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Factors affecting the observed number of young resulting from adjacent-2 disjunction in mice carrying a translocation

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SUMMARY

The frequency of adjacent-2 disjunction in mice carrying the reciprocal translocation T(9; 17)138Ca was studied by mating together animals heterozygous for the translocation and carrying different recessive marker genes, using *Tt* for chromosome 17 and *cwcw* for chromosome 9. The proportion of marked young arising from adjacent-2 disjunction varied according to the markers carried in the two parents. When the female carried *Tt* the frequencies of marked young were always higher than when non-*T* females were used, and when *Tt* and *cwcw* were carried in the same parent there was a shortage of marked young obtaining both copies of the proximal region of chromosome 17 from the father. Both these effects were regarded as probably another example of the phenomenon discovered by Johnson, of inviability of young lacking a maternal homologue of a certain region of chromosome 17. There were other variations in frequency of marked young, among crosses using non-*T* females, which may have been due to differences in transmission ratio of male gametes carrying various *t*-haplotypes or to true variations in frequency of adjacent-2 disjunction.

1. INTRODUCTION

Work on non-disjunction in mice with Robertsonian translocations, or on adjacent-2 disjunction in those with reciprocal translocations, may provide information concerning the factors affecting chromosomal non-disjunction in general, and may therefore be relevant to the question of genetic hazards to man.

In a previous paper concerning adjacent-2 disjunction in mice heterozygous for the reciprocal translocation T(9; 17)138Ca we found an unexpected large difference between the progenies of reciprocal crosses (Lyon, Glenister & Hawker, 1972). The present paper describes further work aimed at ascertaining the cause of this difference.

The principle of the method is to mate together mice heterozygous for the translocation and homozygous for recessive genetic markers in the interstitial segment. If the two mates are homozygous for different markers all the offspring arising by normal disjunction will be heterozygotes and therefore phenotypically wild type. Zygotes formed by union of complementary gametes arising by adjacent-2 disjunction may be homozygous for the markers, however, and so can

be picked out. In the earlier work mice heterozygous for T138 and carrying either the chromosome 9 marker curly whiskers, *cw*, or the chromosome 17 markers Tt^{h2} were mated together (Fig. 1). Offspring arising by normal alternate or adjacent-1 disjunction would all be non-*cw*, and have short ($T/+$) or full length ($+t^{h2}$) tails, whereas offspring arising by adjacent-2 disjunction and receiving complementary gametes from the two parents could be genetically Tt^{h2} *cwcw*, and therefore tailless with curly whiskers.

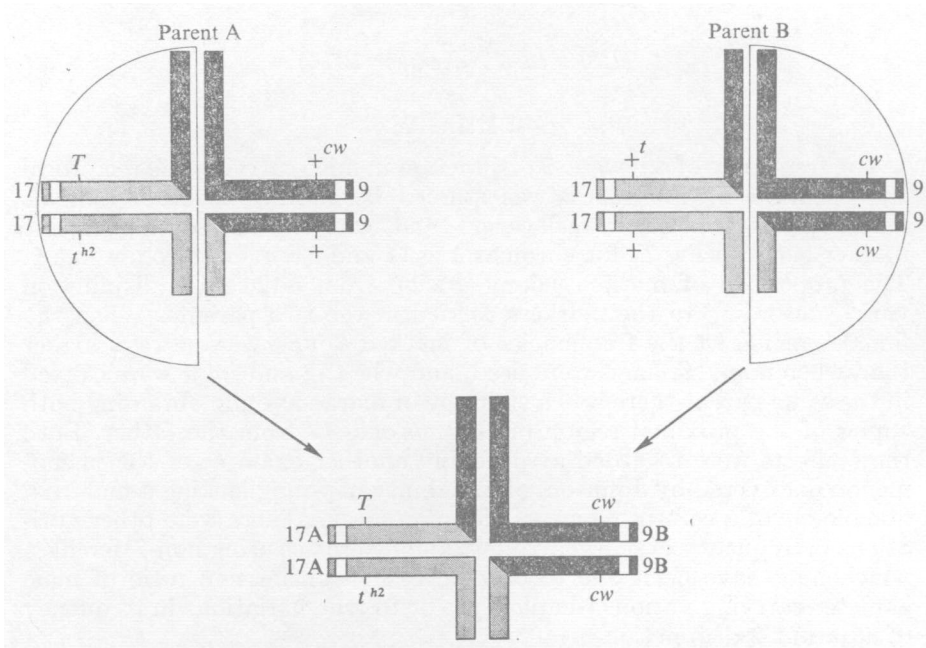

Fig. 1. Origin of marked young by union of complementary gametes arising by adjacent-2 disjunction in both parents, in crosses 1, 1A and 5. In cross 1 the mother is parent A and the father parent B, and in cross 5 the parents are reversed. Cross 2 differs from cross 1 in the substitution of t^6 and T for T' and t^{h2} respectively in parent A, and cross 6 differs similarly from cross 5. In crosses 3 and 7 the *cw* gene is carried in parent A, rather than parent B, leading to two types of marked young.

When the female parent carried Tt and the male *cwcw* the frequency of young resulting from adjacent-2 disjunction was high (*c.* 4%), whereas if the female carried *cwcw* and the male Tt the frequency of marked young was very low (*c.* 0·6%). No such reciprocal difference was reported by Searle, Ford & Beechey (1971) after similar work with other translocations. On the other hand, haplotypes of the *t*-complex, such as t^{h2}, are known to affect male segregation ratio, male fertility, and recombination in both sexes (Bennett, 1975). It therefore seemed possible that the difference between reciprocal crosses involving t^{h2} might be due to some effect of this haplotype on male reproduction or crossing-over and to test this the crosses were repeated, using the same source of *cw*, but a different *t*-haplotype, t^6. There was also a limited amount of data from crosses in which $+ + ^t$ animals were used in place of those carrying Tt.

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Since differences in chiasma frequency in the relevant chromosome arms might affect adjacent-2 disjunction, we attempted to measure this frequency by counting the various types of multivalent configurations found at first meiotic division in male mice heterozygous for the translocation and carrying different markers.

2. MATERIALS AND METHODS

(i) *Breeding*

Translocation T(9; 17)138Ca is a reciprocal translocation (Carter, Lyon & Phillips, 1955, 1956), with one break in chromosome 9, about 28 map units from the *cw* locus and 18 from *d* and *se* (Green & Stimpfling, 1966; Lyon, 1977). The locus of *cw* is thought to be about 2 units from the centromere (Lyon, Butler & Kemp, 1968). In chromosome 17 there is 30–35 % crossing-over between the *T*-locus and the translocation break. However, the presence of the translocation markedly alters crossing-over in the interstitial segment of chromosome 17. Lyon & Phillips (1959) found that recombination between *T* and *tf* was approximately doubled in T138 heterozygotes, and Klein & Klein (1972) found a proportionately similar increase for the *T–H-2* interval. The recombination between the *H-2* locus and the T138 break was only about 3 %.

As genetic markers of the interstitial regions we used the *cw* locus in chromosome 9 and the *T*-locus in chromosome 17. As animals homozygous for *T*-locus alleles either die (*TT*) or are indistinguishable from normal (*tt*), we used heterozygotes, *Tt*, which are viable and tailless. The haplotype t^{h2} is viable when homozygous, has a low segregation ratio from male heterozygotes, and does not suppress crossing-over (Lyon & Meredith, 1964). (In a previous paper t^{h2} was erroneously said to have a normal segregation ratio.) t^6 is lethal, with a high male segregation ratio, and suppresses crossing-over between *T* and *tf*.

The T138 heterozygotes were bred by appropriate crosses between random-bred stocks of the various types of homozygote:

$$\text{T138 } cw/\text{T138 } cw \times +cw/+cw \rightarrow \text{T138 } cw/+cw,$$
$$\text{T138 } T+/\text{T138} + + \times + t^{h2}tf/+t^{h2}tf \rightarrow \text{T138}T+/+t^{h2}tf,$$
$$\text{T138 } T+/\text{T138 } t^6+ \times +Ttf/+ +tf \rightarrow \text{T138}t^6+/+Ttf.$$

In order to detect adjacent-2 disjunction the heterozygotes were then mated together, e.g. T138 $cw/+cw \times$ T138 $T+/+t^{h2}$ *tf*. Two females were mated to each male and the animals were left to breed for their natural reproductive life. The offspring were classified for *cw*, *T* and *t*, and those exhibiting these markers were kept and genetically tested.

(ii) *Cytogenetics*

Males from each of the main stocks used were killed and cytological preparations were made from their testes by the method of Evans, Breckon & Ford (1964). With the slides coded, approximately 100 diakinesis or first meiotic metaphase figures from each male were scored for the type of translocation configurations present. No attempt was made to classify the cells as to the stage of metaphase reached.

Table 1. *Anomalous (or marked) offspring from crosses of heterozygotes for T138*

	Parents		Offspring				
	♀	♂	$T+$ or $++$	*Tt*	*cw*	*Ttcw*	% marked
(1)	Tt^{h2}	*cwcw*	604	—	1*	22	3·67
(1 A)	Tt^{h2}	*cwcw*	447	—	—	25	4·98
(2)	Tt^{6}	*cwcw*	361	—	—	11	2·96
(3)	$Tt^{6}cwcw$	$++$	79	9	0	—	10·2
(4)	Tt^{6}	$++$	182	10	...	...	5·21
(5)	*cwcw*	Tt^{h2}	863	2*	—	2	0·46
(6)	*cwcw*	Tt^{6}	794	1*	—	11	1·49
(7)	$++$	$Tt^{6}cwcw$	195	0	3	—	1·5
(8)	*cwcw*	$++$	251	...	1	...	0·40
(9)	$++$	*cwcw*	97	...	1	...	1·02

—, Class unexpected; ..., class unobtainable.

* These animals were unexpected and the explanation for their occurrence is not known. They could have arisen through numerical non-disjunction, crossing-over between the *T*-locus and the centromere, or misclassification of $T+$ as *Tt*, but all died or became sterile before genetic tests were completed.

Cross 1 A was a repeat of cross 1. Crosses 1 and 5 include data of Lyon *et al.* (1972).

3. RESULTS

(i) *Breeding*

The first point revealed by perusal of the breeding data (Table 1) was that all crosses in which the female was genetically *Tt*, whether Tt^{h2} or Tt^{6} (crosses 1–4), gave a higher yield of marked young than crosses with a *cwcw* or $++$ female (crosses 5–9). This difference is quite clear, there being no overlap in frequency between the two types of cross, and it persists undiminished if one compares crosses using the same type of male. Thus, crosses 1, 1 A and 2 with *cwcw* males and *Tt* females gave a high frequency whereas the cross involving $++$ females and *cwcw* males (cross 9) gave a low frequency. Similarly, when $++$ males were used (crosses 3, 4 and 8) there was a clear difference between the results for *Tt* females and those for *cwcw* females. Reference to Fig. 2 shows that in crosses 1–4 the marked young received two copies of the proximal segment of chr. 17 from the mother (except for *cwcw* young in cross 3, which are considered below), whereas in crosses 5–9 the marked young in most cases received both copies of proximal 17 from the father, the exceptions being *cwcw* young from crosses 7 and 9.

Other interesting points emerge from the two crosses (nos. 3 and 7) in which one parent was genetically Tt^{6} *cwcw*. Here two types of marked young are expected, *Tt* and *cwcw*. By this means two classes of young arising from adjacent-2 disjunction are detected, whereas in all other crosses only one class is detected, all other surviving types being unmarked. Thus, potentially the total frequency of marked young in each of these crosses should be double that in other comparable crosses, and whatever the frequency of adjacent-2 disjunction in either parent, the two types of marked young would be expected to occur with equal frequency. In fact,

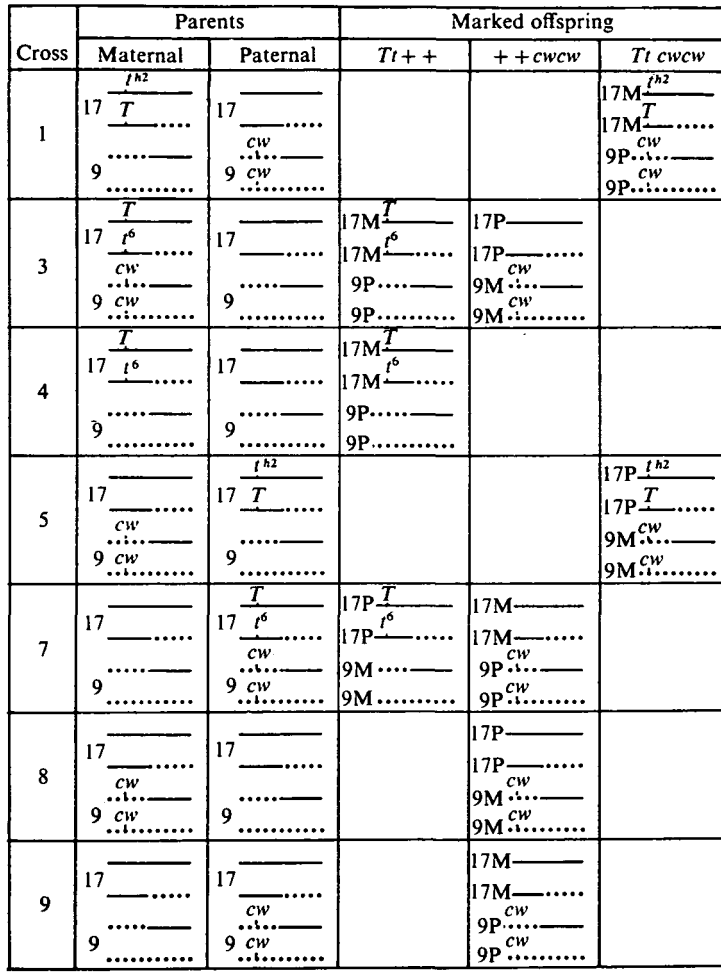

Fig. 2. Parental origin of proximal region of chromosomes 17 and 9 in marked offspring in various crosses, M = maternal; P = paternal. Offspring arising by normal disjunction would receive one each of 17M, 17P, 9M, 9P. As in Fig. 1, crosses 2 and 6 differ from crosses 1 and 5 in the substitution of t^6 and T for T and t^{h2}.

there were 9 *Tt* and 0 *cwcw* young from cross 3 and 0 *Tt*: 3 *cwcw* from cross 7. Although the numbers of animals involved are small, the probability of obtaining by chance such a deviation from equality of the two types of marked young is very low ($P < 0{\cdot}001$). Thus, these results cannot be explained simply by differences in frequency of adjacent-2 disjunction. The two types of young which were found, *Tt* from cross 3 and *cwcw* from cross 7, both received two copies of the proximal region of chromosome 17 from the mother (Fig. 2). Hence, the intra-cross differences in frequency of marked young found here parallel the differences between crosses mentioned above.

Further differences, apparently not related to the parental origin of the marked chromosomes, occurred within the group of crosses involving non-*t*-females

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Table 2. *Viability and genetic tests of marked young from various crosses*

Cross	Total	Died in nest	Died later or sterile	Tested			I.T.
				T138/+	+/+	T138/T138	
1	23	2	3	17	—	—	1
1A	25	—	3	20	1	1	—
2	11	—	1	10	—	—	—
3	9	2	—	7	—	—	—
4	10	1	1	8	—	—	—
Total	78	5	8	62	1	1	1
5	4	1	3	—	—	—	—
6	12	8	3	1*	—	—	—
7	3	—	—	3	—	—	—
8	1	1	—	—	—	—	—
9	1	—	—	1	—	—	—
Total	21	10	6	5	—	—	—

I.T. = incompletely tested.
* This animal was tailless non-*cw*.

(crosses 5–9), particularly among the three crosses involving *Tt* males (nos. 5–7). Those with t^6 males showed a higher frequency of marked young than the one involving t^{h2}, the difference being statistically significant ($\chi_1^2 = 4{\cdot}65$; $P < 0{\cdot}05$). In the cross involving *cwcw* females and + + males only a single marked offspring was born, and this animal died within one day of birth, so that some doubt as to its genotype must remain. If this single animal is accepted as arising from adjacent-2 disjunction then the frequency from this cross is within the general range of values given by the other crosses using *cwcw* females.

As many as possible of the marked young were kept and genetically tested, in order to obtain further evidence concerning their mode of origin. The total offspring from crosses of T138/+ × T138/+ would of course be expected to be in the approximate ratio 1 T138/T138 : 2 T138/+ : 1 +/+. Young arising through adjacent-2 disjunction, however, would be expected to be predominantly T138/+. Table 2 shows that out of 69 marked young from various crosses which were successfully tested 67 proved to be T138/+. Of the two exceptions, both from cross 1A, one was T138/T138 and the other +/+. These two animals could be explained if crossing-over had occurred in the interstitial segment prior to disjunction. When the frequencies of the various chromosomal types of marked young to be expected in the presence and absence of crossing-over are considered, the observation of 2 homozygotes out of 69 is clearly in accord with a reasonable level of crossing-over in the two interstitial segments.

Occasionally, an animal which is genetically *T* +, rather than *Tt*, will show the tailless phenotype, and thus the question arises whether any of the tailless young in our crosses were in fact *T* + rather than *Tt*. In crosses 1, 2, 5 and 6 where the marked young were expected to be *Tt cwcw*, it was thought necessary to test genetically only those tailless animals which did not also have curly whiskers.

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However, of these few animals, the 2 in cross 5 died without being adequately tested, and the animal in cross 6, which incidentally was the only animal in this cross which was proved T138/+, was a female which bred poorly so that its full genotype was not established. Thus, the possibility remains that this animal was genetically $T/+$ and not the product of adjacent-2 disjunction. In cross 3, where the tailless young were expected to show no other markers, as many as possible were tested for *cw*, since if they arose by normal disjunction they should be heterozygous for *cw*, whereas those arising by adjacent-2 disjunction should be homozygous $++^{cw}$. Five animals were so tested and all proved $++^{cw}$. Similarly, in cross 4 six tailless animals were tested for t^6; 5 proved to carry t^6 and the sixth animal was a female not fully tested. Thus, this evidence is all consistent with origin of the marked animals by adjacent-2 disjunction.

It was not possible to test all marked animals, as some died before or soon after weaning age, and others proved sterile. (There is a relatively high incidence of imperforate vagina in this stock.) Table 2 shows that the proportion of animals that died varied among the crosses. In crosses 1–4 in which there was a high frequency of marked young, only 5 of 78 marked animals died before weaning age, whereas in crosses 5–9, with a low frequency, 10 out of 21 died before weaning. This is notwithstanding the fact that the *cwcw* and ++ females in crosses 5–9 were in general better mothers and raised a high proportion of their unmarked young. Since some of the 10 marked young which died did so within only a few days of birth the possibility arises that there were other marked animals which were either stillborn or died within a few hours of birth, and were eaten by the mother before the litter was recorded. Thus, the viability of the marked young must be borne in mind in seeking explanations for the difference in frequency among the crosses.

(ii) *Cytogenetics*

The proportions of cells showing various types of translocation configuration at first meiotic division varied significantly among the groups of males (Table 3). The two groups of Tt males both showed lower frequencies of rings and higher frequencies of cells with no quadrivalent (20II) than the *cwcw* males. The two groups of *cwcw* males were scored at different times, each group concurrently with either the Tt^{h2} or the Tt^6 males, and show good agreement with each other indicating that the results were repeatable. The two types of tailless males Tt^{h2} and Tt^6, also differed from each other in frequency of ring quadrivalents ($\chi^2_1 = 16{\cdot}7$, $P < 0{\cdot}001$).

If one assumes that in a ring quadrivalent there were 4 chiasmata, in a chain 3, and in the absence of a quadrivalent 2 chiasmata in the chromosomes involved, then one may calculate that the mean number of chiasmata in the various groups of males were: *cwcw*, 3·62; Tt^{h2}, 3·23; Tt^6, 2·96. The haplotype t^6 is known to suppress crossing-over in the interstitial segment of chromosome 17, over the region from the locus of *T* to *H-2*, and t^{h2} shows partial suppression, and hence these results could well be due to the effects of these haplotypes in reducing

Table 3. *Translocation configurations at meiosis I in male T138 heterozygotes*

Male type	No. males	Configuration RIV	ChIV	20II	Total	R (%)	Ch (%)	20II (%)
Tt^{h2}	4	233	57	134	424	55·0	13·4	31·6
cwcw	6	539	84	82	705	76·5	11·9	11·6
Tt^{6}	4	162	55	181	398	40·7	13·8	45·5
cwcw	4	284	69	47	400	71·0	17·3	11·7

RIV = ring quadrivalent; ChIV = chain quadrivalent; 20II = no quadrivalent.

chiasma formation (Forejt, 1972). However, since the *cwcw* males came from a different stock, one cannot rule out the possibility of other factors.

4. DISCUSSION

The results show clearly that the most important difference in frequency of marked young was that between tailless and non-tailless females, but there were also further subsidiary differences, such as those between Tt^{6}, Tt^{h2}, or + + males, when all were mated to *cwcw* females.

Numerous possible mechanisms for these effects can be envisaged, including:

(i) variation among crosses in frequency of adjacent-2 disjunction;

(ii) abnormal transmission ratios or selective fertilization of various types of gamete, e.g. sperm nullisomic for the proximal segment of chromosome 9, or disomic for proximal 17 being transmitted with a low ratio;

(iii) differences in viability of the zygotes according to the parental origin of the gametes.

We mentioned that, with some exceptions, the crosses giving a low frequency (crosses 5–9) were those in which the marked young derived both copies of the proximal part of chromosome 17 from the father (Fig. 2). There is already evidence that zygotes derived from female gametes lacking some part of proximal chromosome 17 have reduced viability. Johnson (1974, 1975) showed that heterozygotes for the *T*-allele T^{hp} differed phenotypically depending on whether the mutant allele was derived from the mother or father, with the former type having a very low viability. Bennett (1975) and Biedler (quoted by Bennett) have shown that T^{hp} involves a deletion, genetically covering the locus of *qk* as well as *T*. In our tests, young arising from adjacent-2 disjunction and deriving *Tt* markers from their father and/or *cwcw* from the mother would have received a maternal gamete lacking the whole of the proximal part of chromosome 17, from the centromere to well beyond *qk*. (Fig. 2). Thus, this gamete must have lacked the region whose deficiency causes the low viability of T^{hp} heterozygotes. Clearly then, marked young from some of our crosses would be expected to have low viability. Fig. 2 indicates that marked young from crosses 5, 6 and 8, together with *Tt* young from cross 7 and *cw* young from cross 3, would have this low viability. This fits with our observation (Table 2) that marked young from crosses 5–9 had low postnatal

viability. Indeed, if we consider only the crosses expected to give low viability we see that in crosses 5, 6 and 8 as many as 10 out of 17 marked young died before weaning and in crosses 3 and 7 none of the low viability types were seen. Thus this factor alone could explain a large part of the variation in frequency of marked young in our crosses. However, the *cwcw* marked young from crosses 7 and 9 would be expected to have normal viability, and hence if reduced viability of marked young were the only factor operating, these crosses should give the same frequency as crosses 1 and 2.

It thus seems that other factors in addition to the Johnson effect must have affected the results in some crosses. It is possible that there were true differences among the various parental types in frequency of adjacent-2 disjunction. If $++^{t}$ females gave a lower incidence of adjacent-2 disjunction than Tt females this could explain the lower frequency of marked young from crosses 7 and 9 than from crosses 1–4. We saw there were differences between the Tt and *cwcw* males in chiasma frequency, which might well have affected the likelihood of adjacent-2 disjunction, but we have no evidence concerning the chiasmata in females.

The differences among crosses 5, 6 and 8, in which *cwcw* females were mated to Tt^{h2}, Tt^{6} or $++$ males might again be explained by true differences in adjacent-2 disjunction among the types of males, or perhaps might be due to differential transmission or fertilization of gametes.

Since the haplotype t^{6} has a high male segregation ratio and t^{h2} has a low ratio it might be that genetically Tt^{6} male gametes produced by adjacent-2 disjunction were transmitted at a higher rate than similar but genetically $++$ or Tt^{h2} gametes. This would imply that the effect of the *t*-complex on segregation ratio operated in disomic sperm. However, since we know that the types of males differed in chiasma frequency the data do not differentiate between variations in adjacent-2 disjunction or in male segregation ratio as possible explanations for the differences among types of males in frequency of marked young.

A valuable point from this work is that it provides another instance, and thus confirmation, of the remarkable phenomenon discovered by Johnson (1974, 1975) and Bennett (1975) of the inviability of zygotes receiving only a paternal homologue of a certain region of chromosome 17. Since the chromosome segment involved in our work is larger than the deletion present in T^{hp} our results provide no information on the exact position of the genetic factor involved. Nor do they indicate the time of action in embryogeny. Johnson found that although some of the inviable heterozygotes died soon after implantation a sizeable proportion survived until late foetal stages when they showed oedema and postaxial polydactyly. Our data indicate that some of the relevant marked young could survive birth, and even weaning. Johnson discussed whether the abnormal gene action might occur in the oocyte after meiosis, or whether in view of the relatively late survival of the affected zygotes, the malformation occurred later. Possibly certain genetic material derived from a male gamete might be chromosomally imprinted and inactive unless acted on by a maternal homologue. However, our results provide no information which would test this point.

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