THE GENETICAL AND MECHANICAL PROPERTIES OF THE SEX CHROMOSOMES

V. *CIMEX* AND THE HETEROPTERA

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(With Plates I–III and Eighteen Text-figures)

CONTENTS

1. The problem

Homologous chromosomes within a mating group are, we may say, such as regularly cross over with one another, and such therefore as can be divided into homologous units of crossing-over, or genes. If homologous chromosomes exist in two types, X and Y, distinguished by constant differences in respect of groups of genes, we must suppose that crossing-over is suppressed within these groups when X and Y are brought together in the same individual. In fact each of the chromosomes must consist of two parts, a *pairing segment* in which crossing-over takes place freely and a *differential segment* in which it is suppressed.

The most important example of this principle is the sex chromosome

pair found in the hybrid sex of dioecious plants and animals. In earlier studies I have tried to show how the restriction of crossing-over works in different species. The result is different according to whether the prime mover in restriction is a structural differentiation of X and Y themselves, as appears to be the case in mammals, or a genotypically controlled mechanism which affects autosomes and sex chromosomes differentially, as appears to be the case in *Drosophila*, where crossing-over is suppressed entirely between the autosomes and rigidly restricted between the X and Y. In either case, however, the differentiation is irreversible in detail and culminates, as Wilson concluded from comparative studies of insects, in the extinction of a Y chromosome which has become inert or superfluous.

This climax might be reached in two ways. Either the pairing and crossing-over of X and Y might be reduced to a small inert segment, a position closely approached in *Drosophila*: or pairing and crossing-over might be entirely suppressed between X and Y, which is the condition reached in the Hemiptera-Heteroptera. Here the autosomes continue to pair by chiasmata in the male, while X and Y undergo no crossing-over and achieve regular segregation only by momentary "pairing". We can then see the Y *in statu moriendi*. It is extinct in several families and highly variable in others. But there is another consequence of this suppression of crossing-over. The momentary pairing is achieved by a special spindle structure and a special centromere cycle. And these devices in turn seem to have the effect of preserving systems of unforeseen complexity. It is one of these systems that I want to take as an example for the solution of the special problems of the Heteroptera.

The earlier workers were concerned largely in proving the relationship of chromosome differentiation to sex differentiation. This relationship they established. What is now to be done is to take apart the genetic and mechanical elements in the light of recent knowledge and piece them together in an evolutionary intelligible whole. This I can describe only in a provisional form, but in describing it I think I can show what gaps are left and how they may be filled.

2. Material and methods

I am indebted to Dr C. Johnson of the London School of Hygiene and Tropical Medicine for providing me with all the material, including the reciprocal species crosses which he has made. These crosses he has described elsewhere (1939). The species were derived from the following sources:

C. lectularius:

1. Mass cultures: Beckenham (1927).
   Lister Institute (animal house) (1935).
   Greenwich, Seamen's Hospital (1937).
2. Natural populations: Sheffield, 1938.
   Glasgow, three localities, 1938.
   Mitcham and Lambeth, 1939.
   Cork, three localities, 1939.

C. columbarius: Schmeeda, Gröningen, pigeon house. Mass cultured since 1936.

C. rotundatus: Uganda (human). Mass cultured since 1922.

C. stadleri: Spessart, Germany (bats). Mass cultured since 1935.

The late fifth instar, which lasts from 6 to 11 days, is the most fruitful stage for spermatogenesis.

I am indebted to Mr L. La Cour for making the excellent preparations. The testes were dissected in Ringer's solution, fixed in La Cour's 2 BD or 2 BE solutions (1937) or strong Flemming. To avoid *over-fixation* the testes were removed from the fixative after 1 hr. and placed in 1 % chromic acid solution for 18 hr. Gentian-violet-iodine is the best stain. The sections were cut at 12 μ. The method used for upsetting meiosis will be described later.

In connexion with the methods used the seriation of stages described is not irrelevant. This has apparently given trouble to some workers since Spaul (1922) seems to have been led astray by taking first metaphase for second in *Nepa cinerea*. My own interphase (Pl. I) corresponds with Slack's description (1939) of diakinesis in *Cimex*. These difficulties of seriation I would attribute, in part, to the rhythmical control of the processes of division, mitotic or meiotic, in each follicle and, in part, to the semi-diffuse staining of the autosomes in the diplotene-diakinesis stages. As a rule only first metaphase or second metaphase are found in one follicle. But occasionally they are found together and then there is no interphase with them, although the frequency of occurrence of this stage shows that it is not short. Evidently if cells of a particular group in a follicle fail to enter metaphase with their neighbours they have to wait for a second metaphase. Comparatively few follicles even have mixtures of metaphase and anaphase (Table I).

The greater frequency of second divisions than firsts, even in very young testes, points to a longer duration of the second metaphase. The greater frequency of follicles with first metaphases and anaphases than

with the corresponding second division stages together points the same way. I am inclined to think that the length of second metaphase is due to the extended time required for distribution on the plate, for I find whole follicles occupied by plates that are flat but not finally distributed. So accurate is the timing co-ordination that groups of adjoining second metaphases show the same stage of co-orientation of sex chromosomes. This conclusion needs further testing since it bears on the special properties of the second division spindle to be discussed later.

The same data (Table I) reveal a difference between the *lectularius-columbarius* crosses and all the other bugs. In the former alone is the first metaphase as frequent as the second in the fifth instar fixations used. Presumably the testes of the hybrids develop later than any others. Another problem that deserves attention is the great variation in testis size within the species *lectularius* (cf. Dobzhansky & Boche, 1933).

TABLE I

Cimex spermatogenesis: timing analysis. Frequency of follicles with different stages in different stocks at the fifth instar

	MI	M–AI	AI	MII	M–AII	AII
C. rotundatus	.	.	.	1	.	.
C. columbarius	.	1	.	2	.	.
C. lectularius:						
Beckenham	3	.	.	6	2	.
Glasgow	3[1]	.	.	11[2]	.	1
Cork	1	.	.	5[2]	1	.
Lambeth	2[3]	1	.	4[3]	.	.
Mitcham	4	2	.	4	1	.
Others	5[3]	1	.	13[3]	.	2
C. columbarius × *C. lectularius*	2	.	1	6	.	.
C. lectularius × *C. columbarius*	26[4]	6	.	25[4]	1	2

[1] All of low *X* type.
[2] Including one with interphase also.
[3] Including one with both MI and MII.
[4] Including five with both MI and MII.

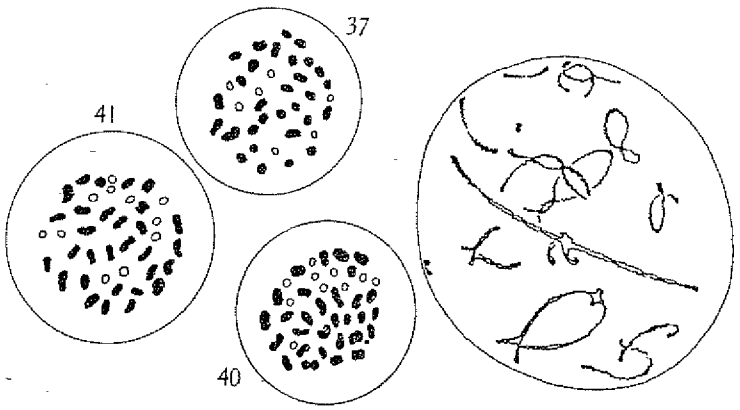
3. Meiosis in *Cimex*

(i) *General*

The chromosomes of *Cimex* are between 1/20th and 1/60th the size of those of most other Hemiptera-Heteroptera that have been described, and for this reason the prophase of meiosis is not to be satisfactorily studied in the male. Between diplotene and first metaphase the chromosomes, apart from *X* and *Y*, are semi-diffuse. In the female the diplotene stage shows the formation of from one to three chiasmata per bivalent (Text-fig. 1). It is therefore comparable with the descriptions of male meiosis in other species by Wilson, Browne, Reuter and others.

At first metaphase in the male the chromosomes form a plate which varies in size and arrangement. Where it is small the chromosomes are evenly distributed. Where it is larger or the chromosomes are fewer they are peripherally distributed and leave an empty centre. Rarely a plate is found with a central group distinct from an outer ring, a distinction suggestive of the second metaphase (Pl. I).

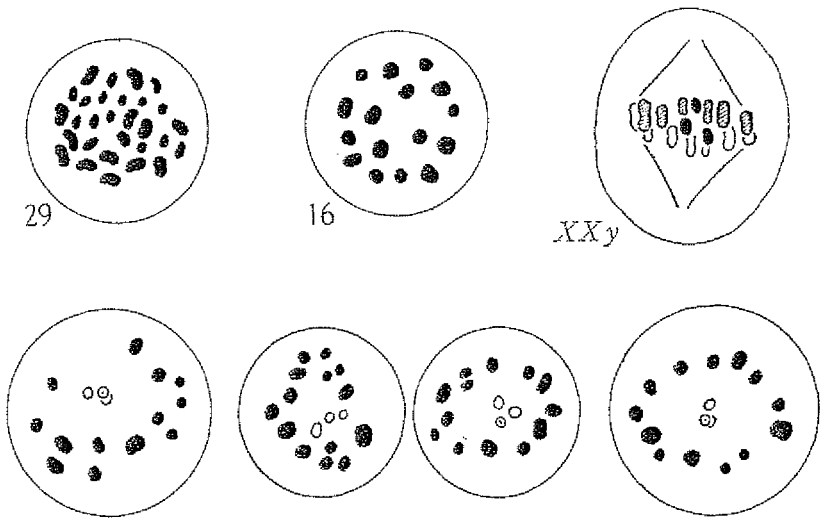
The autosomes consistently form 13 bivalents. The sex chromosomes (*Y* and two to fourteen *X*'s) remain unpaired and seem to lie indifferently on the plate, being grouped neither together nor in any particular relationship to the autosomes. They cannot all be recognized from one point of view. The *Y* exists in two size forms. In *C. lectularius* it is usually larger than the *X*'s, but in four bugs of the Glasgow and



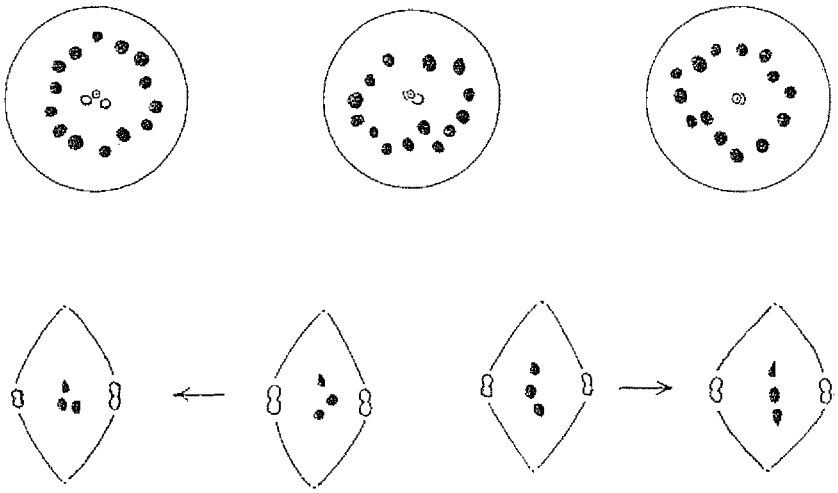
Text-fig. 1. Mitoses from three Beckenham stock females, *C. lectularius*, with 37, 40 and 41 chromosomes. All supernumerary *X* chromosomes in outline. Diplotene nucleus in an egg showing bivalents with one, two and three chiasmata. ×4000.

Beckenham stocks and in one *C. columbarius* it is smaller, and I refer to it as *y* (Text-figs. 2, 16). The *X* chromosomes exist in three forms. First there are the two largest, which are not distinguishable in size. Secondly there are supernumerary *X*'s which are sometimes, perhaps always, slightly smaller than the first two. Thirdly there are supernumeraries which are extremely small, the *x* chromosomes, which are little more than disembodied centromeres, and are distinguishable in polar view as well as in side view. These frequently, and the others occasionally, show a lack of congression and orientation, which is no doubt due to a delay relative to the autosome bivalents. The delay, however, may have the serious consequence of lagging in division, or even of both halves of the univalent sex chromosome passing to the same pole.

Normally the sex-univalents divide at the first metaphase and the



Text-fig. 2. *Cimex columbarius* ♂. Above: mitosis with 29 chromosomes; first metaphase of meiosis with 16 and second metaphase with *y* and two *X*'s co-orientated. Below: stages in distribution of autosomes and co-orientation of sex chromosomes (shown in outline) at second metaphase. ×4000.



Text-fig. 3. Second meiotic metaphase in *C. rotundatus*: $14^{II}A + XXy$. Unstable co-orientations in the middle give stable arrangements at the side. ×4000.

halves of the smallest ones can be recognized in the interphase nucleus (Text-fig. 4). At the second metaphase the autosomes gradually form a ring on the edge of the spindle while the sex chromosomes move to the middle. This arrangement admits of no exception provided that the first division has been regular and the number of X's is small. When, however, the spindle is small the ring may be re-entrant at one or two points. Or, to put it another way, there is a certain minimum distance between successive chromosomes in the ring which crumples, like a half-open umbrella, if the spindle it circumscribes is too small.

The arrangement of the sex chromosomes in the middle is also gradual. It may be divided into two parts. First there is the sorting out of the sex chromosomes and autosomes. Next comes the relative arrangement

TABLE II

Summary of results

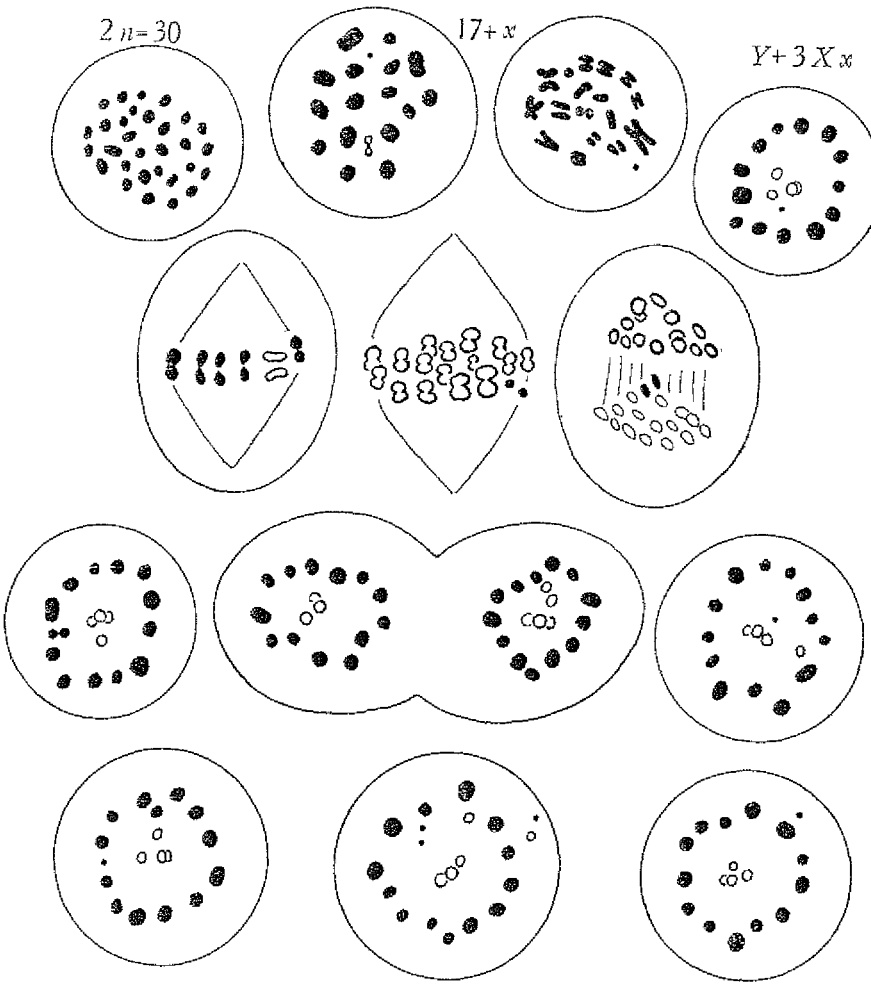
	♂			♀
	Mitosis	MI	MII	Mitosis
C. lectularius (Beckenham)	30	—	—	33 (6X)
	(31)	18	$Y+4X$	36 (9X)
	(32)	19	$Y+5X$	37 (10X)
	33	20	$Y+6X$	39 (12X)
	(33)	20	$y+6X$	40 (13X)
	34	21	$y+7X$	41 (14X)
C. columbarius	29	16	$y+2X$	?30 (4X)
C. columbarius ♀ × *C. lectularius* ♂	(29)	16	$Y+2X$	34 (8X)
C. lectularius ♀ × *C. columbarius* ♂ (extremes)	(29)	16	$Y+2X$	—
	(34)	21	$Y+7X$	—
C. rotundatus	(31)	(17)	$y+2X$	—
C. stadleri	31	—	—	—

Numbers in brackets are inferred. X includes x.

of the Y and the X's. The ordering which seems to be most frequent is that where the single Y chromosome lies to one side of the plate, co-orientated (if we may so describe the relationship) with two X's, while any extra X's lie in the same plane as their fellows, although showing no individual reaction to the Y. I have also seen both a one-to-one co-orientation of Y with one X, the other X's remaining unaffected, and diamond-shaped configurations (Text-fig. 16, Pl. III).

In *C. columbarius* exceptionally the three sex chromosomes may lie in one row. Comparison with *C. rotundatus* shows that *columbarius* is in this most important respect transitional. For in *rotundatus*, of 59 second metaphases classified for co-orientation, eight had the general Reduvioid type of double plate found in *lectularius*, 35 had the linear form of *Thyanta* and *Metapodius*, and 16 were transitional forms which were probably passing into one or the other stable arrangement (Text-fig. 3, Pl. III).

At second anaphase, as a rule, the *Y* chromosome passes to one pole, and all the *X* chromosomes, unless they are very numerous, to the other.

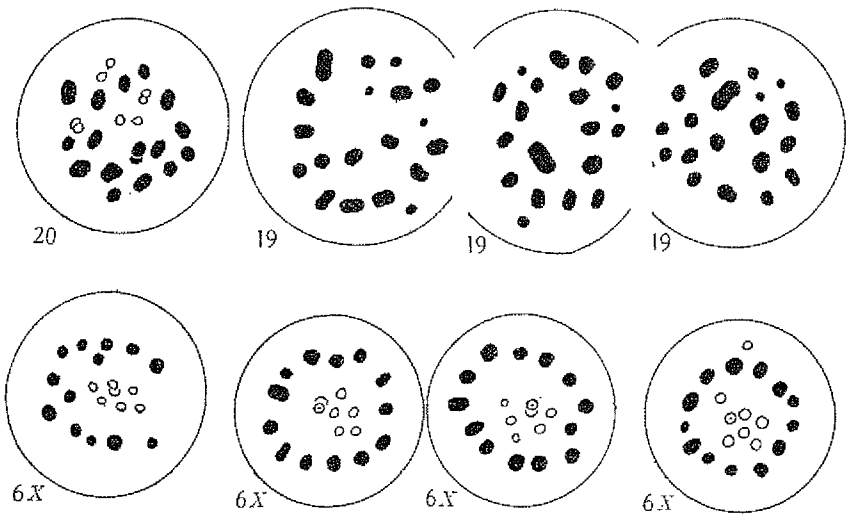


Text-fig. 4. *C. lectularius*, Lister culture. Top row: mitosis, first meiotic metaphase, interphase, second meiotic metaphase. Second row: side views of first metaphase and anaphase. Bottom rows: different combinations of supernumerary *X*'s and *x*'s resulting from non-disjunction at first metaphase. ×4000.

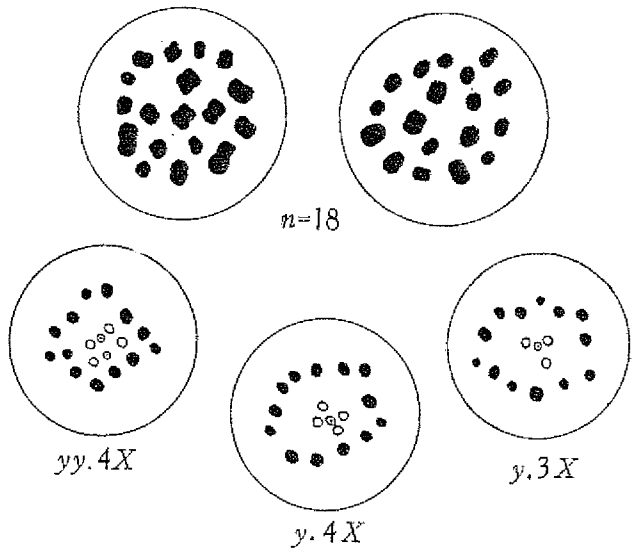
(ii) *Abnormal behaviour*

Abnormalities of meiosis are concerned only with the sex chromosomes and are therefore accentuated in bugs with a high *X*-content. They arise from two causes: non-disjunction of chromatids at first anaphase and irregular co-orientation on the second metaphase spindle.

The first abnormality affects the supernumerary X's and especially the small x's. It causes an obvious variation in the number of chro-



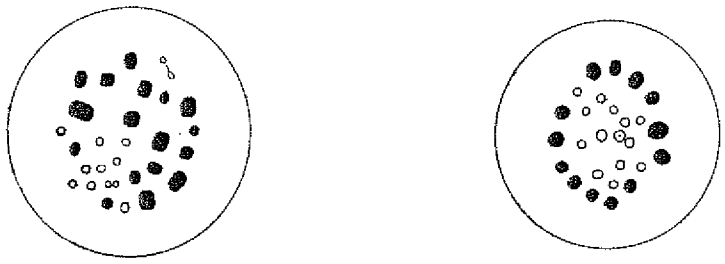
Text-fig. 5. *C. lectularius*, Beckenham culture (except extreme left, Greenwich). Above: first metaphase with 7 and 6 sex chromosomes. Below: second metaphase with 6 X's showing different arrangements. One has a daughter X outside spindle. × 4000.



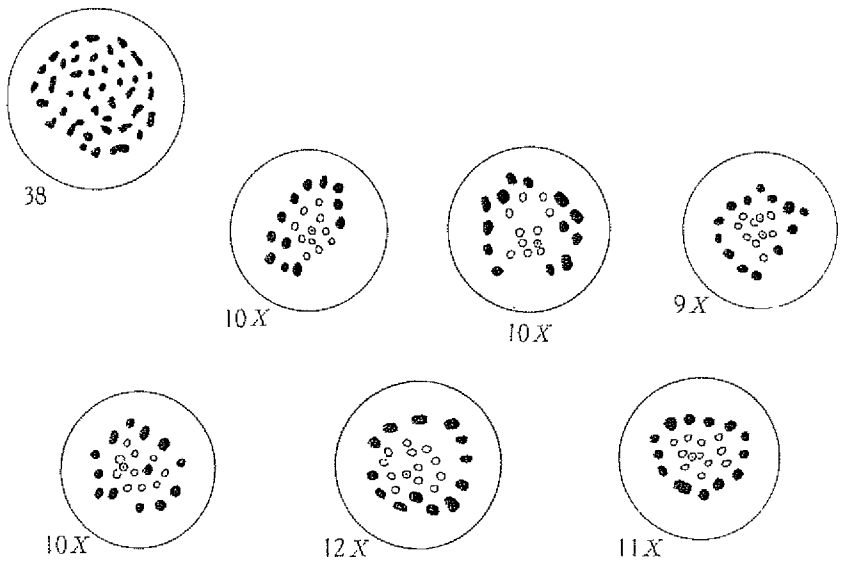
Text-fig. 6. Glasgow stock with $13^{II}A+y+4X$. Above, first metaphase, below second metaphase. y chromosome marked with a point. × 4000.

mosomes within the ring (Text-fig. 9). What is remarkable about this non-disjunction is that the two chromatids do in fact disjoin although they

pass to the same pole, so that plates with too many as well as too few X's are found. The occasional exceptions to this rule are significant, for rarely an extra chromosome is found in the ring, and when large enough it can be seen to be double. This chromosome could always be seen not



Text-fig. 7. Mitcham stock at first metaphase and second metaphase. ×4000.



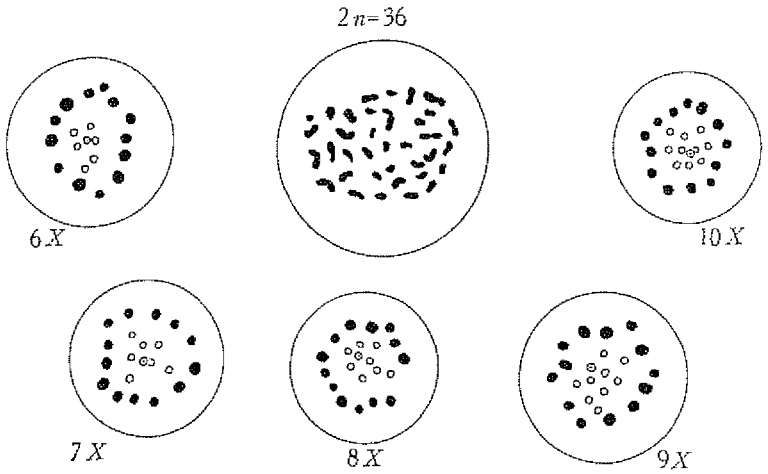
Text-fig. 8. Glasgow stock, mitosis and second metaphase in a bug with 11 X's. Number of X's and stages of distribution vary. ×4000.

to be an autosome, either by its irregular orientation or small size (Text-fig. 4).

The question arises as to whether non-disjunction affects the basic two X's and Y. It is doubtful whether the loss or reduplication of the two X's in the presence of supernumeraries would be recognizable, but the same accidents affecting the Y might well upset the co-orientation system.

Of this I have evidence in occasional cells of two kinds. The first have no signs of co-orientation and the X's are dispersed at random within the ring. These I take to be no-Y cells (Pl. III). The second show, lying off the plate on each side, a single chromosome pointing to its pole in the characteristic manner of the Y. These I take to be two-Y cells. I believe that the exceptional second anaphases in which nearly all the sex chromosomes are lagging, arise from one or other of these two abnormalities (Text-fig. 16, Pl. III).

It is significant on the other hand that the co-orientation is as regular with the small y as with the large Y. The general size of the chromosome is irrelevant in determining the special properties of the Y.



Text-fig. 9. Glasgow stock, mitosis and M_{II} in a bug with 9 X's, showing variation in number owing to non-disjunction at first metaphase. ×4000.

This is also shown by comparing *Sinea* with the other Reduviidae (Text-fig. 17).

The most widespread abnormalities arise at second metaphase in the high-X bugs. They are important in showing that a limit is reached in the capacity of the second metaphase spindle for coping with these chromosomes. At the first division the X's must divide less regularly since the second metaphase plates show (as Slack has pointed out) highly variable numbers of X's together with absolutely invariable numbers of the autosomes. At the second metaphase distribution on the plate takes longer in relation to congression than where there are few X's. Hence many plates are found to be perfectly flat but with autosomes inside the ring and X's still on the edge (Text-fig. 8). And the ring itself

may never close up. The exclusion of autosomes from the centre is quicker than the exclusion of X's from the ring. In a word the movements of the autosomes are more rapid or more advanced in time than those of the sex chromosomes at the second metaphase, just as they are at the first. Those X's which lag in co-orientation in the centre also lag in anaphase movement and are left behind. Those which were lying on the wrong side of the plate may even get pushed towards the Y's pole by the stretching of the spindle, so that XY sperm will be formed as well as X_n and Y.

(iii) *Artificial breakdown*

In order to understand the mechanical conditions of the special XY system in *Cimex* it is necessary to break down the ordinary course of meiosis. The high temperature treatment used by White (1934) on a locust *Schistocerca* was therefore applied. *Cimex* at the fifth instar will survive 40° for 4 hr., although 44° C. is quickly lethal even to *C. rotundatus*. Bugs were kept in a damp chamber at 40° C. for 2 hr. After 13 and 17 hr. at room temperature they were fixed. Controls from the same populations (Cork, Lambeth and Beckenham) showed no abnormalities, but it must be understood that in view of the heterogeneity of the material the only valid control is the whole body of observations on untreated bugs with similar X-contents.

Four significant disturbances were found (Text-fig. 10):

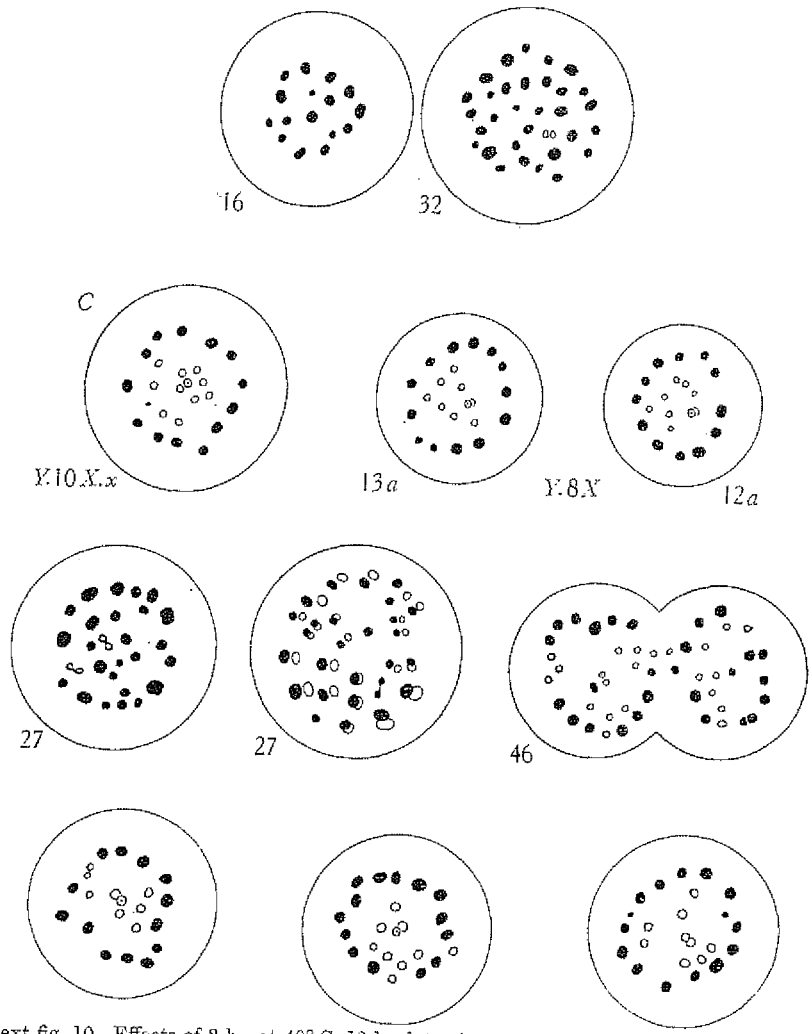
(1) *Mitotic doubling.* A whole follicle, apart from two nuclei, showed 32 chromosomes instead of 16 at first metaphase. The plates were concave, a consequence evidently of the spindle being only double the normal volume, and therefore less than double the area of cross-section. In these tetraploid nuclei the autosomes remained as bivalents and the sex chromosomes, although each was provided with an identical mate, remained unpaired.

(2) *Loss of Y chromosome.* Evidence of non-disjunction of Y was found, as in untreated bugs but more frequently.

(3) *Loss of autosomes.* At second metaphase a ring of 12 autosomes was found instead of the 13 present without exception in all untreated wild and crossed individuals. This is presumably the result of failure of pairing or disjunction of the autosomes at the first division. The number of X's was the normal 8 for this bug.

(4) *Spindle breakdown.* Imperfect spindle development was seen in the same bug both at first and second divisions (Text-fig. 10). At first metaphase, suggestions of the double spindle characteristic of second

metaphase were found. At first anaphase the spindle was unduly broad and the direction of separation was irregular. At second metaphase the



Text-fig. 10. Effects of 2 hr. at 40° C. 13 hr. later (except top row, which is 17 hr. later). Top row: diploids and tetraploid plates adjoining (cf. Pl. III), Cork. Second row: *C*, control, and ring of 12 autosomes due to non-pairing or non-disjunction at first metaphase ,Lambeth. Third row: first metaphase, first and second anaphase following a defect of the spindle, Lambeth. Fourth row: abnormal distributions of sex chromosomes at second metaphase, Lambeth. ×4000.

results are seen in the formation of something like the restitution nucleus commonly seen in plants. The number of chromosomes is too few for all the *X*'s to have divided at the first division. On the other hand we see

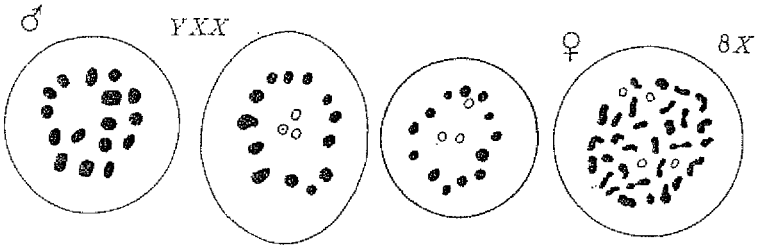
that, although the rings are fairly well defined, there are too many chromosomes lying in them. In several separate second metaphases in the same follicle 17 chromosomes were lying on the ring (Pl. III). It seems that, as in the case of the x chromosomes, the extra numbers are undivided sex chromosomes.

(iv) *Cultures, crosses and populations*

The proof of the distinction between X and Y is given by the chromosome numbers of females, and by the difference between males in the reciprocal species crosses (Table II). The females show a wider range of number than the males in the same broods and reach a number of 39 when their brothers do not exceed 34. The extra chromosomes are therefore X chromosomes, and the corresponding constitutions can be represented as:

C. columbarius		*C. lectularius*	
♀	♂	♀	♂
$4X$ (30)	$Y+2X$	$12X$ (38)	$y+6X$
C. columbarius × *C. lectularius*		*C. lectularius* × *C. columbarius*	
♀	♂	♀	♂
$8X$ (34)	$y+2X$	$8X$ (34)	$Y+6X$

Thus the males produced by crosses between *C. columbarius* with only two X's and *C. lectularius* with from three to seven X's have the same X-constitution as their mothers. *Columbarius* × *lectularius* ♂♂ have the two X's of *columbarius*, *lectularius* × *columbarius* ♂♂ have the large

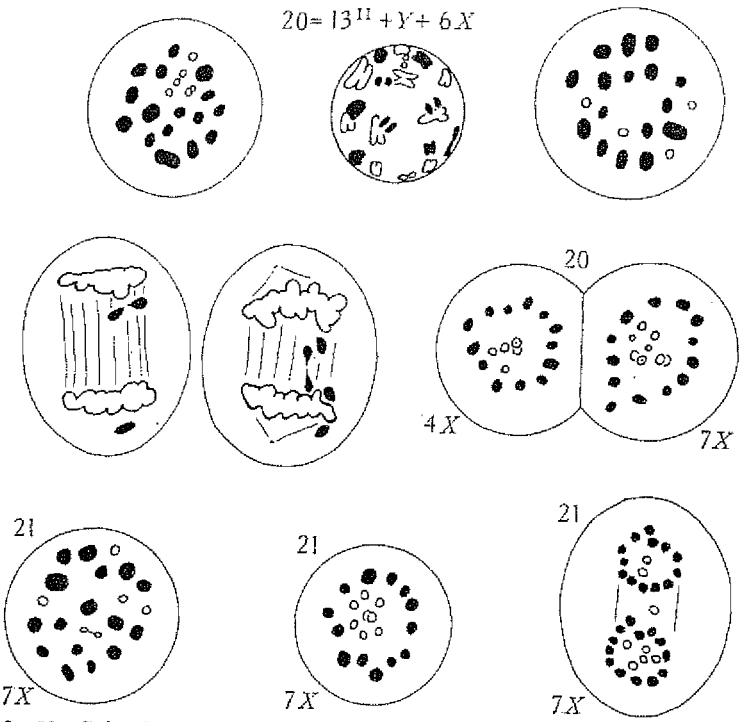


Text-fig. 11. *C. columbarius* ♀ × *C. lectularius* ♂. First and second metaphase in the male, mitosis in the female ($2n=34$, four smallest in outline). × 4000.

number of X's derived from the *lectularius* mother. In the progeny of both these crosses the autosome behaviour at meiosis was consistently regular. What is remarkable about these families is the wide variation in the number of X's contributed by any one mother (Table III). This is the one source of evidence we have of sex chromosome behaviour in egg formation. It seems most likely to be due to the irregular segregation

of multivalent X's, associated in the usual way by chiasmata, at meiosis in the egg.

Such behaviour in the egg indicates that the two X's of the basic complement are dissimilar and complementary rather than similar and supplementary. For if the extra X's, being similar, form multivalents in the female, the four basic X's, being similar, would do likewise and



Text-fig. 12. *C. lectularius* ♀ × *C. columbarius* ♂. Top row: first metaphase and interphase with 20 chromosomes ($Y + 6X$). Second row: first anaphase showing lagging of X's and second metaphase showing consequent unequal assortment of the 6 X's. Bottom row: first and second metaphase and second anaphase with 7 X's. Second anaphase has lagging X's. ×4000.

would then be liable to a like irregularity which would impair the fertility of the species or give single-X males. Whether the extra X's are derived from one or both of the basic X's, we cannot tell.

The natural populations of *lectularius* reinforce the evidence of the crosses in showing that the X-constitution is again variable within each locality and even family. The appearance of the small x chromosome from different sources might suggest that it has maintained itself over considerable periods, since the bed-bug is a species of local tenacity,

endemic and not mobile. Its origin, however, apparently *de novo* in three families of the *L.* × *C.* crosses, makes rather for the opposite explanation of a frequent parallel origin by fragmentation of a larger *X*.

Of these natural populations the most aberrant is that from Cork, individuals of which range from ten extra *X*'s down to none at all. From the same house in Glasgow came one with 13 *X*'s and another with only four. The sporadic appearance of individuals which have suddenly shed, as it were, most of their extra *X*'s agrees with the observation of occasional second anaphases in which most of the *X*'s seem to be lagging. It is these abnormalities which account for the subordinate part of the curve of *X* distribution between two and six in the natural populations.

The Glasgow population included some with the usual large *Y* and some with the small *y* like those seen in *C. columbarius* and *C. rotundatus*, and as supernumeraries in *Metapodius*. Again the *Y* must be largely inert or indifferent to be able to suffer this loss without physiological disadvantage to the organism.

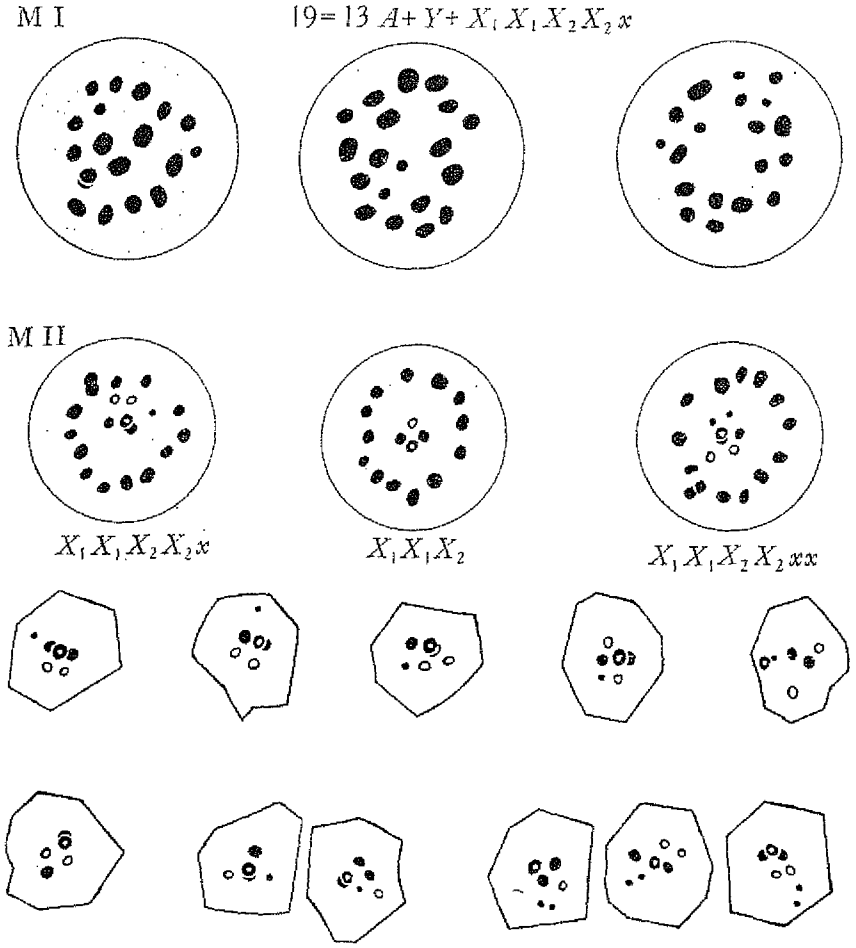
(v) Sex Determination

These observations establish the general agreement of the sex mechanism in *Cimex* with the X_nY type described in other families of *Heteroptera*, e.g. by Payne in the Reduviidae and Galgulidae, by Wilson in the cryptic "B" form of *Thyanta custator* (Pentatomidae) and by Steopoe in *Nepa cinerea* (Nepidae). On the genetical side, however, the problem has been complicated by *Cimex* rather than simplified. In all previous cases the multiple *X* system has been stable, or at least has seemed to be stable. Here it is obviously unstable. This instability must depend on the special genetic character of the sex chromosomes and their mechanical properties at meiosis. More specifically it must depend on a contradiction between what is mechanically feasible and what is genetically desirable. The operation of meiosis favours a low *X*-content. The selection of individuals must therefore favour a high *X*-content.

When we compare the cultures with the natural populations we see a remarkable and unexpected difference in this respect. In the populations selection is somehow forcing up the *X*-content which is maintained, with occasional exceptions, close to the limit of the mechanical capacity of segregation. In the cultures this selection pressure is apparently reduced and the *X*-content has dropped to a level at which very little loss takes place at meiosis, to the level at which we in fact see it in the occasional low-*X* bugs of the natural population. Whether this difference

is due to in-breeding, to sex-ratio selection, or to other cultural conditions remains to be seen.

Whatever the detailed solution of this problem, the conclusion stands that the high-X bugs would not exist if the extra X's were not doing



Text-fig. 13. *C. lecitularius* × *C. columbarius* with three recognizable types of X showing unequal assortment of extra X's and x's at second metaphase. Bottom two rows, all from same loculus, show the autosome positions as polygons. ×4000.

some work. It is worth considering for a moment what this work may be, for it is evidently outside the range of our previous genetic experience.

The argument is as follows. There seem to be no males with two Y's. There can therefore be no females with one Y. Let us label the primary

and necessary X's, X_1 and X_2, and the extras X_3, X_4 and so on. Then $2(X_1X_2)$ and $2(X_1X_2X_3X_4)$ are equally female while X_1X_2Y and $X_1X_2X_3X_4Y$ are equally male. Hence we must suppose that X_3 and X_4 are much less important in sex determination than X_1 and X_2 since Y can scarcely be much more important.

Similarly the great variation in number of extra X's without apparent effect on viability or fertility points to their relative inertness. But this

TABLE III

Numbers of X chromosomes in males of different cultures, crosses and populations of Cimex lectularius

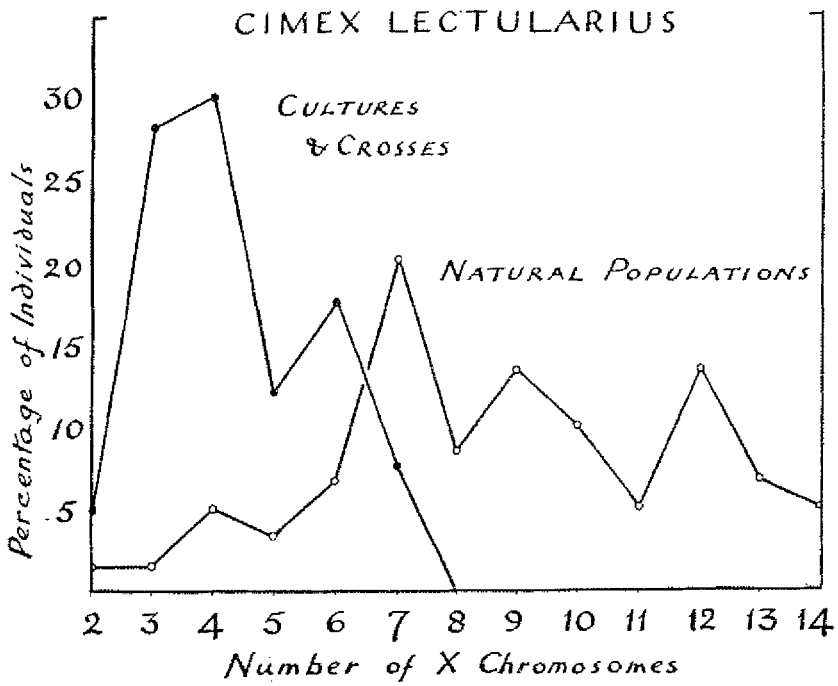
Number of X's	2	3	4	5	6	7	8	9	10	11	12	13	14
Mitotic number	29	30	31	32	33	34	35	36	37	38	39	40	41
Cultures:													
Beckenham (1927) (y)	.	1*	1	1	3	1							
Lister (1935) (x)	.	2	4	.	.	.							
Greenwich (1937)	1	2	1	1	1	.							
Totals (19) Mean 4·3	1	5	6	2	4	1							
Crosses:													
Beckenham × *columbarius* family:													
1 (x)	.	2	1	2	.	.							
2 (x)	.	1	.	.	.	.							
4 (x)	.	1	1	.	.	1							
6	1	2	3	1	2	.							
7 (x)	.	.	1	.	1	1							
Totals (21) Mean 4·3	1	6	6	3	3	2							
Populations:													
Cork	1	.	.	.	1	2	.	.	2	.	.	.	.
Glasgow (y)	.	.	2	.	.	1*	1	.	1	1	1	.	1
Lambeth (x)	.	.	.	.	.	2	.	1	1	1	1	1	.
Mitcham	.	.	.	.	.	.	.	.	1	.	.	1	1
Sheffield	.	.	.	.	.	1	.	.	.	.	.	.	.
Totals (25) Mean 9·0	1	.	2	.	1	6	1	1	5	2	2	2	2
Slack's data† (35) Mean 8·7	.	1	1	2	3	6	4	7	1	1	6	2	1
Total cultures and crosses (40)	2	11	12	5	7	3							
Total populations (60)	1	1	3	2	4	12	5	8	6	3	8	4	3

* Counted from mitosis only.
x Some bugs had x chromosomes which are included in the X's.
y Some bugs had y chromosomes instead of Y.
† Edinburgh, Glasgow and London populations, calculated from "modal number of univalents" at M_{II} less one.

assumption raises other difficulties. These extra X's are little if at all smaller than X_1 and X_2. Moreover, as we have seen, the extra X's are so frequently lost, and their number is consequently so variable within families, that they would certainly disappear altogether from the species if they were entirely inert.

The same principle applies to the M-chromosomes which I shall refer to later. These chromosomes are often variable in size, indicating that

large parts of them are dispensable, but nevertheless they persist throughout the Coreidae and are found in three other families (Text-fig. 18). A similar situation is perhaps to be inferred in the single other example of instability in the X–Y mechanism, viz. in *Metapodius* where males without any Y and with seven Y's were found by Wilson. The loss of Y is consistent with its inertness; its indefinite reduplication is not. Evidently a chromosome, or *a fortiori* a gene, that is superfluous need not be inert.



Text-fig. 14. Graph showing the effects of different kinds of selection on the numbers of X chromosomes in males of *C. lectularius* (from Table III).

The explanation that I would suggest for all these conditions is that such chromosomes are not strictly inert but contain material whose action is so adjusted that relative dosage is almost, and in some autosome combinations quite, immaterial. This implies an extension of the principle of dosage-compensation inferred by Muller (1932*b*) in the X chromosome of *Drosophila*. It would be expected that such a condition of *dosage-indifference* would arise in large related groups by an orthogenetic process and would itself, by parallel changes, give rise to a parallel

multiplication of the *X*, such as is to be inferred from systematic comparison (Text-fig. 18).

But there are other examples of parallel evolutionary changes in the sex chromosomes of the Heteroptera. All of them depend on a second kind of condition, the mechanism of segregation. Any system of sex determination must rest equally on the availability of the genetic materials and of the special mechanical resources for distributing these materials conveniently. We will now return to the mechanical part of the problem.

4. Special systems in the Heteroptera

(i) *Method of argument*

In inferring the mechanical properties of chromosomes from the comparative study of a large group one special precaution must be taken and is perhaps worth mentioning: it is that we must distinguish in considering any particular action of a chromosome between what is spontaneous and mechanically inherent in its special observable conditions on the one hand, and what is hereditary and therefore adaptively inherent in its special observable consequences on the other hand.

To understand the use of this distinction, take the condition of unpaired chromosomes at meiosis. When this condition arises in a new species-cross or in a triploid all of whose chromosomes were paired in its parents we have *spontaneous univalents*. Their behaviour will depend on the conditions inherent in their mechanically abnormal state. As we know, the result may be regular or irregular, although it is always characteristic of the particular hybrid individual under given conditions. Its variations enable us to say that the movements of the univalents depend on the factors of time and space: the time of polarization of its centromere relative to the separation of the bivalents and the size of the chromosomes relative to the space available in the dividing cell (Upcott & Philp, 1939).

Quite otherwise is the position of the hetero-chromosomes, as they used to be called, in the Heteroptera and elsewhere. These chromosomes are descended from a long line of ancestors which have dispensed with the ordinary processes of pachytene pairing and chiasma formation and have secured a more or less regular segregation by the adoption of special devices. Here we are concerned with *hereditary univalents*. In studying them therefore we are not studying mechanisms inherent in meiosis but modifications of these mechanisms which have proved adaptively feasible in the group we are discussing.

(ii) *Anomalous mechanisms*

The special types of segregation found at meiosis in male Heteroptera may be classified as follows:

(1) *XY* and *XO* systems of hereditary univalents with co-orientation at second metaphase or not at all.

(*a*) *X*–*Y* segregation (*Nezara*, *Rhodnius* unpubl.).

(*b*) Supernumerary *Y* chain-segregation (*Metapodius*).

(*c*) Solitary *X* segregation (*Metapodius*, *Protenor*, *Archimerus*).

(*d*) Supernumerary *X* chain- or double-plate-segregation (*Cimex*).

(2) Micro-chromosome (*M*) system of pairing or co-orientation at first metaphase (Coreidae, etc.).

(3) "Heterotropic" system by which spontaneous univalents (unpaired autosomes) pass to one pole at first anaphase (*Banasa*).

These systems have to be considered in relation to four mechanical and two genetical properties of the chromosomes concerned:

(1) Time of division of the chromosome thread.

(2) Potential and actual pairing at pachytene in the prophase of meiosis.

(3) The occurrence of crossing-over.

(4) Congression and distribution on the metaphase plate and the consequent shape of the plate.

(5) Time of division of the centromere.

(6) Genetic activity or inertness and consequent stability or instability of size and number.

(iii) *Pairing and time co-ordination*

The time of division of the three types of chromosome has been described by Reuter in *Alydus*. The *X* chromosome divides first: "Ziemlich früh, ehe noch die Konjugation der Autosomen eingeleitet worden war, wurde es längsgespaltet" (p. 55). The *M*-chromosomes divide next: "in lebendem Material erwiesen sie sich schon in der 'Pachyphase' als längsgespalten" (p. 53). As Table IV shows, this order is correlated both with the capacity for pairing at pachytene and with the capacity for division of the centromere at metaphase. The earlier division of the *X* chromosome prevents its pairing even when in a tetraploid cell of *Cimex* or *Euschistus* an identical partner is provided for it. It also goes with an earlier division of the centromere—at the first instead of the second metaphase. The intermediate timing of the *M*-chromosomes leads to a partial suppression of pachytene, a possibility

of co-orientation at first metaphase and of the division of the centromere being left to second metaphase.

The pairing of the sex chromosomes has been suppressed therefore not by their structural divergence but by the imposition of differential precocity which has restored them to a mitotic cycle in the first meiotic division.

The special properties of the sex chromosomes in the Heteroptera thus arise not by a change in the timing of metaphase for the whole cell

TABLE IV

Related properties of the three chromosome types in the Heteroptera

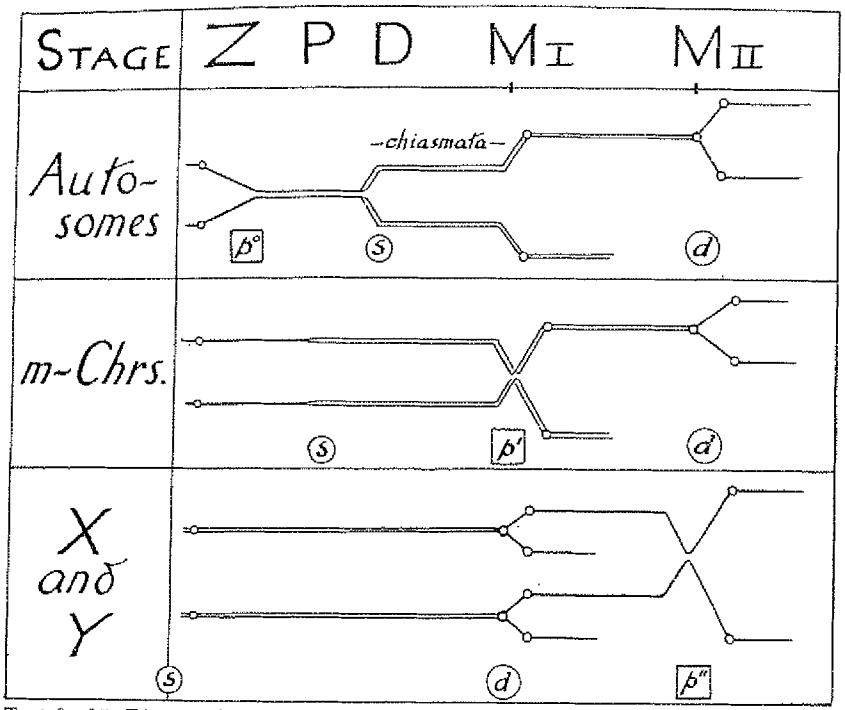
Type	Chr. division[1]	Pachytene	Cm. division		Plate		Function
			M_I	M_{II}	M_I	M_{II}	
Autosomes bivalent (or spontaneous univalent)[2]	Diplotene	Complete. Crossing-over	—	*X*	Ring or solid	Ring or solid	Active
M-chromosomes	Pachytene	Variable. No crossing-over	—	*X*[4]	Centre of ring	Indifferent (solid)	Partly active, partly inert
X in *XO*[3]	?	—	—	*X*	Off plate	Ring	Do.
X in *XO*[5]	Zygotene	—	*X*	—	Indifferent	Edge of plate (no ring)	Do.
X and *Y* in *XY* and X_nY	?	None.[6] No crossing-over	*X*	—	Central if small[7]	Central[8]	Do.

[1] *Alydus* (Reuter, 1930).
[2] *Banasa* (Wilson, 1907).
[3] *Archimerus* (Wilson, 1905*b*).
[4] A third *M*-chromosome, probably non-orientated, occasionally divides at M_I in *Metapodius* (Wilson, 1910).
[5] General type.
[6] Shown in *4x*, *Cimex* and *Euschistus* (Bowen, 1922).
[7] E.g. *Y* in *Nezara viridula* (Wilson, 1911).
[8] But edge of plate if *Y* is lost in *Metapodius* (Wilson, 1909*b*).

(such as is commonly found in plants) but by a change in the timing cycle of the sex chromosomes themselves. This change applies to all the successive stages of meiosis. It begins by a change in precocity which suppresses pachytene pairing. This entails suppression of crossing-over and hence structural divergence of the genetically isolated mates. That such a system survives the suppression of the ordinary mechanism of pairing and segregation by chiasmata is due to the development in the Heteroptera of the special spindle mechanism which we must next consider.

(iv) *Spindle action and centromere valency*

The first and obvious evidence of special spindle mechanisms in this group is seen in the shapes of the first and second metaphase plates. In general it seems that the "hollow spindle" arrangement follows the rule obtaining in *Cimex*: all the chromosomes lie on the edge of the plate provided that there is enough room for them to lie at a certain distance



Text-fig. 15. Diagram showing the timing relationships of autosomes and sex chromosomes in Hemiptera-Heteroptera in respect of (i) pairing at zygotene (p°) and at first and second metaphase of meiosis in the spermatocytes (p' and p''), (ii) the splitting of the chromosomes (s) and the division of the centromeres (d). s and d are correlated, while p° is conditioned by s not coming early enough to interfere with it. The autosomes are held together by chiasmata between diplotene (D) and first metaphase (M_1). Cf. Table IV. (After Wilson, 1912 and Reuter, 1930.)

apart. Hollow spindles therefore appear at both first and second metaphases for no other reason than that the spindle is large enough to allow for it. But there are certain groups in which the spindle is regularly large enough for a more or less regular ring to be developed. This is particularly true of the first metaphase in the Coreidae and in *Notonecta*, and of the second metaphase in the Reduviidae and Cimicidae. And it is with equal regularity in these groups and at these stages that we find

particular chromosomes distinguished from all the rest by not lying in the peripheral ring. The *M*-chromosomes at first metaphase in the Coreidae and the sex chromosomes at second metaphase in the Reduviidae lie in the centre. The significance of this central position at metaphase is shown by the fact that there and then alone are these chromosomes capable of the momentary approximation which allows of their regular segregation.

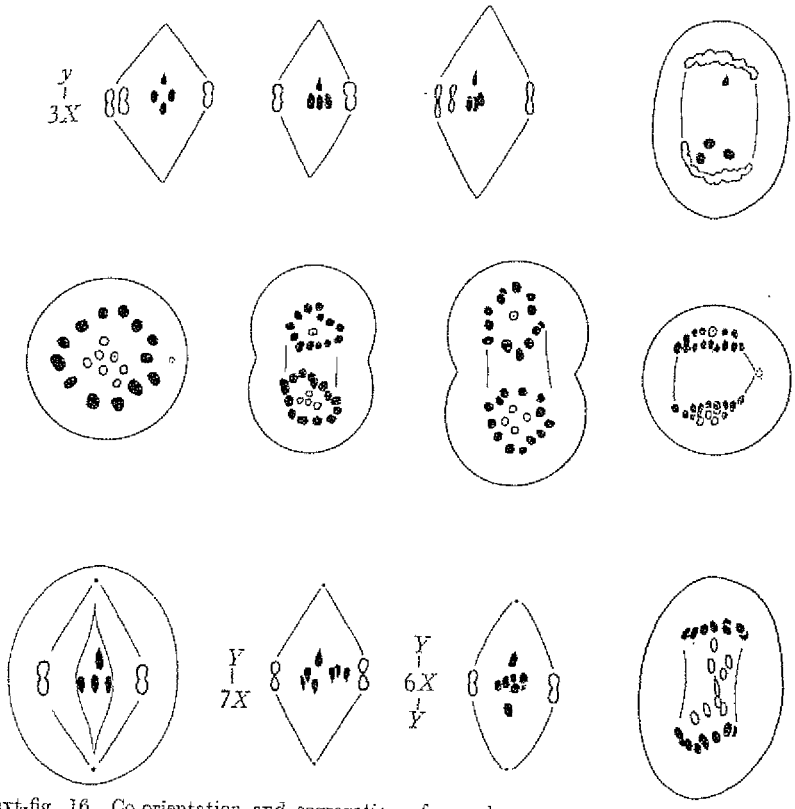
What makes this touch-and-go pairing or co-orientation possible? Three factors seem to be concerned: first the *distribution* of the other chromosomes, secondly the *valency* of the affected chromosomes, and lastly their *size*.

The ring-distribution of the autosomes is clearly necessary for a central position of the special chromosomes to be seen. But it may also be necessary in a more primary sense, viz. for restricting the space in which the special chromosomes can lie. In *Lethocerus* three autosomes are enough to do this (Chickering, 1927). The priority of the autosomes is indicated by the fact that, where there are many *X* chromosomes in *Cimex*, the autosomes make the ring before the *X* chromosomes are forced out of it. The autosomes are *stronger*. This brings us to the second factor, valency. On my view of the centromere, the autosomes have polarized centromeres at second metaphase and the *X*'s have not. Bivalents in the same way are stronger than univalents at first metaphase and, even in *Cimex*, the univalent sex chromosomes are slower in congression and therefore more likely to suffer irregularity than the bivalent autosomes.

It would seem therefore that a difference in the valency of the centromere—unpolarized in the daughter univalent *X*'s and polarized in the daughter bivalent autosomes—is responsible for the distinction between central and peripheral chromosomes at the second metaphase. This conclusion is borne out by the daughter *x*'s being able to lie in the ring in *C. lectularius* although they are the smallest chromosome in the set. Presumably they had passed without division to one pole at first anaphase and therefore had the valency of a daughter bivalent. This conclusion obviously needs testing with material in which some failure of autosome pairing followed by pre-division has been induced by hybridity or abnormal temperature.

On these views the behaviour of the *M*-chromosomes becomes intelligible. They pass to the middle of the spindle because they have unpolarized centromeres like the second metaphase chromosomes, and this movement is assisted further by their small size, since, other things

being equal, small chromosomes with the smallest body repulsions always pass to the middle of the plate. The exceptionally small *y* passes to the middle of the plate in *Metapodius* and sometimes the larger *Y*, which is however smaller than any of the autosomes, may lie just inside the ring, while the very large *X* always lies outside the ring of autosomes.



Text-fig. 16. Co-orientation and segregation of sex chromosomes at second metaphase and second anaphase in *Cimex* with different numbers of *X*'s. Top row: *XXXy*, Glasgow; fourth *X* not shown. Second row: segregation of four and five *X*'s from *Y*, Greenwich (second metaphase shows exceptional 6 *X*'s). Bottom row: left, *lectularius* × *columbarius* *XXXY* showing hypothetical central region of spindle within which co-orientation can occur; centre, co-orientation of one and two *Y*'s with 6 *X*'s (Mitcham); right, consequent failure of segregation at second anaphase (Glasgow). ×4000.

Thus the polarized centromeres of the *X* and *Y* univalents at first metaphase arrange themselves almost in the same way as the co-oriented centromeres of the bivalent autosomes. The half-univalent (or semi-valent) *X* and *Y* at second metaphase do not arrange themselves like the half-bivalent autosomes. This is to be related to the fact that the *X* and *Y* centromeres cannot divide again while the autosome

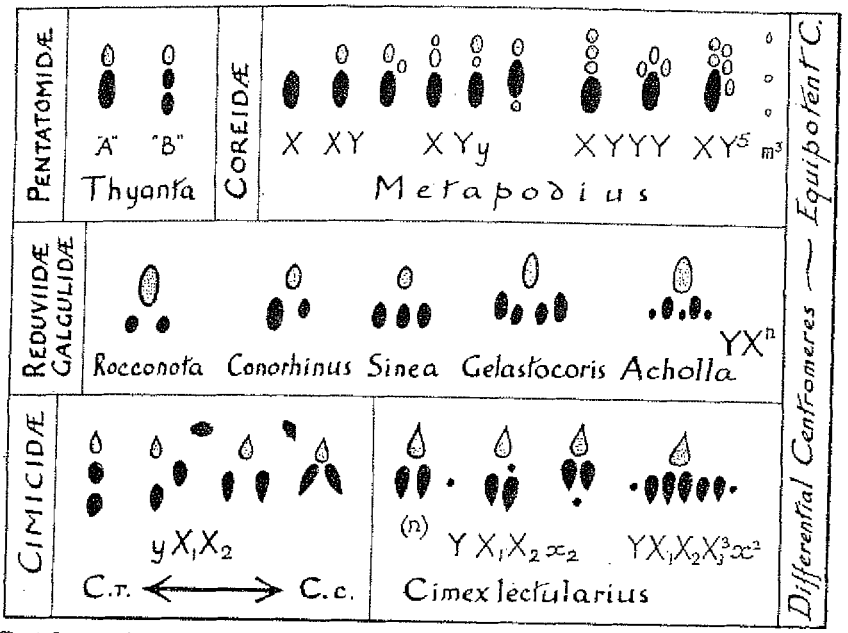
centromeres can do so. In short, when allowance is made in particular cases for the rules governing distribution of chromosomes of different sizes the principle is established that the special distribution of chromosomes in the Heteroptera is governed by the three valency conditions found in their centromeres—co-oriented, polarized, and unpolarized.

We have now to consider what the touch-and-go pairing has to do with the special position of the chromosomes concerned. In the first place it might seem that a co-orientation or approximation of the chromosomes was requisite for their movement. The X, in exceptional XO individuals of *Metapodius*, may remain outside the ring at second metaphase. This, however, is not usually the case. Both M-chromosomes in *Anasa* and X and Y in *Cimex* may move towards the middle without any previous relationship. On the contrary it is their movement to the middle which makes this possible. This is very well shown in *Cimex*, where the series of stages in arrangement are prolonged by the presence of so many X's. And yet ultimately a regular arrangement in a double plate can be achieved.

In order to understand this co-orientation of either M's or X's in the centre of the plate two further properties must be borne in mind—first its non-specificity in all cases, and secondly its differential possibilities with the double-plate arrangement. Both these properties differentiate touch-and-go pairing from the continuous pairing established by chiasmata or secondary attraction.

What I mean by non-specificity may be illustrated by two examples. In *Metapodius* an X, Y and y group may lie end-to-end equally well in the orders XyY and yXy (Text-fig. 17). Such a system is of course unstable in evolution, as Wilson found. In *Cimex* similarly the position of the small x chromosome in the configuration is variable, although usually furthest from the Y. The result however is less unstable on account of another peculiarity which distinguishes all the stable X_n forms from the unstable Y_n forms of *Metapodius*. In *Metapodius* the XY configuration forms a single or double chain. In the Reduviidae, and in *Cimex* too, a double plate is formed. In *Metapodius* the position of the Y is indifferent, in *Cimex lectularius* it is opposed to all the X's. This system may break down where there are more than a dozen X's, but such a break-down does not alter the significance of the rule for lower numbers. Further, Y can cope with many X's even when it is smaller than any of them. The body of the chromosome is irrelevant; and, since the position on the spindle is an indispensable condition, the centromere must be the effective agent acting through the spindle and at a distance.

The view that the "pairing" of *M*-chromosomes at first metaphase and of sex chromosomes at second metaphase are both conditioned by centromere-spindle relationships is reinforced by a comparison of the arrangements of new or spontaneous extra chromosomes of the two kinds. Where a third *M*-chromosome is present in *Metapodius* (Wilson, 1910) or an extra *X* in *Thyanta* (Wilson, 1911) the centromeres are as we may say, *equipotent* and the three chromosomes are of equal size; the chain configuration formed is therefore symmetrical. In the two



Text-fig. 17. Diagram showing the relationship of size and position at second metaphase of the sex chromosomes in Hemiptera-Heteroptera. Above, the chain arrangement; below, the double-plate arrangement. *X*, solid, *Y*, stippled. (After Wilson, 1909; Payne, 1910 and present study.)

cases we then find that the middle member of the group commonly lags on the equator. It is only by a difference in properties of one chromosome from all the others that it can regularly segregate from all the others in a multiple group. And this difference could reside only in the centromere.

The development of the double-plate system from a chain system is shown by the transition in behaviour between *C. rotundatus* with predominantly chain formation and *C. lectularius* with an almost consistent double plate—which has obviously been the condition of extensive

reduplication of X's. This transition helps us to understand the development of the *lectularius* system. An increase in the differential character in *columbarius* and *lectularius* would increase the frequency of the double plate as opposed to the simple chain found with equipotent centromeres. This increase would permit the survival of the high-multiple X type of segregation which has actually developed in *lectularius*.

The crux of the problem is therefore the nature of the differential character of X and Y. That many are repelled from one leaves no doubt that the difference is quantitative, not qualitative. That the size of the Y is irrelevant leaves no doubt that the body of the chromosome is not concerned. We are left with the conclusion that the difference is a quantitative difference between the centromeres. We may say that in a bipolar spindle the easiest arrangement will then be for the stronger centromere of the single Y to oppose the weaker centromeres of the many X's.

The material basis of such a differentiation of centromeres is provided by the observation that a centromere can divide longitudinally to give two functional halves, each bound to be weaker than the parent centromere (Darlington, 1939*b*). Such misdivision would provide for the evolution of the X_nY system in the Heteroptera. Whether it has done so can readily be tested by repeating the present observations and experiments on a type with larger chromosomes.

Recapitulating, four assumptions seem to be required by the double-plate system of multiple X segregation:

(i) That the relationship between X's and Y can be established only in the centre of the spindle.

(ii) That they are driven to these positions by their centromeres being unpolarized, and not by any specific affinities.

(iii) That the Y opposes all the X's because its centromere is stronger than theirs.

(iv) That double-plate formation as opposed to chain formation is favoured by an increase in the strength of the odd chromosome's centromere.

5. The evidence of evolutionary change

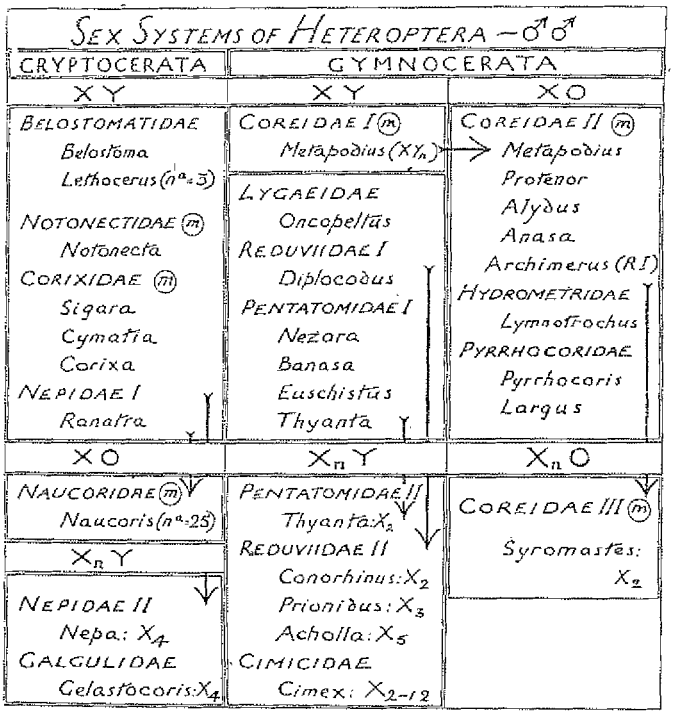
In most groups of insects as well as vertebrates the corresponding sex chromosomes, X and Y, have not merely a relationship of ancestry, but an exact correspondence of genes in parts of their length between which crossing-over regularly takes place. These interchangeable parts or pairing segments must have existed in the ancestors of the Heteroptera.

They have now disappeared throughout the group and they must therefore have disappeared long ago. Indeed there is no reason to suppose that any relationship now exists between the linear sequences of genes in *X* and *Y*. The two chromosomes are homologous only as chromosomes. Their historical relationship however still expresses itself in two entirely different ways: their common adaptation, on the one hand, to the genotypic requirements of the two sexes and, on the other hand, to the mechanical requirements of their own segregation. They have the same time co-ordination of chromosome splitting and centromere division, which distinguishes them from the autosomes. How this has arisen we need not consider for the moment. Its evolutionary consequences however are clear.

The sex chromosome mechanism has reached an extreme of differentiation. A comparative study of the less extreme cases in *Drosophila* species indicates that such systems are liable to be replaced by, or combined with, new systems derived from the autosomes. In the Heteroptera we find an instability of the sex chromosomes, but it is a highly standardized instability. The reason is evident. The sex chromosomes have developed a special mechanism of segregation that renders their replacement impossible. It is easy enough, as is seen in mammals, birds and fishes as well as in *Drosophila*, to switch the differential sex mechanism from one chromosome to another, provided that the ordinary method of pairing and segregation is retained; but where the segregation depends on special properties of the centromere itself, which cannot be lost, and is not readily susceptible to genotypic control and adaptation, the system becomes inevitably rigid—hence its mechanical constancy throughout the Heteroptera. This evolutionary deadlock seems to have resulted in the restriction of the active differentials of the sex chromosomes to a very small part, leaving them, *X* as well as *Y*, for the greater part inert, whence arises their variability in size and number, a variability whose common condition is revealed by the parallel changes in different families where independently the *X* has been multiplied or the *Y* lost (Text-fig. 18).

The most obvious aspect of this instability is the breaking up of the *X*. In most families where the *Y* has already been lost this has not happened. Where it has happened the *Y* has always been retained. It has been retained not, I suggest, on account of its genetic activity but on account of its mechanical function in the segregation of the multiple *X*'s. Where the *Y* has recently been lost, as in the Coreidae, the *X* usually lags on the second anaphase spindle. Its movement leaves no margin of safety

in segregation, and two X's without a Y would scarcely pass regularly to one pole. In this respect the position of *Syromastes* with an X_2O system is exceptional and interesting, for the break-up of the X has here



Text-fig. 18. *Notes on Heteroptera diagram* (after Imms, 1925). There are two well-known methods of dividing the Heteroptera. The division by antennae into Gymnocerata and Cryptocerata followed here agrees with the distinction between dry and wet habitats. The division by genitalia into Reduvioid and Pentatomoid groups (cf. China, 1933) cuts clean across the first one, as will be seen from the names. It will be noticed however that on either system the distinction between single X's and multiples, and equally the distinction between Y and no Y, must have arisen independently, i.e. by parallel change. The third possibility of course is that this distinction by X and Y chromosome types is the natural one. This possibility can, it seems, be set aside in view of the independent changes within families and even genera as shown in the table by arrows.

Notonectidae. I do not regard the incipient "fragmentation" of the X in *N. indica* (Browne, 1916) as anything more than a symptom of unequal spiralization like that described by Koller (1938) in the hamster. (*m*): family with *M* chromosomes.

Archimerus, with first division reduction of X (RI), shows reversion to the centromere timing system of the autosomes.

come after, not before, the loss of the Y. The two X's adhere and lie to one side of the plate. Preceding the autosomes to the poles, they avoid dispersal by a method entirely different from that of the double plate.

The mechanical stability required by the spindle mechanism has entailed a further mechanical stability of the whole chromosome system.

This secondary stability is shown by the limited variation in chromosome number in the Heteroptera. We might ascribe such a property, as Prokofyeva has suggested, to "tiefergreifende innere Verschiedenheiten der Chromosomen" but we find in fact that the two forms of *Thyanta calceata* with 7 and 12 bivalents, and many other examples, show that the capacity for variation is present in this group as much as in others. The limitation is due to the fact that a hollow spindle at first metaphase is necessary for the pairing of the *M*-chromosomes and equally a hollow spindle at second metaphase is necessary for the segregation of the sex chromosomes. As we have seen in *Cimex*, the chromosomes need not form a good ring for this purpose, and in *Lethocerus* they cannot do so. The highest number of autosomes, 25 pairs, is found in *Naucoris*, where the chromosomes are small enough to form a ring leaving a hollow spindle at first metaphase and where the absence of the *Y* makes a ring unnecessary at second metaphase. Where the chromosomes are suddenly increased in size as in *Macrocoryxa* as compared with *Coryxa* they are no longer confined to a ring at first metaphase, and the small *M*-chromosome has disappeared. Similarly the second metaphase ring loses its regularity in the *XO* Coreidae where the co-orientation of *X* and *Y* is no longer necessary.

The parallel changes of *X* and *Y* within the Heteroptera are symptoms of what I have expressed in the most general terms as the evolutionary disequilibrium of all *XY* systems of sex-determination (1939*a*). A permanent adaptive balance is impossible between sex chromosomes whose crossing-over frequencies and mutation requirements are all necessarily different from those of the autosomes in the same nucleus. Hence the continual reconstruction of the system within the limits that are mechanically feasible. The importance of *Cimex lectularius* is in showing this reconstruction taking place under conditions of exceptional evolutionary stress.

6. SUMMARY

A. *Cimex*

1. *C. columbarius* has in the male the chromosome formula $13^{II}A + X_2Y$. *C. lectularius* has 0–12 extra *X*'s, the average number of *X*'s being higher in natural populations (9·0) than in mass cultures (4·3).

2. A variable number of extra *X*'s pass to the female-producing sperm in high *X* males owing to loss at meiosis.

3. Males of *Cimex columbarius* ♀ × *C. lectularius* ♂ have no extra *X*'s. A variable number of *X*'s pass into the egg-nuclei of *C. lectularius* ♀♀ since full brothers from the reciprocal cross *C. lectularius* ♀ × *C. columbarius* ♂ vary in number of *X*'s.

4. The size of the *Y* chromosome varies within both species; the smaller type, *y*, agrees with that in *C. rotundatus*.

5. Some of the extra *X*'s in certain populations have also suffered loss to give a smaller *x*.

6. The chain co-orientation of *X*'s and *Y* at second metaphase is commoner in *C. rotundatus*, the double plate in *C. lectularius*; *C. columbarius* is intermediate.

B. *The Heteroptera*

1. The cycles of chromosome and centromere division are correlated in three types of differentially precocious chromosomes recognizable in the group: autosomes, *M*-chromosomes and sex chromosomes (Table IV).

2. The "pairing" of the sex chromosomes depends not on specific attractions of genes in the nucleus but on balanced reactions of centromeres in the spindle. These act by determining co-orientation without chiasma formation, a condition which depends on the chromosomes occupying a central position in a hollow spindle.

3. The double-plate system is a condition of the regular segregation of numerous *X*'s from one *Y* and itself demands quantitative differences between centromeres, a strong *Y* and weak *X*'s.

4. The similarity of *X* and *Y* chromosomes is a similarity of mechanical behaviour and gene ancestry but not necessarily of gene content.

C. *New problems*

1. The central spindle-co-orientation of *M*-chromosomes at first metaphase and sex chromosomes at second metaphase seems to depend on two factors, the state or valency of the centromere and the relative size of the chromosome. This can be tested by studying the behaviour of unpaired chromosomes produced by temperature shocks in a hollow second metaphase spindle.

2. The extra *X*'s of *C. lectularius* are presumably active, since they are preserved by selection, but relatively dosage-indifferent since they can be reduplicated indefinitely. Variations in body size, testis size and time of maturity occur within and between natural populations. These are possibly related to *X*-content. A closer study of the sex-ratio and of the effects of inbreeding are necessary to test these possibilities.

APPENDIX I

GLOSSARY FOR THE HEMIPTERA-HETEROPTERA

A. *Old terminology*

1. *Hetero-chromosomes*: any other than a normally pairing autosome, i.e. *M*, *X*, or *X* and *Y* or an autosome univalent.

2. *Idio-chromosomes* or differential chromosomes: members of an *X*–*Y* pair.

3. *Accessory chromosome*: unpaired *X* in an *XO* male.

4. *Heterotypic chromosome*: usually as (3) but in *Banasa*, an unpaired autosome.

5. *Micro- or minute chromosome*: *M*-chromosome.

6. *Supernumerary chromosomes*: extra *Y* or *y* chromosomes in *Metapodius* species.

7. *Compound chromosome*: a group of chromosomes independent at mitosis but segregating as a unit at second meiotic anaphase and functioning as an *X* chromosome.

B. *Present terminology*

X and *Y*: chromosomes whose segregation determines sex (as opposed to autosomes).

M: other chromosomes which do not pair and form chiasmata at pachytene.

x, *y* and *m*: centric fragments arising by loss from *X*, *Y* and *M*.

n^a: haploid number of autosomes.

APPENDIX II

ON PREFERENTIAL SEGREGATION

The segregation of multiple *X*'s from *Y* in *Cimex* may be described as preferential segregation, being preferential in an extreme degree, 100 % for the first three or four *X*'s and 70 or 80 % for the fourteenth. This makes one suspect that the same kind of assumption would account for other cases of preferential segregation, notably in the case of the triploid fourth chromosomes in *Drosophila melanogaster* so precisely recorded and analysed by Sturtevant (1936). This suspicion is strengthened by the fact that here, as in *Cimex*, similar chromosomes are less likely to go to the same pole. I say, as in *Cimex*, for it would be absurd to suppose, in *Cimex*, that twelve chromosomes go to the same pole because

they are all more like a thirteenth than they are like one another. Now Sturtevant has suggested that it may be the relative distances apart of the three centromeres of the fourth chromosomes that determine their fixed preferences of segregation. This view agrees with the observation in plants that trivalents arrange themselves most frequently so that the pairs of centromeres which have been closest at diakinesis lie on an axis of the spindle, i.e. the pairs between which there is the greatest repulsion. The same *scalene* conditions that arise in ordinary trivalents owing to asymmetrical positions of chiasmata or attractions between chromatids might equally well arise from asymmetrical strengths of the centromeres. This view, however, is untenable in the *Drosophila* case for two reasons: (i) the centromeres of differently behaving chromosomes are often of closely related origin, and (ii) the inert regions at both ends of the chromosomes play a part in determining preferences. This effect of the distal inert regions of the chromosomes indicates that the ordinary conditions of trivalent co-orientation apply to the fourth chromosome in *D. melanogaster*. The preferences are determined, in the female, by the chances of chiasma formation which is probably confined to the distal inert regions and, in the male, by the relative attractions of the three chromosomes for which these regions again are largely responsible. The reduction of preferences in the male is due to an aggregate attraction having a less determinate effect in the absence of the all-or-nothing difference of chiasma formation. The means of co-orientation is of course always the centromere repulsion, but its variable effect is due to variations in the chromosome attractions. Sturtevant's observations make it all the easier to suppose that the regular segregation X_1X_2–Y in *Drosophila miranda* is in fact due to the convergent co-orientation of three chromosomes associated by chiasmata as found in comparable cases in plants.

Known systems of apparently controlled segregation may therefore be provisionally classified in the following manner:

1. *Selective fertilization.*
   (*a*) *Habrobracon* and other haplo-diploids.
   (*b*) Self-sterility systems.
2. *Selective viability.*
   All 3 (*a*) systems in part.
3. *Directed segregation.*
   (*a*) Convergent co-orientation of multivalents, e.g. X_1X_2Y sex systems, *Oenothera* and probably *D. melanogaster* triplo IV and *miranda*.

(*b*) Special spindle mechanism:

(i) *Sciara.*

(ii) *Blaps, Ascaris.*

(iii) *Acholla, Nepa, Cimex.*

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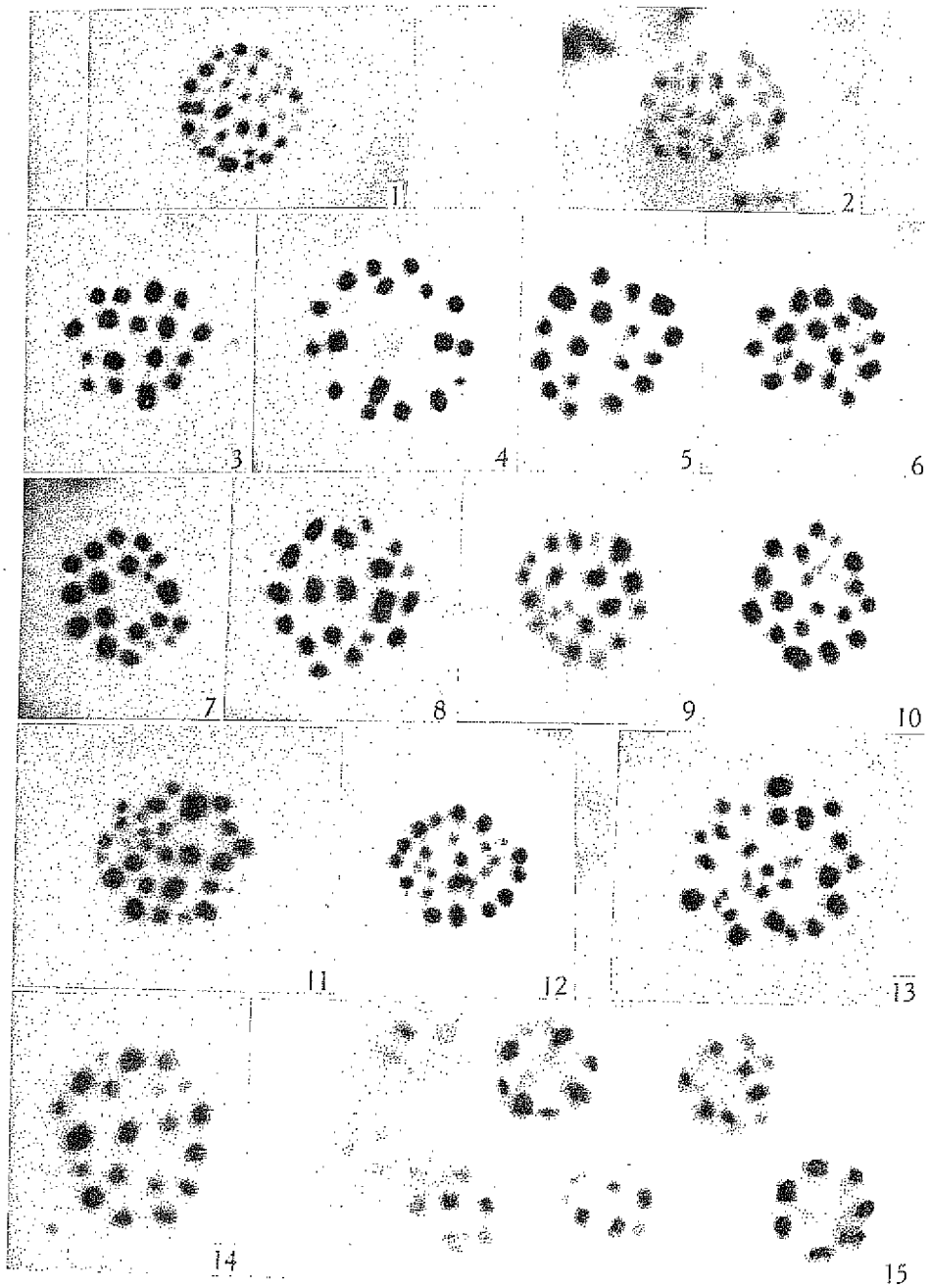
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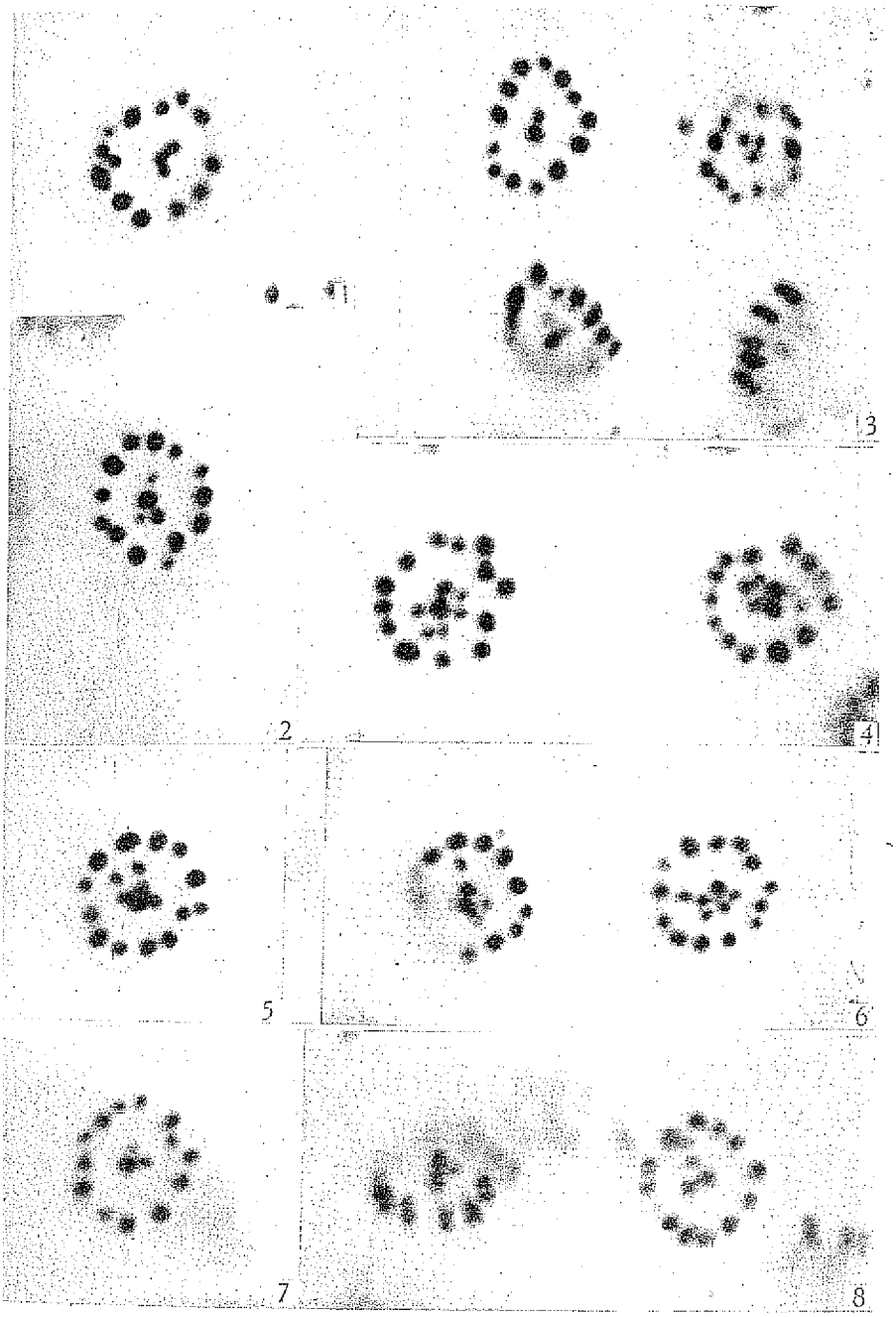
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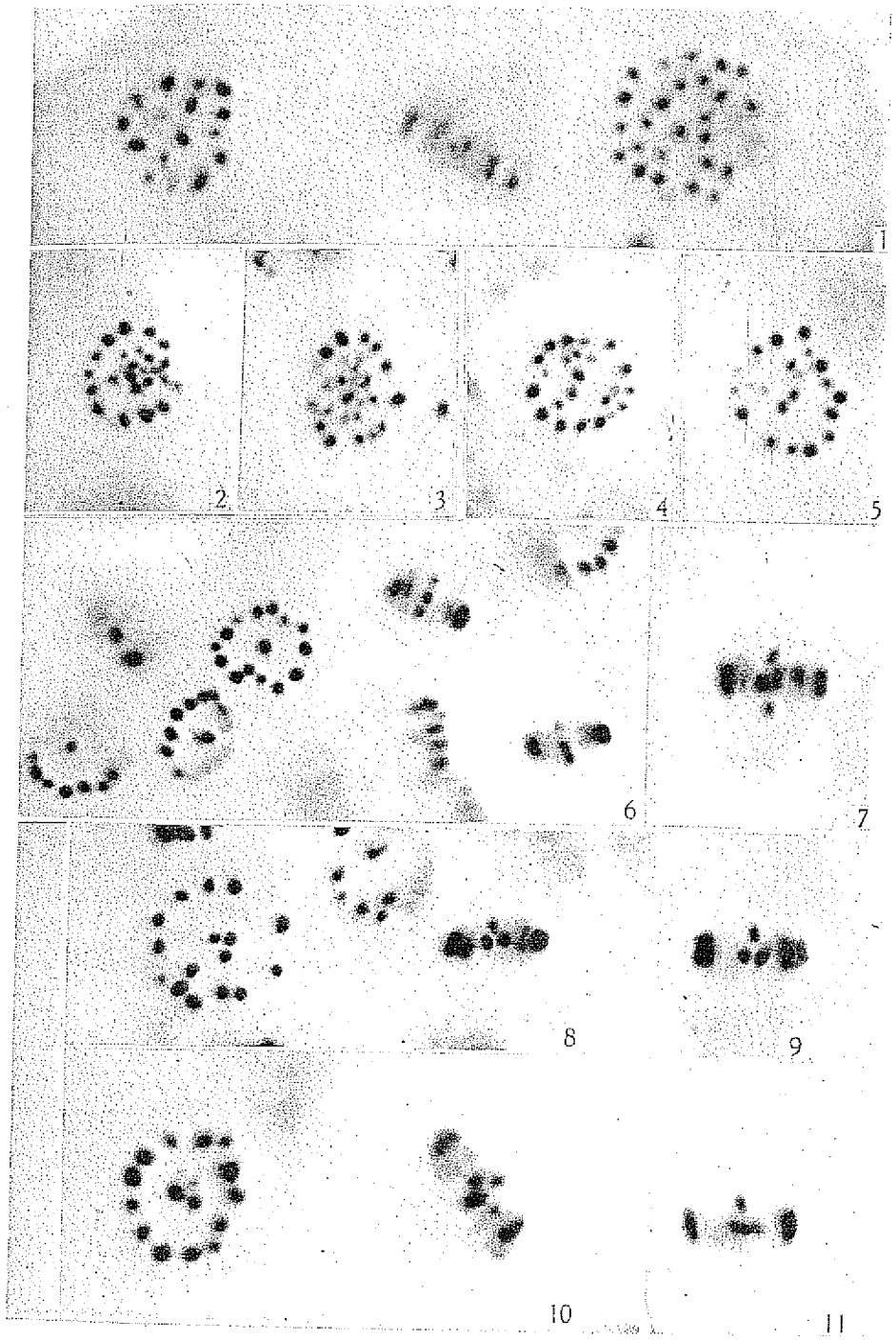
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EXPLANATION OF PLATES I—III

Microphotographs by H. C. Osterstock and L. La Cour.

PLATE I

Fig. 1. Mitosis in ovary, *C. lectularius*, Greenwich. $2n=34$.

Fig. 2. Mitosis in testis, *C. lectularius*, Lister Institute. $2n=30$.

Figs. 3–11, 13 and 14. First metaphase of meiosis in spermatocyte. Figs. 3 and 4. *C. lectularius* × *C. columbarius*, $n=17$. Figs. 5 and 6. Lister, $n=17$ and 18. Figs. 7 and 8. Beckenham, $n=19$. Figs. 9, 10 and 14. *Lectularius* × *columbarius*, $n=20$, 21 and 21. Figs. 11 and 12. First metaphase, $n=28$, and second metaphase, 13 X's, Mitcham. Fig. 13. $n=26$, Lambeth. Fig. 15. Interphase (*lectularius* × *columbarius*, $n=21$).

Note. Hollow spindle in Fig. 4, double spindle in Fig. 13. Non-orientation of X's in Figs. 6, 13 and of x in Fig. 14. × *ca.* 3000.

PLATE II

Second metaphases.

Fig. 1. *C. columbarius*, $13^{II}A+XXy$.

Fig. 2. *C. lectularius*, Beckenham culture, $13^{II}A+XXXY$.

Fig. 3. *C. columbarius* × *lectularius*, $13^{II}A+XXY$.

Fig. 4. *C. lectularius* × *columbarius*, $13^{II}A+X_7Y$.

Figs. 5 and 6. Greenwich culture X_6Y. Fig. 6 shows extra x outside ring, found in patches in one testis.

Figs. 7 and 8. Lister culture, X_3xY, showing x inside (7) or on (8) the ring. × *ca.* 3000.

PLATE III

Fig. 1. Diploid and tetraploid first metaphase, after heating; Cork (Text-fig. 10).

Figs. 2–11. Second metaphases. Figs. 2 and 3. 11 and 14 X's (Glasgow). Figs. 4 and 5. Dispersed X's, after heating (Lambeth). Fig. 6. *C. rotundatus*, linear co-orientation. Fig. 7. Triple plate, probably with two Y's (Mitcham). Fig. 8. *C. columbarius*, double plate, XXy. Fig. 9. Same, XXY (Lister). × *ca.* 2000. Fig. 10. $XXxY$ (Lister), × *ca.* 3000. Fig. 11. Double plate X_6Y (Greenwich), × *ca.* 2000.