

CHANGES IN TISSUE POLYPLOIDIZATION DURING DEVELOPMENT OF WORKER, QUEEN, HAPLOID AND DIPLOID DRONE HONEYBEES

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Cytophotometric measurements were made of DNA content in nuclei stained by the Feulgen method and direct measurements of length and width of nuclei were also made. There was an enormous increase of polyploidization in larvae 4 days old, from an average of 110 n in workers through 112 n and 200 n in haploid and diploid drones to 320 n in queens. Mean DNA content in nuclei of haploid drone, diploid drone, worker and queen larvae were in the ratios 1·0 : 1·7 : 1·1 : 3·1. Since diploids began with twice as much DNA as haploids, polyploidization was about twice as fast in haploid drone larvae as in worker ones and only a little slower in diploid drone larvae than in haploids. A drastic decrease of polyploidization was found in early pupae from 5 n in haploid drones and workers to 8 n in diploid drones and queens. Some increase of average polyploidization was found in imagines, from 7 n in haploid drones through 8 n in diploid drones and workers to 10 n in queens. Relative polyploidization in imagines as determined by both DNA content and volume of cell nuclei may be presented in the following succession: workers, 1·0; haploid drones, 1·0; diploid drones, 1·2; queens, 1·5. Since diploids began with twice as many chromosomes as haploid drones, the relative rate of polyploidization up to the imago may be presented in the following sequence: workers, 1·0; diploid drones, 1·2; queens, 1·5; haploid drones, 2·0. Diploid drones showed sexualities ranging from intersex to female.

Introduction

In the honeybee, drones arise normally from unfertilized eggs, and workers and queens from fertilized ones. Consequently, the youngest male larvae are haploid with 16 chromosomes and the female are diploid with 32 chromosomes.

In the development of many insects, however, endopolyploidization of somatic cells occurs (Geitler, 1939) and a high degree of polyploidization is reached by somatic tissues in varying degrees in different tissues.

Several studies on tissue polyploidization in haploid drone, worker and queen honeybees have been made. Risler (1954) counted the chromosomes and measured the size of cell nuclei and of the spindle in dividing cells in five successive larval instars of drones and workers. He concluded that during the first instar the cells of drones were haploid and those of workers were diploid. In later instars, polyploidization occurred in both drone and worker tissues. As a result, the degree of polyploidization of tissues of older drone larvae was similar to that of workers.

Other authors determined the DNA content of cell nuclei and measured their size. Mittwoch et al. (1966), investigating larvae 5–20 h old, found mitoses to be all diploid in young female larvae and almost all haploid in male larvae. But DNA values of interphase nuclei showed a high level of polyploidization (up to 32 n) in drone larvae. The data suggested that polyploidization might be faster in drone larvae than in worker larvae. Stekolščikov (1970) found a high level of polyploidization in workers up to the 5th larval instar.

Mello and Takahashi (1971) found that the volume of nuclei and DNA content in the Malpighian tubules of the last larval instar was higher in drones (DNA = 128 n) than in workers (32 n and 64 n) but was still higher in queens (512 n).

In adults, Merriam and Ris (1954) found that the class peak of the volume of nuclei in the small intestine and Malpighian tubules of haploid drones was 1·3–1·4 times that of workers. In both workers and drones the principal class of DNA content in Malpighian tubules was 16 n whereas in queens it was 32 n. In the small intestine the principal class was equal (16 n) in all three castes and the salivary glands uniformly showed 128 n and 256 n classes in all three castes. The authors concluded that adult male tissues have the same chromosome number as comparable female tissues.

Merriam and Ris (1954), Stekolščikov (1970) and Mel'nichenko and Kapralova (1971) concluded that there is a high positive relationship between the degree of polysomaty and the physiological activity of the cells.

Woyke (1963) discovered diploid drones and later reared their imago (Woyke, 1969). Woyke and Skowronek (1974) showed that the process of spermatogenesis in diploid drones was similar to that in haploids. Reduction in the number of chromosomes during spermatogenesis does not occur in drones, so diploid ones produce diploid spermatozoa. A head of a diploid spermatozoan contains twice as much DNA as a head of a haploid one (Woyke, 1975) and the volume of a diploid spermatozoan is about twice that of a haploid one (Woyke, 1984).

Thus a very interesting question arises. Is the polyploidization rate of somatic tissues of diploid drones the same as that of haploid ones? If this is so, somatic cells of adult diploid drones should have double the number of chromosomes found in the corresponding cells of haploid drones (i.e. twice as many as those in females) since adult haploid drones reach the same degree of polyploidization as females.

Woyke (1971) found that diploid drones were larger than haploid ones. The diploid showed some supermale characters but also some intersex, female and intercaste characters. These findings were confirmed by Chaud-Netto (1975), Chaud-Netto and Duarte (1975) and Woyke (1977, 1978a, 1978b), but Chaud-Netto (1975) and Chaud-Netto and Duarte (1975) concluded that diploid drones were 8% more masculine than haploid ones (they called them 'meta-males'), showing that the maleness genes were additive. However, Woyke (1980) concluded that the main difference between diploid and haploid drones is the larger size of the diploids rather than any shift of sexuality, and that the size of diploid body parts is the result not of genic balance but of higher polyploidization. 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, showing different sexuality.

Hitherto, polyploidization of body tissues during the development of diploid drones has not been studied.

Materials and Methods

Four types of *Apis mellifera carnica* bees (haploid and diploid drones, workers and queens) were investigated in four development stages. The stages were 4th-instar and spinning larvae, pupae with white eyes and emerging imagines. Six tissues were investigated in each of five individuals from each of the four types of bee but not in all three developmental stages. Altogether 80 bees were investigated. Measurements were made on 30 nuclei from each tissue of each of the bees. Thus, 7800 cytophotometric measurements and 15 600 measurements of the length and width of cell nuclei were made. Additionally, 2400 cytophotometric measurements were made to construct the hydrolysis curves necessary to find the optimal time of hydrolysis of nuclei for the Feulgen reaction.

Diploid drones were reared by Woyke's method (1969). The organs were dissected in 0.9% saline solution, squashed between a slide and cover-slip and frozen on dry ice. The cover-slip was removed and the tissues fixed in Carnoy's fluid for 1 h. Hydrated tissues were subjected to Feulgen reaction procedures: they were hydrolysed for 3 min in 1 N HCl at 58-60°C and treated in Schiff's reagent for 1 min. The optimal hydrolysis time was determined empirically. Dehydrated tissues were mounted in Euparal.

The DNA content of cell nuclei was determined in a Barr and Stroud integrating microdensitometer in green light ($\lambda = 550$ nm). Extinction of cell nucleus as well as of background was measured. The difference between the values of the two extinctions gave the DNA content in arbitrary units.

The degree of polyploidization of the nuclei of each of the six tissues was calculated in relation to the DNA content of heads of spermatozoa (Woyke, 1975).

Analysis of variance was applied to the results and the new Duncan's multiple-range test was used to detect significant differences between the means. In Table 1 and Table 2 different letters against two means indicate that they are significantly different ($P < 0.05$). A correlation coefficient was also calculated.

Results

Ventriculus

Significant differences in the DNA content of nuclei of the ventriculus were found by the 4th larval instar in diploid drones, workers and queens (Table 1), despite the fact that all of them

TABLE 1. Mean DNA content (expressed in arbitrary units) and degree of polyploidization n (in relation to heads of haploid spermatozoa) in tissues of four types of the honeybee at four stages of development. All means are of 150 measurements.

Tissue in organ and development stage	Haploid drones		Diploid drones		Workers		Queens	
	DNA \pm SE	n	DNA \pm SE	n	DNA \pm SE	n	DNA \pm SE	n
Ventriculus:								
larva (4-day)	432.07 \pm 11.70a	*368	698.50 \pm 16.42b	595	415.80 \pm 12.92a	354	892.17 \pm 17.54c	761
pupa	9.37 \pm 0.19a	8	19.67 \pm 1.04c	17	11.67 \pm 0.22b	10	19.84 \pm 0.56c	17
imago	10.13 \pm 0.31a	9	14.31 \pm 0.32b	12	19.34 \pm 0.30d	16	17.25 \pm 0.42c	15
Small intestine:								
larva (4-day)	3.44 \pm 0.06a	3	3.55 \pm 0.08a	3	3.61 \pm 0.09a	3	3.89 \pm 0.13a	3
pupa	4.28 \pm 0.09b	4	3.26 \pm 0.07a	3	3.48 \pm 0.09a	3	4.27 \pm 0.14b	4
imago	5.61 \pm 0.16a	5	7.33 \pm 0.15b	6	7.55 \pm 0.13b	6	7.09 \pm 0.17b	6
Malpighian tubules:								
larva (4-day)	79.89 \pm 2.28a	68	235.20 \pm 5.31c	201	147.87 \pm 4.43b	126	426.00 \pm 11.94d	363
pupa	3.25 \pm 0.06b	3	4.19 \pm 0.09c	4	2.82 \pm 0.06a	2	5.58 \pm 0.09b	3
imago	6.61 \pm 0.20a	6	9.19 \pm 0.21b	8	7.35 \pm 0.13a	6	12.61 \pm 0.28c	11
Silk gland:								
larva (4-day)	101.15 \pm 2.35b	86	175.91 \pm 4.18c	150	24.17 \pm 0.26a	21	451.03 \pm 11.13d	385
spinning larva	386.53 \pm 11.50b	330	550.97 \pm 12.25c	470	70.02 \pm 2.18a	60	814.67 \pm 24.74d	695
Fat body:								
larva (4-day)	38.49 \pm 1.10a	33	53.30 \pm 1.93a	45	52.04 \pm 1.17a	44	97.03 \pm 3.74b	83
Postcerebral glands:								
imago	8.83 \pm 0.25c	8	7.35 \pm 0.19b	6	6.16 \pm 0.16a	5	8.67 \pm 0.21c	7

* Different letters indicate significant differences between means ($P < 0.05$) of different types of the same development stage.

TABLE 2. Mean volume (μm^3) of nuclei in tissues of four types of honeybee at four stages of development. All means are of 150 measurements.

Tissue in organ and development stage	Haplod drones		Diploid drones		Workers		Queens	
	Volume	SE	Volume	SE	Volume	SE	Volume	SE
Ventriculus:								
larva (4-day)	43559.96	± 2552.95	55609.39	± 4449.10	19207.56	± 753.44	79608.27	± 4225.22
pupa	281.76	± 14.79	459.79	± 21.70	257.32	± 11.13	393.25	± 17.13
imago	229.34	± 10.96	267.12	± 11.68	190.23	± 6.38	337.90	± 16.56
Small intestine:								
larva (4-day)	105.92	± 6.22	65.31	± 3.56	95.07	± 3.29	82.47	± 4.83
pupa	110.90	± 5.19	76.70	± 4.35	71.89	± 1.51	70.29	± 3.06
imago	112.41	± 5.66	93.59	± 4.99	98.65	± 2.71	113.43	± 6.30
Malpighian tubules:								
larva (4-day)	5643.11	± 315.04	14033.33	± 784.27	9983.25	± 369.63	20980.76	± 925.42
pupa	43.90	± 2.08	90.27	± 3.12	54.72	± 1.35	49.90	± 2.54
imago	121.54	± 5.47	149.72	± 5.48	65.82	± 1.59	226.42	± 10.03
Silk gland:								
larva (4-day)	4199.07	± 170.25	5723.85	± 315.93	886.00	± 28.72	13580.63	± 586.72
spinning larva	27688.29	± 1557.72	39668.98	± 1346.35	1337.58	± 60.93	58173.13	± 2820.90
Fat body:								
larva (4-day)	1477.90	± 102.79	1398.54	± 63.15	1047.15	± 21.66	5524.44	± 322.58
Postcerebral glands:								
imago	231.66	± 17.21	351.70	± 17.77	89.29	± 1.96	268.02	± 5.98

* Different letters indicate significant differences between means ($P < 0.05$) of different types of the same development stage.

initially contained the same number of chromosomes. The DNA content of nuclei of diploid drone larvae was intermediate between workers (lowest) and queens (highest).

The DNA content of nuclei of the ventriculus in diploid drone larvae (698·50) was 1·6 times as high as that of haploids (432·07), and in workers (415·80) it was similar ($\times 0\cdot96$) to that of haploids. Since at the beginning of development the diploids had twice as much DNA in their nuclei as the haploids, it appears that polyploidization in haploid drones was faster than in both diploids.

In pupae a drastic decrease in DNA content was found (e.g. in workers from 415·80 in larvae to 11·67 in pupae). This is caused by histolysis of larval organs and development of new ones. Diploid drones and queens had the highest DNA content and were similar to each other.

In imagines, DNA content increased only in haploid drones and workers. The highest amount was found in workers, probably because of their higher ventricular physiological activity.

The volumes of cell nuclei in the larval ventriculus were lowest in workers and highest in queens (Table 2). No significant differences were found between haploid and diploid drones.

In imagines, the diploid drones had larger mean nuclear volumes than the haploid ones, but the difference was not significant.

Small intestine

The DNA content in the small intestine of 4th instar larvae (Table 1) was several hundred times lower than that in the ventriculus, probably due to the very different physiological activity of the two organs. Similar amounts of DNA were found in nuclei of all four types of individuals, indicating a polyploidization rate twice as high in haploid drones as in diploid ones. In the imagines, the DNA content was significantly lower in haploids than in the three types of diploid, but since the DNA amount in diploid drone nuclei (7·33) was only 1·3 times as high as that in haploid nuclei (5·61), polyploidization is faster in haploid drones than in diploid drones or in either female caste.

The volume of nuclei in the small intestine in haploid drone pupae was actually higher than in all three types of diploid individuals (Table 2), but in imagines no significant differences were found between the four types of individual. Thus polyploidization is twice as fast in haploid drones as in the three diploids.

Malpighian tubules

The DNA content in the nuclei of Malpighian tubules of diploid drone larvae was intermediate between those in workers and queens (Table 1). The DNA content in diploid drones (235·20) was 2·9 times as high as that in haploid drones (79·89). Since at the beginning of larval development diploids have only twice as many chromosomes as haploids, polyploidization of the tissue of Malpighian tubules was faster in diploid drones than in the haploid ones, up to 4th-instar larvae. A drastic decrease of DNA content occurred in pupae because of the histolysis of the four larval tubules and their replacement by about 150 new imaginal ones.

In imagines, diploid drones had 1·4 times as much DNA (9·19) as haploids (6·61) and queens had 1·9 times as much (12·61) as haploids. Thus polyploidization of the adult Malpighian tubule epithelium was faster in haploid drones than in diploid drones and similar to that in queens.

The volume of nuclei in the Malpighian tubules was 2·5 times as great in diploid drone larvae as in haploids (Table 2). Thus polyploidization was faster here in diploids than in haploids. There was a drastic decrease in pupae, just as with DNA content.

Adult diploid drones had larger mean nuclear volumes than haploid ones, but the difference was not significant. Queens had nuclei 1·9 times as large as those of haploid drones—a similar relationship to that for DNA content. Thus the polyploidization rate of the volume of nuclei in the Malpighian tubules of adult haploid drones is greater than that in diploid drones, and similar to that in queens.

Silk glands

Silk glands are present in the larva only. DNA content in the nuclei of silk glands was similar in the 4th-instar larva and in the spinning one. The lowest content was in workers and the highest in queens. Spinning diploid drones had 1·4 times as much DNA, and queens had 2·1

times as much DNA, as haploid drones. Thus polyploidization was faster in haploid drones than in diploid drones and similar to that in queens. Relationships in volume of silk gland nuclei in all four types of bee were similar to those for DNA content in these castes. So there seems to be some relationship between nucleus volume and DNA content.

Fat body

In larvae four days old, haploid drones had a lower mean nuclear DNA content than diploid drones or workers, but the differences were not significant (Table 1). So here, also, faster polyploidization in haploid drones than in diploid drones and workers is suggested. However, since the degree of polyploidization in queen larvae (83 *n*) was 2.5 times as great as that in haploid drones (33 *n*), polyploidization was faster in queens than in haploid drones. Relationships in nuclear volume between the four types of bee (Table 2) were roughly similar to those for DNA content.

Postcerebral glands

These exist only in imagines. The lowest DNA content was in workers' nuclei (Table 1). Haploid drones had a significantly higher DNA content than diploid drones, but the mean was similar to that found in queens. Thus here, polyploidization in haploid drones was twice as fast as in queens.

The smallest nuclei were found in workers. Haploid drones had nuclei similar to those found in queens (Table 2), but diploid drones had the largest nuclei of all. A similar relationship was not noticed in any other tissue of adult bees.

Correlation between DNA content and volume of cell nuclei

Correlation between 52 means of DNA content in cell nuclei (Table 1) and the corresponding 52 means of the volume of those nuclei (Table 2) gave a very high correlation coefficient ($r = 0.96$) that was highly significant.

Degree and dynamics of tissue polyploidization in larvae and pupae

The mean DNA content in nuclei of tissues (expressed in arbitrary units) was divided by that in heads of haploid spermatozoa to give the integer *n*, indicating the degree of polyploidization (Table 1). Averages of *n* degrees of polyploidization for all investigated tissues together, were calculated separately for each development stage of all four types of bee. Fig. 1 shows the huge increase of mean polyploidization degree in larvae, from 110 *n* in workers, through 112 *n* in haploid and 200 *n* in diploid drones, up to 320 *n* in queens.

To compare the degree and dynamics of tissue polyploidization between those four types of individuals, the DNA content in nuclei and the nuclear volume in tissues of three diploid types of bees were divided by those values in haploid drones (Table 3).

DNA content in tissues of juvenile forms (larva or pupa) of diploid drones was in three cases twice as high or higher (2.1; 2.9; 1.7) than in haploids. This indicates similar or greater rates of tissue polyploidization in juvenile forms of diploid drones than in haploids.

In worker larvae, only nuclei of Malpighian tubules contained about twice as much DNA as those of haploid drones. This high level in workers probably reflects their different physiological development stage; worker larvae at this age are close to excreting the content of those tubules. DNA content in all other tissues of both forms of juvenile worker was close to that of haploid drones, indicating that tissue polyploidization was twice as fast in the juvenile forms of haploid drones as in those of workers. In four tissues from queen larvae, DNA content was more than twice as high as in haploid drones. The much higher DNA content in the Malpighian tubules ($\times 5.3$) and silk glands ($\times 4.5$) of queen larvae four days old than in haploid drones is related to the much quicker development of queens than of drones. Although females had twice as many chromosomes as haploids at the beginning of development, most tissues of queen larvae had more than twice as much DNA as those of haploid drones.

The average DNA content in all tissues of larvae four days old of haploid and diploid drones, workers and queens was in the ratio 1.0 : 1.7 : 1.1 : 3.1 (Table 3 and Fig. 1). Thus by the larval stage haploid drones had reached the same degree of polyploidization as workers. This means that DNA increased almost twice as fast in the larval stage of haploid drones as it

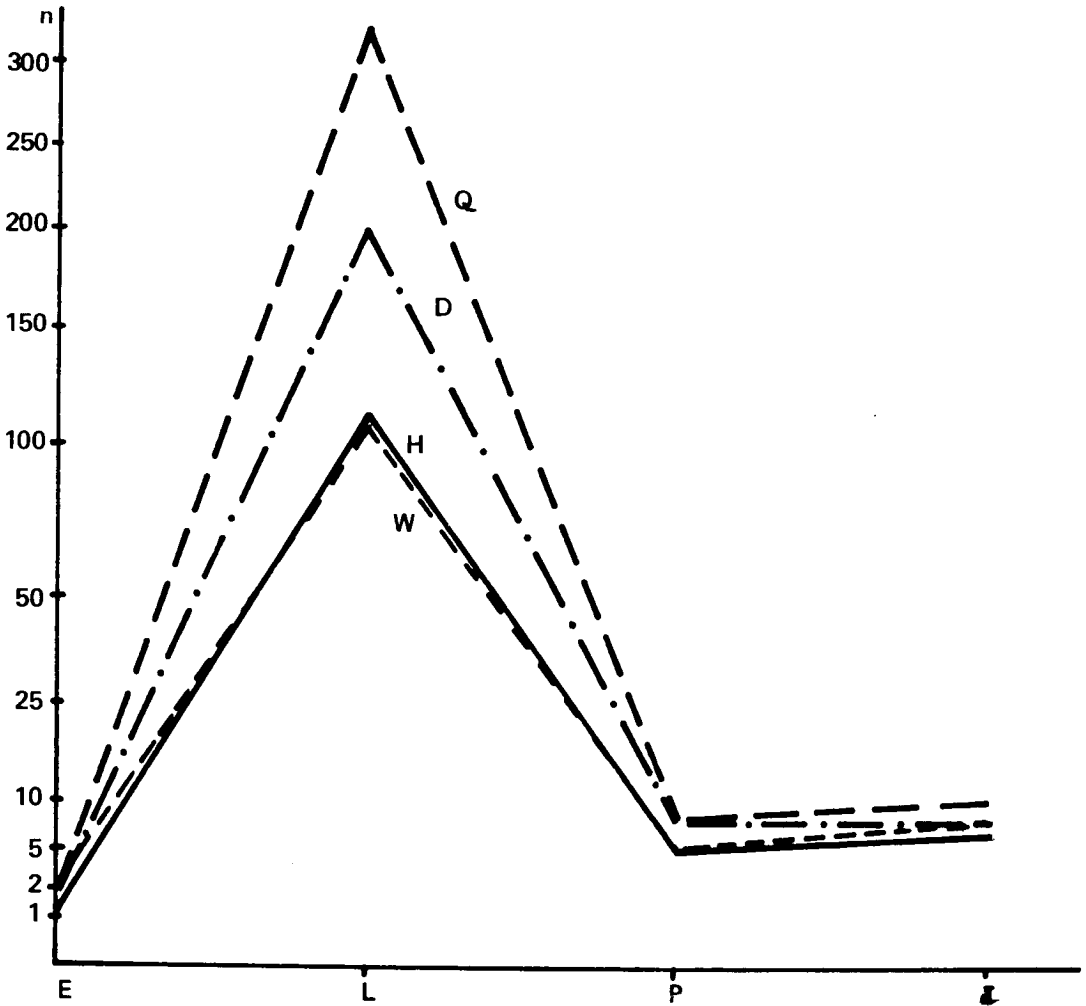


FIG. 1. Dynamics of tissue polyploidization (n multiplication of DNA) in four types of honeybees: workers (W), haploid drones (H), diploid drones (D) and queens (Q), detected during developmental stage of egg (E), larva 4 days old, early pupa (P) and imago (I). Ordinate $y = \sqrt{n}$.

did in workers, and in diploid drones only a little slower than in haploids. DNA increased in queen larvae 1.6 and 1.8 and 2.8 times as fast as in haploid and diploid drones and workers respectively.

With regard to the volume of nuclei (Table 3) in juvenile forms of diploid drones, only the Malpighian tubules have nuclei more than twice as large as those in haploid drones, and show a faster volume increase than that in haploids.

In worker larvae and pupae, the volume of nuclei, like DNA content, was mostly similar to or smaller than that of haploid drones. A volume of nuclei more than twice as large as that in haploid drones was found in three tissues of queen larvae 4 days old, and almost twice as large ($\times 1.8$ in the ventriculus) in one tissue (Table 3).

The average nucleus volume in investigated tissues of larvae 4 days old in haploid drones, diploid drones, workers and queens was in the ratio 1.0 : 1.3 : 0.7 : 2.6 respectively, which shows a faster increase in haploid drone larvae than in workers and diploid drones, although the increase in queen larvae was faster still.

TABLE 3. Mean DNA content and volume of nuclei in tissues of three diploid honeybee types in relation to haploid drones.

Organ	DNA			Volumes of nuclei		
	Diploid drone	Worker	Queen	Diploid drone	Worker	Queen
			<i>Larva (4 day)</i>			
Ventriculus	1.6	1.0	2.1	1.3	0.4	1.8
Small intestine	1.0	1.0	1.1	0.6	0.9	0.8
Malpighian tubules	2.9	1.9	5.3	2.5	1.2	3.7
Silk glands	1.7	0.2	4.5	1.4	0.2	3.2
Fat body	1.4	1.4	2.5	0.9	0.7	3.7
Overall mean	1.7	1.1	3.1	1.3	0.7	2.6
			<i>Pupa with white eyes</i>			
Ventriculus	2.1	1.2	2.1	1.6	0.9	1.4
Small intestine	0.8	0.8	1.0	0.7	0.6	0.6
Malpighian tubules	1.3	0.9	1.1	2.1	1.2	1.1
Overall mean	1.4	1.0	1.4	1.5	0.9	1.0
			<i>Imago</i>			
Ventriculus	1.4	1.9	1.7	1.2	0.8	1.5
Small intestine	1.3	1.3	1.3	0.8	0.9	1.0
Malpighian tubules	1.4	1.1	1.9	1.2	0.5	1.9
Postcerebral glands	0.8	0.7	1.0	1.5	0.4	1.2
Overall mean	1.2	1.3	1.5	1.2	0.7	1.4

A drastic decrease of tissue polyploidization was found in the early pupal stage, from 5 n in haploid drones and workers to 8 n in diploid drones and queens (Fig. 1). This was presumably caused by histolysis of larval organs in the prepupal stage, and build-up of new imaginal organs. The average DNA content in tissues of early pupae of haploid and diploid drones, workers and queens was in the ratio 1.0 : 1.4 : 1.0 : 1.4; hence, in haploid drones it was equal to that in workers, and in diploid drones to that in queens.

The degree and dynamics of tissue polyploidization up to the imago stage are analysed separately to enable further conclusions to be reached.

Adult bees reached mean DNA degrees of polyploidization in tissues from 7 n in haploid drones through 8 n in diploid drones and workers to 10 n in queens (Fig. 1). Thus the degree of polyploidization was higher in imagines than in early pupae, but much lower than in larvae.

Table 3 shows that DNA content in nuclei of one tissue of adult diploid drones was lower ($\times 0.8$), and in three other tissues higher ($\times 1.3$ – $\times 1.4$), than in haploid drones. Average DNA content in all investigated tissues of adult diploid drones was 1.2 times as high as in haploid drones.

Adult workers had a similar amount of DNA to haploid drones (Table 3) in most tissues, but the DNA content of the ventriculus was 1.9 times as high as that in haploids. On the average, DNA content was 1.3 times as high in tissues of adult workers as in those of haploid drones.

Adult queens contained more DNA than haploid drones in nuclei of three tissues out of four. Average DNA content in all investigated tissues was 1.5 times higher in queens than in haploid drones.

Thus the overall mean DNA content in tissues of adult haploid and diploid drones, workers and queens was in the ratio 1.0 : 1.2 : 1.3 : 1.5 (Table 3 and Fig. 1).

Diploid drones had smaller nuclei than haploids in one tissue only; in all other tissues the nuclei were larger (Table 3).

Adult workers had smaller nuclei than haploid drones in all investigated tissues. This seems very strange, since the workers began as diploid and the drones as haploid.

Queens had nuclei like those of haploid drones in the small intestine only. Nuclei in all other queens' tissues were larger than those in haploid drones. Adult queens also had larger nuclei than workers in all investigated tissues, and larger nuclei than diploid drones in three tissues.

Average volumes of nuclei in investigated tissues in adult haploid and diploid drones, workers and queens were in the ratio 1.0 : 1.2 : 0.7 : 1.4.

The above values of both characters, DNA content and nucleus volume, may be combined, since the results are only relative. Thus relationships in polyploidization level determined by both characters may be presented in the following succession: workers, 1.0; haploid drones, 1.0; diploid drones, 1.2; queens, 1.5. Since the diploids began with twice as many chromosomes as the haploid drones, the rate of tissue polyploidization up to the imago stage, determined by both characters, may be presented in the following relative succession: workers, 1.0; diploid drones, 1.2; queens, 1.5; haploid drones, 2.0.

Sexuality of diploid drones

The sexuality of diploid drone characters was determined by comparing their values with those of haploid drones and females, as was done by Woyke (1971), Chaud-Netto (1975) and Woyke (1980). The DNA content (Table 1) and volume of nuclei (Table 2) in tissues of diploid drones (D) were compared with those of haploid drones (H), workers (W) and queens (Q) (Table 4). In one case out of eight (DNA content of ventriculus) diploid drones showed an intersex character, located between haploid drones and females ($H < D < Q < W$), and in two cases (nuclear volume of ventriculus and of Malpighian tubules) they showed a caste character close to queens, whereas haploid drones were closer to workers ($W < H < D < Q$). In two cases diploid drones showed a female character; in one (DNA of Malpighian tubules), they were located between workers and queens ($H < W < D < Q$), in the other (DNA of small intestine), they had the same DNA content as both females ($H < D = W = Q$). It should be noticed that the same tissues could show different sexuality depending upon the character studied, the DNA content or the volume of nuclei. In no case did diploid drones show a supermale character ($D < H < W < Q$ or $W < Q < H < D$).

TABLE 4. Sexuality of adult diploid drones determined by relationship of DNA content and volume of nuclei in tissues of haploid (H) and diploid (D) drones, workers (W) and queens (Q)

Organs	DNA content	D character	Volume of nuclei	D character
Ventriculus	$H < D < Q < W$	intersex	$W < H < D < Q$	caste
Small intestine	$H < D = W = Q$	female	$D < W < H = Q$?
Malpighian tubules	$H < W < D < Q$	female	$W < H < D < Q$	caste
Postcerebral glands	$W < D < H = Q$	caste	$W < H < Q < D$?

Discussion

Risler (1954) found that cell nuclei in 1st-instar larvae were haploid in haploid drones and diploid in females. In larvae of the 5th-instar, 8–16 n nuclei were found in tissues of female larvae and 8–32 n ones in haploid drone larvae. Since Risler counted chromosomes, polyploidization degrees given by him are mostly lower than in cases where polyploidization in resting nuclei is determined. It is known that endopolyploidization in interphase nuclei is faster than in dividing ones (Mittwoch et al., 1966).

A huge amount of polyploidization during larval development was found in this investigation and in investigations by Stekolščikov (1970) and Mello and Takahashi (1971). The most polyploidization occurred in tissues with the highest physiological activity.

According to Merriam and Ris (1954), DNA content in nuclei of the Malpighian tubules and the small intestine was similar in workers and adult drones and higher in queens, but the

volume of nuclei was higher in haploid drone tissues than in workers. In the present investigation, adult workers contained slightly more DNA in cell nuclei than haploids, but the relative volumes of nuclei in the three types of bee were similar to those found by Mello and Takahashi (1971).

Polyploidization in diploid drones has not been investigated by other authors. The results now presented show that polyploidization levels determined for both characters (DNA content and cell volume) were in the ratio 1.0 : 1.2 in haploid and diploid drones. The sexuality of diploid drones determined by different tissues varied; this was foreseen by Woyke (1980). Nothing suggested that a diploid drone is a super-male.

Conclusions

An enormous degree of average polyploidization in tissues was reached in the late larval development period, from 110 n in workers, through 112 n and 200 n in haploid and diploid drones, to 320 n in queens. The highest level of polyploidization was found in the most physiologically active tissue (354–761 n in the ventriculus). The DNA content in nuclei of some tissues of larvae 4 days old was almost twice as high in diploid ($1.6\times$: $1.7\times$) as in haploid drones, and it was 2.9 times as high in nuclei of larval Malpighian tubules.

Due to histolysis of larval organs in prepupae and the build-up of new imaginal organs there was a drastic decrease of polyploidization in the early pupal stage of all four types of bees. Although, however, there was a renewed increase of polyploidization in imagines, the level of DNA content in their nuclei was many times lower ($1/12$ – $1/52$) than the maximum found in the larvae.

Since many body parts are larger and polyploidization is higher in adult queens than in adult workers, we conclude that a queen's body parts are larger because of greater polyploidization. Similarly, since body parts are larger and polyploidization is higher in diploid drones than in haploid ones, we conclude that diploid drone body parts are larger because polyploidization is higher. In the latter case, the question remains as to what extent greater polyploidization is due to diploidism and to what extent to different rearing conditions.

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