

Beermann, W. 1956. Inversions-heterozygotie und fertilitat der mannchen von
Chironomus.

(Inversion heterozygozity and fertility of the males in *Chironomus*)

Chromosoma 8: 1-11.

Inversion polymorphism is very common in Diptera (Da Cunha 1955). Its occurrence is generally interpreted as an effect of heterosis. The inversion heterozygotes have a selective advantage over the homozygotes. The relative selective value of the genotype depends on the viability and fertility of the phenotype it determines. Hitherto in the discussion of the inversion heterosis the problem of relative viability has stood in the foreground. The problem of relative fertility has on the other hand been previously neglected, because in the chromosomes of *Drosophila* and *Sciara*, the genera chiefly investigated, no chiasmata are formed in the male meioses. In the female meioses the chromatid bridges (from a simple interchange in the region of the heterozygous inversion) can indeed occur, however one of the chromatids not concerned in the interchange is always seen to be retained in the presumptive egg nucleus at Anaphase II (Carson 1946; Sturtevant and Beadle 1936); the fertility of the females is therefore not affected in inversion heterozygotes.

Drosophila and *Sciara* embody the achiasmatic type of spermatogenesis: in many Nematocera on the other hand there are also chiasmata in the paired meiotic bivalents of the male sex, eg. in the majority of the Tipulidae, the Limoniidae, Chironomidae and Culicidae. In these groups the formation of bridges and fragments through crossing over in the inversion heterozygotes must also be expected in spermatogenesis. In fact Wolf (1941) has found bridges and fragments

in almost all Anaphase I's of most males of the midge *Dicranomyia trinotata* and completely described their behaviour (see below). The problem of the possible influence which the formation of such bridges exerts on the fertility of the males of *Chironomus* is discussed in Philip (1942), and is quite plain in view of the broad distribution of inversion polymorphisms in Chironomidae. Philip believes that possibly sperm with aberrant chromosome constitution, which must result following bridge formation, are at a disadvantage against the normal in insemination and fertilization of the egg - though one naturally thinks of a simple compensation for the fertility loss by raised viability in order to understand inversion heterosis. Previously there remained the possibility that the chiasmata in the spermatogenesis of male chironomids were outside the inversion and thus, in spite of the meiotic chiasmata, bridges were not formed. However they are found in large numbers in the corresponding meiotic pictures of *Chironomus dorsalis*

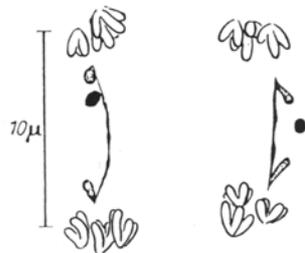
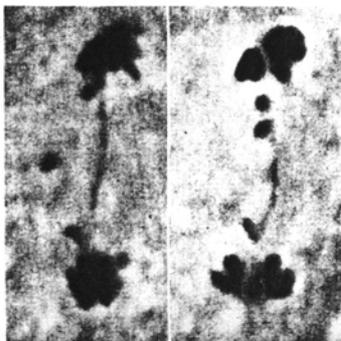


Abb. 1. Dikineticische Brücken mit akinetischem Fragment in der Anaphase I der Spermatogenese von *C. tentans*. Die Brücken sind durch Austausch innerhalb der heterozygoten Inversion *In 4—1* des telokinetischen kurzen Chromosoms 4 entstanden. Brücke und Fragment in den Zeichnungen schwarz, die beiden normalen Chromatiden punktiert. Orcein-Eisessig-Milchsäure (OEM)

(Acton 1956) and *Chironomus tentans* (Fig. I), so that the latter explanation does not hold. It remains therefore still a problem, whether and to what degree the regular formation of dicentric bridges in spermatogenesis can decrease

the fertility of the males.

Chironomus tentans possesses 4 haploid chromosomes, 3 long metacentrics and 1 short almost telocentric. In all chromosome arms inversions occur (Beermann 1955). For the accomplishment of the investigation the inversion *In(t)*

4-1 (in the following discussion shortened to "In4") of the short 4th chromosome (an accurate description of this inversion is found in Bauer and Beermann (1952) and Beermann (1955)) was found as the best suited. The meiotic bivalent 4 is easily and reliably identified from the other 3 bivalents on account of its shorter length: a confusion of the In4 bridge with bridges due to the other inversions is not in question. Moreover, In4 is almost as long as the whole chromosome; thereby the formation of chiasmata outside the inversion would be very improbable (see Table 1). Finally, for the 4th bivalent it can already be determined with certainty in prometaphase I, whether or not a chiasma is formed within the inversion (Figs. 2 and 3). Therefore one is not dependant on the rare anaphase figures for the statistics. In itself must simple exchange in every other simple inversion, also shown in Figs. 2 and 3 shown, give partly asymmetrical configurations (see

Darlington (1937), Fig. 9).

However the asymmetry would clearly only be in a long inversion in

telocentric chromosomes

and is therefore as yet

scarcely observed.¹

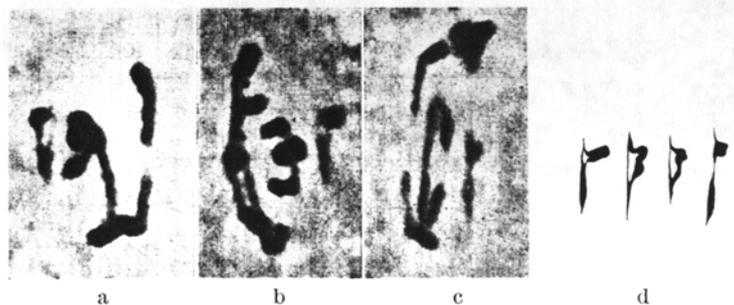


Abb. 2a—d. Prometaphasische Bivalente des 4. Chromosoms von *C. tentans* bei Heterozygotie für *In4—1*. a, b, c Ganze Prometaphasen mit dem 4. Bivalent (stets am Rande der Platte) in der typischen Gestalt, die durch Bildung eines Chiasma innerhalb der Inversion zustande kommt; in d einzelne Typen von heterozygoten Inversions-Bivalenten des 4. Chromosoms. OEM, 2000fach

The gonads and the salivary chromosomes of the same male prepupae (larvae, shortly before they pupate) were examined, and indeed in sibs from a cross: In4/In4 female X In4/St male (St = standard arrangement of the chromosome). Besides In4, in the other chromosomes of these animals always

¹ Note in correction. The formation of asymmetrical diakinesis bivalents through inversion chiasmata in *Lilium* are described and discussed in a just published work of Brown and Zohary (1955) .

occurs heterozygotes for the complex "Y"-inversion In(t)1-k1 (see Beermann 1955)

and occasionally also the simple inversion In(t)3L-1; both can not influence the result of the investigation.

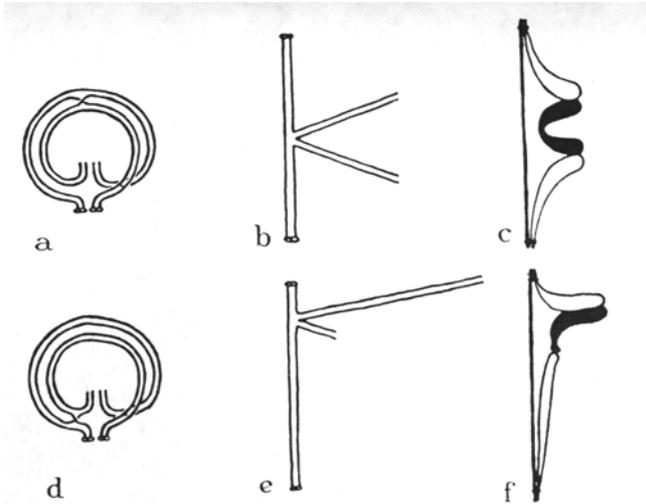


Abb. 3 a—f. Schematische Darstellung des Zustandekommens der in Abb. 2 gezeigten Formen von Bivalenten des Chromosoms 4. a, b, c Chiasma in der Mitte des invertierten Bereiches; d, e, f Chiasma an der Grenze der Inversion. a, d Pachytän; b, e Übergang vom Diplotän zur Prometaphase (eine Diakinese gibt es bei Chironomus nicht); c, f Prometaphase. Brücke und Fragment schwarz

As is clear from Table 1, heterozygosity for In4 results in no decrease in the chiasma frequency as a consequence. As well, in the hetero- as also in the homo-zygotes (In4/In4), univalent chromosome 4's were found in only 5% to 10% of the meioses.

Tabelle 1. Häufigkeit der Chiasmen im Bivalent 4 von *Chironomus tentans* bei Inversions-Heterozygotie und -Homozygotie

Bezeichnung des Individuums ¹	Konstitution	Metaphasen mit einem Chiasma im Bivalent 4		Metaphasen ohne Chiasma im Chr. 4	Chiasmenfrequenz im Bivalent 4
		innerhalb der Inversion	außerhalb der Inversion		
S 1	} $\frac{In\ 4 - I}{st}$	89	0	11	0,89
S 31		93	0	7	0,93
S 34		29	0	1	0,97
S 38		94	0	6	0,94
S 40		90	0	10	0,90
S 43		100	0	7	0,94
S 44		93	0	7	0,93
SP 1/1		85	0	6	0,81
S 2	} $\frac{In\ 4 - I}{st - 4}$	86	86	14	0,86
S 12		96	96	4	0,96
S 21	} $\frac{st - 4}{st - 4}$	90	90	10	0,90
41 C 2		53	53	47	0,53

¹ S = Stamm Schweden; SP1/1 = F₂ von Schweden × Plön; 41C2 = vor mehreren Jahren gehaltener Plöner Stamm.

From a single observation on other material (see also Table 1) it is of course known, that this value is occasionally - independent of the inversion heterozygosity - can increase up to almost 50% (through general lowering of the chiasma frequency in all chromosomes). Secondly the meiosis statistics show that in line with expectation, all inversion heterozygotes for the 4th bivalent form chiasma lying within the inversion, thus in every case resulting in the formation of the dicentric chromatid with acentric fragment. The rare case - only detected in one case - of double crossing-over in chromosome 4 was not considered here.

On the basis of these results a precise answer to the original question is possible. When in meiosis of heterozygous In4 males a dicentric bridge results with a probability of 90%, so must 45% of all spermatids (in usually normal course of the maturation division) possess an unbalanced chromosome constitution; here can only 55% normal sperm be formed. And even when a chiasma in the 4th chromosome only occurred in 50% of all meioses (see above) - without regard for

Tabelle 2. Prozentuale Häufigkeit und cytologische Konstitution der normal entwickelten Individuen aus Kreuzungen mit Inversions-heterozygotem (In4-1) Vater

Kreuzung ¹ ♂♂ stets $\frac{In\ 4 - 1}{st}$	Normal entwickelte und geschlüpfte Eierlarven ²	Anzahl der zur Aufzucht ange-setzten Larven	Überlebende Larven					
			Anzahl im Stadium		Cytologische Konstitution			
			II - III	III - IV	Anzahl der untersuchten Larven	$\frac{In\ 4 - 1}{st}$	$\frac{st}{st}$ oder $\frac{In\ 4 - 1}{In\ 4 - 1}$	Haplo-4 bzw. Brücke
1. SP5 ♀ × SP6 ♂	> 99%	100		71	0	—	—	—
2. SP5 ♀ × SP6 ♂ ³	~ 20% ⁴	160		105	96	46 ⁵	50	0
3. EES ♀ × SP6 ♂	> 90%	50		47	0	—	—	—
4. EES ♀ × SP6 ♂	> 99%	{ 100 100	90 ⁶	— 82	— 82	— 40	— 42	— 0

¹ E = Stamm England, weitere Abkürzungen s. Fußnote Tabelle 1; die ♀♀ in Kreuzung 1 und 2 sind homozygot *st*, die ♀♀ in Kreuzung 3 und 4 homozygot *In4-1*.

² Anzahl der durchschnittlich in einem Gelege vorhandenen Eier ~1500.

³ Gleiches ♂ wie in Kreuzung 1.

⁴ Rest unbefruchtet, vgl. Text.

⁵ Darunter 7 Triplo-4-Individuen, deren Vorkommen mit der Inversion in keinem Zusammenhang steht.

⁶ Zucht nicht weitergeführt.

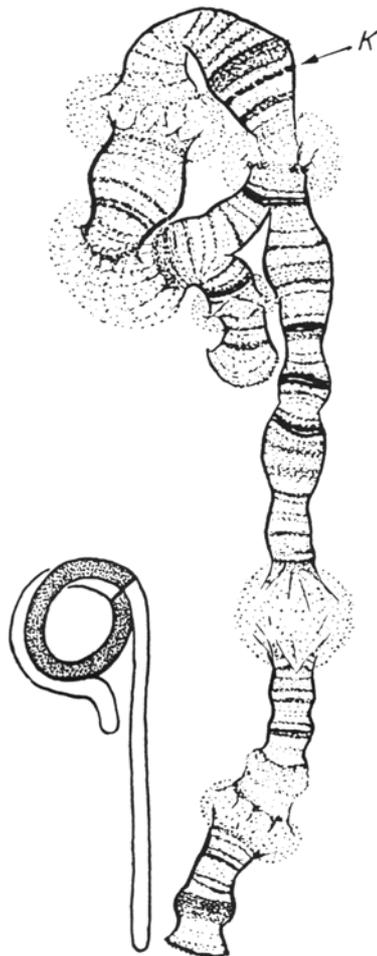


Abb. 4. Überzähliges dikinetisches 4. Chromosom in der Speicheldrüse eines F_2 -Individuums der Kreuzung *C. tentans* \times *C. pallidivittatus*. Das Brückenchromosom (im Schema punktiert; zu seiner Entstehung vgl. Text) ist lateral mit einem 4. Chromosom von *C. pallidivittatus* und außerdem mit dem proximalen Bereich eines invertierten 4. Chromosoms von *C. tentans* gepaart. In der Kinetochorregion (K) terminale Paarung (vgl. BAUER und BEERMANN 1952). Karmin-Essigsäure, etwa 900fach

the possibly aberrant behaviour of the univalents - still 25% of gametes with an unbalanced genome are expected. The chromosomally aberrant spermatids differentiate in the same manner as the euploids to fully functional sperm, so may one expect in the descendants of an In4 male 25% to 50% chromosomally unbalanced, and hence probably sub-vital, zygotes.

During a study in the past year with another aim, again and again (in at least 20 cases) it was observed that more than 95% of the F_1 embryos of heterozygous In4 males pass through a normal development and all simultaneously hatch, and recently an enquiry of these conspicuous facts has begun. In the laboratory every *Chironomus* female deposits about 1500 eggs united in a jelly mass, which develop synchronously. The chorion is fully transparent, and hence all disturbances in

development can immediately be recognized. In the presently considered singly-mated, structurally homozygous female with heterozygous In4 males the older observations were confirmed (Table 2); the unhatched eggs in trial 2 had generally not developed - no polar cell constriction - and must hence be distinguished as unfertilized. Now in order to test whether among the normally developing and

hatching embryos perhaps some with an abnormal chromosome constitution were present, the larvae were cytologically investigated. The analysis of the salivary gland chromosomes can, of course, be accomplished only beginning toward the end of the third instar and in the 4th instar; therefore the possibility that the perhaps existing individuals with an unbalanced genome died at an early stage must be excluded.² Besides, for this a limited quantity of freshly hatched larvae for rearing must be obtained and be counted again when grown up (see Table 2). Normally the mortality in *Chironomus* cultures is considerably high due to uncontrollable bacterial and protozoan development. Therefore the 3 experiments, in which it has hitherto succeeded in rearing over 80% of the larvae set up, are especially important.

The cytological investigation of the grown up larvae must aim first of all for the presence of "haplo 4" individuals, then dicentric bridges or their eventual breakage products are mitotically unstable and could be eliminated in the course of the cleavage divisions or later.

In this connection may a peculiar, single older observation be mentioned. In many hundred cytologically analyzed F₂ animals of the species cross *C. tentans* X *C. pallidivittatus* (see Beermann 1955) was found once a larva whose salivary gland nuclei as well as the normal diploid chromosome constitution, from the combined chromosomes of both species, additionally showed a dicentric chromosome 4 (Fig. 4). The "bridge-chromosome" must have resulted through simple crossing-over between a In4 chromosome of *C. tentans* and the uninverted homologous segment of chromosome 4 of *C. pallidivittatus*; whether the dicentric chromosome in the

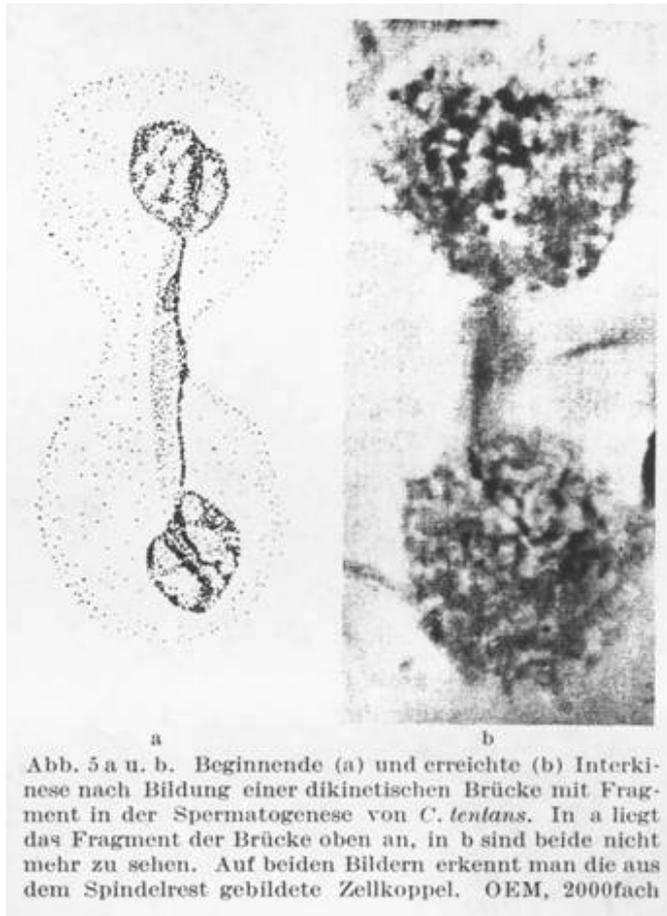
² At least for the haplo-4 genotype this possibility can be estimated in spite of the smaller relative length of the 4th chromosome, since haplo-4 individuals have still never been found in numerous experiments with *C. tentans* and *C. pallidivittatus* (over 3000 salivary gland preparations), in contrast to the repeated appearance of triplo-4 animals (see also note 5 in Table 2)

zygote came from the female or the male pronucleus cannot be determined. It remains unclear above all, in what manner the bridge can succeed when a supernumerary element in a euploid sperm or egg nucleus. As well it is noteworthy, that the bridge has been maintained over several mitoses - until the establishment of the salivary gland in the embryo. The fact that only about half of the salivary gland cells in the animal concerned contained the bridge, shows nevertheless that the elimination as such actually occurs and plays a part.

The cytological analysis of the F_1 of heterozygous In4 has as yet only in two crosses as specified above been performed; the findings (the experiments continued) are clear (Table 2): The haplo-4-genotype was not found, and just as few individuals with the dicentric bridge or their derivatives were found.

Since in the breeding experiments, in the best case, not even 10% of the zygotes died and since the mortality also in other aspects is less than the expected value, the result of the cytological study can only signify, that sperm without 4th chromosome and sperm with a dicentric bridge, or their breakage products, have not contributed to the insemination of eggs. One conclusion can be advanced already on the basis of the entirely normal developmental behavior of all offspring of heterozygous In4 males, when one considers that haplo-4 individuals, when noting their reduced viability, probably should show at least a retardation of the embryonic development.

Wolf (1941) has accurately investigated the behaviour of the dicentric chromatid bridge in the spermatogenesis of *Dicranomyia* and made observations therefrom, that have direct relevance to the problem formulated here:

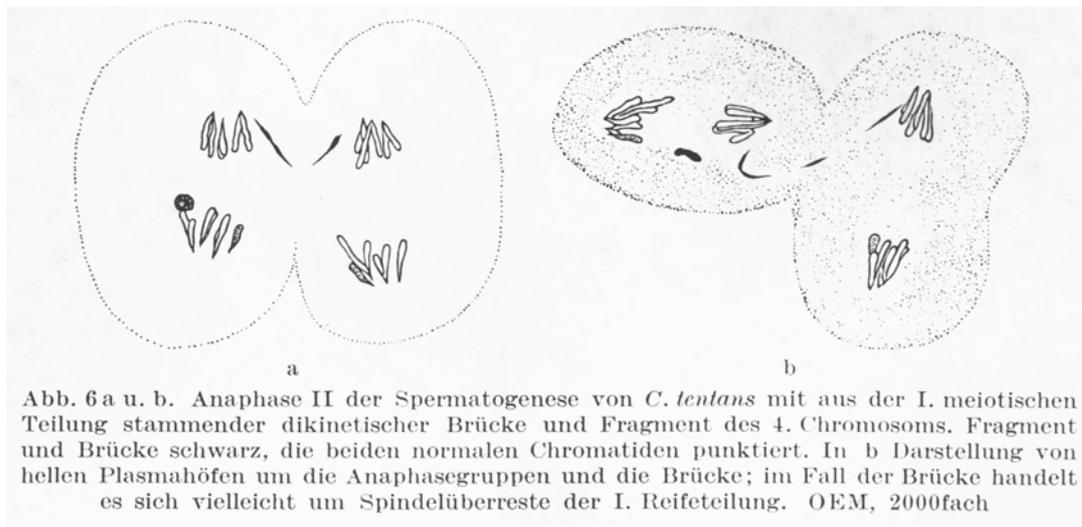


In *Dicranomyia* different length bridges were formed from different inversions. In first place the behavior of the longest bridge is interesting. As far as it is not broken was in the majority of cases appear not to be the case, it appears that it remains stretched between the daughter nuclei of the last maturation division during the interkinesis. Wolf showed that the sperm joined by a bridge fuse and become a double-nucleated sperm. Other observations, on short bridges, confirm the supposition that quite generally the cytoplasmic separation of the remnants of the spindle bodies

(“cell couplings”) ceases or is not complete, when chromatid bridges are stretched between the daughter nuclei.

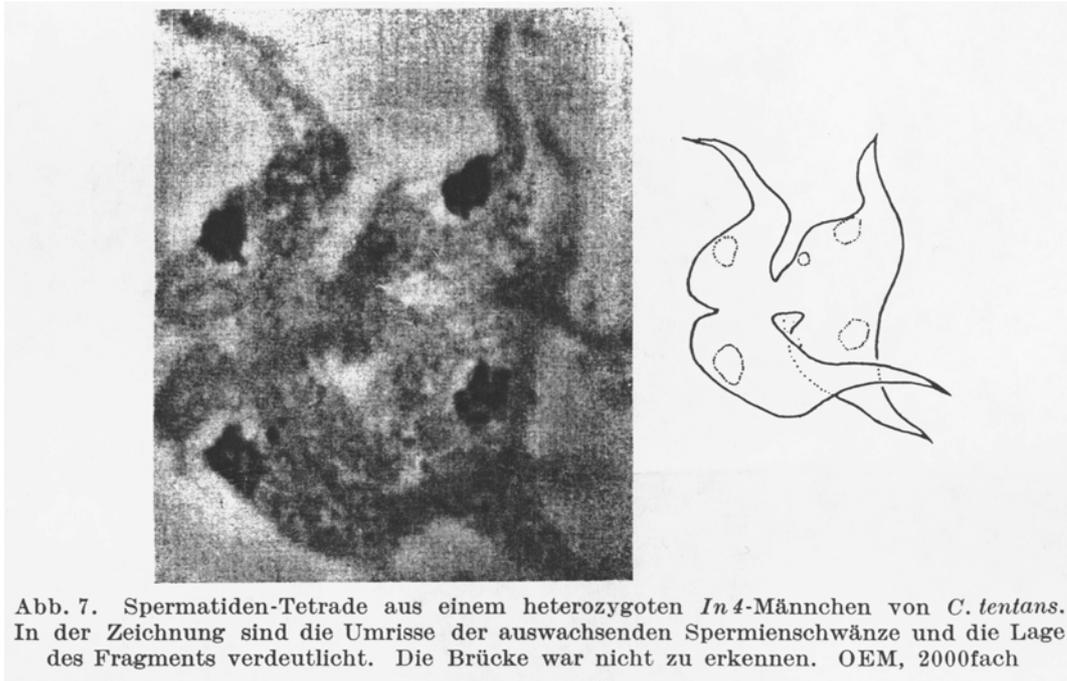
In *Chironomus* the spermiogenesis is not so clear. As in *Dicranomyia* – the area especially for analysis is a survey of the chromosome constitution of sperm nuclei. However the previous observations on *Chironomus* indicate an extensive conformity with *Dicranomyia*, both in the behavior of the long chromatid bridges and also the fragments. The long bridges formed by crossing over in a heterozygous In4 bivalent prevent the formation in diakinesis nuclei not separated; the bridge is stretched between these nuclei (Fig. 5). Both spermatocyte II's remain joined by a cell coupling. In anaphase II the picture is similar to that in *Dicranomyia*: always two of the spermatids are bound by a chromosomal and cytoplasmic bridge (the remains of the spindle from 1st meiotic division?) (Fig. 6). In telophase II the

bridges are invisible. Accurate data concerning the ultimate fate of the joined



spermatids is still missing: double-nucleated sperm were observed; whether these however also form two tails (see below) remains to be proved.

Also, the present investigation of the F1 of heterozygous In4 males of *Chironomus tentans* show that the aberrant sperm eventually resulting from a double-spermatid has no chance of insemination. The morphological or physiological cause of this phenomenon must still be clarified. The composition of the genome should not affect the mobility and efficiency of animal sperms, as extensive genetic results with deficiencies and duplications show; nevertheless it is possible that hyper- and hypo-ploidy can influence the duration of the differentiation of spermatid to a greater extent, and that the sperm of young males initially contain preponderantly normal sperm. Very closely related however is the already indicated possibility that doubling or malformation of the motion apparatus, possibly developed through the presence of two centrosomes in the same spermatid, causes the unsuitability of the double sperm. It can be observed that a spermatid tetrad with 4 nuclei and a fragment (however also with a possibly unbreakable bridge) form four separate



motion apparatus (Fig. 7). Further investigation should show whether such an interpretation is correct. In any case, these and Wolf's findings probably show that the inversion heterozygous males, in a wholly similar manner as their female companions, always produced only normal euploid gametes although inversion bridges regularly form in meiosis. Bauer and Timoféeff-Ressovsky (1943) have already shown this in *Dicranomyia*.

Formally shown when the above expressed conclusion proved correct, inversion heterozygous males are certainly also then not unrestrictedly fertile; then in no case can (*ceteris paribus*) the absolute number of functionally efficient sperm produced by heterozygous males be the same as from structurally homozygous males. This difference can however for this reason scarcely play a role, because the number of sperm produced by a *Chironomus*-male certainly amounts to far over 10,000, from which are utilized on average at most 3,000 for insemination (under the supposition that each male only copulates once, every female has at most 3,000

eggs, and that polyspermy does not occur).³ The selective value of inversion heterozygous males would however scarcely be affected by the theoretically necessary numerical loss of normal sperm. First, when several inversions in different chromosomes are present, this could be altered: each additional bridge raises of course the probability that all 4 partners of a spermatid tetrad simultaneously are aberrant. Since however the chiasma frequency in each arm of the 3 long V-chromosomes of *C. tentans* amounts to only 0.4 - 0.5 (Beermann, unpublished), inversion bridges in the larger bivalents of *C. tentans* could occur at best in 50% of all meiosis (as long as both arms are not inversion heterozygotes). As well, the most inversions are shorter than In4, so that not every chiasma must fall within their length. Therefore even in heterozygotes for 3 or 4 inversions the actual fertility of *Chironomus* would scarcely be reduced.⁴

Summary

1. Bivalents of chromosome 4 of *C. tentans*, even when heterozygous for inversion *In(t)4-1*, form a chiasma during spermatogenesis with a frequency of 95%.
2. Already at prometaphase it is possible to see with certainty that in heterozygotes for *In(t)4-1* the chiasma is always within the inversion -, so that therefore in up to 95% of all meioses in the male, a dicentric chromatid bridge and an acentric fragment will be formed.
3. Experimental crosses show - in combination with controlled rearing and cytological analysis - that inversion heterozygote males (*In4-1*) produce a fully viable and cytologically normal F1.

³ The finding that in cross 2 (Table 2) about 80% of the eggs remain infertile, could perhaps be interpreted as a consequence of an exhaustion of the father's sperm reserve, as they must occur when one and the same male copulates more females; whether the sperm reserve of a heterozygous In4 male lasts not even for 2 occasions (in total about 4,000 eggs) appears doubtful, but was not investigated. Possibly the male used in the cross 1 and 2 had already copulated before isolation.

4. These results are best interpreted, that the spermatids bound by a chromatid bridge are totally or partially fused with one another, so that they are seen as a single object in spermiogenesis (Wolf 1941). The double sperm arising in this manner are probably inferior and not capable of insemination. The decrease in the total number of normal sperm can hardly maintain the fertility and thereby the selective value of the heterozygous male in nature.

⁴ The investigation was continued. A full presentation of the meiosis in *Chironomus* females is in preparation.

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