——— (1907) Spermatogenesis of the honey bee (*Apis mellifica*). Correction. *Anat. Anz.* 31(6) : 168–169

HACHINOE, Y. & ONISHI, N. (1952) [On the meiosis of the drone of honeybee (*Apis mellifica*).] *Bull. nat. Inst. agric. Sci. Ser. G.* (3) : 83–87. *In Japanese*

HOAGE, T. R. & KESSEL, R. G. (1968) An electron microscope study of the process of differentiation during spermatogenesis in the drone honey bee (*Apis mellifera* L.) with special references to centriol replication and elimination. *J. Ultrastr. Res.* 24(1/2) : 6–32

KERR, W. E. (1951) Sex-chromosome in honey-bee. *Evolution* 5(1) : 80–81

MACKINNON, F. A. & BASRUR, P. K. (1970) Cytokinesis in the gonocytes of the drone honey bee (*Apis mellifera* L.). *Can. J. Zool.* 48(6) : 1163–1166 + 4 plates

MANNING, F. J. (1949) Sex determination in the honey bee. *Microscope* 7(7) : 175–180

MARK, E. L. & COPELAND, M. (1906) Some stages in the spermatogenesis of the honey bee. *Proc. Am. Acad. Arts Sci.* 42(5) : 103–111

MEVES, F. (1903) Über Richtungskörperbildung im Hoden von Hymenopteren. *Anat. Anz.* 24(1) : 29–32

——— (1907) Die Spermatocytenteilungen bei der Honigbiene (*Apis mellifica* L.), nebst Bemerkungen über Chromatinreduktion. *Arch. microsc. Anat. u. Entwegesch.* 70(3) : 414–491 + 5 plates

NACHTSHEIM, H. (1913) Cytologische Studien über die Geschlechtsbestimmung bei der Honigbiene (*Apis mellifica* L.). *Arch. Zellforsch.* 11(2) : 169–241 + 4 plates

ORSKA, J. (1939) Badania cytologiczne nad spermatogeneza pszczoły domowej (*Apis mellifica* L.) [Cytological investigations on spermatogenesis in the honeybee]. *Arch. Tow. Nauk. Lwów* O III 10(1) : 1–82 + 6 plates

RIS, H. & KERR, W. E. (1952) Sex determination in the honey-bee. *Evolution* 6(4) : 444–445

SANDERSON, A. R. & HALL, D. W. (1948) The cytology of the honey bee (*Apis mellifica* L.). *Nature, Lond.* 162(4105) : 34–35

——— (1951) Sex determination in the honey-bee. *Evolution* 5(4) : 414–415

——— (1951) Sex in the honey bee. *Endeavour* 10(37) : 33–39

WOLF, B. E. (1960) Eine Nachuntersuchung zur Cytologie der Honigbiene (*Apis mellifica L.*). *Zool. Beitr.* 5(2/3) : 373–391

WOYKE, J. (1963) Drone larvae from fertilized eggs of the honeybee. *J. apic. Res.* 2(2) : 73–75

——— (1969) A method of rearing diploid drones in a honeybee colony. *J. apic. Res.* 8(2) : 65–74

WOYKE, J. & SKOWRONEK, W. (1967) Spermatogenesis in diploid drones. XXI *Int. Beekeep. Congr.* : 470–471