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'The selective value of the different types of chromosomal structure from Chironomus nuditarsis Str.'

Archiv der Julius Klaus-Stiftung 43 (1968) pp31-40

Chironomus nuditarsis is a species polymorphic for inversions. There is frequently a large inversion in each of the chromosome arms, A and B, of one of the three long chromosomes. Also the small G chromosome occurs in two structural types. In the report presented here we deal only with the inversions of the AB chromosome, and we do not consider separately the three smaller inversions of arm-A which always appear in conjunction with the large inversion.

Both inversions of the AB chromosome are very old, since they also occur in the closely related, but clearly separate species Ch. plumosus (Keyl and Keyl 1959, Fischer and Rosin 1967). Obviously we deal with here a case of balanced polymorphism which is being constantly stabilized by effective mechanisms of selection.

Inversion polymorphisms have been studied till now mainly in species of Drosophila. How these systems of biologic factors react to give local and periodic variations in the frequency of inversions (ie. varying with time and place) has been shown impressively by Dobzhansky and his school in particular (reviewed by Ford 1964). In these cases temperature and population density have been found chiefly to be the important selection factors. However there is little known about the period of action of the selection force, and in the case of Chironomus these questions have still not been completely settled. Since we have been engaged with the Chironomus nuditarsis population at Wohlen Lake near Bern for several years, our observations of material have been tested to see whether some contribution can be made to these questions.

A polymorphic system with three genotypes is most securely stabilized when the heterozygotes have a distinct superiority over both homozygotes. This over-dominance can be obtained in the most differing periods of life and in a different way. In addition to vitality differences with embryo, larva, pupa or imago, the speed of development, the readiness to mate, the fertility and the fecundity can be different. This way also a mating selection mechanism may be arranged, yet only then if the probability of reproduction of certain genotypes will be altered. Polymorphic systems can remain to be preserved without the heterozygote advantage, when both genes are alternately at a disadvantage. An unequal splitting up in meiosis (meiotic drive) or selection of gametes can, combined with a selection of genotypes in the opposite direction, lead to the preservation of a detrimental gene, as this has been shown for the t-gene of the domestic mouse by Dunn and Morgan (1953). Periodic fluctuating environmental conditions and small area environmental differences can, moreover, lead to biologic polymorphisms of various types, without the heterozygotes being possibly advantaged (refer Mayr 1967, page 193 and following).

Every selection acts on the Hardy-Weinberg equilibrium. Consequently our material shall be tested above all in this regard. As will be shown in 1., no certain variation can be determined here. But since chance deviations easily overplay weak selection effects, individual components of selection which are applicable in all cases shall nevertheless be investigated.

The catching of spawning females in the open air, and the rearing and analysis of offspring, has yielded contributions to the following points : mating selection, vitality up to the prepupa inclusive of meiotic drift and gamete selection, as well as the speed of development. Larvae captured in the lake in a year of severe parasitization allowed us to examine the material for variable susceptibility. Questions of fecundity were investigated by laboratory crossings.

1. Frequency of different chromosomal configerations.

The results of larval and imaginal captures from the lake up to now are compiled in Table 1. With the exception of the larval sample from 1961, all come from the same region of the lake. The chromosome configerations of the imagines were found out from the offspring of the spawning females. In doing so however, on account of the repeated exchange, only the structure of the chromosome arms, not that of the whole chromosomes, could be comprehended. Standard homozygotes are labelled with 11, heterozygotes with 12, and inversion homozygotes with 22. Result : Both with the larval samples, and with the imagines, no certain variations from a Hardy-Weinberg distribution can be determined. Not even a tendency to an increased frequency of heterozygotes is perceptible. Consequently, by comparison with the Hardy-Weinberg distribution, no selection is evident either for the larval period or for the imaginal period. Beerman (1955) has, in the species Camptochironomus tentans and C. pallidivittatus equally polymorphic for inversions, found with the larvae of the forth stage no variations either, and concluded from this that a critical stage falls in the period during and after metamorphosis.

2. Mating Selection.

The mating combinations comply, within the bounds of chance variations, to the composition of the self-reproducing population(Table 2). As is shown by the signs of the differences and from the 'sum' numbers, there are no indications for a mating selection, according to the chromosome configerations present.

3. Survival rate of different types up to pupation.

On numerous batches of common offspring, we have raised a portion of the larvae up to the prepupa, and found out the chromosome structures. All cases with two classes of offspring are presented in figure 1.

They must originate from a crossing of homozygote X heterozygote, and should have indicated a 1 : 1 analysis, when no disturbing influences have acted. The rearing conditions varied very much, partly good, partly so bad that only few larvae survived. From figure 1. it is obvious that no systematic deviation from 1 : 1 in favour of the heterozygotes exists. Disturbances by meiotic drift, gamete selection or vitality differences in the larval stage are thus not evident in our experiments.

4. Speed of development.

With the larvae from one laying, a considerable variation in relation to the speed of development is regularly to be observed. At room temperature the imaginal moultings can be distributed over 10 to 14 days. The male hatches about three days earlier than the female. Consequently the sexes must be considered separately. By the utilization of breeding, all prepupae were in turn sought out and prepared at intervals of 3-4 days. These procedures allowed the speed of development of the different chromosome types to now be compared with one another. To allow a simple analysis, the material was classified as uniformly as possible into a more rapidly developed and a more slowly developed portion, and examined with the help of a four-field test (ie. a 2X2 contingency test) to see whether the structural types in both portions showed similar frequencies. Such a four-field test looks as follows eg.

	B 11	B 12	
1.	34	66	100
2.	80	43	123
	114	109	223

The homogeneity test yields a $\chi^2_{(1)}$ of 21.3 with $P \ll 1\%$. Consequently the heterozygotes (B 12) have developed more rapidly than the homozygotes (B 11). The four-field correlation coefficient $\left(r = \sqrt{\chi^2/N}\right)$ serves as a criterion for the size of the relationship. In our example r= 0.31. In figure 2. the r-values are presented separately for both the large inversions and for the sexes.

In many rearings it is seen that no certain differences appeared in the speed of development of the different structural types, but that in seven cases the heterozygotes, in three cases the homozygotes however, were faster developing. Factors which influence the speed of development are detected within the region of the A-inversion and also the B-inversion, but both are effective with the heterozygotes and also the normal homozygotes. Whether the heterozygotes show a more frequent and more distinct developmental advance must be tested on an additional experimental datum. Moreover the developmental speed factors plays a minor part in the maintenance of polymorphisms. Differences are only then effective when the chance of survival will be influenced, over the duration of development, but according to section 3. no indication exists for that. Otherwise a quicker development of certain genotypes may appear only to result in periodical fluctuations in the gene frequencies - for example, the case that not all developmental stages endure the winter equally well.

5. Susceptibility towards parasitization by Nematodes.

In 1965 the population had to suffer under a very severe infection of Mermis. In Table 3. the chromosome configerations of parasitized and healthy larvae are compared to each other. The distributions permit no sort of preference of the parasites for certain structural types to be detected.

6. The relation between structural type, size and fecundity.

These problems have been investigated first of all by the offspring of certain laboratory crossings. For the A- or B-inversion heterozygote males, in which the AB chromosome is not the sex chromosome (Rosin and Fischer 1966), were crossed with heterozygote females of the same kind. The three structural types must consequently appear in the offspring in the ratio 1 : 2 : 1. With the females of these batches of brothers and sisters, a test-crossing was then always carried out, the spawn photographed, the eggs counted, and a specimen from them raised up to discover the structural type of the female. In order to obtain simultaneously a relation to the size, the front tibia of the examined females were measured.

In figure 3. each such experiment for the A-inversion and for the B-inversion is presented. A positive correlation between number of eggs and size is distinct for both inversions. For the two cases with remarkably lower number of eggs, it could have been a question of incomplete hatching. Since the females could be only partly recorded, and because a stratification appeared by means of the variable speeds of development, it makes no sense to make an issue of the small existing differences between the structural types. The experiment shows however that it is possible to determine a possible correlation between structural type and fecundity by means of a common relationship to the size.

The relation between size and structural type was more precisely investigated by way of a part of the larval material from similar rearings. For the measurement of the size, the width of the head capsule in the final larval stage was selected. From Table 4. it is obvious that in this material the homozygotes are smaller than the heterozygotes and the normal homozygotes for both the A-inversion and for the B-inversion, and between the latter no difference is detectable. Since however our material comes from only a batch of brothers and sisters, the relationships found here must not be generalized. It is planned to analyse them against an additional independent sample from the population. Nevertheless it can be said that a continuous and in every case effective heterozygote advantage is not present with reference to the fecundity. The possibility of an advantage on the average is still undecided.

Table 1.
Frequency of the different chromosome configerations, and comparison with the distribution according to Hardy-Weinberg

	Time	Chr.-Arm	Configeration 11	12	22	N	Frequency of Inv. (%)	Het.	$\chi^2_{(1)}$
Larvae									
	1961	A	257	123	16	396	19.6	-	0.06
	(ref. Frank '61)	B	193	170	33	396	29.8	+	0.27
	1965	A	71	34	1	106	17.0	+	2.04
	12th August	B	55	42	9	106	28.3	-	0.06
	1967	A	155	86	15	256	22.7	-	0.45
	20th May	B	128	108	20	256	28.9	+	0.18
	1967	A	239	110	10	359	18.1	+	0.39
	13th September	B	206	136	17	359	23.7	+	0.84
	1967	A	158	94	17	269	23.8	-	0.35
	25th September	B	195	67	7	269	15.1	-	0.18
Imagines									
	1963	A	38	20	2	60	20.0	+	0.10
	October/November	B	35	24	1	60	21.7	+	1.88
	1964	A	79	49	4	132	21.6	+	1.24
	whole season	B	78	48	6	132	22.7	+	0.16
	1965	A	233	130	29	392	24.0	-	3.23
	whole season	B	226	142	24	392	24.2	-	0.08
	1967	A	166	85	11	262	20.4	0	0.00
	10-12th October	B	182	69	11	262	17.4	-	1.78

Table 2.
Mating Combinations

	1963		1964		1965		1967		Sum	
Matings	30		66		196		131		423	
Arm A	b	d	b	d	b	d	b	d	b	d
11 × 11	10	—2,0	20	—3,6	70	+0,8	53	+0,4	153	— 4,5
11 × 12	16	+3,3	36	+6,7	80	+2,7	52	—1,9	184	+10,9
11 × 22	2	+0,7	3	+0,6	13	—4,2	8	+1,0	26	— 1,9
12 × 12	2	—1,3	6	—3,1	19	—2,6	15	+1,2	42	— 5,8
12 × 22	0		1		12	—2,4	3		16	+ 0,7
22 × 22	0	—0,7	0	—0,5	2	+1,0	0	—0,8	2	+ 0,6
χ^2 (d.f.)	2,87	(2)	3,48	(2)	3,71	(3)	0,49	(2)	1,93	(3)
P	24%		17%		29%		65%		57%	
Arm B										
11 × 11	9	—1,2	20	—3,1	71	+5,9	64	+0,8	164	+2,4
11 × 12	16	+2,0	34	+5,6	74	—7,9	47	—0,9	171	—1,2
11 × 22	1	+0,4	4	+0,5	10	—3,8	7	—0,6	22	—3,6
12 × 12	4	—0,8	6	—2,7	28	+2,3	9	—0,1	47	—1,3
12 × 22	0		2		12	—3,3	4		18	—3,8
22 × 22	0	—0,4	0	—0,3	1	—0,3	0	+0,9	1	—0,1
χ^2 (d.f.)	1.27	(2)	2.47	(2)	3.92	(3)	0.32	(2)	1.64	(3)
P	50%		29%		27%		85%		65%	

b = observed values
d = difference : observed - expected values

Figure 1.
1 : 1 analysis with offspring of crossings of heterozygotes with normal homozygotes. Filled circles = inversion A, open circles = inversion B
The 99% confidence limits for the 1:1 distribution is on the basis of the values for the binomial distribution marked in Geigy tables, page 105.

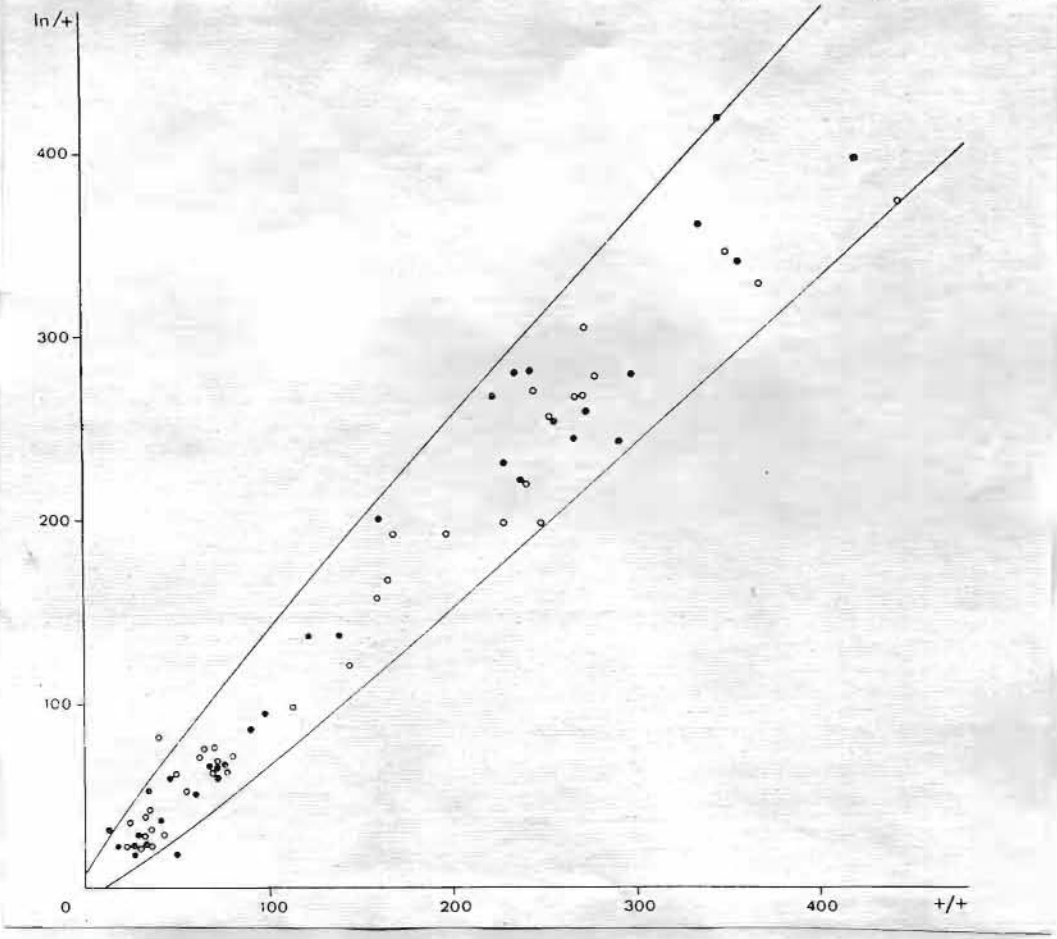
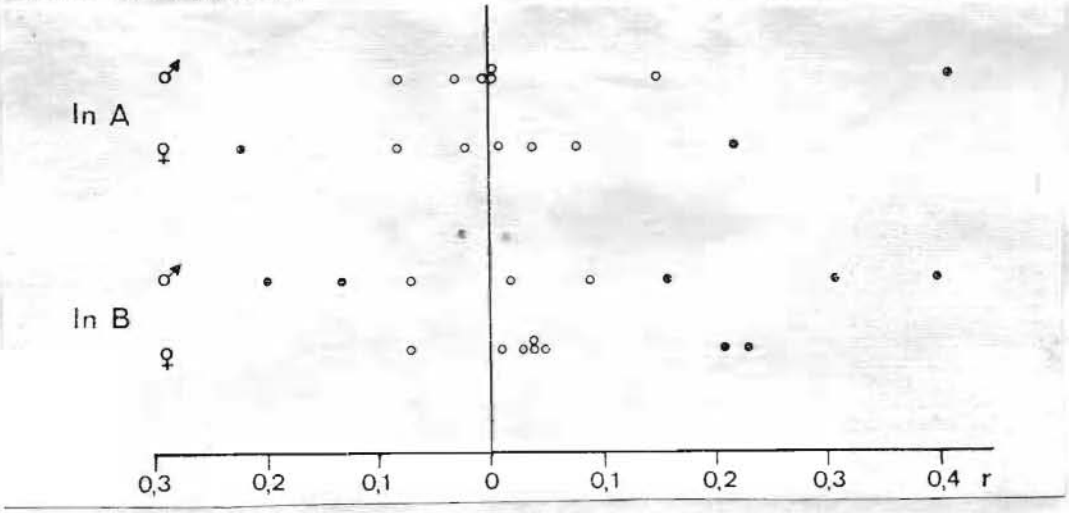


Figure 2.
Speed of development of inversion heterozygotes in comparison with normal homozygotes.

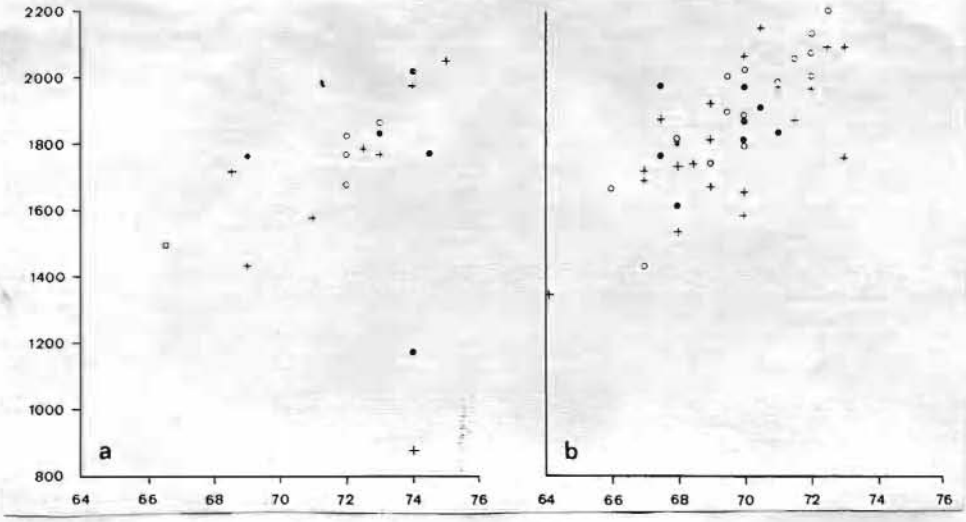


Abscissa : four-field correlation coefficient.
filled circles : certain difference (P<5%); open circles : difference not certain. Right side : In/+ faster developing than +/+. Left side : In/+ slower developing than +/+.

Table 3.
Parasitism by Mermis.

1965	normal	Mermis	Sum
11 11	8	30	38
11 12	9	23	32
12 11	4	13	17
12 12	3	7	10
11 22	–	1	1
12 22	1	6	7
22 22	–	1	1
Sum	25	81	106

Figure 3.
Relation between body size and number of eggs, in the case of the different structural types



Abscissa : tibia length (unit = 25.8 μ).
Ordinate : Number of eggs from first hatching.
open circles : standard homozygotes; crosses : inversion heterozygotes;
filled circles : inversion homozygotes.

Table 4.
Relation between structural types and larval size.

		N	$\bar{x}$	S_{xx}	$s_{\bar{x}}$	t[1]	Significance[1]
In A ♀	11	53	79,09	227	0.287	0.24	–
	12	127	79,00	674	0.205	3.27	* *
	22	57	77,89	171	0.232	3.23	* *
In A ♂	11	56	72,87	92	0,185	1,15	–
	12	111	72,58	295	0,155	5,11	* *
	22	51	71,35	156	0.247	4,33	* *
In B ♀	11	44	81,48	115	0.246	1,52	–
	12	105	81,02	302	0,166	2,75	* *
	22	57	80,58	149	0.216	1,59	–
In B ♂	11	51	74,94	103	0.201	0,45	–
	12	97	75,06	246	0.163	1,76	–
	22	64	74,45	144	0.189	2,42	*

[1] t-Test : first line : comparison of 11 with 12; second line : comparison of 11 with 22; third line : comparison of 12 with 22.

Summary :

1. Both the larvae and the imaginal captures of Chironomus nuditarsis show in their distribution of chromosomal structural types no deviations from Hardy-Weinberg expectations.
2. A mating selection mechanism is not detectable.
3. Indications of a preference for heterozygotes by meiotic drift, gametic selection or vitality differences in the stages of development up to the prepupa were not found.
4. The speed of development of inversion heterozygotes is often greater than that of normal homozygotes, but also in a few cases is definitely smaller.
5. With reference to parasitism by Mermis, the different types show no variation in susceptibility.
6. A correlation between structural type and fecundity could be present by way of a common relation to the body size.