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LENGTHS OF HAPLOID AND DIPLOID SPERMATOZOA OF THE HONEYBEE AND THE QUESTION OF THE PRODUCTION OF TRIPLOID WORKERS

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Summary

Altogether the whole lengths of 3575 spermatozoa from 59 diploid and 84 haploid drones, and the lengths of the nuclei of 450 diploid and haploid spermatozoa, were compared.

The shortest diploid spermatozoa were 15 μm longer than the longest haploid ones produced by drones originating from the same queen. The average length of diploid spermatozoa was 313 μm and that of haploid spermatozoa 242 μm.

The head length of diploid spermatozoa averaged 7·4 μm and of haploids 4·8 μm. Thus length may be used to distinguish haploid from diploid spermatozoa, and consequently haploid from diploid drones.

Reports of production of triploid worker bees are considered questionable.

Introduction

Spermatozoa are normally haploid cells produced by reduction of the number of chromosomes during spermatogenesis. In the honeybee, however, the drones are haploid, the spermatocytes are haploid from the beginning, and no reduction in the number of chromosomes occurs during spermatogenesis (Meves, 1907).

Woyke (1969) elaborated a method of rearing diploid drones, and he and Skowronek (1974) demonstrated that in these drones also the number of chromosomes is not reduced during spermatogenesis. Thus diploid drones produce diploid spermatozoa, which are very rare in the animal kingdom. Woyke (1975) found that the average amount of DNA in both the nuclei of diploid spermatocytes and the heads of diploid spermatozoa was twice that in haploid spermatocytes and spermatozoa, respectively. The amount of DNA was variable, however, and the extreme values in haploid and diploid cells overlapped. Thus it was possible that diploid drones produced some haploid spermatozoa as well as diploid ones. This possibility makes if difficult to establish that triploid worker bees can be produced (Chaud-Netto, 1972), and to decide whether a queen inseminated by one drone can produce diploid as well as triploid workers. Without genetic markers it is difficult to say whether a drone used to mate a queen was diploid or haploid, even if the drone was reared by the method producing diploid drones.

The lengths of spermatozoa produced by haploid and diploid drones have now been investigated, to see whether they can be used to distinguish haploid from diploid spermatozoa.

The lengths of haploid spermatozoa have been reported by Smirnov (1953), Cruz-Hoffling et al. (1979) and Lensky et al. (1979), but diploid spermatozoa have not previously been measured until now.

Materials and Methods

The investigation was conducted in two different years. Haploid and diploid spermatozoa of Carniolan bees were measured in the first year and those of hybrid Caucasian/Carniolan bees in the second year. Different people measured the spermatozoa in the two years.

In each year haploid and diploid drones whose spermatozoa were compared were the progeny of the same queen; three queens were used for these comparisons. Spermatozoa of free-flying haploid drones originating from several queens were also investigated.

Altogether the whole length of 25 spermatozoa from each of 84 haploid drones and from 59 diploid drones were measured. Later the lengths of 250 *heads* of spermatozoa from haploid drones and of 200 from diploid drones were measured.

To obtain the material, virgin queens were inseminated instrumentally using semen from their brothers. The survival rate of brood was monitored, and queens producing brood of low survival rate were used as mothers of haploid and diploid drones. The diploid drones were reared by Woyke's (1969) method.

Spermatozoa of haploid and diploid drones were collected from the testes of young drones. Drones were killed and dissected in 1% physiological solution, and the testes smeared in that solution on a microscope slide, so that the spermatozoa were released. A coverslip was put on the slide, and the spermatozoa were measured using a phase contrast microscope with a micrometer eyepiece. Because the spermatozoa were mostly curved they had to be measured in segments.

The *heads* of spermatozoa were measured to obtain information on the amount of chromatin substance in them. For that purpose smears of spermatozoa on microscope slides were fixed in Carnoy fluid (3 parts absolute alcohol to 1 part glacial acid) and then treated by the Feulgen reaction, which stains DNA in the nuclei. The smears were hydrolysed for 15 min in 1-N HCl at 60°C, and then stained in Schiff's reagent. Staining was done with basic fuchsin, after which the smear was dehydrated, embedded in Canada balsam and protected with a cover glass. The nuclei in the heads were measured with a ×100 oil-immersion objective and ×25 micrometer eyepiece.

Results

Whole lengths of spermatozoa

The length of 2100 spermatozoa from 84 haploid drones (Table 1) ranged from 221 to 270 μm, mean 241·7 μm. The mean for each drone ranged from 230·2 to 247·0 μm, and for each of the three queens from 241·0 to 243·0 μm. Thus the mean lengths of haploid spermatozoa produced by drones reared at different times and from different queens showed little variation.

The coefficients of variation calculated for spermatozoa of different haploid drones collected at random from various queens (2·5%–5·3%) were similar to those of drones originating from one queen only and investigated under similar conditions: 2·2%–5·4%.

The length of 1475 spermatozoa originating from 59 diploid drones ranged from 266·0 to 370·0 μm, mean of 312·7 μm. The mean for each drone ranged from 292·6 to 333·9 μm. The mean length of all spermatozoa from all diploid drones from Carniolan queen 1978 was 301·0 μm, whereas that of spermatozoa from drones from the two hybrid queens was 317·0 and 320·0 μm, respectively. The small number of queens used, however, makes it impossible to establish whether there was a heterosis effect.

TABLE 1. Lengths of spermatozoa of haploid (H) and diploid (D) drones (25 spermatozoa from each drone).

			Length (μm)			
Queen no.	*Type of drone*	*No. drones*	*Range for all spermatozoa*	*Range of mean for individual drones*	*Coeff. of variation (%) for individual drones*	*Overall mean ± SE*
			ENTIRE SPERMATOZOA			
1st period, Carniolan bees						
1978	H	9	229–255	237·9–245·3	1·9–2·7	241·9 ± 0·38
1978	D	9	266–343	292·6–308·1	2·2–3·7	301·0 ± 0·77
2nd period, hybrid Carniolan/Caucasian bees						
2890	H	25	221–267	239·0–246·0	2·2–5·4	243·0 ± 0·39
2965	H	25	222–270	230·2–247·0	2·8–5·0	241·6 ± 0·40
various	H	25	222–267	238·2–244·0	2·5–5·3	241·0 ± 0·30
2890	D	25	282–365	308·0–333·0	1·6–6·7	317·0 ± 0·59
2965	D	25	288–370	307·0–333·9	2·8–6·3	320·0 ± 0·50
Total						
	H	84	221–270	230·2–247·0	1·9–9·8	241·7 ± 0·19
	D	59	266–370	292·6–333·9	1·6–6·7	312·7 ± 0·45
			NUCLEI OF HEADS OF SPERMATOZOA			
1978	H	10	4·25–5·75	4·7–5·0	5·7–9·6	4·8 ± 0·02
1978	D	8	6·25–8·50	7·2–7·6	4·1–7·7	7·4 ± 0·04

The greatest length of a haploid spermatozoon (270 μm) overlapped the smallest length of a diploid one (266 μm) by 4 μm. This occurred in the comparison between spermatozoa of drones of different races, measured by different people. When haploid and diploid spermatozoa produced by drones from a single Carniolan queen (1978) were compared there was no overlap, the difference between the longest haploid supermatozoon (255 μm) and the shortest diploid one (266 μm) being 11·0 μm. The corresponding difference for spermatozoa of drones produced by a hybrid queen was 15 μm (2890) and 18 μm (2965). Thus the mean difference between the longest haploid and the shortest diploid spermatozoa produced by drones from the same queen was about 15 μm.

Length of the nucleus in the spermatozoon head

The extreme values for haploid (4·25–5·75 μm) and diploid spermatozoa (6·25–8·50 μm) did not overlap, the shortest nuclei of diploid spermatozoa being 0·5 μm longer than the longest ones of haploid spermatozoa.

The average length of diploid and haploid nuclei was 7·4 and 4·8 μm, respectively.

Discussion

The average length of all haploid *spermatozoa* measured (241·7 μm) was similar to lengths given by Smirnov (1953) and Cruz-Hofling et al. (1970): 240 to 250 μm and 250 μm, respectively. The average length of all haploid *nuclei* measured (4·8 μm) was similar to lengths given by Cruz-Hofling et al. (1970) and Lensky et al. (1979): 5·5 μm and 5 μm, respectively.

The length of spermatozoa from diploid drones differed considerably from those of spermatozoa from haploid drones, and this supports the earlier finding that diploid drones produce only diploid spermatozoa (Woyke & Skowronek, 1974). To understand the significance of this finding for unanswered questions concerning triploid workers, consider the investigations of Chaud-Netto (1972). He inseminated 4 queens with semen collected from drones reared by the method for diploid drones. He concluded that:

1. Diploid drones produce at least some diploid spermatozoa.
2. Segregation of *La* and *la* during spermatogenesis does not occur in diploid drones.
3. Out of 18 pupae resulting from the above insemination, 11 showed 48 chromosomes in their ovaries and 7 showed 32 chromosomes.

The present investigation shows that queens inseminated with semen from diploid drones cannot at once produce diploid and triploid workers, because all spermatozoa produced by those drones are diploid. I think the only explanation of Chaud-Netto's results is that the queens were inseminated by haploid spermatozoa from haploid drones, reared by the method for diploid drones. This is supported by the fact that out of 22 drones reared by this method only 4 had semen (because they were haploids). Although genetic markers were used, the phenotypes of drones did not permit discrimination between haploid and diploid drones.

I was able to verify by genetic markers, that among the diploid drones produced by experimentally sibling-mated queens, haploid drones also are often reared. The cause of this is the small amount of semen used for these queens (1 mm^3 instead of 8 mm^3), which makes the queens lay some unfertilized eggs among the fertilized ones.

The fact that 48 chromosomes are found in ovaries of some workers does not prove their triploidy. I investigated the number of chromosomes in the ovaries of diploid workers by the same method and found even higher numbers of chromosomes, which must have been produced by endopolyploidization.

So far no report of the production of triploid workers has provided evidence that the drones used to mate the queens were without doubt diploids, and not haploids reared by the method for diploids. Consequently the production of triploid workers so far must be regarded as doubtful.

Conclusion

The average length of entire diploid spermatozoa is 129% of the length of haploid spermatozoa. But the head length of diploid spermatozoa is 154% of the head length of haploid spermatozoa.

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J51