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DNA CONTENT OF SPERMATIDS AND SPERMATOZOA OF HAPLOID AND DIPLOID DRONE HONEYBEES*

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Summary

Testis smears from haploid and diploid pupae were stained with gallocyanin or subjected to the Feulgen reaction. The DNA content of 720 nuclei of spermatids or heads of spermatozoa was measured with an integrating microdensitometer. Twice as much DNA was found in the spermatid nuclei and in the heads of the spermatozoa of diploid drones as in those of the haploids, and it is concluded that the diploid drones produced diploid spermatozoa. No clear evidence was found that diploid drones could produce haploid or tetraploid spermatozoa, or that haploid drones produced diploid spermatozoa.

Introduction

Normally, honeybee drones develop from unfertilized eggs, but they may also develop from fertilized eggs which are homozygous in the sex locus (Woyke, 1963*a*). These diploid larvae are eaten by the worker bees within 6 hours of hatching (Woyke, 1963*b*), but a method of rearing them in the colony has been worked out (Woyke, 1969*a*, 1969*b*). A most important question arises: whether these drones produce haploid or diploid spermatozoa.

Spermatogenesis in haploid drones was described in detail by Meves (1907) and spermiogenesis by Orska (1939). Spermatogenesis in diploid drones was studied by Woyke and Skowronek (1967, 1974) while the present investigation was in progress; there was some difficulty in counting the exact number of chromosomes in the anaphase of haploid and diploid drones, although strong evidence was found that the number in the diploids was twice that in the haploids. However, as the end-product of spermatogenesis is the most important stage for further breeding work, it needs to be definitely established whether the spermatids and spermatozoa of diploid drones are diploid or haploid. An investigation of the desoxyribonucleic acid (DNA) content of resting nuclei is another method for providing evidence on this point, and the present work was therefore undertaken. The DNA content of the nuclei of various tissues of the honeybee has already been studied by Merriam and Ris (1954), Mittwoch, Kalmus and Webster (1966), Stekol'shchikov (1970) and Mello and Takahashi (1971).

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Materials and Methods

Queens of *Apis mellifera* were artificially sibling-mated, and those producing brood of 50% survival rate were used for further investigations. Diploid drones were reared by the method described by Woyke (1969*a*), with the modification that larvae in the incubator were fed with brood food taken from worker cells containing young larvae (Woyke, 1969*b*).

Haploid and diploid drone pupae with light pink eyes (about 15 days after hatching) were used for the investigation of spermatids, and pupae due to emerge in 1–3 days for investigation of spermatozoa. The pupae were dissected in physiological saline solution, and the testes were then squashed and smeared on slides. The presence of spermatids was confirmed with a phase-contrase microscope. The smears were fixed for 30 minutes in a mixture of 9 parts 80% alcohol with 1 part formalin, and then dried.

To detect DNA, two staining techniques were used. In the first, the smears were digested for 2 h in 10% ribonuclease solution in Sorensen buffer adjusted to pH 6·5, and then were stained for 48 h in gallocyanin. In the second technique (Feulgen reaction), the smears were hydrolysed for 13 or 15 minutes in 1-N HCl at 65°C and then stained in Schiff's reagent. Fuchsin-pararosaniline was used for staining the spermatids, and basic fuchsin (made in Poland) for the spermatozoa.

The quantitative measurements of the intensity of the reaction representing the amount of DNA were made on an integrating microdensitometer (Barr and Straud Ltd). First, the extinction of the field filled with a spermatid nucleus or spermatozoon head was measured, and then the same measurement was made on an empty area nearby. The difference gave the extinction of the specimen. The extinction was measured at green light ($\lambda = 550$ nm); it is expressed in arbitrary units.

The DNA content of 150 spermatids (6 × 25) and 210 spermatozoa (7 × 30) from 13 diploid drones was compared with that of the same numbers of spermatids and spermatozoa from 13 haploid drones (samples of 25 or 30, respectively, being taken from each drone). However, in the main investigation, the spermatids were measured at a different time from the spermatozoa, using stains of different origin. Thus, although the results from haploid and diploid drones were comparable, those from spermatids and spermatozoa were not. To rectify this, a third set of measurements was made under identical conditions on 75 spermatids from 3 haploid drones (25 each) and on 75 spermatozoa from another 3 haploid drones (25 each).

Results and Discussion

Table 1 shows that the results did not differ much according to whether the smears were stained with gallocyanin or with fuchsin, or according to whether hydrolysis continued for 13 or 15 minutes during the Feulgen reaction.

The mean DNA content in the round nuclei of the spermatids from diploid drones was twice that from the haploids. The absolute deviation from the mean was twice as high in the diploids (3·15) as in the haploids (1·52), but the—rather high—variation in relation to the mean was almost the same in the diploids as in the haploids (coefficient of variation 18·72% and 17·90%, respectively).

The mean DNA content in the heads of spermatozoa from the diploid drones was also twice that of the haploids, but the coefficient of variation in the haploids was twice that of the diploids. The very high coefficient of variation in the spermatozoa of the haploid drones is accounted for by two factors. First, the mean value in the arbitrary units used was very low (2·10), so a deviation of only 1 unit gave a 50% variation. Secondly, the heads of haploid spermatozoa were smaller than those of

the diploids (detailed results to be published), so they covered only a very small area of the field of the microdensitometer, resulting in greater interaction of additional factors.

TABLE 1. Mean DNA content (expressed in arbitrary units) of 25 spermatids or 30 spermatozoa from different haploid and diploid drones.

Treatment	*Haploids*			*Diploids*		
	Mean ± SE	*Standard deviation*	*Coeff. of variation (%)*	*Mean ± SE*	*Standard deviation*	*Coeff. of variation (%)*
			Spermatids			
Gallocyanin	8·00 ± 0·34	1·58	19·75	15·90 ± 0·71	3·53	22·20
Feulgen HCl 13 min	8·28 ± 0·29	1·43	17·27	15·40 ± 0·42	2·12	13·77
„ „	9·72 ± 0·28	1·38	14·20	17·64 ± 0·48	2·38	13·49
„ „	8·16 ± 0·21	1·07	13·11	17·74 ± 0·91	4·56	25·70
„ „	8·56 ± 0·33	1·64	19·16	18·16 ± 0·61	3·04	16·74
„ 15 min	8·24 ± 0·29	1·45	17·60	16·14 ± 0·35	1·74	10·78
Average	8·49 ± 0·12	1·52	17·90	16·83 ± 0·26	3·15	18·72
			Spermatozoa			
Feulgen HCl 13 min	2·40 ± 0·16	0·89	37·08	4·27 ± 0·18	0·98	22·95
„ „	2·33 ± 0·14	0·76	32·62	4·53 ± 0·17	0·90	19·87
„ „	1·90 ± 0·15	0·80	42·11	4·67 ± 0·18	0·97	20·77
„ „	2·43 ± 0·18	0·92	37·86	4·53 ± 0·18	0·97	21·41
„ „	1·80 ± 0·15	0·85	47·22	4·53 ± 0·12	0·68	15·01
„ „	1·90 ± 0·15	0·80	42·11	4·56 ± 0·16	0·90	19·74
„ „	1·93 ± 0·13	0·69	35·75	4·30 ± 0·20	1·12	26·05
Average	2·10 ± 0·02	0·86	40·95	4·49 ± 0·02	0·94	20·94

In Table 1 the DNA content appears to be much higher in spermatids than in spermatozoa. This difference could have been caused by chemical changes during spermiogenesis, or by different staining and measuring conditions during the two investigations (*see* Materials and Methods). A third investigation on 25 spermatids from each of 3 haploid drones gave mean DNA contents of 1·78, 1·68 and 1·65 units (mean 1·70), which were very similar to comparable means of 25 spermatozoa from 3 other drones: 1·68, 1·74 and 1·78 units (mean 1·73). Thus, the differences between spermatids and spermatozoa found in the main investigations were probably due to differing experimental conditions.

The measurable extinction in Feulgen-stained bee spermatids and spermatozoa should be discussed here. During spermatogenesis in many species, there is a transition from a somatic, lysine-rich histone to a highly arginine-rich type, which is often followed by a further alteration to protamine in the mature spermatozoa. Gledhil et al. (1966) found a drastic reduction in the Feulgen reactivity of nuclear DNA when bull spermatids differentiated into spermatozoa. They interpreted this as an alteration of the spermatid histone into an arginine-rich protein, and further as an increase in the strength of the electrostatic binding of nuclear protein to the DNA molecule. Das et al. (1964) found in *Drosophila* a transition from a lysine-rich to an arginine-rich histone, but no further alteration into protamine. Verma (1972) indicated that honeybee sperm cells contained histones similar to somatic cells and did not show any transition to an arginine-rich type. The equivalence of DNA

levels found in the 75 spermatids and 75 spermatozoa stained under identical conditions in the present investigation suggests that the chemical changes found during spermatogenesis in many other species do not occur in the honeybee.

The relatively high variation found in spermatids (as well as in spermatozoa) of haploid and diploid drones raises another question. Did one drone perhaps produce haploid and diploid spermatozoa? Fig. 1 shows that the highest values for the DNA content of spermatids from haploid drones (13 units) was more than twice the lowest value (5 units). Mello and Takahashi (1971) found in testes of white-eyed haploid pupae (spermatocytes) a DNA content ranging in one group from 52 to 195 units and in another one from 85 to 552 units, although these results may have included measurements of polyploid nuclei in the testes sheath. The ratio of the highest value to the lowest was 1 : 3·8 in their first group and 1 : 6·5 in their second. But since there is no evidence that spermatocytes of haploid drones can be diploid, the variation in DNA content was more likely to have been caused by staining and measurement conditions.

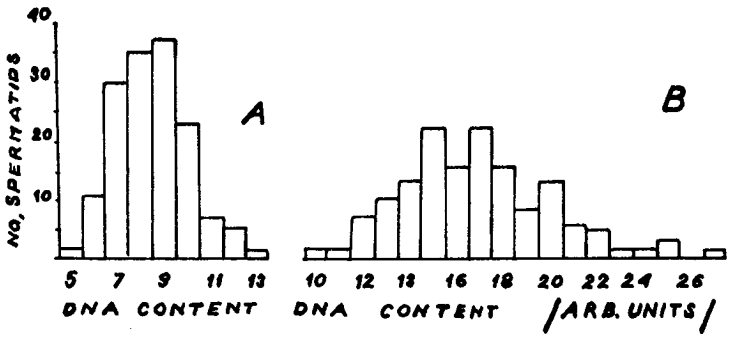

FIG. 1. DNA content of spermatids. A haploid drones; B diploid drones.

The ratio of the highest to the lowest DNA content in the spermatids of haploid drones found in the present investigation (1 : 2·6) was lower than that found by Mello and Takahashi. Fig. 1A is unimodal, with no indication of a combination of two populations of different ploidy (haploid and diploid). Since the mean DNA content of haploid spermatids was 8·5, the mean for the diploids should be about 17 units, and no spermatid from the haploid drones gave a value as high as this.

The coefficient of variation of the DNA content in spermatids from diploid drones was almost the same as that in the haploids. Since the mean value was 16·8 units for the diploid spermatids, the value for the haploids would be about 8·4 units. No spermatids with such a low value were found in diploid drones (Fig. 1B).

Fig. 2 shows much less differentiation in DNA contents of spermatozoa. With a mean of 2·1 units for haploid drones and 4·5 units for the diploid drones, some spermatozoa of haploid drones could be classified as diploid. On the other hand no spermatozoa from a diploid drone could be classified as haploid or tetraploid. It is difficult to believe that diploid spermatozoa could have been produced by haploid drones from haploid spermatids; it is more likely that the extreme values were caused by factors which led to the high variation discussed earlier. Thus, because the mean DNA contents (as well as the extreme values) in the spermatids and spermatozoa of diploid drones were approximately twice as high as those in the haploids, it can be concluded that the diploid drones produced diploid spermatozoa.

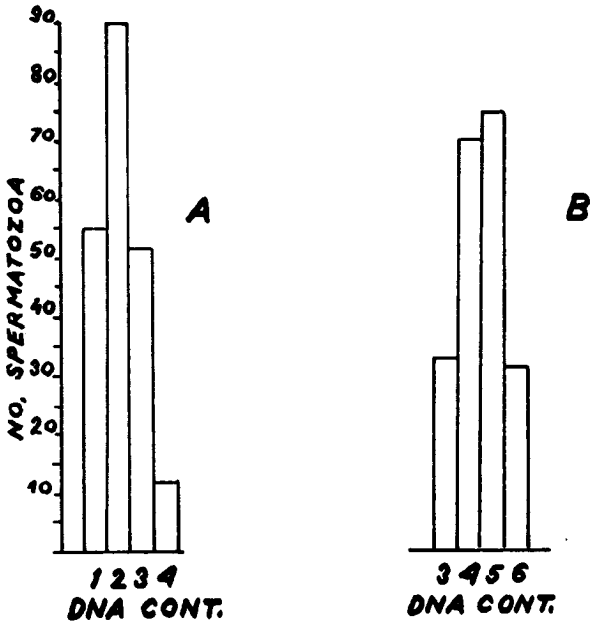

FIG. 2. DNA content of spermatozoa. A haploid drones; B diploid drones.

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