A TRANSLATION OF: "POLYMORPHISM FOR THE MALE SEX REALISERS IN *CHIRONOMUS*"

by S. Rosin and J. Fischer[1,2]

from: Revue Suisse de Zoologie 79: 119-141 (1972)

Up till now little is known of sex determination in Chironomus: BEERMANN (1953,1955a,1955b) has found sex linked inversions in Camptochironomus tentans and C. pallidivittatus and thereby demonstrated genotypic sex determination. A relationship between inversions and sex has been furhter established by ACTON (1957) in Chironomus plumosus and Ch. luridus[3] and by KEYL (1962) in Ch. obtusidens. We have found the same state of affairs in Ch. nuditarsis (ROSIN and FISCHER, 1965).

Heterogamety of the male sex has been demonstrated in C. tentans, C. pallidivittatus and Ch. nuditarsis. In the chironomid Polypedilum nubifer, however, the female has been shown to be heterogametic (MARTIN, 1966).

Already BAUER (1936) has shown that no morphologically distinguishable sex chromosomes exist in Chironomus. When sex linked inversions occur, these in general are linked partly to the male and partly to the female sex. However when certain inversions are found only in the male sex, one can not speak of true sex chromosomes in Chironomus. In the salivary glands all chromosome pairs are structural homologues.

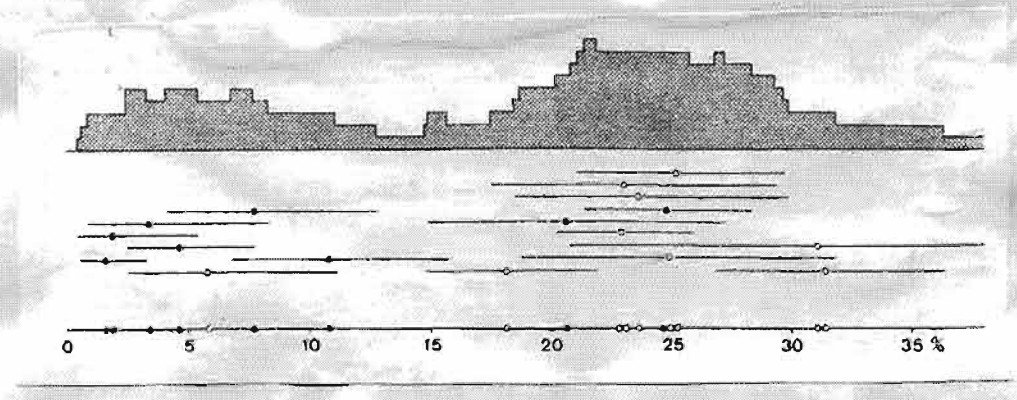

Fig. 1. Crossover values between M and in B of the AB chromosome of Ch. nuditarsis (Table 1). Individual values with 95% confidence limits. Above: Number of confidence limites which a particular crossover value included. The values fall in two groups - Filled circles: A-arm of the male structurally homozygous - Empty circles: A-arm inversion heterozygote

1,2 - not translated

3 The species called Ch. dorsalis by ACTON should be known as Ch. luridus according to KEYL (1959). (TRANSLATORS NOTE).

Sex determination is a cytologically not definable but genetically localisable region which, according to the existing results, can be considered as a point gene locus. As BEERMANN (1955b) has showed, the weakspot differentiator for the male sex can be produced from the background of a balance of male and female determiners "additively, through the addition of a weight from the scale pan of the other sex". The dominant differentiator for male sex is here considered as an individual (locus) and shall be considered in the simple style as additive working, and he signified with the letter M.

BEERMAN (1955b) has found in C. tentans that in the same population two different chromosomes can carry an M-factor. TOKUNAGA (1958) and MAINX (1959,1962,1964) have established a similar condition in breeding the Phorid Megaselia. MAINX has introduced the idea of alternative sex determiners for this. When more than two positions of the M-factor can occur as in the Megaselia case, the idea of "alternative" is not wholly proved. When males which differ in relation to the locus of their M-factor occur side by side in a population, this may be labelled as an M-polymorphism. The formerly designated "Y-locus" (ROSIN and FISCHER 1965) will be replaced by "M-locus", while the letter Y will be reserved for the heterosome.

M-POLYMORPHISM IN *CHIRONOMUS NUDITARSIS* STR.

M_1 lies in the AB chromosome

In Table 1 are summarised the hitherto obtained cases in which a cross-over value between M and In B can be calculated. As Fig. 1 shows the crossover values fall into at least two groups. We will investigate whether the grouping of crossover values indicate several M-loci or whether another explanation can be found fot it. The occurrence of crossovers can be influenced by different factors: temperature, age of the animal, genetic background, presence of inversions outside the studied area in the same chromosome or even in other chromosomes. We have made some studies on the problem of the importance of such factors.

Influence of temperature on crossing over in the AB chromosome

Inversion heterozygore males from a sister stock were allotted to the desired temperature in the third larval instar in each case, for further rearing, and crossed with inversion free females. The crossover values were

Table 1

Crossover value, f, between M and InB in Ch. nuditarsis (with Fig. 1)

Material	Ohne Austausch		Mit Austausch		f		A-Arm
	♀	♂	♀	♂	%	%	
4.804.6	205	188	49	38	18,1	53	ho
5.430.2 [1]	32	98	1	7	5,8	24	he
5.430.6	191	246	4	3	1,6	44	ho
5.711.1	64	72	25	20	24,9	49	he
5.711.13 [1]	16	35	15	8	31,1	44	he
5.804.6	135	132	60	62	31,4	50	he
5.830.9 *a*	159	129	8	6	4,6	55	ho
5.1014.10 *a* [1]	24	132	1	2	1,9	16	ho
Cr 2 F_1	59	54	1	3	3,4	51	ho
Cr 3 F_1	119	79	11	13	10,8	59	ho
Cr 5 P	86	71	6	7	7,7	54	ho
Cr 8 F_2	81	62	24	13	20,6	58	ho
Temp. 1	311	402	80	132	22,9	42	he
Temp. 2	172	306	51	107	24,8	35	ho
Temp. 3 *a*	86	82	30	22	23,6	53	he
Temp. 3 *b*	75	86	22	26	23,0	46	he
Temp. 4	164	144	43	61	25,2	50	he

The first eight cases and Cr5P are bred from wild layings for the years 1964 to 1966, the rest come from laboratory crosses. Temp: from Table 2, collected from 15°C and 20°C — ho: A-arm homozygous - he: A-arm heterozygous

[1] These crosses probably contain a lethal factor

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ascertained from mass rearings of the offspring. The crosses can thus be symbolised as follows: M/InB ♂ +/+ ♀

The results are listed in Table 2. The data cannot be used without restriction for the calculation of the crossover values. The first two series of experiments show a disturbed sex ratio virtually throughout. Since they come from rearings from single layings, we accept that both parents possess a common lethal factor, e.g. M/ℓ InB ♂ +/ℓ ♀. Depending on the position of ℓ the proportion of females can vary between 33% and 66%. For the calculation of the crossover value the presence of a lethal factor to be sure, does not affect it. In experiments 15°C and 25°C of the second series,

however, the proportion of females certainly lies outside the span of 33% to 67%. Such an extreme sex ratio requires at least two identical lethal factors in both parents. The assymetry of numbers of crossover and non-crossover animals, when females and males are examined separately, shows that certainly not just an unspecified inhibition of development of one sex exists. In the case of two lethal factors, one sex can be almost entirely absent if they lie very near to M. If several lethal factors are at work the usual calculation of the crossover value produces a false result. In Table 2 therefore experiments 15°C a and 25°C a of the second series are excluded in the evaluation. In the remaining cases with deviating sex ratios there are no compelling grounds for not considering them.

Table 2

Influence of temperature on the crossover value, f, between M and InB in Ch. nuditarsis

Versuchsserie	Temp.	Ohne Austausch ♀	Ohne Austausch ♂	Mit Austausch ♀	Mit Austausch ♂	f ‰	♀ %	G.V.	As.
1	15°	131	199	39	71	25,0	39	**	—
	20°	180	203	41	61	21,1	45	—	—
	25°	156	193	70	79	29,9	45	*	—
2	10° *a*	54	107	20	58	32,6	31	**	—
	10° *b*	39	98	19	40	30,1	30	**	—
	15° *a*	22	107	7	80	40,3	13	**	*
	15° *b*	66	104	16	33	22,4	37	**	—
	20° *a*	54	127	14	50	26,1	28	**	—
	20° *c*	52	75	21	24	26,1	42	—	—
	25° *a*	208	8	2	34	14,2	83	**	**
	25° *b*	82	113	23	34	22,6	42	**	—
3	15°—20° *a*	86	82	30	22	23,6	53	—	—
	15°—20° *b*	75	86	22	26	23,0	47	—	—
4	10°	64	71	40	31	34,5	50	—	—
	15°	82	68	24	31	26,8	52	—	—
	20°	82	76	19	30	23,7	49	—	—
	25°	160	145	52	67	28,1	50	—	—

G.V.: sex ratio, deviation from 1:1. As: assymetry. Different crossover values for females and males. Experimental series: 1= material 1968; 2= 1968/69; 3 and 4 = 1971.

In all four temperatures the three or four existing experiments are homogeneous. In summary we obtain the following crossover values between M and InB:

Temperature	Crossover value f(M - InB) %	N
10°C	32.4	641
15°C	24.8	864
15°C-20°C	23.3	429
20°C	23.4	1109
25°C	27.65	1174

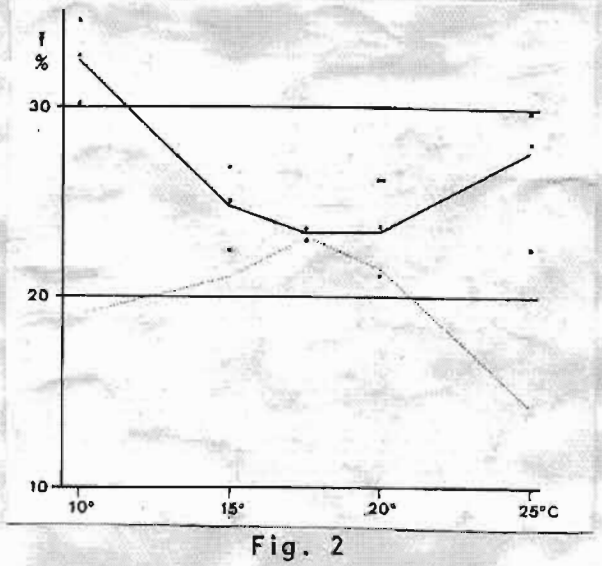

Fig. 2

Crossover value f in the AB chromosome of Ch. nuditarsis in relation to temperature. Points: crossover values between M and InB (Table 2)
Solid line: mid point curve to these data
Dotted line: crossover between InA_2 and M (each one value)

The crossover value has a minimum at about 20°C and is significantly higher at 25°C and also at 10°C. In the experimental series 3 and 4 the crossover value between inversion A_2 and M_1 was simultaneously determined. Interestingly a completely reciprocal result is seen here:

Series	Temperature	Crossover value f(InA_2-M) %	N
4	10°C	19.0	206
4	15°C	21.0	205
3	15-20°C	23.1	429
4	20°C	21.3	207
4	25°C	14.1	213

On account of the small numbers these differences are not significant. The experimental series 3 (without thermostat, mostly 17°C-18°C) fits into the picture in both cases.

Summarising we can hold that in Ch. nuditarsis the crossover frequency in males in the middle region of the AB chromosome is influenced by temperature. The temperature conditioned differences are however relatively small: at 10°C the cross over value between M and InB reaches about 1.4 times the minimum value at 20°C. Crossover values of only 3% instead of 23%-32% (Table 1) cannot be explained by temperature differences unless temperatures under 10°C would unexpectedly lower recombination strongly. However perhaps the heterogeneity between the two crossover groups (Fig. 1) is at least partly temperature conditioned.

Influence of genetic background on recombination in the AB chromosome

We have followed the crossover value between M and InB over several generations in different strains. The temperature in these experiments was held steady at 16.5°C. As is evident from table 3, the crossover value between InB and the same M locus can have statistically different values in different generations. In Cr5 a moderate recombinate of 7.65% was first obtained; in the second and fourth generation the crossover value however, with 24% and 33%, belongs to the higher group in Fig. 1. Also in Cr2, one of the four values lies outside the confidence limits of the other three. Such differences show that the existence of different groups of crossover values can not be due to different M-loci in the AB chromosome. The data under consideration indicate rather that modifier genes can have a considerable influence on the crossover values.

Also the presence of a heterozygous inversion in arm A appears to affect recombination between M and InB. (In Drosophila such an influence has been detected CARSON, 1953). In the cases with a homozygous A-arm the crossover values are smaller on average than the values from experiments in which the A arm was heterozygous for an inversion (Table 1, Fig. 1). The X-test (VAN DER WAERDEN) shows a significant difference, however our material for this question is not completely representative.

Different crossover values in two-point-crosses therefore still do not prove that different M loci exist in the same chromosome. Cases in which the chromosome is marked by two inversions can however show whether M always lies in the middle or perhaps outside at the A or B end. The crossover classes of our three-point experiment have been arranged in Table 4 on the

Table 3

Crossover value f between M and InB of Ch. nuditarsis in different generations of two male-lines

		Ohne Austausch		Mit Austausch		
		♀	♂	♀	♂	f %
Cr 2.6.610.4	F_1	59	54	1	3	3,4
	F_3	76	71	12	14	15,0
	F_4	78	73	3	2	3,2
	F_7	70	80	3	2	3,2
Cr 5.6.908.40	P	86	71	6	7	7,7
	F_2	56	61	18	19	24,0
	F_4	71	50	34	27	33,5

assumption that M lies between the two inversions (Fig. 3). Cases 4 to 7 demonstrate the correctness of the assumption because the supposed double crossover class is much smaller than the classes with a single crossover. In cases 6 and 7 the simple inversion InA_2 is present. M lies here approximately in the middle between the two inversions. The two cases, 4 and 5, with the complex inversion ($In3 = InA_{2+4+5}$) reaching almost to the middle of the chromosome show that M lies near the proximal end of this inversion, therefore in the centromere region.

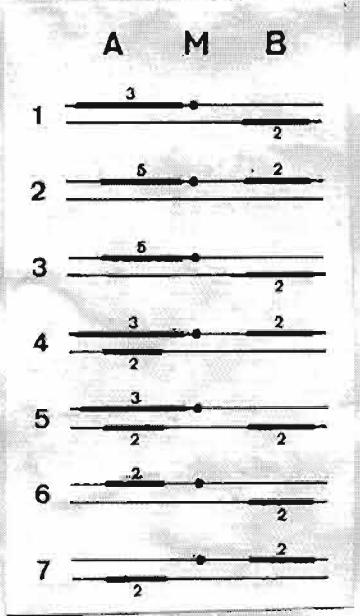

Figure 3

Chromosome configuration of the males whose performance is listed in Table 4. The complex inversions 3 and 5 differ only in the left extremity

The remaining three cases each show only one animal resulting from recombination between the complex inversion and M. Here it is not impossible that these animals may be the direct result of a double crossover. These cases give no information about the position of M. (For case 2, if no interference is assumed, it is as probable that M lies at the distal end of the A arm as in the middle. AnM in the middle with little crossover to the A inversion can however also produce these results with the relatively high probability of 25%).

Table 4

Three-point localisation of the M_1 factor in Ch. nuditarsis (see Fig. 3)

		Ohne Austausch		einfacher Austausch In A-M		einfacher Austausch M-In B		Doppel-Austausch		Total
		♀	♂	♀	♂	♀	♂	♀	♂	
1	5.430.2	32	98	1	0	1	7	0	0	139
2	5.711.1	64	71	0	0	25	20	0	1	181
3	5.711.13	15	35	1	0	15	8	0	0	74
4	5.804.6	126	125	9	7	60	62	0	0	389
5	Temp. 1	439	583	28	12	148	208	2	3	1423
6	Temp. 3 *a*	61	56	25	26	30	22	0	0	222
7	Temp. 4	160	162	68	53	80	89	3	3	618

M_2 lies in the G-chromosome

Crossing experiments in which the AB chromosome was well marked by two long inversions and a translocation have lead to the conclusion that M does not always lie in this chromosome (ROSIN and FISCHER, 1966). Further work on such M_2-lines has lead to the identification of the G chromosome as the second possible sex chromosome. The short G-chromosome is inversion-polymorphic in the Ch. nuditarsis population from the Wohlensee in Bern, whereby a marker is already given. In this connection it is sufficient only to distinguish heterozygotes from homozygotes. In Table 5 is shown a case where the sex determining factor lies in the G-chromosome. In this rearing chromosome arms A, F and G are polymorphic for inversions at the same time. Only the G inversion however shows a relation to the sex. The data shows

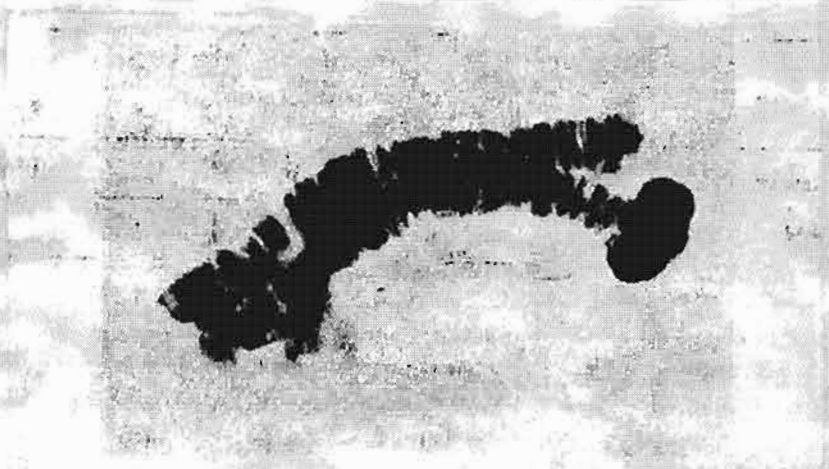

ABB. 4.

G-Chromosom von *Ch. nuditarsis*.
Heterozygote, nur bei Männchen aufgetretene Heterochromatinmutation G (h).
Nukleolus und Kinetochor liegen am andern Ende des Chromosoms.

that M_2, as also M_1, lies in an inversion polymorphic chromosome, not however in the inversion itself. The statement made by BEERMANN (1962) that the short chromosome pair are autosomal, is thus not supported. The G-chromosome can also carry the decisive sex determining factor.

Table 5

Sex-correlation of an inversion in the G chromosome of Ch. nuditarsis

In G d 5.68	A		F		G	
	♀	♂	♀	♂	♀	♂
Homozygot	30	52	57	77	98	9
Heterozygot	77	86	50	61	9	129

$f(M_2\text{-InG}) = 7.3\%$. InA and InF are sex-independent

G-chromosome with extra heterochromatin

In the material for 1970 a heterochromatin mutation has appeared in the G-chromosome of three independent wild egg masses (5-34). This large terminally located heterochromatin block was always heterozygous and only occurred in the male sex. No recombination has appeared in 714 individuals from sister stocks of G(h) ♂ + ♀. Whether this mutation has occurred in the immediate vicinity of an existing M_2 or whether it itself accomplishes male sex determination cannot be decided. It is not obligatory for M_2 males since in 16 other cases with M_2 nothing similar was observed. It is also not applicable to speak of a cytologically recognisable Y chromosome here. It is noteworthy that G(h) was found in three independent rearings of eggs found on two different days, so that at that time there must have existed a considerable frequency of G(h) in the population. MARTIN (1966) has made a similar observation in Polypedilum nubifer: an only heterozygously occurring heterochromatic end here marks the fourth chromosome of the obviously heterogametic female.

The frequency of both M-factors in Ch. nuditarsis

In order to obtain more precise information about the numerical participation of both factors in respect to these M polymorphisms, we have collected and reared a quantity of egg masses from the population of the Wohlensee. The inversions which occur in considerable frequency in the AB and G chromosomes give the opportunity to eventually find the relation between sex and inversion already in these, designated the F_1 generation, namely when the eggs have been fertilized by a male whose sex chromosomes were heterozygous for an inversion. Such a rearing we called a "primary correlation". In many cases however no correlation is shown in the first place. Here one can proceed as follows: a male from the F_1 is crossed with a female which possesses the complex inversion A_3. Half of the F_2 males then have this inversion. In cases where M is on the AB chromosome, this complex inversion, which now marks the X-chromosome, must occur in most female offspring but not in most male offspring. If however no correlation is obtained in this generation, then M is certainly not in the AB chromosome. Now it must be attempted to get a positive association to the G or the CD-EF complex by a cross of a CD-EF translocation and a G inversion. In all these cases a "secondary correlation" is recorded.

A first rearing series for the year 1968 has given the following:

M_1 (in AB)	primary 20	
	secondary 7	27
M_2 (in G)	primary and secondary	12
not M_1		19
M primary not in AB, further crosses failed		4
1968	total	62

Thus M_1 is concerned in about half the M-polymorphism.

If the frequency of a particular inversion is known one can calculate the number of cases in which a primary sex correlation would be expected provided the M-factor lies only in this chromosome. A comparison with the observed number of cases of primary correlation shows then whether, and in what frequency, other M-loci must occur. The calculation is only significant

when the material is large enough that the deviation of the calculated values is considerable. In species with only few inversions however it can lead indirectly to the discovery of an M-polymorphism. In the present analysis it has been possible to calculate from the cases primarily sex linked with A or B inversions, that 42% of M-loci should not be located in the AB chromosome. The observed fraction is 35/62 = 56%, which agrees well with the calculated value.

In a second series from the year 1970, not only has the AB chromosome been systematically studied, but we have taken the trouble to positively relate also the cases which were AB-independent. (Unfortunately in this series the rearings of the wild egg masses often only sparsely survived, so that in some rearings further crosses had to be made directly without being able to investigate whether a primary sex correlation was present or not). The 38 studied egg mass rearings gave the following:

	M_1	M_2	Not M_1, later lost	Total
Autumn 1970	18	19	1	38

Up till now can only the twomale determiners M_1 and M_2 be demonstrated. A correlation with the CD-EF translocation has never appeared. If the very similar results of both collections are pooled, we obtain:

	M_1	M_2 and perhaps others	Total
1968 and 1970	45	51	96

The two determiners for male sex are thus about equally frequent in the Wohlensee population.

M-factors in two other Chironomus-species

To obtain a first indication of the position of the sex determining factor in other Chironomus species, we have caught a large number of spawning (inseminated) females and reared their egg masses. The species of each egg mass was determined through cytological diagnosis and by the sex association of the prepupae on the basis of the gonads, it was possible to prove whether one of the randomly present inversions was correlated with sex.

The egg masses collected from the 6-10 May 1971 derived from the following species:

Ch. plumosus	59
Ch. nuditarsus	4
Ch. spec. 1	106
Ch. spec. 2	4

The species which is here tentatively designated as Chironomus spec.1 belongs cytologically to the chromosome type AD BC EF G and is not yet described. It was examined by Fraulein A.M. KLÖTZLI. The determination of the adult leads to the species Ch. winthemi Goetg. The morphological characterization is not sufficient here for the cytologically very different species, Chironomus spec. 2, also fits this description to some extent. (A.M. KLÖTZLI, personal communication)[1]

Chironomus spec. 1

We have reared 106 egg masses of this species. First of all 10-12 larvae from each of 62 rearings were dissected. 51 of these rearings contained some larvae with a heterozygous inversion in arm F as well as structurally homozygous larvae. With regard to the sex they give the following numbers: InF/+: 289 males, no females; +/+: 296 females, no males. InF is thus only found in the male sex. In 585 studied was no recombinant animal found. However M is not obligatorally linked with this inversion. This is shown by the remaining 11 rearings with a total of 79 females and 78 males which were all free of the F-inversion. After the absolute linkage of InF with M was established, only 1 male was dissected from 44 further rearings. Thereby can the frequency of F- and +- males in population be better comprehended.

	With InF	Without InF	Total
Random sample of about 10	51	11	62
Single males	38	6	44
Total	89	17	106

1. Translator's note: Chironomus spec. 1 was subsequently described as Ch. bernensis by Wülker and Klötzli (1973)

   Ref. Wülker, W. and Klötzli, A.M.: Revision der Gattung Chironomus Meig. IV. Arten des lacunarius-(commutatus-)complex. Arch. Hydrobiol. 72: 474-489 (1973).

Besides InF which occurred only in males, a much lower frequency of a sex independent inversion in arm A was found. In the 62 larger random samples seven egg mass rearings with InA heterozygous animals occurred, four of these with InF at the same time, three in sister stocks without InF. This species is therefore only to a limited extent polymorphic for inversions. The very frequent inversion F is however a direct sex label.

Chironomus plumosus

This species has inversion polymorphism in all chromosomes. An initial series of 18 rearings showed that none of the many inversions is tightly sex linked. Since the carriers of different chromosome types can have unequally rapid development, the material is mostly stratified and, in cases with larger crossover values, small random samples do not yield reliable results. We have therefore extracted a random sample of 40-60 larvae from each of 35 further rearings and completely worked up these partial rearings. The foregoing had the advantage that at the same time we could obtain information about the sex ratio in sister strains of Ch. plumosus.

Because the small G-chromosome of Ch. plumosus is almost always unpaired, inversion in this chromosome can only be recognised through protracted study. The G-chromosome is therefore not considered in our analysis.

In 11 of 35 sister strains the inversions of the AB chromosome, in two cases those of the CD chromosome, show a clear relation to the sex (Table 6)

The crossover value between M and InA of 12% and between M and InB of 11% indicate that also in this species the male determiner probably lies in the middle of the chromosome. Both cases with a correlation to the inversions of the CD chromosome are highly assymetrical. We interpret this as a result of a lethal factor.

The sex ratio in Ch. plumosus (Fig. 5)

In the 35 partial rearings the sex ratio is certainly away from 1:1 in 14 cases. In eight cases too few males occur, however in six too few females occur. We are of the opinion that lethal factors are responsible for these abnormal ratios, however the presence of a distorter gene, as has been proved for Aedes (CRAIG and HICKEY, 1967) can not yet be disproved. The examination of this question is intended. The situation that the sex displacement

Table 6

Sex linked inversions in Ch. plumosus, partial rearings from wild collected egg masses

	1971	Ohne Austausch		Mit Austausch		Total
		♀	♂	♀	♂	
In A	507.45	21	23	2	3	49
	510.3	9	9	1	0	19
	510.4	21	32	7	5	65
	510.12	14	9	1	1	25
	510.25	15	15	2	3	35
	510.29 [1]	26	15	5	11	57
	510.34	9	8	1	0	18
	510.53	9	5	0	2	16
	Total	124	116	19	25	284
	In A-M	Austausch 15,5%				
In B	510.11	25	35	1	2	63
	510.16	23	38	3	9	73
	510.30	27	21	2	3	53
	Total	75	94	6	14	189
	M-In B	Austausch 10,6%				
In C	510.54 [1]	15	50	16	14	95
	In C-M	Austausch 32%				
In D	510.38 [1]	29	21	11	0	61
	M-In D	Austausch 18%				

1. Assymetrical values, possibly caused by a lethal factor.

occurs to both sides speaks for the presence of a lethal factor, which to be sure must be unusually frequent. Further a considerable embryonic lethality has been observed in some wild egg masses. With identical lethal in both parents, 25% lethality is to be expected. With two lethal factors the rate of lethality lies between 25% and 50% according to the position of the lethal factors. In regard to the sex ratio, if a lethal factor

linked to the female portion occurs, it lies between 33.3% and 66.7%. Only in the case of two identical lethal factors in both sexes can all the remaining sex ratios arise and in the extreme case one sex can be completely absent. One of the 14 cases with abnormal sex ratio belongs here: the 56 studied prepupae were exclusively female.

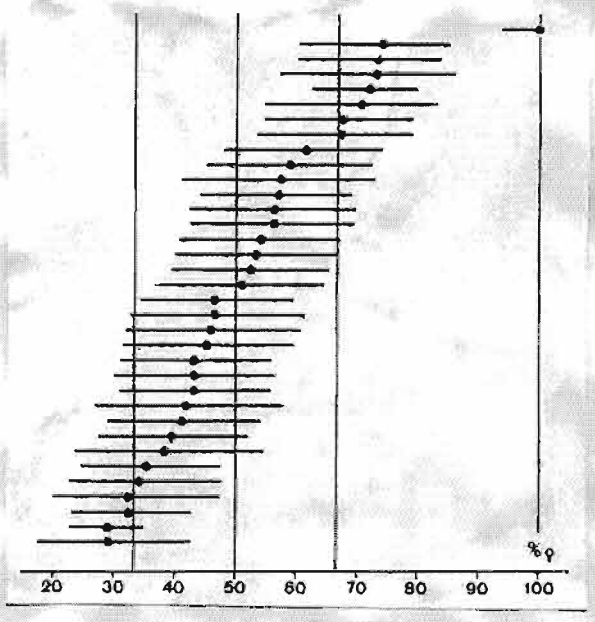

Fig. 5

Sex ratio in Ch. plumosus

Female fraction with 95% confidence limits

Rearings from egg masses from the wild population

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THE POSITION OF THE M-FACTOR IN CHIRONOMUS

As the following compilation shows, up to now a relationship between sex and an inversion has been found in seven Chironomus species. In four cases the inversions are exclusively limited to the males (♂ inversion). The M factor here lies in the inverted region or near to it. The remaining data rely on localisations, which in parts are considerably uncertain.

Species	Sex Chromosome	Position of M	Author
C. tentans	B	at the end	BEERMANN (1955b)
	F	♂ inversion	BEERMANN (1955b)
C. pallidivittatus	B	at the end	BEERMANN (1955b)
Ch. annularius	F	♂ inversion	BEERMANN (1955b)
Ch. obtusidens	F	♂ inversion	KEYL (1961)
Ch. plumosus	AB		ACTON (1957), KEYL (1962)
	AB	probably in the middle	ROSIN and FISCHER, preceding work
	CD	probably in the middle	ROSIN and FISCHER, preceding work
Ch. nuditarsis	AB	in the middle	ROSIN and FISCHER (1965)
	G	at the end	ROSIN and FISCHER, preceding work
Ch. spec. 1	F	in the inversion	ROSIN and FISCHER, preceding work

The homology of the chromosome arms is adapted from KEYL (1962). An M-polymorphism is identified by it for Camptochironomus tentans, Chironomus nuditarsis and Ch. plumosus.

DISCUSSION

Position of the M-factor

BEERMANN (1955b) was the first to find an M-polymorphism. He has shown for Camptochironomus tentans that the sex differentiator can lie in two different chromosomes, and that both male types occur side by side in the population. On the basis of the linkage found he came to the conclusion: "It is conceivable that the differentiators in 2L and 1R are both at the chromosome end, possibly they are even localised in the small segment of terminal heterochromatin, the 'telomere'". According to KEYL (1962) 2L corresponds to the B-arm and 1R to the F-arm of Chironomus. The possibility mentioned by BEERMANN in this connection, that the origin of the two non homologous Y-chromosomes is the result of a translocation of the terminal differentiators has been put up for the phorid Megaselia scalaris as the best interpretation of the M-polymorphism obtained in rearings of this fly (TOKUNAGA 1958; MAINX 1959, 1962, 1964, 1966). As BEERMANN himself indicated, his data for the terminal location of the differentiator are

however not compelling. It is based in one case on the comparison of crossover values of two series of experiments. According to our results however a large difference in the same interval can occur through differences in the genetic background. In our opinion therefore the communicated facts do not contradict the supposition that also in C. tentans the M-factor lies in the proximal part of the B arm and thus in the centromere region. Also for the second M-factor the terminal position is not proven. The differentiator in the F-arm is inseparably bound with the complex inversion 1-k1, and thus lies most nearly in this inversion and not at the chromosome end.

Our results show that the M-factor certainly does not always lie at the end. A cytologically undetectable translocation of the M-factor is, however, only possible when it sits in the telomere. With the finding of an interstitial M-factor, the translocation hypothesis for the origin of the M-polymorphism is unlikely.

Closer it appears to us, is the possibility that M-factors newly arise through mutation, as ULLERICH (1963) has at least taken into consideration for Calliphora erythrocephala. One can imagine a relatively unspecific mutation in the heterochromatin, which mostly only occurs in large quantity in the vicinity of the spindle attachment (BEERMANN 1962). In Ch. nuditarsis M_1 certainly lies in this region of the AB-chromosome, and probably also in Ch. plumosus. A sex-linked unequal development of the homologues is however not seen in the AB chromosome. However this happens for the heterochromatin mutation found only in males on the G-chromosome. Unilateral increase in heterochromatin in combination with a male sex-linked heterozygous inversion has also been described by BEERMANN (1955a) and KEYL (1961). A causal relationship between quantity of heterochromatin and sex determination, to be sure, can not be derived from these cases.

M-factors and inversions

Between M-factors and inversions the following degrees of relationship were observed:

1. A specific inversion is sex independent
2. An inversion is transmitted as sex linked, shows however more or less large recombination with the M-factor. It marks in part the chromosome with the M-factor, the 'Y-chromosome', in part however the one without the M-factor, the 'X-chromosome'.
3. An inversion marks only the 'Y-chromosome' and never occurs in the 'X-chromosome'. However it gives in addition unmarked 'Y-chromosomes'.

4. An inversion marks only the 'Y-chromosome' and every 'Y-chromosome' is marked by this inversion.

Case 4 has been found in Ch. annularius by BEERMANN (1955b) (90 larvae investigated from a wild population). Provided no exceptions are found in further investigations, there is here, as BEERMANN has enlarged on, a prerequisite for the establishment of the degeneration of the Y to take place. Indications of any effective change in the chromosome are however not present. An M-polymorphism is not possible in case 4.

Case 3 possibly occurs in Ch. tentans and indeed for the complex inversion in the B-arm as well as the one in the F-arm (BEERMANN, 1955b). KEYL (1962) has reported the same relation for an InF in Ch. obtusidens and we have found the same in Ch. spec. 1, also for an InF. Also here no recombinant animals were found in the sister strains, as in the fourth case. This case is of interest especially in reference to the origin of the M-polymorphism. The two following possibilities can be considered:

On the one hand one can assume that the inversion is relatively young and has originated in an M-chromosome. It must lead to a selective advantage so that the In-M-chromosome can become frequent (in the example of Ch. spec. 1, 84% of the 'Y-chromosomes' carry the inversion). The M-inversion polymorphism however stands to command no balance mechanism through heterozygote advantage because no inversion homozygotes at all can occur. It can act here only as a transient polymorphism and must sooner or later vanish. The species would then belong to the annularius-type (case 4); or the balance occurs through ecological polymorphism.

On the other hand the sex determination can first of all be of the annularius-type, with M_1 in the F-inversion. When in such a population a factor M_2 arises by mutation at some place on the chromosome, outside the inversion, this leads to males which do not carry the inversion. Here a genetic balance is possible, whereby a selective disadvantage of the inversion free animals opposes the value of the new mutations of M-factors lying outside the inversion.

Case 2, with inversions not completely sex-linked, is realised in Ch. plumosus, Ch. nuditarsis and possibly in C. tentans and C. pallidivittatus. A transition situation with rare recombination and the rare occurrence of

the inversion in the female sex has been described for the complex inversion A_3 of Ch. nuditarsis (ROSIN and FISCHER 1965). This complex inversion consists of the long InA_2, an overlapping InA_4 and an adjacent InA_5. In the progeny of females coming direct from the population, some A_3-recombinant females occurred (ROSIN and FISCHER 1965), and in the summer of 1967 we captured an inseminated female in whose offspring the complex inversion A_3 was independent of sex (FISCHER 1969). When females of this strain are crossed with M_2-males (M in the G-chromosome), the segment A_3 remains autosomal. If on the other hand they are crossed with M_1-males, A_3 becomes the 'X-segment'. Through the capture of these types in nature we were able to combine the complex inversion A_3, which was functioning normally as a 'Y-segment' with a structurally similar 'X-segment'. The homozygous A_3 embryoes were however lethal, and also an individual with this combination has never yet occurred in the wild. Crossing experiments have shown that the component inversion A_4 homozygotes can be viable. Thus the inversion A_5 at least must contain a lethal. As long as InA_3 is linked to M, the lethal factor in the inversion is protected by the M-factor. In large numbers, as M is separated from InA3 by recombination, the A_3 inversion gets into the female sex. The mating of two midges heterozygous for the inversion is then possible and ¼ of the offspring, the inversion homozygotes, are lost. In this selection process always the same number of A_3 inversions without M are lost as those with M, which works out more heavily on the frequency of the few A_3 without M. A_3 therefore remains frequent in the male sex. The disadvantage through the occasional crossover between InA_3 and M can only be compensated through heterozygote advantage. By these examples will become clear how or account of the linkage of an inverted region with an M factor, the way becomes open for this region to become an independent differentiation in the direction of a Y-chromosome. When however the inversion brings clear advantage to both sexes, a position for M outside the inversion is favourable Both appear to occur in Chironomus. Plainly in the case of the inversion-rich species Ch. nuditarsis and Ch. plumosus the M factors found up till now lie outside the inversions, whereas in Ch. spec. I the only frequently occurring inversion is completely sex linked.

SUMMARY

1. Two different sex determining factors for male realisation (M-factors) are shown to exist in Ch. nuditarsis. M_1 is located in the middle of the AB-chromosome, M_2 on one end of the G-chromosome, in some cases certainly on the end opposite the kinetochor. Both

factors occur parallel to one another in the Wohlensee population near Berne. This unisexual polymorphic system is termed M-polymorphism.

2. Ch. plumosus is M-polymorphic, too.

3. In a further species (Ch. spec. 1), still to be described, a heterozygous inversion in the F-arm is found in 84% of the males. 16% of the males and all the females do not have this inversion.

4. An absolutely sex-linked heterochromatin mutation, located on the G-chromosome, is described.

5. The cross-over frequency in the male of Ch. nuditarsis is only slightly influenced by temperature. More pronounced differences, however, may be caused by different genetic background.

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