Cytological Studies on the Interspecific Variability.
The behaviour of the chromosomes with their
metrical analysis in the male germ-cells of
Halys dentatus and *Halys* sp.

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Abstract

Cytologically *Halys dentatus* and *Halys* sp. differ in the staining reaction of the nucleolus which is, however, formed by the heteropycnotic ends of the autosomes in both the species. It appears that the staining difference between the two is of the same nature as that of the heterochromatin which shows positive or negative reaction at different stages during the life of a particular cell. The metrical analysis of the chromosomes at metaphase I reveals that the difference in the chromosome sets of the two species is of the nature of a single homozygous deletion or reduplication in the longest pair of the chromosomes. It has further been observed that there is a continuous decrease in the total length of the monoploid genome during the successive spermatogonial generations which is also accompanied by the decrease in the area of the cell. There does not, however, appear any correlation between the two.

Introduction

The comparison of the actual size of the chromosomes has been used by many workers as a means to trace the evolution of the otherwise similar karyotypes. Rao (1937) and Powers (1942) have demonstrated the usefulness of this method in providing evidence of structural changes in the Acrididae. Ray-Chaudhuri and Dutt (1947) and Dutt (1948) have extended these data to different subfamilies while more recently Manna (1951), Dutt (1955) and Parshad (1956) have used this method to find out karyotype reorganisations even within different genera. The present investigation has been confined to two species of the genus *Halys*. The species, *Halys dentatus* and *Halys* sp., collected from the mango and *Acacia* trees respectively, were originally thought to be the two varieties of *H. dentatus* which was supposed to be the only representative of the genus *Halys* recorded from the plains of India. The strict gregarious habit of these insects leading to reproductive isolation (actual observation during 1953-54), however, aroused a doubt in our minds as to their actual systematic nature and consequently their specimens were sent to the Entomological Section of the Zoological Survey of India and the British Museum, London. The two types have now been unanimously described as two different species, but, due to the lack of sufficient literature on the genus, no specific name could be given to the new species. The present paper deals with a detailed comparative study of the mitosis and

meiosis in addition to the inter- and intra-specific variations in the length of the chromosomes.

Material and technique

The two species, *Halys dentatus* and *Halys* sp., were procured from their respective host plants, mango and *Acacia* trees, dissected out in normal saline and the testes subsequently fixed in the various chromosome fixatives like Bouin's Allen, Sanfelice and Carnoy. The testes from the different individuals were treated separately. Sections cut varied from $7\frac{1}{2}\mu$ to 15μ in thickness and these were stained with 0.5% Iron-haematoxylin and Feulgen.

For the measurement of the chromosome lengths the selected metaphase plates were drawn with the help of a camera lucida at the table level with 2 mm apochromatic oil immersion objective and $15\times$ eye-piece, giving a total magnification of 2150 times. This was followed by the measurement of these chromosome figures with the help of a travelling microscope to finally calculate their actual dimensions. The method seems to be very accurate as is evident by the frequency with which the homologous chromosomes come to be equal in size and whenever a difference was observed the particular chromosome was drawn and redrawn and the results checked carefully.

Observations

Resting spermatogonium: The spermatogonia, which occupy the extreme free ends of the testes, are arranged singly or in small groups, not surrounded by a membrane in the form of a cyst. The single cell shows that it is of the first spermatogonial generation, while the group represents the subsequent ones, corresponding to the number of the cells in the group. There are, in all, seven spermatogonial generations, as determined by the total number of cells in a group at the final gonial stage.

The two species show a difference in the disposition of the chromatin in the resting nucleus of the spermatogonium. In *Halys dentatus* (Fig. 1) the chromatin is in the form of granules which vary in size and are distributed in the substance of the nucleus. In addition to these granules the sex-chromosomes mass lies in association with the oxyphilic nucleolus which appears as a pinkish body in the haematoxylin slides counter-stained with eosin (Fig. 2). In *Halys* sp., however, the fine chromatin granules are arranged in the form of a coarse net-work which has a peripheral position in the nucleus, with a small area free from it wherein lies the basophilic nucleolus (Fig. 3). No sex-chromatin mass can be made out as such and it seems to have indistinguishably fused with the nucleolus.

Spermatogonial mitosis: During the early prophase, the sex-chromo-

somes mass becomes disassociated from the nucleolus, basophilic or oxyphilic, and resolves itself into two unequal darkly-stained bodies which finally differentiate into X and Y (Figs. 4, 5, 6 and 7). In *Halys* sp. the basophilic nucleolus is represented by a diffused area. This then becomes lobed and finally divides

into three to five elements (Fig. 5)

which are seen at a later stage, attached to the differentiating autosomal threads (Figs. 7 and 7a).

As the autosomes thicken and stain more deeply the nucleolar elements become more and more diffused to disappear finally. The exact nature of the oxyphilic nucleolus in *Halys dentatus* could

not be traced, but it persists till the late prophase as a diffused and irregular

body to which the heteropycnotic ends of the autosomes are applied (Fig. 8).

The chromosomes during the prophase are elongated. Each one of them is divided into two chromatids which are coiled round each other. In Fig. 4a are drawn three large chromosomes to show their double structure. In the polar view of the metaphase plate (Fig. 9), there are present fourteen

Figs. 1-14. 1 and 2, spermatogonial nuclei, *Halys dentatus*. 3, spermatogonial nucleus, *Halys* sp. 4 and 6, early prophase nuclei, *H. dentatus*. 5 and 7, early prophase nuclei, *H. sp.* 8, prophase, *H. dentatus*. 9, spermatogonial metaphase. 10, spermatocyte nucleus, *H. sp.* 11, spermatocyte nucleus, *H. dentatus*. 12 and 13, early leptotene. 14, contraction stage (ch. n.-sex-chromatin mass; N₁-oxyphilic nucleolus. N₂-Feulgen positive nucleolus. n₁, n₂, n₃, -fragments of N₂) × 2550.

chromosomes, twelve of them being the autosomes and the remaining two, the sex-chromosomes, X and Y. The Y is always identified as the smallest in the complement, while the X cannot be distinguished from the autosomes except by the metrical study. In the later generations of the spermatogonium, however, it does not usually stretch ideally on the equatorial plate and so becomes easily distinguishable from the autosomes of which only a single pair can be identified as the largest.

During anaphase all the chromosomes split and move simultaneously towards their respective poles without any differential behaviour of the sex-chromosomes. By telophase the chromosomes reach the poles of the spindle and the cell divides by a simple constriction without the appearance of any mid-body.

Meiosis: The pre-meiotic resting stage is of a very short duration and is represented by a rounded cell with a differential behaviour of the nucleolus in the two species—basophilic in *Halys* sp. and oxyphilic in *Halys dentatus* (Figs. 10 and 11). The general chromatin is, however, in the form of dark granules in both the species. During the leptotene stage these granules unravel themselves into lightly-staining fine threads which cross and intercross each other (Fig. 12). The sex-chromosomes condense into two compact bodies one of which is larger than the other. Before the leptotene threads become prominent these bodies become applied to each other (Fig. 13) and finally fuse to form a single heteropycnotic body which remains as such till the diplotene when the two elements again disassociate (Figs. 14 to 21). During the contraction stage (Fig. 14) the autosomes form an undifferentiated mass in which are also entangled the fused sex-chromosomes.

The diffused stage represents the complete “resting” condition of the nucleus wherein the heteropycnotic sex-chromosomes mass lies in close association with the nucleolus which is oxyphilic in *Halys dentatus* (Figs. 16 and 17) and basophilic in *Halys* sp. (Fig. 18). The basophilic nucleolus may sometimes be represented by more than one body (Fig. 19). During the diffused stage the nucleus undergoes an extensive growth and is, therefore, very favourable for the study of the nucleolus. It has been clearly observed that in the beginning of this stage both the basophilic and oxyphilic nucleoli always appear in association with the autosomes (Fig. 15a, 15b) of which they seem to be the enlarged ends. In the later stages, as the autosomal bivalents emerge out of the diffused stage, the oxyphilic nucleolus begins to show a very interesting behaviour inasmuch as it becomes basophilic and directly contributes its substance to the differentiating chromosomes, being connected to the latter (Fig. 20).

The diplotene bivalents (Fig. 21) are rods, rings and double crosses which arrange themselves along the periphery of the nucleus. The sex-chromosomes become disassociated and lie as heteropycnotic bodies which vary in size corresponding to X and Y. During diakinesis (Fig. 22) the bivalents undergo

a good deal of contraction along with the terminalization of most of their chiasmata. The sex chromosomes divide equationally and form diads which are the smallest components of the diakinetid complement.

The polar view of the metaphase plate of the first meiotic division (Fig. 23)

shows eight

elements

out of

which the

six larger

are the

autosomal

bivalents

while the

two smaller

ones are the

diads, X &

Y. All the

bivalents,

except the

rings, are

arranged at

right angles

to the equa-

tor of

the spindle

and parallel

to the

spindle

fibres, the

rings, how-

ever, being

parallel to

the equator.

Unlike

the mem-

bers of the

families

Coreidae,

Reduviidae

and Noto-

nectidae

there is no definite arrangement or group pattern displayed by the chromosomes at metaphase I. The following types of chromosomal arrangements have been observed:

Figs. 15-19. 15a and 15b, early diffused stages of *H. sp.* and *H. dentatus* respectively. 16 and 17, diffused stages, *H. dentatus*. 18 and 19, diffused stages, *H. sp.* $\times 2550$.

- a, X and Y in the ring of autosomes.
- b, Y in the ring of autosomes and X.
- c, Y and one autosome in the ring.
- d, All the chromosomes, including X and Y, forming a ring.
- e, One autosome in the ring.
- f, Two autosomes in the ring.

Figs. 20-28. 20, bivalents reappearing after the diffused stage, *H. dentatus*. 21, diplotene. 22, diakinesis. 23, metaphase I (polar view). 24, anaphase chromosomes. 25, Late anaphase elements. 26, Interkinetic stage. 27, metaphase II. 28, anaphase II. $\times 2550$.

In addition to the six types of patterns, the individuals of *Halys dentatus* have been observed to display what may be called a stage in between a and b, i.e. the X-chromosome which has a tendency to lie in the ring itself actually lies just inner to it-a'. An exact count of the different types of metaphase patterns has been made in the four individuals, two belonging to each species and is represented in Table 1.

From this (Table 1) it is

evident that in addition to the differences in the patterns themselves the individual frequencies also vary a good deal, even in the 'Mode pattern' which

may itself show variations within the limits of the same species for example individuals 1 and 2.

During anaphase the homologous chromosomes are simply pulled to the opposite poles of the spindle without any change in their pattern or the differential behaviour of the sex-chromosomes. The movement of the chromosomes parallel to the spindle fibres, at right angles to the equator (Fig. 24) suggests their acrocentric nature. During the late anaphase there appears in each autosome a cleft (Fig. 25) which represents the line of division along which the two chromatids will separate during anaphase II.

Table 1. Frequency distribution of the chromosome patterns at metaphase I

No. of Individuals	No. of cells studied	Metaphase patterns						
		a'	a	b	c	d	e	f
<i>Halys dentatus</i>								
1	30	5	8	15	2	—	—	—
2	30	—	1	6	5	2	14	2
<i>Halys</i> sp.								
3	30	2	—	9	8	1	7	3
4	30	—	—	24	4	—	1	1

The interkinetic stage is of a very short duration with the characteristic anaphase grouping of the chromosomes (Fig. 26). Associated with the absence of the resting stage there is no prophase of the second meiotic division and the interkinetic chromosomes are directly transferred first to the prometaphase and finally to the metaphase II.

During metaphase II (Fig. 27) there are seven elements out of which six are the autosomal diads while the remaining one is the pseudobivalent formed by the sex-chromosomes. The latter usually lies in the centre of the ring of autosomes. During anaphase II (Fig. 28) the autosomes divide equationally, while the sex-chromosomes divide reductionally, each one of them going to the opposite poles of the spindle.

Relation of the cell-size, chromosomes length and the spermatogonial generation: It was Morse (1909) who, perhaps for the first time, observed that both the general appearance and the length of the metaphase chromosomes vary from one spermatogonial generation to the next. White in 1935 further noticed that at metaphase of the first of the six divisions, in *Locusta migratoria*, the chromosomes are very long and thin but the degree of metaphase contraction increases with each successive generation so that at the last they are very short and thick. Powers (1942), after a comparison of the total length of the monoploid genome during all the seven spermatogonial generations in *Dactylotum pictum*, however, concludes that the individual complexes, though show considerable variation in the length of their chromosomes, their lengths remain remarkably constant while the cell size decreases to about one-

third. He is of the opinion that the impression of the decrease in the lengths of the chromosomes is simply due to this reduction in the cell size and consequent crowding of the chromosomes on to a smaller equatorial plate.

The present observations on the different spermatogonial generations from a single testis of *Halys dentatus* clearly give the idea of a continuous decrease in the length of the chromosomes in the successive generations. The metrical study has also been undertaken and the results are given in Table 2.

Table 2. Chromosome lengths and the area of the cell in the metaphase of different spermatogonial generations

No. of chromosomes No. of spermatogonial generations	I	II	III	IV	V	VI	X	Y	Total length of monoploid genome	Area of the cell in sq. micra
1st	2.33	2.22	1.91	1.55	1.50	1.46	1.48	0.95	13.40	115.73
2nd	2.20	1.87	1.79	1.49	1.40	1.33	1.53	1.05	12.57	—
4th	1.82	1.65	1.39	1.28	1.21	1.13	1.63	0.74	10.85	69.01
5th	1.71	1.51	1.28	1.16	1.09	1.05	1.73	0.66	10.19	—
6th	1.49	1.28	1.09	1.00	0.93	0.90	1.13	0.64	8.46	33.31
7th	1.38	1.13	1.04	0.88	0.80	0.77	1.11	0.57	7.68	24.87

From this table it is evident that there is a continuous decrease both in the total length of the monoploid genome and the cell area during the successive spermatogonial generations though there appears no correlation between the two. While the cell area decreases by 78.6 per cent that of the first generation, the length of the monoploid genome decreases only by 42.7 per cent. Further the sex chromosomes are odd in their behaviour in that they increase in their size during the second and perhaps also the third generation in the case of Y and during the second, third, fourth and the fifth generations in the case of X and decrease during the subsequent ones.

Comparison of the chromosome lengths in the two species: Since the length of the monoploid genome or that of the individual chromosomes varies in the different spermatogonial generations, it is necessary that the same spermatogonial generation should be taken in the two species for the comparison of their lengths. This is, however, difficult because of the small number of division stages in an individual. This difficulty has been overcome by undertaking the metrical analysis of the chromosomes at metaphase I. For this purpose ten metaphase plates from each species have been selected, each one with six chiasmata, one in each bivalent, so that in side view all the chromosomes lie at right angles to the equator, parallel to the spindle fibres. The length of the chromosomes in these side views of the metaphase plates formed the basis of the whole statistical analysis.

a. *Length of chromosomes at metaphase I*: The absolute length of the chromosomes along with the total length of the monoploid genome, at metaphase I, has been tabulated in Table 3. From this table it is quite evident that both the length of the monoploid genome and that of the individual elements vary greatly from cell to cell even within the same individual. The difference is sometime very great as is seen between cells 2 and 8 (from

Table 3. Absolute length of the chromosomes and the total length of the monoploid genome at metaphase I

No. of chromo- somes	I	II	III	IV	V	VI	X	Y	Total length of monoploid genome
No. of cells									
<i>Halys dentatus</i>									
1	1.99	1.56	1.35	1.25	1.19	1.12	0.92	0.70	10.08
2	2.11	1.65	1.44	1.17	1.15	1.02	0.74	0.62	9.90
3	2.12	1.62	1.46	1.21	1.16	1.05	0.92	0.66	10.20
4	2.14	1.65	1.44	1.38	1.26	1.16	0.87	0.71	10.61
5	1.87	1.52	1.35	1.31	1.17	1.13	0.68	0.48	9.51
6	2.20	1.73	1.39	1.35	1.26	1.16	0.84	0.67	10.60
7	2.04	1.55	1.35	1.17	1.12	1.05	0.85	0.57	9.70
8	1.92	1.44	1.26	1.21	1.14	1.00	0.74	0.63	9.34
9	2.19	1.77	1.49	1.42	1.33	1.26	1.02	0.77	11.25
10	1.94	1.48	1.31	1.15	1.09	1.05	0.82	0.64	9.48
<i>Halys</i> sp.									
1	1.93	1.65	1.47	1.27	1.20	1.14	0.75	0.52	9.93
2	2.21	1.99	1.71	1.45	1.37	1.29	0.92	0.64	11.58
3	1.74	1.45	1.25	1.19	1.13	1.09	0.82	0.60	9.27
4	1.82	1.59	1.36	1.30	1.20	1.09	0.88	0.69	9.93
5	1.55	1.34	1.29	1.20	1.16	0.98	0.88	0.70	9.10
6	1.95	1.67	1.40	1.26	1.13	1.08	0.83	0.74	10.06
7	1.96	1.71	1.48	1.38	1.26	1.16	0.86	0.74	10.55
8	1.70	1.49	1.31	1.28	1.10	1.01	0.79	0.65	9.33
9	1.91	1.64	1.40	1.21	1.16	1.12	0.83	0.63	9.90
10	1.72	1.41	1.22	1.16	1.05	0.96	0.80	0.59	8.91

different individuals) in *Halys* sp., where it is as much as 2.25μ (between the lengths of the monoploid genomes) i.e. even more than the length of the chromosome No. I. The difference between cells 1 and 2 (from the same individual) is, however, 1.65μ only. But, when we compare the relative or the percentage lengths (the length of the monoploid genome being regarded as equal to 100), this difference becomes insignificant as is evident by the comparison of their percentage length in cells 1 and 2, when it never exceeds 0.57% for the individual chromosomes. Even in cells 2 and 8 the difference in the percentage length of the individual chromosomes never exceeds 1.22% of the total.

Powers (1942) regards the difference in the absolute chromosomes length (monoploid genome) during the spermatogonial generations of *Dactyloctenium aegyptium* as probably due to the various factors induced by cell growth and the microtechnique, but the similarity of the relative length of the chromosomes in the different individuals suggests a genotype control similar to all other parts of the organism, as is suggested by Rao (1937) and Darlington (1946) who regard the difference in the chromosome sizes of the related species as the visible sign of this influence.

b. *Comparison of the mean length of the chromosomes.* The statistical analysis of the lengths of the chromosomes at metaphase I is given in Table 4. The calculations have been carried out on the same basis as that used by Powers (1942) except that the value of 't' has been calculated for each one of the chromosomes which are numbered in their descending size order.

Table 4. Metrical analysis of the lengths of the chromosomes at metaphase I

Chromosome No.	Mean length				Standard deviation		t
	<i>Halys dentatus</i>		<i>Halys</i> sp.		<i>Halys dentatus</i>	<i>Halys sp.</i>	
	in micra	in per- centage	in micra	in per- centage			
I	2.05	20.36	1.85	18.76	0.0139	0.0228	3.301
II	1.60	15.89	1.59	16.13	0.0113	0.0346	0.147
III	1.38	13.71	1.39	14.10	0.00531	0.0203	0.197
IV	1.26	12.51	1.27	12.88	0.00933	0.00806	0.239
V	1.19	11.82	1.18	11.96	0.00442	0.00804	0.283
VI	1.10	10.92	1.09	11.06	0.00534	0.00931	0.261
X	0.84	9.34	0.84	8.52	0.0080	0.00253	—
Y	0.65	6.45	0.65	6.59	0.00641	0.00487	—

From Table 4 it is evident that the mean length of the chromosomes is practically the same in both the species except that of the chromosome No. I where the difference is 0.20μ . The difference in their relative lengths, however, varies from 0.14 to 0.39% in all the chromosomes except that of No. I where it is 1.60%.

'n' being equal to 18 the value of probable 't' at 5% significance level is 2.101. For all the chromosomes except No. I the value of calculated 't' is much less, falling in between 0.147 and 0.283, so that the difference between the results is definitely insignificant with respect to these elements. For chromosome No. I, however, the value of 't' is very high, falling even beyond 1% significance level, thus showing that this component differs significantly in the two species.

Discussion

Nature of the interspecific variability: From the comparison of the

morphology of the different stages in the germ line it may be concluded that the two species differ in the nature of the nucleolus during the resting period of the nucleus. In *Halys* sp. it is basophilic and Feulgen positive, formed by the heteropycnotic ends of the autosomes. In *Halys dentatus*, however, it is essentially oxyphilic except during the late diffused stage when it suddenly changes into a basophilic one.

It appears that both the types of the nucleoli are of the same nature as is evident from their common origin during the beginning of the diffused stage in the form of terminal enlargements of the early diplotene bivalents to which they remain attached till the latter lose their staining capacity. This is further proved by the fact that simultaneously with the reappearance of the bivalents out of the diffused stage the oxyphilic nucleolus of even *Halys dentatus* starts behaving in the same way as that of *Halys* sp., thus contributing its substance to the differentiating chromosomes as formed bodies. In fact the nucleolus in both the species is constituted by the heteropycnotic ends of the autosomes and its staining difference in the two species is simply due to the chemical change in the nucleic acids which is perhaps of the nature of polymerization or depolymerization. This staining difference appears to be similar to that which the chromosomes themselves show during their nucleination or denucleination or even to that of the sex-chromosomes which change from negatively heteropycnotic to positively heteropycnotic bodies during the life of one and the same cell in the germ-line (Dutt, 1950).

The metrical analysis of the chromosomes at metaphase I points to the conclusion that the chromosome sets in the two species differ only with respect to the mean length of the chromosome No. I, the difference being statistically significant. This would mean that the chromosome No. I in *Halys dentatus* is longer than that in *Halys* sp. which can be accounted only as the result of a single homozygous deletion or reduplication since no significant change is apparent in the rest of the complement. It appears that such structural changes have played a great role in evolution and that it is quite true of heteroptera is evident by the metrical analysis of the chromosomes undertaken by Manna (1951). Manna concludes that inter-chromosomal changes have played a large part in the evolution of the karyotypes and that the largest and the X and Y chromosomes are involved more often than the other chromosomes and the same appears to be true of the genus *Halys*.

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