AN ANALYSIS OF CHROMOSOMAL POLYMORPHISM IN TWO SPECIES OF *CHIRONOMUS*

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(With Plate 6 and Two Text-figures)

The genic pattern of chromosomes is less stable than had been assumed in earlier work. New arrangements appear by means of segmental interchange and inversions, and these may persist in the populations side by side with the original ones. This constitutes a polymorphism comparable with the one affecting external features, and its geographical distribution will follow laws similar to those postulated for morphological characters. Chromosomal mutants possessing survival value under certain climatic conditions will have habitats along a cline where these conditions prevail, whereas neutral forms will occur at random throughout the area inhabited by the species. The mode of achieving this random distribution depends on the ecology of the species which may be expressed in terms of migration, population size, or mutation. It has not yet been possible to observe the speed with which a chromosomal change spreads in a species.

Chromosomal variations may be decisive for the evolution of a new species. It is doubtful, however, whether the well-established polymorphism described in this paper fulfils any such function. The incidence of inversions in isolated breeding groups of two species of *Chironomus* will be correlated with spermatogenesis.

Since the systematics of the group is in a state of confusion due to the high variability of the male adults, on which classification is based, it is difficult to assign a given individual with certainty to any one species. The existing keys (Goetghebuer in the *Faune de France* and Edwards in his paper on the non-biting midges of Britain) take external features only into account. But internal anatomical differences do exist (Melland, 1941) and will certainly be helpful in any future classification of the species. Table I gives the list of the species I have observed. It must be treated with caution, since no expert has checked it owing to the war.

Table 1. Chironomus *species cytologically examined*

Species	Mitosis	Meiosis	Polytene nuclei
Ch. Dorsalis	×	×	×
Ch. Thummi	×	×	×
Ch. riparius	—	×	×
Ch. longipes	—	—	×
Ch. cingulatus	—	—	×
Cryptochironomus sp.	—	×	×

The amount of intrachromosomal variability in this genus is surprising in view of the interspecific similarity in the mitotic, meiotic and polytene complements. The basic number in the Chironomidae is four. In my material I have observed the spermatogonia of *Chironomus dorsalis*

and *C. Thummi*. There are three pairs of long chromosomes with a more or less median centromere and one pair of short ones where it is subterminal. The chromosomes at metaphase show somatic pairing typical of most Diptera.

The mitotic chromosomes of *C. plumosus* in the cells of the suboesophageal ganglion, as drawn by Melland, are indistinguishable from those of *C. dorsalis* and *C. Thummi*. Unfortunately Bauer has been unable to find any mitotic divisions.

The polytene complement was investigated in the salivary glands. There are four elements corresponding to the four pairs of mitotic chromosomes. Of these, three are long and one short, and they lie free inside the nucleus unconnected in a chromocentre. The short chromosome IV is characterized by the nucleolar organizer and the granular Balbiani ring, a structure whose function is as yet unknown. The clear, kidney-shaped nucleolus often forces the chromonemata on both sides of the organizer apart, so that the chromosome appears to lose its continuity.

Text-fig. 1. Spermatogonial metaphase in *Chironomus Thummi*. Beck microscope, obj. $\frac{1}{12}$, ocul. × 35.

The nucleolus in these Diptera is formed at a particular locus of the chromosome, as in plants. The organizer may lie in the euchromatic sections, as in *Chironomus*, or in the heterochromatic portions, as in *Drosophila*.

The salivary glands were fixed and stained with orcein or lacmoid indicator in acetic acid. The slides were made semi-permanent by surrounding them with Zirele's gelatine solution, the carmine in his recipe being substituted by orcein or lacmoid indicator. Permanent preparations were made as follows: The temporary slides were soaked in 10% acetic acid, transferred to absolute alcohol, and after two changes of cedarwood oil, embedded in thick cedarwood oil. I wish to thank L. La Cour for constant help in adapting his method to this material. The preparations were photographed with a Beck microscope using a 1/12 objective and ×25 ocular. I should like to thank H. Osterstock and L. La Cour for their kind help in making the photographs shown on Pl. 6.

Analysis of the populations

I investigated two isolated populations of *C. dorsalis* and one of *C. riparius* for the incidence of inversions. Bauer's photographs suggest that his species *Chironomus* I is identical with my *C. riparius*. This conclusion is based on the similarity of the aberrations in the two samples.

I have therefore included his data in my paper. The larvae were collected irrespective of size, but preparations were made from fourth-instar larvae only, preferably just prior to the prepupal stage, when the nuclei have reached their optimum development. In earlier stages they fail to spread well on the slide, while in the later stages the gland may have started to degenerate.

One population of *C. dorsalis* came from a small lily pond in the Manor House garden at Merton, Surrey (forty-two individuals cytologically analysed), while the second was from a water butt in Chesham Bois, Buckinghamshire (thirty individuals).

I had hoped to follow up the Merton population throughout the summer. Collection began on 14 May 1941, but by 6 June the supply was exhausted, the pond remaining free of *Chironomus* during the following months. The last collection was attempted towards the end of September. The winter had been mild but prolonged and the summer exceptionally wet, and this abnormal weather may have been responsible for the absence of larvae during the summer. Larvae of other species were found within a few hundred yards of this pond. The Chesham Bois population was caught during August and September 1941.

In both populations each of the four chromosomes were found in two sequences. Two of the long chromosomes (II and III) had subterminal inversions, while the third (I) had one almost terminal loop and a second flat loop in the distal third of its length. The distal loop often failed to pair, so that the chromosome appeared branched. Dobzhansky & Sturtevant (1938) interpreted configurations of this type as a small inversion within a larger one.

The normal sequence *A : B C D E F G H I J : K* has been inverted from *B* to *J*. In this new sequence *A (J : I H G F : E D C B) K*, the segment *I–F* has again undergone inversion, giving rise to a chromosome with the sequence *A (J (E F G H I) D C B) K*. The original chromosome may pair with the doubly inverted one as follows:

A B C D E F G H I K
A J E F G H I D C B K

The temporal order in which the inversions occur is irrelevant. This configuration is a fairly stable one, since it can only revert to each of the two original inversions if crossing-over occurs simultaneously in sections *J* and *B C D*.

A similar configuration with two loops may appear if a short segment has been translocated within the chromosome.

In the normal sequence *A : B C D : E F G H I J K*, the segment *B–D* is inserted the wrong way round between *J* and *K*. The configuration in the heterozygote therefore becomes

A B C D E F G H I J K
A E F G H I J D C B K

Thus when pairing is complete *B C D* should form a perfect loop over the space between bands *A* and *E*, as in Bridges's (1938) illustrations. In the double included inversion the chromosomal segment opposite the loop is genetically quite different. It is impossible to distinguish the two abnormalities if one of the points of breakage of the first inversion coincides with either one of the second. If the smaller inversion lies approximately in the middle of the larger one, the unpaired segments will not form loops. Inversions of this type may be responsible for the lack of pairing in *Drosophila miranda-pseudoobscura* hybrids.

Since in chromosome I of *Chironomus dorsalis*, the loops, though rather small, are always flat, I assume that it has undergone double inversion but not translocation. The intermediate forms with one small

Table 2. *The composition of two populations of* Chironomus dorsalis

No. of inversion	Type	Merton population			Chesham Bois population		
		Obs.	Exp.	χ^2	Obs.	Exp.	χ^2
0	—	6	9·35	1·20	5	3·99	0·25
1	I	6	2·92	3·25	1	2·76	1·08
	II	—	0·23	0·23	3	3·99	0·24
	III	7	7·01	0·00	2	1·71	0·04
	IV	11	8·50	0·73	1	2·31	0·74
2	I, II	—	0·07	0·07	6	2·76	3·80
	I, III	2	2·19	0·00	—	1·14	1·14
	I, IV	1	2·66	1·04	3	1·51	0·06
	II, III	1	0·23	2·57	2	1·71	0·24
	II, IV	—	0·05	0·05	2	2·31	0·04
	III, IV	7	6·38	0·06	2	0·99	1·03
3	I, II, III	—	0·05	0·05	—	1·14	1·14
	I, II, IV	—	0·06	0·06	—	1·51	1·51
	I, III, IV	1	2·01	0·51	1	0·66	0·17
	II, III, IV	—	0·15	0·15	1	0·99	0·00
4	I, II, III, IV	—	0·05	0·05	1	0·66	0·17
Sum		42	—	10·02	30	—	12·56
15 degrees of freedom				$P=0·8$			$P=0·6$

Long chromosomes: I, double included inversion; II, subterminal inversion; III, terminal inversion.
Short chromosome: IV, median inversion.

or one large inversion, do not occur in my material. Bauer found a similar, and possibly identical, double inversion in *C. dorsalis* from northern Germany, which he states is rare, but he gives no figures.

In chromosome IV the middle section, which includes the nucleolar organizer and the Balbiani ring, is inverted. This chromosome has two euchromatic regions, the larger one with a constriction half-way down its length. The nucleolus may lie near one or other of these regions, that is to say, it may be submedian or subterminal. In the heterozygote a typical inversion loop is formed. Because the inverted region is relatively small, and pairing near the nucleolus often incomplete, the inverted middle portions are frequently unpaired.

It has not yet been possible to score the two homozygotes of two such sequences separately. Usually the pattern along the chromosome is not sufficiently striking to permit classification with any certainty. It is possible, in chromosome IV, to determine the incidence of the two

arrangements by direct observation. If equal viability is assumed, the ratio of the two types of chromosomes can always be calculated from the proportion of homozygotes to heterozygotes, but deviation from this assumption cannot be detected unless the number of heterozygotes exceeds that of homozygotes.

Table 2 gives a detailed survey of the two populations of *C. dorsalis* arranged according to the type and number of inversions present. Expectations were calculated on the assumption that the chromosomes would combine at random. There is no significant difference between observed and expected values in a single class. χ^2 in combination I, II is 3·8. This result is to be expected in one case out of twenty on the basis of chance alone. The actual number of observations was 32. The deviation in the I, II class is of no importance, since χ^2 taken for the whole Merton population is 10·02, $P=0{\cdot}8$ for 15 degrees of freedom, and for Chesham Bois population $\chi^2=12{\cdot}56$, $P=0{\cdot}6$. Thus within each breeding group the different types of chromosomes associate at random.

More information may be gained by investigating the association of any two chromosomes in single heterozygotes, double heterozygotes, and double homozygotes. Values of χ from 2×2 contingency tables for any pair of chromosomes, with the probabilities, are given in Table 3.

Table 3. *χ of contingency tables for any two inversion heterozygotes in* Chironomus dorsalis

	Merton population			Chesham Bois population		
Combination	χ	P	Excess	χ	P	Excess
I, II	—	—	—	0·3728	0·71	—
I, III	0·5753	0·57	—	0·7308	0·46	—
I, IV	1·6407	0·11	—	0·0775	0·94	—
II, III	—	0·43*	—	0·0000	—	—
II, IV	—	—	—	0·7576	0·45	—
III, IV	0·0447	0·97	—	0·9920	0·32	—

* P estimated directly from the marginal totals.

In the combination II, III the probability has been estimated directly from the marginal totals, as two of the classes are very small (Fisher, 1935). The variation of χ is due to random sampling.

The distribution of classes in which the alternative sequences may occur must now be tested to determine whether this polymorphism is maintained as a result of a specially viable heterozygote. In *C. dorsalis* the three classes for chromosome IV can be scored directly, and the ratio of the two sequences, u, can be calculated by maximum likelihood,

$$u=\frac{2a+b}{b+2c},$$

where a is the homozygote with the median nucleolus, b is the heterozygote, and c is the homozygote with the subterminal nucleolus (Haldane, 1938).

Table 4 gives an analysis of the data. The observed numbers agree well with the expectations calculated on the assumption of random mating. Both classes of homozygotes are as viable as the heterozygotes.

Table 4. *Incidence of the two homozygotes and the heterozygote of two sequences of chromosome IV in* Chironomus dorsalis

	Median nucleolus (*a*)			Heterozygotes (*b*)		
	Obs.	Exp.	χ^2	Obs.	Exp.	χ^2
Merton population	7	6·5	0·15	8	9·9	0·36
Chesham Bois population	15	14·01	0·07	11	12·98	0·30
	Subterminal nucleolus (*c*)					
	Obs.	Exp.	χ^2	$\Sigma\chi^2$	P	u
Merton population	5	4·05	0·22	0·73	0·4	$1·22 \pm 0·45$
Chesham Bois population	4	3·01	0·33	0·70	0·4	$2·16 \pm 0·69$

As the difference between the homozygotes was not recognized until later in the analysis only twenty out of the forty-two individuals were properly scored. It is unlikely that the other twenty-two differ much in the ratio of the homozygotes. Hence a more accurate estimation of u was made as follows:

The sample falls into two groups: (1) homozygotes scored jointly (twenty animals) and (2) homozygotes scored separately (twenty-two animals). The classes in group 1 are the same as above (a, b, and c). In group 1 the homozygotes are put as d and the heterozygotes as e.

The estimates for the five classes are:

$$E(a) = \frac{u^2}{(u+1)^2}(a+b+c), \quad E(d) = \frac{u^2+1}{(u+1)^2}(d+e),$$

$$E(b) = \frac{2u}{(u+1)^2}(a+b+c), \quad E(e) = \frac{2u}{(u+1)^2}(d+e).$$

$$E(c) = \frac{1}{(u+1)^2}(a+b+c),$$

The logarithm of likelihood is

$$L = 2a \log u + b \log u - 2(a+b+c) \log (u+1) + d \log (u^2+1) + e \log u - 2(d+e) \log (u+1);$$

therefore

$$\frac{dL}{du} = \frac{2a+b+e}{u} + \frac{2du}{u^2+1} - \frac{2(a+b+c+d+e)}{(u+1)} = 0,$$

and

$$u^3(a+2c+e) - u^2(2a+b+2d+e) + u(b+2c+2d+e) - (2a+b+e) = 0.$$

The variance of the expression is

$$\frac{-(u+1)^3}{4u(a+b+c+d+e)}.$$

In this sample $a=7$, $b=8$, $c=5$, $d=10$, $e=11$. By approximation $u=1·18$.

Though it is possible to estimate the ratio u for the various forms of the long chromosomes, this estimate cannot be checked, since the two

homozygotes must be scored together. The frequency p of the prevalent sequence and the frequency of the heterozygote were used to compare the two populations.

To estimate p put the sum of the homozygotes $AA+BB=m$ and the number of heterozygotes $AB=l$. The expectation for $AA+BB$ becomes $np^2-n\,(1-p)^2$ and for AB $2np\,(1-p)$:

$$L=\log p^2-(1-p)^2m-\log 2p\,(1-p),$$

$$\frac{dl}{dp}=-\frac{2m\,(1-2p)}{p^2-(1-p)^2}-\frac{1\,(1-2p)}{p\,(1-p)}=0;$$

$$p=0{\cdot}5\,\frac{n-2}{4n}.$$

The frequency p is listed in Table 5.

Table 5. *Estimate of frequency p of the commoner sequence in* Chironomus dorsalis

	I	II	III	IV
Merton population	0·8619	0·9879	0·6892	0·6091
Chesham Bois population	0·7236	0·5000	0·8162	0·7583

p varies from 0·5 to 0·99, thus covering the whole possible range. Although each population appears to be a completely balanced entity, the absolute frequencies of the different sequences vary greatly. The incidence of the two types of chromosome IV is similar in both populations, 1·556 for 2 degrees of freedom, $p=0{\cdot}5$ (Table 6).

Table 6. *Comparison between two populations for the three configurations of chromosome IV in* Chironomus dorsalis

	Homozygote (*a*) Medium nucleolus	Heterozygote (*b*)	Homozygote (*c*) Subterminal nucleolus	χ^2	P
Merton population	7	8	5	—	—
Chesham Bois population	15	11	4	—	—
Total	22	19	9	1·556	0·5

There is little difference in the incidence of the heterozygotes in chromosomes I and III, but the difference for chromosome II is highly significant. The probability had to be estimated from the marginal totals (Table 7).

Table 7. *Comparison between two populations of* Chironomus dorsalis

	Merton population $n=42$		Chesham Bois population $n=30$			
Inversion	Obs.	Exp.	Obs.	Exp.	χ	P
I	10	12·8	12	9·2	1·2124	0·23
II	1	9·3	15	6·7	—	$1{\cdot}6\times10^{-6}$
III	18	15·7	9	11·3	0·8660	0·38
IV	20	18·1	11	12·9	0·2168	0·83

The discrepancy of the two populations depends solely on chromosome II. This is probably accidental and therefore of little importance,

since the number of parents in the two communities must needs be limited.

The population of *C. riparius* was collected from the same rain-water butt in Chesham Bois during August and early September 1941. The chromosomes of sixty-three individuals were scored.

The shape of the chromosomes and the types of inversion found suggested the identity of this species with *Chironomus* I, in one population of which Bauer has analysed seventy-seven individuals cytologically. I have treated his data in such a way as to make them comparable with my own. Bauer's colony was from a pond in northern Germany.

Both populations contain five inversions. Chromosome I has one subterminal inversion in each arm, chromosomes II and III both have a single subterminal inversion. In chromosome IV the section containing the nucleolar organizer and the Balbiani ring is inverted. Here again both ends are roughly equal in length, but whereas one is perfectly straight, the other is constricted half-way down its length (cf. Pl. 6, figs. 1, 3). In one homozygote the nucleolus lies near the straight segment, in the other near the constricted segment. As in *C. dorsalis* the heterozygote can be recognized by the typical inversion loop or by the unpaired segment (cf. Pl. 6, fig. 2).

Table 8 analyses the two populations in detail. In the Chesham Bois population the combination I*r*, II, IV occurs in excess of expectation with $\chi^2 = 6{\cdot}91$. χ^2 for the whole of the population is 23·85, P for 31 degrees of freedom is 0·36.

In Bauer's population association is at random with the exception of combination I*r*, III, IV, where $\chi^2 = 5{\cdot}51$, and combination I*l*, II, III, IV, where $\chi^2 = 21{\cdot}62$, which is highly significant. χ^2 for the whole is 47·25 with $P = 0{\cdot}08$ for 31 degrees of freedom.

The tables in Bauer's paper dealing with this population are slightly inconsistent, the frequencies of chromosome II differing in the two tables. This may be due to a misprint.

In Table 9 the different inversions are treated in pairs. It is remarkable that the distribution of I*r* and I*l* in respect of each other is not disturbed in either population. They are in equilibrium and appear to be segregating independently. The figures given in this table serve as a useful check on those in the previous one. In the Chesham Bois population combination I*r*, II, IV was found in excess of expectation. If any of the combinations possessed real advantage, this should be apparent in the contingency tables I*r*, II, or I*r*, III, or II, IV. There is, however, no indication of this.

In Bauer's population the combinations I*r*, III, IV and I*l*, II, III, IV were in excess. This excess reappears in the contingency tables for II, III and III, IV, where there are significantly too many heterozygotes. The meaning of this is not clear, but it suggests that the heterozygotes are somewhat favoured in this species.

An analysis of the incidence of the three classes of chromosome IV

was only possible for the Chesham Bois population. Here the agreement with random mating is still apparent, though not so close as in *C. dorsalis*,

Table 8. *Composition of two populations of* Chironomus riparius

No. of inversion	Type	Bauer's population Obs.	Exp.	χ^2	Chesham Bois population Obs.	Exp.	χ^2
0	—	18	11·8	3·23	10	7·67	0·71
1	*Ir*	4	4·73	0·12	3	5·05	0·83
	Il	6	5·68	0·02	2	1·45	0·21
	II	2	4·15	1·11	—	0·38	0·38
	III	2	6·02	2·68	7	4·41	1·51
	IV	5	6·38	0·30	7	11·66	1·77
2	*Ir*, *Il*	1	2·76	0·71	1	0·95	0·00
	Ir, II	3	1·66	1·08	—	0·25	0·25
	Ir, III	1	2·41	0·83	2	2·90	0·28
	Ir, IV	4	2·56	0·82	10	7·67	0·71
	Il, II	3	2·00	0·51	—	0·07	0·07
	Il, III	3	2·90	0·00	—	0·83	0·83
	Il, IV	1	3·07	1·40	5	2·20	1·57
	II, III	3	2·12	0·37	—	0·22	0·22
	II, IV	1	2·51	0·91	—	0·58	0·58
	III, IV	2	3·26	0·49	6	6·70	0·07
3	*Ir*, *Il*, II	—	0·80	0·80	—	0·14	0·14
	Ir, *Il*, III	1	1·16	0·00	—	0·55	0·55
	Ir, *Il*, IV	2	1·23	0·50	—	1·45	1·45
	Ir, II, III	—	0·85	0·85	—	0·14	0·14
	Ir, II, IV	—	0·90	0·90	2	0·38	6·91
	Ir, III, IV	4	1·31	5·57	5	4·41	0·08
	Il, II, III	1	1·02	0·00	—	0·07	0·07
	Il, II, IV	1	1·08	0·00	—	0·11	0·11
	Il, III, IV	2	1·57	0·12	—	1·26	1·26
	II, III, IV	1	1·15	0·03	1	0·34	1·28
4	*Ir*, *Il*, II, III	1	0·41	0·86	—	0·03	0·03
	Ir, *Il*, II, IV	—	0·43	0·43	—	0·07	0·07
	Ir, *Il*, III, IV	—	0·63	0·63	2	0·83	1·65
	Ir, II, III, IV	1	0·46	0·64	—	0·02	0·02
	Il, II, III, IV	4	0·55	21·62	—	0·06	0·06
5	*Ir*, *Il*, II, III, IV	—	0·22	0·22	—	0·04	0·04
Sum		77	77·06	47·25	63	62·90	23·85
31 degrees of freedom				$P=0·08$			$P=0·36$

Table 9. *χ of contingency tables for any two chromosomes in* Chironomus riparius

Combination	Bauer's population χ		P	Excess	Chesham Bois population χ	P	Excess
Ir, *Il*	1·0296		0·30	—	0·3302	0·74	—
Ir, II	0·2832		0·78	—	1·1045	0·27	—
Ir, III	0·1924		0·85	—	0·1000	0·39	—
Ir, IV	1·4724		0·14	—	1·8000	0·07	—
Il, II	1·3036		0·19	—	—*	0·59	—
Il, III	1·4900		0·14	—	0·8240	0·41	—
Il, IV	0·0233		0·98	—	0·3286	0·74	—
II, III	2·0119	>	0·01	Double heterozygotes	—*	0·70	—
II, IV	0·3040		0·76	—	—*	0·21	—
III, IV	2·0290	>	0·01	Double heterozygotes	0·1000	0·39	—

* P estimated directly from marginal totals.

$\chi^2=3·34$, $P=0·07$ for 1 degree of freedom. The ratio of homozygotes to heterozygotes is 25:38, a fairly large excess of heterozygotes, though not significantly different from equality ($p=0·5$) (Table 10).

The frequency p is set out in Table 11. p for chromosome IV is taken as 0·5.

The difference between the two populations of *C. riparius* is more pronounced than in those of *C. dorsalis*. P for chromosomes I and IV is significant, for chromosome II almost so (Table 12).

Table 10. *Incidence of the two homozygotes and the heterozygote of two sequences of chromosome IV in* Chironomus riparius

	Homozygote (*a*) nucleolus near straight segment			Heterozygote (*b*)		
	Obs.	Exp.	χ^2	Obs.	Exp.	χ^2
Chesham Bois population	18	21·46	0·67	37	30·03	1·52

	Homozygote (*c*) nucleolus near constricted segment					
	Obs.	Exp.	χ^2	$\Sigma\chi^2$	P	u
Chesham Bois population	7	10·49	1·15	3·34	0·07	1·43 ± 0·039

Table 11. *Estimate of frequency p of the commoner sequence in* Chironomus riparius

	I*r*	I*l*	II	III	IV
Bauer's population	0·8271	0·7850	0·8376	0·7733	0·7612
Chesham Bois population	0·7272	0·9135	0·9754	0·7598	0·5 +

* Deviation from $p = 0·5$, $\chi^2 = 2·091$, $P = 0·1$.

Table 12. *Comparison between two populations of* Chironomus riparius

	Bauer's population $n = 77$		Chesham Bois population $n = 63$			
Inversion	Obs.	Exp.	Obs.	Exp.	χ	P
I*r*	22	25·85	25	21·15	1·2042	0·23
I*l*	24	18·70	10	15·30	2·9866	<0·01
II	19	12·10	3	9·90	1·9026	0·06
III	26	26·95	23	22·05	0·1613	0·87
IV	28	36·30	38	29·70	2·6552	0·01

The differences between the populations of each species of *Chironomus* is of the same order as that found in contiguous populations of *Drosophila pseudoobscura*. They can almost certainly be regarded as accidental.

Discussion

In two species of *Chironomus* I have found a very wide distribution of chromosomal patterns. In *C. dorsalis* the same inversions appeared in localities about 50 miles apart. There is some indication also that one of these, an included inversion, was present in a population in northern Germany. Similarly, in *C. riparius* five inversions were found in two widely separated places, England and northern Germany, and these were almost certainly of identical type. This cannot be regarded as in any way unusual. Polymorphism for inversions in the races and subraces of *Drosophila pseudoobscura* covers a comparable area with geographical obstacles almost as severe as the English Channel.

However, the persistence of inversions in a species in the heterozygous form constitutes a real problem. The formation of dicentric, monocentric and acentric fragments with duplications and deficiencies after an uneven number of cross-overs means that fertility is reduced. This necessarily places an inversion heterozygote at a disadvantage. In the genus *Drosophila* the effect of inversions has been neutralized in a special manner. There is no crossing-over in the male, and in the female the nucleus reaching the terminal position in the egg is fertilized. Nuclei held together by inversion bridges (dicentric fragments) lag behind and never reach the terminal position. As a result fertility is not reduced in a female heterozygous for an inversion.

Little is known of the effect of inversions on gamete formation and fertility in other organisms. Theoretically crossing-over within the inversion may affect spermatogenesis in the following way: (*a*) No spermatozoa with abnormal complement are formed, or if formed, they are not functional. (*b*) Functional spermatozoa with abnormal complement are formed. This means that there will be a greater number of inviable zygotes, constituting a serious danger to the fitness of the species. (*c*) All crossing-over takes place outside the inversion, or the inversions lie outside the chiasma-forming regions. This would lead to a reduction in fertility.

Inversions segregating throughout the species, like those described above, must have been subjected to rigorous selection over a wide area for a long time. Thus they could only have established themselves if they were advantageous, or at least neutral. The structure of the population suggests no obvious adaptation for either sequence.

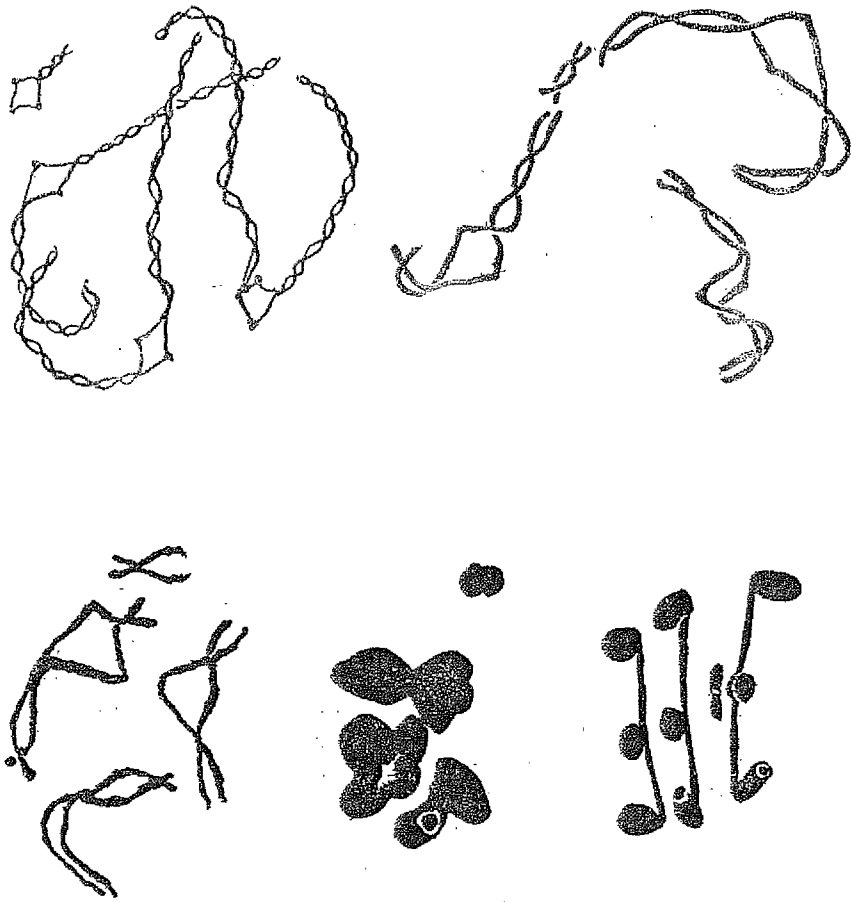
If crossing-over occurred inside the inversion loop and all unbalanced nuclei perished, the reduction in the number of spermatozoa would be considerable. If one inversion were present, instead of all spermatocytes developing into spermatozoa, only half would reach maturity. This reduction would be even more pronounced with four or more inversions, since only one-sixteenth of the gametes would develop. As spermatozoa are produced in such abundance, it is debatable whether a reduction of this order is a serious disadvantage. Work on artificial insemination in mammals suggests, however, that the number of spermatozoa is an adaptation to the anatomical condition of the female. At the time of mating, the spermatozoa are in different stages of maturity, so that only a fraction of those present is able to effect fertilization, and of these, only a few reach the egg. Besides fertilization, the spermatozoa may have some additional function, as for instance in the mare, where they have to free the egg from the surrounding tissue.

In the horse the number of spermatozoa in 1 c.c. of semen varies from 22,500 million (the highest number counted) to 500 million. A reduction to 500 million reduces fertility considerably.

Probably not more than a million spermatozoa are produced in an insect of the size of *Chironomus*. Most of the 2000–3000 eggs laid hatch.

With four inversions and the number of spermatozoa reduced to one-sixteenth, there will only be some 60,000 present, that is to say, 20–30 per egg, one of which must be the right age to effect fertilization. This leaves a very small margin.

The two species could not have accumulated so many inversions without some mechanism to counter the deteriorating effect of crossing-over on the fertility of the inversion heterozygotes.



Text-fig. 2. Meiosis in *Chironomus dorsalis* ♂ (homozygous animal). Beck microscope, obj. 1/12, ocul. ×35.

Preliminary observations on meiosis in *C. dorsalis* and *riparius* show that in diakinesis at metaphase and early anaphase each of the long chromosomes has one terminal and one subterminal chiasma. The longest one occasionally has a terminal chiasma in the other arm. Chromosome IV has one terminal chiasma. There is no terminalization.

As yet it has not been possible to analyse the diplotene and pachytene chromosomes to determine the actual position of the chiasmata in relation to the inversions. We can determine indirectly whether there is any crossing-over inside the inversions by examining the anaphase and telo-

phase of both meiotic divisions for inversion bridges or fragments. In larvae, the salivary glands of which had been fixed, and which were heterozygous for as many as four inversions, no dicentric or acentric fragments were found. I therefore conclude that the inversions in *C. dorsalis* and *riparius* lie outside the chiasma-forming regions. This means that only such inversions where no crossing-over occurs can persist in the population.

Inversions of this type do not represent the beginning of a new line of evolution, but are the neutral end-product of a selection for the most advantageous sequence in the species.

SUMMARY

Two populations of *Chironomus dorsalis* from two English localities and two of *C. riparius*, one from England (my collection) and one from northern Germany (Bauer's collection), were analysed for the incidence of inversions.

The chromosomes of different sequences are combined in individuals at random.

The two homozygotes of chromosome IV could be distinguished from the heterozygote by means of the position of the nucleolus. Analysis of the frequency of the three types showed that mating in the population was at random and that no single type was favoured at the expense of the other.

The difference in the frequency of some of the sequences found is probably accidental, due to the limited size of the populations.

No acentric or dicentric fragments were found in the meiosis of males known to have inversions. It was therefore concluded that they were situated outside the chiasma-forming regions and had in consequence no deleterious effect on fertility.

I wish to thank the director of the John Innes Horticultural Institution, Dr C. D. Darlington, for his help and hospitality during the two years I spent in Merton owing to war conditions. I am grateful to Prof. J. B. S. Haldane for his advice in the mathematical treatment of the data; and to Mrs J. F. Edwards for letting me collect the larvae from her garden in Chesham Bois.

REFERENCES

BAUER, H. (1936). Beiträge zur vergleichenden Morphologie der Speicheldrüsenchromosomen. (Untersuchungen an den Riesenchromosomen der Dipteren. II.) *Zool. Jb.*, Abt. allg. Zool. Phys. Tiere, **56**, 239–76.

DOBZHANSKY, TH. & QUEAL, M. L. (1938). Genetics of natural populations. I. Chromosome variation in populations of *Drosophila pseudoobscura* inhabiting isolated mountain ranges. *Genetics*, **23**, 239–51.

DOBZHANSKY, TH. & STURTEVANT, A. H. (1938). Inversions in the chromosomes of *Drosophila pseudoobscura*. *Genetics*, **23**, 28–64.

Edwards, F. H. (1929). British non-biting midges. *Trans. Ent. Soc. Lond.* **77**, 219–30.

Fisher, R. A. (1935). The logic of inductive inference. *J. R. Statist. Soc.* **18**, 39–82.

Goetghebuer, M. (1932). Diptères chironomidae. III. Chironomaria. *Faune de France.*

Haldane, J. B. S. (1938). Indirect evidence for the mating system in natural populations. *J. Genet.* **36**, 213–20.

King, R. L. & Beams, H. H. (1934). Somatic synapsis in *Chironomus* with special reference to the individuality of the chromosomes. *J. Morph.* **56**, 577–92.

Koller, P. C. (1939). Genetics of natural populations. III. Gene arrangements in populations of *Drosophila pseudoobscura* from contiguous localities. *Genetics*, **24**, 22–33.

Melland, A. (1941). Ph.D. Thesis to Univ. Lond.

Sadler, W. O. (1934). Biology of the midge *Chironomus tentans* Fabricius and methods for its propagation. *Mem. Cornell Agric. Exp. Sta.* no. 173.

EXPLANATION OF PLATE 6

Fig. 1. Chromosome IV in *Chironomus riparius*. Nucleolus near straight euchromatic segment.

Fig. 2. Chromosome IV in *C. riparius*. Heterozygote.

Fig. 3. Chromosome IV in *C. riparius*. Nucleolus near constricted segment.

Fig. 1.

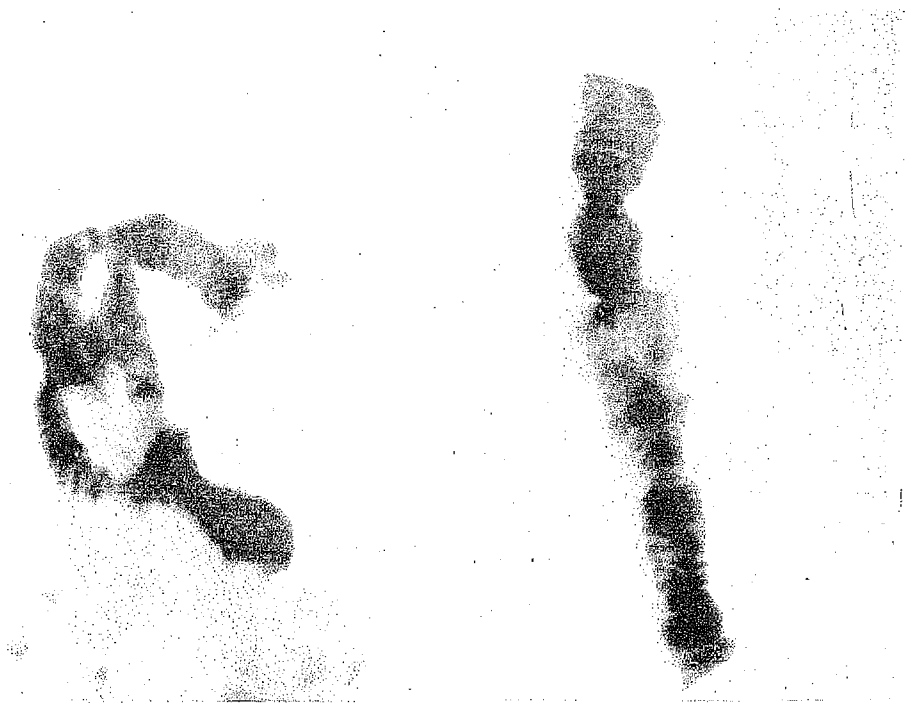


Fig. 2.

Fig. 3.