

# FURTHER DATA ON LINKAGE IN *GAMMARUS CHEVREUXI*; AND ITS RELATION TO CYTOLOGY.

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### I. Introduction.

THE work of Morgan and his pupils has finally proved for the Dipteran *Drosophila* (1) that the factors of Mendelian heredity are contained in the chromosomes; (2) that they are disposed within the chromosomes in a constant and linear arrangement; (3) that a special mechanism exists for ensuring that factors within the same chromosome can become separated from each other—the mechanism of crossing-over. Of these three statements, we may regard (1) as universally valid; (2) as probably also valid universally, although it will take much work before this can be definitely established. As to (3), we know that wherever factors have been found to be linked—*i.e.* in the same chromosome—they have always been found to exhibit crossing-over, at least in one sex. However, in no organism except *Drosophila* do we know anything accurate as to the detailed mechanism of the process, although it appears probable that in other organisms, *e.g.* the Sweet Pea (Punnett, 1923), the mechanism is not dissimilar.

There is, however, no reason to suppose that the mechanism *need* always be similar. Our present knowledge indicates that one at least of the prime functions of sexual reproduction is to afford the possibility of recombining in the largest number of ways the different mutations which crop up in the genes of a species, so providing a far larger raw material on which natural selection may work. Any mechanism which ensures

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that the large majority of genes can be separated from each other, and therefore that a large proportion of the theoretically possible combinations of the genes existing in an organism shall be found in its offspring, will be biologically satisfactory.

In *Drosophila*, three of the four sorts of chromosomes are elongated bodies, containing at a minimum computation several hundred genes each. Morgan's theory of crossing-over for this species (and it is as well to state at the outset that no alternative theory has been proposed which satisfies the facts) is that—probably in the early growth-stages of the germ-cells, when the chromosomes are in the elongated thin-thread stage, and certainly during the synaptic period when paternal and maternal chromosomes are closely paired—a break occurs in a certain number of the pairs, in homologous positions or loci in the two members of the pair; and that then each broken-off piece attaches itself to the main piece of the other member of the pair. In a smaller number of germ-cells there are in the same pair of chromosomes two such breaks, and three breaks in a very small number; while in a fairly large proportion no break occurs at all. He supposes that the two homologous chromosomes loop round each other, and that breakage, when it occurs, occurs at the nodal points of the loops. Crossing-over is measured (cross-over value—c.o.v.) as the percentage of breaks between particular genes. The chromosomal distance between genes is measured in terms of units each equivalent to a c.o.v. of 1 per cent. Fifty per cent. c.o.v. between two genes is equivalent in its results to completely free assortment—*i.e.* to what would be found if they were in different chromosomes.

In *Drosophila* a considerable amount of crossing-over is only found in the elongated chromosomes. In the small dot-like 4th chromosome the highest c.o.v. yet found is about 1. The Crustacean which is the subject of the present paper also has small ovoid chromosomes, and yet a c.o.v. of over 40 per cent. is to be found between two of the genes discussed.

There are various reasons for thinking that this cannot be explained by postulating a mechanism of the same nature as in

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*Drosophila*; and evidence for other possible types of mechanism is reviewed. Work is being continued on the subject, and it is hoped that further conclusions may soon be reached from genetic and cytological studies already begun.

### 2. The Linkage of B and C.

In a previous paper (Huxley, 1921) I reported some results of breeding experiments with *Gammarus chevreuxi*, which indicated that linkage existed between the factors for colour or its absence (C and c) on the one hand, and for black or red (B and b) on the other. The following is a continuation of the work there reported; the data *include* most of those previously published (some few having been excluded as being unsatisfactory, *e.g.* when the totals were too small).

Pairs of animals heterozygous for all the three factors B—b, C—c, and W—w (white versus no-white) were mated together. It was impossible at the time to make back-crosses with the double or triple recessives, as the pure stocks had died out. The same male and female were left together throughout their lives. In a few cases, the male died and was replaced by a second; usually, however, the mating was terminated by the female being eaten by the male. Sometimes the females were already ovigerous (eggs in the brood-pouch) when taken from stock. These eggs would in every case represent the first brood, as evidenced by the known age and size of the individuals of the stock culture.

When heterozygotes of constitution  $\frac{B C W}{b c w}$  are mated *inter se*, six classes are expected. If there were no linkage, they would be expected in the following ratios:—B C W (black with white), 27; B C w (black no-white), 9; b C W (red with white), 9; b C w (red no-white), 3; B (b) c W (albino), 12; B (b) c w (colourless), 4.

If linkage occurs, there should be an excess of blacks (B C W and B C w), a deficiency of reds (b C W and b C w). The excess of b c and the deficiency of B c types is masked through both of them being albinos. Where, therefore, the sum of the black classes is more than three times the sum of the

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red classes, linkage is present, the more intense the greater is the  $\frac{\text{black}}{\text{red}}$  ratio. The results are as follows:—

TABLE I.

In this and subsequent tables, the numbers at the heads of columns have the same significance; this, however, is only given in Table I.

1.	2.	3.	4.	5.	6.	7.	8.	9.	10.	11.	12.	13.
♀ No.	No. Broods.	B C W.	B C w.	b C W.	b C w.	B (b) c W.	B (b) c w.	Total.	$\frac{B}{b}$ Ratio.	$\frac{W}{w}$ Ratio.	$\frac{B W}{b W}$ Ratio.	$\frac{B w}{b w}$ Ratio.
N. 1	7	61	18	10	4	26	10	129	5.64	3.03	6.10	4.50
N. 4	10	86	34	25	6	48	12	211	3.87	3.06	3.44	5.67
N. 7	3	19	5	5	1	6	1	37	4.00	4.29	3.80	5.00
N. 10	7	84	33	22	7	45	13	204	4.03	2.85	3.82	4.71
N. 15	5	40	22	11	6	11	6	96	3.65	1.82	3.64	3.67
N. 18	6	59	20	17	3	21	5	125	3.85	3.46	3.47	6.67
Q. 5	7	44	22	10	2	27	5	110	5.50	2.79	4.40	11.00
Q. 6	8	33	12	10	1	22	7	85	4.09	3.25	3.30	12.00
Q. 10	4	42	19	11	3	15	6	96	4.36	2.43	3.82	6.33
R. 1	6	43	21	9	4	33	3	113	4.92	3.04	4.77	5.25
R. 3	10	160	48	43	9	67	27	354	4.00	3.21	3.72	5.33
R. 5	9	110	44	32	13	47	11	257	3.42	2.78	3.44	3.38
S. 1	3	21	10	3	1	5	1	41	7.75	2.42	7.00	10.00
S. 2	8	117	41	33	12	50	15	268	3.51	2.94	3.55	3.42
S. 3	2	12	9	2	2	5	4	34	5.25	1.27	6.00	4.50
S. 4	5	81	24	17	11	36	10	179	3.75	2.98	4.76	2.18
T. 1	6	22	6	3	4	6	5	46	4.00	2.07	7.33	1.50
N. 12	7	40	18	12	7	24	13	114	3.05	2.00	3.33	2.57
N. 14	6	61	20	19	8	24	8	140	3.00	2.88	3.21	2.50
Q. 2	6	16	9	6	2	8	8	49	3.12	1.58	2.67	4.50
Q. 8	8	76	19	25	4	48	12	184	3.27	4.26	3.04	4.75
R. 7	4	44	3	10	6	18	7	88	2.94	4.50	4.40	0.50
V. 2	2	52	15	19	2	23	9	120	3.19	3.62	2.74	7.50
M. 1	6	22	8	9	2	12	7	60	2.73	2.53	2.44	4.00
M. 7	6	47	23	19	9	29	7	134	2.50	2.44	2.47	2.55
N. 2	9	102	37	43	14	50	20	266	2.44	2.75	2.37	2.64
N. 3	10	135	44	38	24	67	20	328	2.88	2.73	1.55	1.83
N. 9	10	97	32	34	10	45	18	236	2.93	2.93	2.85	3.20
N. 16	5	30	14	12	5	13	5	79	2.59	2.29	2.50	2.80
Q. 3	2	19	5	4	5	5	2	40	2.67	2.33	4.75	1.00
Q. 7	3	15	4	7	1	14	2	43	2.37	5.14	2.14	4.00
Q. 11	7	29	16	17	6	10	8	86	1.96	1.87	1.71	2.67
R. 2	8	66	25	28	13	36	8	176	2.22	2.83	2.36	1.92
33 matings *		1885	680	565	207	896	295	4528	3.32	2.83		
		2565		772		1191		...	...	...		

\* Offspring per mating, 137.2.

If we calculate the cross-over value according to Haldane (1919), we find it to be 44.69 per cent. (Since the cross-over

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values may differ somewhat in ♂ and ♀, all that we find is the geometric mean between ♂ and ♀ value.)

The lower linkage intensity for the totals than that previously found (Huxley, 1921: c.o.v., 37.45 per cent.) is due to two causes: (1) the fact that linkage is less in late broods than in earlier, and that the present data include a greater proportion of these; (2) to the fact that linkage is less at about 25° than at about 15° (Huxley, 1921), and that many of the broods whose figures are here presented for the first time were produced during the very hot summer of 1921, during which room-temperature was often over 25°.

Some of these females were already ovigerous with their first brood when taken out of the stock-pot (of triple heterozygotes); one or two were remated on the death of their mate after several broods. The above figures should therefore be corrected if it is desired to have only the offspring of the same pairs throughout. However, the correction makes no appreciable difference to the result, as is seen below:—

*Total corrected so as to include only the offspring of single pairs remaining mated for the course of the experiment.*

	3.	4.	5.	6.	7.	8.	9.	10.
33 matings . .	1817	660	541	197	859	282	4356	3.36
	2477		738		1141			

A glance at the individual  $\frac{B}{b}$  ratios shows that they vary from very high figures such as 7.75 and 5.64, apparently indicative of intense linkage, to very low ones like 1.96 and 2.29, apparently indicative of more than 50 per cent. crossing-over.

By applying statistical methods,\* however, it was discovered that the diversity of the ratios was not significantly greater than what is to be expected as a result of random sampling.

A 2 × 33 table was formed of the 33 families divided

\* I have to thank Mr R. A. Fisher of Rothamsted Experimental Station for his kind help with the statistical treatment.

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into reds and blacks, and the value of  $\chi^2$  is calculated for the table. The details of this test for goodness of fit are due to Mr R. A. Fisher, who has not yet published them.

If random sampling errors are at work,  $\chi^2$  for the whole series should be close to  $n-1$ , where  $n$  is the number of determinations (here families); and when  $n$  is large  $\sqrt{2\chi^2}$  should be within  $\pm 2$  of  $\sqrt{2n-3}$ . Actually (1)  $n-1=32$ ,  $\chi^2=35.620$ , and (2)  $\sqrt{2\chi^2}-\sqrt{2n-3}=8.4404-7.9373=0.5031$ . Thus the apparent differences in linkage-intensity among the different families, including the apparent cross-over values of over 50 per cent. in the last ten, are not significant. That such divergencies based on families with

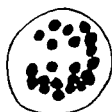


FIG. 1.—First maturation division,  $\delta$ .  
Equatorial plate. 21 or 22 chromosomes.

$\times$  about 1750. Note spheroidal shape of the very small chromosomes similar to the 4th chromosome of *Drosophila*. From an unpublished drawing by A. F. Dence.

such considerable totals should not be significant is due to the fact that the random sampling error really depends on the total number of (b C W + b C w), which is quite small. The back-crosses will be better in this respect: however, the presence of only one cross-over and one non-cross-over class respectively is a great drawback. The figures illustrate, however, the

importance of statistical treatment before drawing conclusions. One further fact, however, may be worth mentioning. A glance at the last two columns of Table I. shows that when we divide the  $\frac{\text{black}}{\text{red}}$  figures into with-white and no-white classes, both ratios are usually similar, *i.e.* both high or both low. This would indicate that possibly we have also real differences in the material which the present figures do not bring out. This again will, it is hoped, be settled by back-cross tests.

Harland, in a recent paper (1922), brings forward evidence which may mean that in *Ricinus* certain factors may sometimes show more than 50 per cent. of crossing-over; but he does not regard his experiments as terminated. The matter in *Gammarus* is now being pursued further in back-cross tests,

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so that I will here confine myself to mentioning the facts. I would like to add that unfinished investigations on the cytology of the species by Mr A. F. Dence show that the haploid number of chromosomes is about 20, and that they are small and short, none of them at all like the elongated X, 2nd or 3rd chromosomes of *Drosophila*, but much more like the 4th chromosome.

This adds to our difficulties; since the large number of chromosomes makes the discovery of linkage between two of the three mutant characters so far found a remarkable coincidence, and their small size renders a large cross-over value very curious.

The  $\frac{W}{w}$  ratio  $\left( \frac{\text{with-whites} \times \text{albinos}}{\text{no-whites} \times \text{colourless}} \right) = \frac{3346}{1182} = 2.83$ , or 26.1 per cent. *ww*.

The ratio  $\frac{C}{c}$   $\left( = \frac{\text{blacks and reds}}{\text{albinos and colourless}} \right)$  for the total is 2.80. There is thus an excess of the pure *cc* recessives (26.3 instead of 25 per cent.). The differences are not statistically significant, but show the good viability of the *ww* and the *cc* animals.

### 3. The Effect of Age on Crossing-Over.

Finally it remains to consider the possible effect of age upon cross-over value. *Gammarus* provides admirable material for dealing with this problem, since it continues to grow long after it has begun to reproduce, and produces in favourable circumstances 10 or more broods in a lifetime.

Taking the matings we have already considered, and finding the totals for the first 3 broods, the second 3 broods, and the 7th to 10th broods, we obtain the following results:—

TABLE II.

	2.	3.	4.	5.	6.	7.	8.	9.	10.	11.
Broods 1 to 3	94	588	206	164	41	206	86	1353	3.87	3.06
„ 4 „ 6	74	832	295	255	115	368	126	1991	3.05	2.73
„ 7 „ 10	38	470	182	147	52	265	86	1202	3.22	2.76

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The difference in linkage intensity between the second and the third class is not significant. If we take these two classes together, we find the following figures:—

	All blacks.	All reds.	Per cent. reds.
Broods 1 to 3 . . .	794	205	20.5
„ 4 „ 10 . . .	1779	569	24.2

For these percentages  $\chi = 2.33 \pm 1$ , which indicates that the difference is significant.

It is noteworthy that in the later broods there is very slight linkage.

This increase of cross-over value with age is the reverse of what has been found for *Drosophila* (see Discussion).

If we take in addition 9 matings, comprising 13 broods, which we have not so far considered because their totals were so small, we find the same thing as regards high linkage-intensity in early life. Only one of this set went beyond the 3rd brood; here only broods 1 to 3 are considered.

	2.	3.	4.	5.	6.	7.	8.	9.	10.	11.
Broods 1 to 3	13	55	18	11	7	15	5	111	4.06	2.70

There is thus a real difference in linkage according to age, the intensity being greater in youth. Since most of the matings came to an end on account of cannibalism, and not through old age or disease, those which produced many broods are not necessarily more vigorous than those which only produced few. Accordingly we find no significant difference in the linkage intensity between the first 3 broods of those who produced 3 broods only and the first 3 of those which produced 7 or more ( $\frac{\text{black}}{\text{red}}$  ratios 3.58 and 3.67 respectively).

### 4. Discussion.

The work of Plough (1917, 1921) and Bridges (1915) on the effects of age and temperature on crossing-over in *Drosophila* must be briefly mentioned. At optimum temperatures (22° to 27°) the c.o.v. is lowest. It is slightly higher at 17.5° and 29°, and beyond these limits rises very rapidly to about three times its previous value: these figures hold for chromosome II., genes black-purple, whose normal c.o.v. is



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6.2 per cent. (Bridges and Morgan, 1919). When, however, a longer region was taken, *e.g.* purple-curved, map-distance 20.8 units, the increase of c.o.v. with sub- and super-optimum temperatures, although comparable at 17.5° and 29°, was not nearly so marked with extreme temperatures, the highest values being 27.2 at 13° and 26.7 at 31°. This is almost certainly due to an increase in double cross-overs largely neutralising the increase in single cross-overs, which alone are visible. This was in chromosome II. In chromosome III., a similar result was obtained with high temperature for the genes sepia-spineless, the normal c.o.v. being much increased (28.7 to 34.2 per cent.; 26.1 to 39.0, 25.7 to 33.5 per cent. in three successive lots of offspring from the same control and experimental pairs).

Careful experiments by Plough (1917), Bridges (1915), etc., on the effects of age (summarised in Bridges and Morgan, 1919, p. 194), show that in the black-purple region of chromosome II. the initial c.o.v. is about 8 per cent.; this falls at first rapidly, then more slowly to about 5 per cent. on the ninth day, remains constant till about the sixteenth day, rises again to 8 per cent. on the twenty-first day, and sinks to its minimum of 3.5 per cent. on the thirtieth day. Similar effects probably hold for a number of genes. In any case the *initial drop* has been noted for many c.o.v.'s.

We now have to consider the relation between the genetic and cytological facts so far as it concerns the mechanism of crossing-over in *Gammarus*. For our present purpose we may sum up the facts in *Drosophila* as follows.

Crossing-over between genes in the same chromosome in *Drosophila* is brought about by breaks which may occur at any point within the length of the chromosome.

Although there is a modal value for loop-length—*i.e.* for the distance between two successive breaks in one and the same chromosome, yet this fact does not lead, as might be supposed, to mean c.o.v.'s of over 50 per cent. for genes whose distance from each other is just greater than the mean loop-length.\*

The reason for this is (1) that the loop-length is very variable; but much more (2) *that a large number of pairs of*

\* Jennings (1923) points out that if interference extended to more than 30 units, cross-over values of over 50 per cent. would be expected.

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*chromosomes do not exchange portions at all before gamete-formation*, but pass unchanged into the gamete nuclei. For instance, the moderately long X- or 1st chromosome, according to Morgan (1922, p. 192), only breaks in 57 cases out of every 100, the other 43 per cent. of chromosomes passing unbroken into the gametes. Müller (1916, p. 366); in an actual experiment involving 57 units out of the 68 at present known as the "length" of the 1st chromosome, obtained 54.4 per cent. of cases when there were apparently no breaks. Although he employed no fewer than 12 genes within this distance, some of the distances between successive genes were large enough to allow a few undetected double cross-overs, so that the percentage of non-cross-overs should be reduced to probably just over 50. To avoid confusion, it should be remembered that as some chromosomes break twice and even three times, the *number of breaks per 100 chromosomes* will be larger than the *percentage of chromosomes which break*—e.g. the total breaks per 100 chromosomes in the above experiment was 50.0 per cent. as against 45.8 per cent. of broken chromosomes, and in the whole chromosome-length is 73 as against 57 per cent.

The 2nd and 3rd chromosomes are longer, and therefore show fewer non-cross-overs (absences of breakage). Müller (*loc. cit.*, p. 422), dealing with nine loci covering 105.5 units of distance in chromosome II., found 32.5 per cent. of unbroken chromosomes, 67.6 per cent. cases of chromosomes breaking, and 85.4 breaks per 100 chromosomes. Because of undetected double cross-overs, the percentage of non-cross-overs must be slightly reduced, but by certainly well under 5 per cent.

In the 3rd chromosome, Plough (1921), working with five loci covering 61.2 units out of the total distance of just over 100 units, obtained 50.2 per cent. of cases of non-cross-over (in the first ten days of his controls).

In *D. obscura* the sex chromosome has a length of about 170 units. Lancefield (1922) in a cross involving four loci found a correspondingly low proportion of non-cross-overs—21 per cent. (p. 348).

Finally, the short 4th chromosome of *D. melanogaster* probably passes unbroken into the gametes in *at least* 90 per cent. of cases.

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I emphasise these facts, because I do not think they are sufficiently realised in this country; and they obviously have an important bearing on our ideas of the cytological basis of crossing-over.

What are the consequences for our purpose? Since (a) even in the long chromosomes, in about a third of the cases, all factors remain coupled in chromosomes which pass unbroken through to the gametes, and (b) the length of the loop between successive cross-over points, although it has a modal value, is extremely variable, it follows that in a population unselected for factors modifying loop-length, even if two genes are taken which are separated by a distance just greater than this modal length, yet they will remain linked (1) in at least 30 per cent. of cases through failure of the chromosome to break, (2) in another considerable percentage of cases in which a break occurs, but not between the loci in question, and (3) in still further cases through double crossing-over when the loop is considerably below its modal value.

Finally, since the modal loop-length so far as known at present is less than 50 units (46 units in chromosome I. (Weinstein, 1918), 25 to 30 units in chromosome III. (Gowen, 1919)), it follows that so soon as the percentage of crossing-over equals the distance of the modal loop-length, any further increase will be checked by double crossing-over, which will *reduce* the percentage of times that the two genes are actually separated.

Even if the modal loop-length were over 50 per cent., it is almost certain that this would have as effect a large increase in the number of non-cross-overs (these being under one aspect cases of extreme plus variation in loop-length), so that here again it would be impossible to separate a pair of factors more often than they remained together.

C.o.v.'s of 50 per cent. are only found for loci separated by a considerable distance—*e.g.* in chromosome III. by 70 to 80 units of map-distance (Morgan, Sturtevant, and Bridges, 1922). It is probable that the recorded observations of c.o.v.'s greater than 50 per cent. are due to errors of random sampling. This at least appears to be the opinion of Morgan and his associates, to judge from fig. 2 of Morgan, Sturtevant, and Bridges (1922). These authors introduce the term "re-

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combination percentage" for the actual c.o.v. between any two genes, as opposed to the map-distance between them. Their figures show the recombination percentage for all pairs of genes approximating to 50 per cent. as a limiting value.

The highest cross-over value so far found for genes located in the 4th chromosome is about 1 per cent.

The main fact which emerges from this discussion is that in *Drosophila*, genes must be separated by a considerable length of chromosome before high cross-over values are found: linkage is almost complete for genes located in small dot-like chromosomes such as the 4th.

Now *Gammarus chevreuxi* possesses only small dot-like chromosomes not unlike the 4th chromosome of *Drosophila melanogaster*; and yet it shows high cross-over values for a pair of linked genes.

This might mean that the condensation of chromosomes was very great and that they did exist at other stages in an elongated thread-form permitting marked crossing-over. But, since this does not hold in the properly investigated case of *Drosophila*, we are justified for the present in assuming that some mechanism for linkage and crossing-over is here at work which is in important respects different from that so brilliantly worked out for *Drosophila* by the Morgan school. It remains to ask what other mechanism can be postulated? We cannot at the present stage do more than hint at possibilities. It is, however, important to notice that Seiler has demonstrated a cytological mechanism in two species of moths which might serve as the basis of a modified form of crossing-over, although it would not serve to explain the facts in *Gammarus*.

In *Lymantria monacha* (Seiler and Haniel, 1922), the male and female differ in their chromosomal behaviour. For both,  $n = 31$  in embryonic cells and gonads, and presumably in all intermediate stages. The chromosomes are small or moderate in size, never elongated. In the male, the spermatocytes show only 28 chromosome pairs, and 28 remains as the haploid number throughout maturation. This reduction in number appears definitely to be effected by the end-to-end union of 4 of the 31 chromosomes existing in earlier stages into one large compound chromosome, which is a prominent object

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during maturation. In the female, however, the original haploid number of 31 is retained through the 1st maturation (reduction) division; but in interkinesis a similar large chromosome to that seen in the male appears, and the number is found to have been reduced to 28 for the 2nd division.

In *Solenobia pineti*, on the other hand, Seiler (1922) finds three different haploid chromosome numbers in both male and female maturation—30, 31, and 32. Only one number is found in any one specimen, with rare exceptions in the ♀. There is no unpaired X, nor a visibly different XY pair, in the female. In the embryos he found the diploid number, out of 48 certain cases, to be 61 (18 cases), 62 (29 cases) or 63 (1 case). Although all the embryos are invariably the product of a single copulation, yet in each brood there are at least two different diploid numbers. Occasionally the same embryo will show two or even three different diploid numbers in different blastoderm mitoses. Some of these had 60 as the number for some of their mitoses. Seiler's cytological work is so careful that we may feel confidence in his facts. What is the explanation? He supposes that there are three "chromosome races" of the species, with  $n = 30, 31,$  and  $32$  respectively (the latter was rare in his material, and 31 the commonest figure—24 specimens with 30, 51 with 31, 6 with 32).

A similar state of affairs he had previously found in *Phragmatobia fuliginosa* (Seiler, 1917), where there are two chromosome-races with  $n = 28$  and  $29$  respectively. But in this species, although the two races interbreed freely, segregation then takes place *at reduction* into cells with 28 and 29 chromosomes, and "hybrid" tetrads can be recognised in the pre-maturation stages.

Seiler advances the hypothesis, which certainly seems the simplest covering the facts, that in *S. pineti* (1) the three "races" differ from each other in respect of 3 chromosomes, which may be all separate ( $n = 32$ ), all united ( $n = 30$ ), or 2 united and 1 separate ( $n = 31$ ); (2) that when members of different races cross, one type of chromosome union is dominant over the other during maturation, so that all the tetrads look perfectly normal, with similar homologous halves, and there is no segregation into the original two chromosome numbers

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during 1st or 2nd maturation divisions. When, however, somatic mitoses start in the embryo, the influence exerted by the dominant grouping comes to an end, and this "segregation" becomes apparent in the fact that different embryos from the same pair may have different chromosome numbers.

It may be mentioned that the note on p. 191 of Morgan (1922), while apparently referring to this piece of work, is not correct in its statement of Seiler's facts. It would be more pertinent if it referred to the work on *Lymantria*.

It is interesting to find an essentially similar state of affairs in a wholly different organism. Kuwada (1919), in a paper apparently unknown to Seiler, has shown that in different races of maize (*Zea mays*) the haploid number of chromosomes as seen at reduction may be 10, 11, or 12. The diploid number in root-tip mitoses may be 20, 21, 22, 23, or 24. Chromosome measurements indicate that, as Seiler supposes for his moth, the different haploid numbers are due to different degrees of end-to-end fusion. Three races appear to exist: one (X) in which 4 chromosomes (call them *a*, *b*, *c*, and *d*) are separate, and  $n = 12$ ; one (Y) in which these are inseparably united into two longer chromosomes (*ab* and *cd*); and one (Z) in which this same union has occurred, but is not so intense.

If race X is crossed with race Z, the X condition usually dominates, and 12 gemini are formed. If X is crossed with Y, the Y condition dominates, and there are 10. Plants with 11 gemini arise as recombinations after crossing. Kuwada also makes the point that his results might be applied to the explanation of linkage phenomena.

Seiler points out that in *Solenobia*, if one only considered the reduction division, there would be complete linkage between factors in the three chromosomes in question in the 30-chromosome race, free assortment between these three factor-groups in the 32-chromosome race, partial linkage in the 31-chromosome race. Given certain percentages of the three races in the general population, random mating would give, as statistical result, a certain percentage of "cross-overs" and the rest "non-cross-overs." However, it is important to emphasize a fact that seems to have escaped Seiler's notice, that no crossing-over would be obtained in offspring of a single pair, but crossing-over

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would only be a statistical phenomenon observed in the offspring of a number of genetically distinct pairs.

In *Lymantria*, provided that union of the small chromosomes to form the large one in the male were more likely to occur in certain ways than in others, then we should get free assortment in the female between the four factor-groups concerned, but in the male a certain degree of linkage, which would be complete if the two compound chromosomes contained only paternal and only maternal constituents respectively.

Seiler wishes to establish a general theory of linkage and "crossing-over" on this sort of basis. It is worth, however, pointing out quite definitely at this stage that such a theory cannot possibly apply to the genetic facts known in *Drosophila*, since in that species the breaks may occur at any point along a factor-group, whereas Seiler's hypothesis, without the addition of modifications which would seriously reduce its cogency, would imply that breaks should only occur at a few definite points. It could further not account for the facts of interference, or the *graded* way in which the frequency of double crossing-over increases as one passes away from a first point of breakage, as does the theory of Morgan. When Seiler (1922, p. 213) adduces the fact of interference in *Drosophila* in his favour, which is based on the observation that not single genes but whole blocks of genes are exchanged at crossing-over, he does not take into account that these blocks of genes are not always the same, but that the chromosome may be cut up into a varying number of such blocks in an unlimited number of ways. Finally, that part of it based on the *Solenobia* work fails to explain crossing-over as observed in offspring from a single pair.

It does, however, warn us to admit that phenomena similar in their general genetic results to linkage and crossing-over in *Drosophila* could quite well be brought about by other methods.

The reduplication theory of Bateson and Punnett, and the variable force hypothesis of Goldschmidt (1917) are the only other theories propounded to account for the genetic facts. They both lack experimental or observational basis. The former has been shown to be untenable on many grounds (see *e.g.* Morgan, 1919); the latter will not work except with several subsidiary

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hypotheses (see Bridges, 1917). However, neither the Seiler nor the Morgan scheme appears to be tenable for Gammarus. On the other hand, if (as a pure speculation) numbers of paternal and maternal chromosomes were in Gammarus united end-to-end into large compound units at the leptotene stage, and the subsequent arrangement upon the spindle were determined partly by this previous arrangement (which would give "non-cross-overs") and partly by attractions or repulsions between the various smaller units (attraction between paternal unit-chromosome A and maternal unit-chromosome B, e.g. giving "crossing-over" for the factor-groups concerned), we should have a scheme which would provide the possibility of linkage and high cross-over values for genes within these *chromosome-groups*.

However, it is not at present profitable to discuss the theoretical possibilities further.

The variation of crossing-over with age is also of interest. Bridges (1915) has found in *Drosophila* exactly the reverse of what I have in Gammarus—in the Crustacean, linkage is most intense in the first three as against the subsequent broods, in the insect it is more intense in the second than in the first. In view of the great differences in development, length of life, etc., between the two forms, this is perhaps not surprising. A similar state of things has been found in fowls by Haldane (unpublished). Mr Haldane informs me that so far as his data now go, linkage-intensity is much reduced in the sex chromosome of the fowl after the first year.

Since the above was in print, I have found that Goldschmidt (1923, *Arch. f. Zellforsch.*, 17, 167) has amplified Seiler's facts and theoretical ideas. He finds that in *Lymantria dispar* the above-mentioned existence of one compound chromosome in the male's maturation is merely the remains of a more extensive similar process in the prophase of maturation, whereby the 31 bivalents become grouped in a small number (9 or 10) of compound chromosomes. These arrange themselves on the spindle and then split up into single dot-like chromosomes. Somewhat similar behaviour is shown in the female and (although to a lesser extent) in other species. This might obviously have important genetic consequences.



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Goldschmidt draws the same general conclusions as Seiler, so that the same criticisms apply to both (in particular, failure of the mechanism to account for crossing-over at any point—unless the further assumption be made that the single chromosomes are not homologous from individual to individual, in which case it is difficult to understand why the number should be constant). It is, however, clear that the possibility of a different type of linkage from that in *Drosophila* is given by such a mechanism.

Goldschmidt also refers to another paper bearing on the subject, which I had missed—viz., Gates and Rees, 1921, *Ann. Bot.*, 35. The authors find occasional end-to-end union of chromosomes in *Lactuca*, and also draw attention to the bearing of the fact on theories of crossing-over.

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### 5. Summary.

1. In the Amphipod *Gammarus chevreuxi*, the cross-over value between the loci of two mutant genes (red and albino), for the 4528 offspring of 33 pair matings continued for 3 to 10 broods, was 44.69 per cent.

2. The haploid number of chromosomes is about 20; they are small, round, or ovoid.

3. In view of the small size of the chromosomes in *G. chevreuxi*, it is concluded that the cytological basis of crossing-over in this species is probably different in essential respects from that found in *Drosophila*, where it appears that a considerable actual length of chromosome must separate loci before they can show high cross-over values. Work is being continued on the subject.

4. In addition to the effect previously reported to be exerted by temperature on crossing-over in this species, it is

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found that age also modifies the process. The percentage of crossing-over is low in the first three broods, and rises later. This is the reverse of what is found in *Drosophila*.

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