INTERRELATION OF INVERSION SYSTEMS IN THE MIDGE *CHIRONOMUS INTERTINCTUS* (DIPTERA: NEMATOCERA)

I. A SEX-LINKED INVERSION

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Summary

One of the inversions in *Chironomus (Kiefferulus) intertinctus* Skuse, the Corio inversion in chromosone III, is closely linked to the sex-determining region. Different selective values in males and females result in the Standard sequence being more common in the Y-chromosomes while the Corio sequence is more common in the X-chromosomes. In a population from Echuca, Vic., only the Standard sequence occurs in the Y-chromosomes.

The sex ratio in the larvae differs significantly from a 1 : 1 ratio in all but one collection. In all collections the females were more numerous than males.

I. INTRODUCTION

In recent years an increasing number of cases have been reported which indicate that the various inversions in a population may not be independent of each other or of other factors in the genetic make-up of the population. The most numerous of these cases have been non-random associations of inversions in the same chromosome. A review of these cases is given by Levitan (1958). A somewhat similar phenomenon occurs in those groups, such as the Chironomidae, in which there are no cytologically distinct sex chromosomes and sex determination appears to be due to single genes or groups of genes. In some of these the sex-determining regions are either included in, or closely linked to, an inversion. Cases of this kind have been reported in chironomids (Beermann 1955a, 1955b; Acton 1957a, 1957b, 1957c) and simuliids (P. K. Basrur 1959; Dunbar 1959). The third type of lack of independence has been reported in the Australian grasshopper *Moraba scurra* (White 1957, 1958; Lewontin and White 1960). In this species two inversions on different chromosomes interact. These associations may all be favoured for the reason suggested by White (1958) for the last-mentioned type—i.e. because they improve the viability of certain homozygous classes and therefore increase the reproductive capacity of the population as a whole. If each inversion is independent then the reproductive capacity of the population may be lowered considerably.

The cases so far reported have all been single instances but this series of papers reports a population of *Chironomus (Kiefferulus) intertinctus* Skuse (Diptera: Nematocera) in which the three most common inversions are all tied up in associations. This paper deals with a sex-linked inversion, the Corio inversion on chromosome III, in which the sequences have different frequencies in males and females. The Standard sequence is more common in males and the Corio sequence is more common in females. This effect may be due to epistasis or to a position effect (organization effect). In either case selection probably acts largely to reduce

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crossing over between the inversion and the sex-determining region. It also appears that this may be one stage of an evolutionary series, the ultimate stage being reached when the sex chromosomes become cytologically distinguishable due to one sequence occurring on the X-chromosomes and the alternative sequence on the Y-chromosomes.

II. MATERIALS AND METHODS

Most of the larvae used in this study were collected from a large pool between Wallington and Ocean Grove, on the Bellarine peninsula, about 60 miles south-west of Melbourne. This pool has been permanent at least since May 1959 when it was first sampled. Even following the exceedingly dry summer of 1961–62 the pool did not dry out. Most of the larvae were to be found on bark or leaves which had fallen into the water from a large gum tree in the south-west corner of the pool, or on the leaves of the swamp lily (*Otelia ovalifolia*), especially as it died back in autumn.

TABLE 1

DISTRIBUTION OF THE CORIO INVERSION IN THE FEMALES AND MALES OF THE OCEAN GROVE POPULATION

Method of calculation of expected values is described in the text

Genotype	Pooled Data for Six Collections		Corrected Results			
			Females		Males	
	Females	Males	Observed	Expected	Observed	Expected
St^{Co}/St^{Co}	37	69	37·252	32·164	70·746	70·957
St^{Co}/Co	170	203	170	180·263	203	202·577
Co/Co	256	10	257·748	252·574	10·254	10·465
Unscored homozygotes	2	2	—	—	—	—
Total	465	284	465*		284†	

* $\chi^2 = 1·495$, $P = 0·25$, not significant.   † $\chi^2 = 0·006$, $P > 0·9$, not significant.

The swamp lily was also in the south-west corner of the pool. The sample from Ocean Grove is the pooled result of six collections from November 1960 to January 1961 and January–February 1962. Homogeneity (χ^2) tests on the results for males and females separately showed no significant differences between the collections for the Corio inversion (χ_5^2 for females $= 3·70$, $P = 0·5$; χ_5^2 for males $= 5·05$, $P = 0·4$). There were four homozygous individuals in the early collections which could not be completely identified (Table 1, columns 2 and 3), so for the purposes of analysis these were divided proportionately between the two homozygous classes (Table 1, columns 4 and 6).

Other populations came from Echuca (about 120 miles north of Melbourne) in September 1961, and Chiltern (about 170 miles north-east of Melbourne) in March 1961. Both pools are surrounded by trees which drop leaves and bark into the water, and both appear to be semipermanent.

The living larvae were sexed by examination of the ventral invaginations of the twelfth segment which are sufficiently distinct to enable accurate sexing (Miall and Hammond 1900). This method appears to be much easier than the method used by Acton (1957*b*) of dissecting out the gonads. The salivary glands were dissected, fixed, and stained by the method outlined in Keyl and Keyl (1959) with some slight modifications. After fixation the glands were transferred to 1N HCl for 5 min to reduce cytoplasmic staining (Chu 1946). It was also found that a synthetic orcein (Gurrs) reduced cytoplasmic staining. To enable the slides to be kept for several weeks a drop of 10% glycerine in 45% acetic acid was placed at the margin of the cover-glass and allowed to flow under as the aqueous portion evaporated (Rattenbury 1956).

TABLE 2

OBSERVED AND EXPECTED NUMBERS OF GENOTYPES FOR THE CORIO INVERSION IN THE CHILTERN AND ECHUCA POPULATIONS

Genotype	Chiltern				Echuca			
	Females		Males		Females		Males	
	Obs.	Exp.	Obs.	Exp.	Obs.	Exp.	Obs.	Exp.
St^{Co}/St^{Co}	2	1·78	13	10·27	2	3·8	7	6·4
St^{Co}/Co	24	24·44	65	70·46	22	18·5	15	15·6
Co/Co	84	83·78	3	0·27	21	22·7	—	—
Total	110		81		45		22	

Where it was not possible to use the larvae immediately, they were sexed, fixed whole in the alcohol–acetic acid fixative, and then refrigerated. Material kept at 5°C for 12 months still gave quite good preparations although the fine bands were obscured and the chromosomes did not stretch as readily. The material kept better at temperatures below 0°C. A lactic–orcein stain was found to give better results on chromosomes stored in this manner for more than a week or so. The lactic–orcein stain was the same as that used by Dr. M. Wasserman of this Department in his work on *Drosophila* salivary gland chromosomes. The glands were dissected out and hydrolysed in 1N HCl and then stained for about 15 min. The salivary secretion, which becomes extremely hard after prolonged fixation, must be carefully removed, before squashing the glands.

III. RESULTS

The chromosomes of *C. intertinctus* are numbered in order of size, chromosome I being the longest and chromosome IV the shortest. Chromosome maps, descriptions of the chromosomes, and their inversions will be published in a later paper. The

Corio inversion, near the left end of chromosome III (Fig. 1), has been quite common in all populations so far investigated from Victoria, and also in a sample from Western Australia. None of these populations have shown agreement with Hardy–Weinberg expectation for this inversion in the males, because they show well over 50% heterozygotes. At Ocean Grove 71% of the males, at Chiltern 80%, and at Echuca 68% of the males were heterozygotes. It was also found that while the Corio sequence (Co) was the most common in females, the Standard sequence (St^{Co}) was more common in males (Tables 1 and 2).

The most likely explanation of these results is that the Corio inversion is closely linked to the sex-determining factor. Chironomids have no cytologically recognizable sex chromosomes, but sex determination appears to be due to single genes or groups of genes (Beermann 1955b). The male is the heterogametic sex and certain inversions have been found to occur only in males (Beermann 1955a, 1955b;
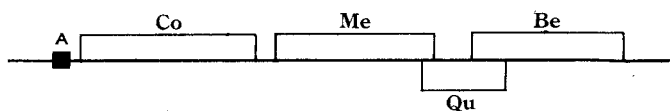

Fig. 1.—The inversions of chromosome III of *C. intertinctus:* *Co*, Corio inversion; *Me*, Melbourne inversion; *Qu*, Queenscliff inversion; *Be*, Bellarine inversion. The probable position of the sex-determining gene is represented by *A*.

Acton 1957a, 1957c). Therefore it is assumed that chromosome III of *C. intertinctus* is the sex chromosome. The frequency of St^{Co} and Co in the X-chromosome can be calculated directly from the females. From these frequencies the frequencies in the male Y-chromosome can be calculated as follows. At Ocean Grove 0·6065 of all chromosomes III in males carry the St^{Co} sequence. From the females it can be estimated that 0·1315 of these carry the sequence in an X-chromosome. Thus 0·4750 will carry the St^{Co} sequence in a Y-chromosome. The estimated frequencies of the sequences in the X- and Y-chromosomes at Ocean Grove were:

	X	Y
St^{Co}	0·263	0·950
Co	0·737	0·050

When these values were used to calculate the expected genotype frequencies they showed very good agreement with the observed values (Table 1, columns 4–7).

The results from the Chiltern and Echuca populations are shown in Table 2. The estimated frequencies for these populations, calculated as above, were:

		X	Y
Chiltern	St^{Co}	0·127	0·996
	Co	0·873	0·004
Echuca	St^{Co}	0·29	1·00
	Co	0·71	0·00

These results also show good agreement between observed and expected genotype frequencies (Table 2).

IV. Discussion

The linkage of the Corio inversion with the sex-determining region appears similar to the case reported for inversions *1R* and *1S* in *C. dorsalis* (Acton 1957b, 1957c). Acton found that *1R* had a high frequency in the Y-chromosome while *1S* had a high frequency in the X-chromosome. He postulated that selection favours the case where *1R* is linked to the male-determining gene and acts against the case where it is linked to the female-determining gene. This would be similar to the phenomenon, called an organization effect by Dobzhansky (1959), reported for the second-chromosome inversions in *Drosophila robusta* (Levitan 1954a, 1958). This would imply that the two types of heterozygous males would have different selective values. Heterozygous males of genotype $X–St^{Co}$, $Y–Co$ would carry two unfavourable combinations, while the $X–Co$, $Y–St^{Co}$ heterozygotes carry two favourable combinations. An alternative explanation is that the presence of St^{Co} in males and of Co in females is favoured by selection. This then would be epistasis. In this case both types of male heterozygote would have the same selective value. Since it has not been possible to breed *C. intertinctus* in the laboratory, it has not been possible to perform experiments to distinguish between the two hypotheses. In either case little or no crossing over would occur between the inversion and the sex-determining factor. If no crossing over occurs at present then it must be assumed that at least one crossover has occurred at some time in the past. The fact that apparently only St^{Co} occurs in the Y-chromosome at Echuca would seem to indicate that in this population, at least, crossing over does not occur at the present time. Selection would presumably act to reduce crossing over since crossing over would tend to equalize the frequencies of St^{Co} and Co in the X- and Y-chromosomes. It seems unlikely that selection acts to regulate the frequencies of the genotypes so that there is a minimum of individuals in which effective crossing over can occur (Levitan 1958), since the only genotypes in which this is possible, the heterozygous males, constitute the bulk of the male genotypes.

A further possible explanation of these results would be that the inversion is on an autosome but selection is different in males and females, similar to the case reported in *D. robusta* by Levitan (1951). This would mean that the same frequencies of each genotype would be produced in the eggs of both sexes in each generation and selection would then act strongly against St^{Co}/St^{Co}, St^{Co}/Co females and Co/Co males in the egg or early larval stages. This would mean that the populations were carrying an extremely heavy genetic burden, far heavier than that carried by the *D. robusta* populations. Since the sex-linkage hypothesis involves only a very slight genetic burden it seems a far more likely explanation. Further evidence that chromosome III is the sex chromosome comes from its other inversions (Fig. 1). The Melbourne inversion has been found only in 2 males, the Queenscliff inversion only in 13 males, while the Bellarine inversion, near the other end of chromosome III, shows no significant deviation between males and females. This suggests that the sex-determining region is near the Corio inversion and may be immediately distal to it (Fig. 1).

A further example of this type appears to occur in *Glyptotendipes barbipes* since inversion *1S–1* has a higher frequency of heterozygotes in males than in females,

in a small sample (V. R. Basrur 1957). Basrur found no *1S–1/1S–1* individuals and so assumed that this genotype was lethal. However, on the assumption of sex linkage, this could be due to an extremely low frequency of *1S–1* in the females.

There appears to be an evolutionary series of stages in the linkage of inversions with the sex-determining regions, from inversions which occur equally in both sexes to the condition where the *X*-chromosome carries one sequence and the *Y*-chromosome the other. Examples of all stages can be found in the literature on Simuliidae and Chironomidae. The examples from *C. intertinctus*, *C. dorsalis*, and *G. barbipes* are the first stage of differentiation, showing only differences in frequency between the sexes. In *Eusimulium aureum* form D (Dunbar 1959) and in *C. tentans* (Acton 1957a) there is only one sequence in the *X*-chromosome but the *Y*-chromosome carries two sequences. The alternative of this stage is shown by *Prosimulium hirtipes* (P. K. Basrur 1959) and the Echuca population of *C. intertinctus*, where there are two sequences in the *X*-chromosome but only one in the *Y*-chromosome. *C. annularius* (Beermann 1955b; Acton 1957c), *P. inflatum* from Scotland (Dunbar 1959), and *C. tentans* from Sweden (Beermann 1955a) show the final condition of having one sequence in the *X*-chromosome and the other in the *Y*-chromosome. The later stages of the series could also arise if the inversion included the sex-determining region, so that actually three evolutionary series could be involved:

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                                       One sequence
                                       eliminated from
(1) Apparent   →  Different fre-    ↗  X-chromosome      ↘  One sequence
    autosomal      quencies in X- and                        in X-, other in
    inversion      Y- chromosomes   ↘  One sequence      ↗  Y-chromosome
                                       eliminated from
                                       Y-chromosome
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(This is the series if the inversions are linked to the sex-determining region.)

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(2) No inversion  →  Inversion in Y-chromosome  →  Standard sequence
                     includes sex genes,            eliminated from
                     Standard sequence              Y-chromosome
                     still present
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(3) No inversion  →  Inversion in X-chromosome  →  Standard sequence
                     includes sex genes,            eliminated from
                     Standard sequence              X-chromosome
                     still present
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Unless the stage showing different frequencies in males and females is present it is impossible to say if the inversion includes the sex-determining region or is closely linked to it. Also there is no way of knowing whether these populations are balanced or whether in time all will proceed to the final condition. Under certain conditions this may happen but in others they may remain in one of the intermediate stages until some environmental change occurs. Different stages have been found in different populations for some of these inversions. Beermann (1955a) found a population of *C. tentans* in Sweden in which all *Y*-chromosomes carried one sequence and all *X*-chromosomes carried the other, i.e. all males were heterozygous, while

Acton (1957*a*, 1957*c*) in England found that only some of the males were heterozygous so that there were still two sequences in the *Y*-chromosome. This inversion could either include the male-determining region or be just linked to it. Two stages are also shown by the populations of *C. intertinctus* and it is possible the other stages may yet be found.

It appears that the deviation from a 1 : 1 sex ratio shown by the populations of *C. intertinctus* is a real effect. All populations show an excess of females over males and in all except the small sample from the Melbourne Botanical Gardens, the deviation is significant (Table 3). This is the reverse of the situation found in

TABLE 3

NUMBER OF MALES AND FEMALES IN COLLECTIONS OF C. INTERTINCTUS

Population	Males	Females	Total	χ_1^2
Ocean Grove				
Observed	413	598	1011	33·86***
Expected	505·5	505·5		
Chiltern				
Observed	81	110	191	4·40*
Expected	95·5	95·5		
Echuca				
Observed	22	47	69	9·06**
Expected	34·5	34·5		
Botanic Gardens				
Observed	7	16	25	1·9†
Expected	12·5	12·5		

* $P = 0·04$.   ** $P = 0·003$.   *** $P > 0·0001$.   † $P = 0·15$, not significant.

C. dorsalis (Acton 1957*b*). Whether these deviations are still present in the adults is unknown, so it may be that selection against males occurs at a different stage of the life cycle from selection against females. Levitan (1954*b*) showed the presence of such differences in sexual selection in *D. robusta*.

V. ACKNOWLEDGMENTS

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