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Genetic studies on male sterility of hybrids between laboratory and wild mice (*Mus musculus* L.)

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SUMMARY

The genetic control of the sterility of male hybrids between certain laboratory and wild mice (*Mus musculus* L.) is investigated. The observed sterility is, by definition, hybrid sterility since both parental forms (i.e. wild and laboratory mice) are fully fertile, their male offspring displaying small testes with arrest of spermatogenesis at the stage of spermatogenesis or primary spermatocytes. Results of genetic analysis as well as the failure to detect any chromosomal rearrangements point to a genic rather than a chromosomal type of hybrid sterility.

Fifty-three wild males were classified into three sets, after mating with C57BL/10 inbred females, according to the fertility of their male progeny (set I – only sterile sons; set II – only fertile sons; set III – both fertile and sterile sons). The wild males of set I, which yield only sterile male offspring with C57BL/10 females, sire sterile sons also with females of the following inbred strains: A/Ph, BALB/c, DBA/1, and AKR/J, whereas the same wild males produce fertile offspring with females of C3H/Di, CBA/J, P/J, I/St and F/St inbred strains.

The described hybrid sterility seems to be under the control of several independently segregating genes, one of them (proposed symbol *Hst-1*) being localized on chromosome 17 (linkage group IX), 6 cM distally from dominant *T* (*Brachyury*). A chance to search for the mechanism of hybrid sterility is provided by the finding of two laboratory inbred strains, C57BL/10 and C3H/Di, differing with respect to the *Hybrid sterility* genetic system only at the *Hst-1* gene.

Hst-1 is closely linked but apparently not identical with the sterility factor of recessive *t* alleles of the *T* locus.

1. INTRODUCTION

Hybrid sterility is one of the reproductive isolating mechanisms safeguarding the integrity of species. Infertility of interspecific hybrids belongs to the oldest biological problems to which human attention has been paid. The sterility of the mule had been already discussed by Aristoteles, and infertility of various plant and animal hybrids had been recognized long before Mendel established the first principles of genetics (Mayr, 1963; Dobzhansky, 1951, 1972).

Present knowledge of the genetic control of hybrid sterility comes mainly from studies on Drosophila species (Dobzhansky, 1951; Mayr, 1963). It is generally

accepted that hybrid sterility is either chromosomal, if the parental species display gross structural non-homology of their chromosomal complements, or genic if ensured by interaction of specific genes.

Neither the sequence of events leading to hybrid sterility nor the products of 'isolating genes' have been hitherto identified in any species. The nature of hybrid sterility should be most easily elucidated in species which are easily accessible not only to genetic analysis but also to biochemical and physiological investigations. In mammals such a premise is met by the house mouse (*Mus musculus*). Recently incomplete sterility of the hybrids between *Mus musculus* and *Mus poschiavinus* (tobacco mouse) has been observed (Gropp *et al.* 1972). There is a drastic change in the karyotype of the tobacco mouse caused by the occurrence of the 14 Robertsonian translocations, nevertheless it appears that the reduced fertility of hybrids is associated with some genic differences between the two species (Cattanach & Moseley, 1973).

The other example of sterility of hybrids between laboratory and wild mice has been described in this laboratory (Iványi *et al.* 1969; Iványi & Micková, 1971). It was found that certain male wild mice sired completely sterile sons when mated with females of the C57BL/10 inbred strain, but the same males yielded fertile progeny with C3H inbred strain females. The genetic factor associated with the *Histocompatibility-2* (*H-2*) complex on chromosome 17 (linkage group IX) was found to be the major cause of the sterility of male hybrids.

The present paper describes further genetic studies aimed to localize this gene (proposed symbol *Hst-1*) on chromosome 17, and to ascertain its relationship to the recessive *t* alleles of the *T* locus. An opportunity for the investigation of the mechanism of the observed hybrid sterility arises from the finding of two unrelated inbred strains of mice which differ, with respect to the *Hybrid sterility* genetic system, at only a single gene – *Hst-1*. The G-banding of mitotic chromosomes as well as C-bands of meiotic chromosomes were studied in sterile males in order to distinguish between the chromosomal and the genic type of hybrid sterility.

The term 'wild mice' as used in this paper refers to the wild-living feral mice (*Mus musculus* L.) captured at specified localities. The term 'hybrid mice', if not stated otherwise, refers to hybrid progeny of wild males and females of laboratory (inbred) strains.

2. MATERIALS AND METHODS

(i) *Mice*

Altogether 91 wild male mice were tested for fertility of their male progeny obtained from the cross of wild males with females of several inbred strains. The wild males were trapped at 14 localities of Central Bohemia. Of these, eight localities were in Prague Zoological Garden, where the trapping places were from 10 m to 1 km apart. Three male mice from the Jutland Peninsula (Denmark) and five males from Great Gull Island (Connecticut, U.S.A.) were obtained through the courtesy of Professor L. C. Dunn, Columbia University, N.Y. These mice have been already explored for distribution of H-2 antigens (Micková & Iványi, 1971),

protein polymorphism (Selander, Hunt & Yang, 1969) and polymorphism of the *T* locus (Dunn & Bennett, 1971). The female wild mice are poor breeders in captivity; this was the reason why only wild males were used in the study.

The following inbred strains of mice maintained at this Institute were employed: C57BL/10ScSnPh (abbreviated B10); C3H/Di (C3H); A/Ph (A); AKR/J; BALB/c; CBA/J; DBA/1; I/St; F/St; and P/J.

For testing the interaction between *t* alleles and *Hst* genetic system, five non-inbred balanced lethal stocks were used: T/t^6; T/t^{12}; T/t^{w1}; T/t^{w12}; *and* T/t^{w8}.

(ii) *Plan of routine testing of wild male mice*

A routine testing programme for each wild male consisted of mating to (1) B10 females, (2) C3H females and (3) (B10 × C3H) F_1 hybrid females. The aim of the cross (1) was to find out whether the wild male produced sterile sons, that of cross (2) to obtain 'control' fertile sons from the same wild male, and that of cross (3) to obtain data on segregation of sterility factor(s).

Not all wild males underwent the complete testing programme. The shortened scheme consisted of crosses (1) and (3), and some males were tested only by cross (3).

(iii) *Testing of fertility of hybrid males*

The fertility of hybrid males was estimated from the number of offspring per mating unit (OMU). One mating unit consisted of one male caged with one female for 1 month. Each hybrid male was tested for at least 10 mating units. We soon realized that the impairment of fertility corresponded well to the size of the testes (see Results); therefore in recent experiments only testes weight was used as a criterion of fertility.

The wet weight of paired testes was scored to the nearest milligramme in males aged 2–4 months. In hybrid males we found no significant correlation between body and testes weights, hence the latter was presented in absolute values. From this viewpoint, some of our earlier data (Iványi *et al.* 1969; Iványi & Micková, 1971) are re-evaluated in the present paper.

(iv) *Hormonal assays*

Androgen activity of sterile male hybrids and their fertile sibs was judged from the relative weights of seminal vesicles (ratio of 10^3 × wet seminal vesicle weight to whole body weight). Plasma testosterone concentrations in both types of hybrid were determined by immunoprecipitation by means of TESTOK kits (Cea Ire, Sorin).

(v) *Serotyping of H-2 antigens*

The H-2 antigens were determined by PVP haemagglutination (Stimpfling, 1961). The *H-2* genotype was estimated in wild mice through the *H-2* analysis of their offspring. The detailed data on H-2 antigens in wild mice and the serological technique were published elsewhere (Micková & Iványi, 1971).

(vi) *Cytogenetic methods*

The C-banding method for the air-dried meiotic and mitotic preparations was described in previous papers (Forejt, 1972*a*, 1973). The trypsin-induced G-banding of mitotic chromosomes (Seabright, 1972) was done using a modification of Burkholder & Comings (1973).

3. RESULTS

(i) *Fertility of hybrid male offspring from crosses – wild male × B10 inbred strain female*

As shown in previous papers (Iványi *et al.* 1969; Iványi & Micková, 1971) certain wild male mice (W) yielded sterile male progeny when crossed with female mice of the B10 inbred strain. In accordance with Haldane's rule (Haldane, 1922), the impairment of fertility was limited only to the male sex, hybrid females being always fertile.

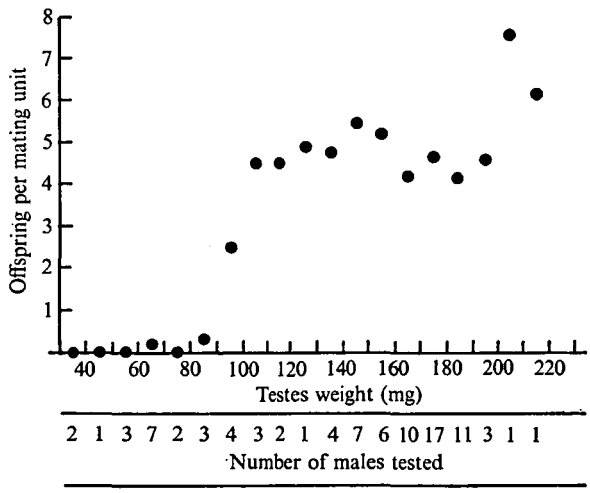

Fig. 1. Relationship between testes weight and fertility (OMU) in (B10 × W) hybrids. Normal fertility corresponds to OMU values above 4.

Fertility of hybrid males was determined quantitatively as the number of offspring per mating unit (OMU). Fig. 1 shows the dependence of OMU value on the testes weight; hybrid males with testes weight lower than 75 mg are sterile, whereas the normal fertility is ensured by testes heavier than 120 mg. Testes weight values in the range of 75–120 mg reflect the continuous variation from complete sterility to fertility.

Generally, three types of wild male were distinguished, after crossing with B10 females, as suggested from the presence in their litters of (*a*) only sterile, (*b*) only fertile or (*c*) both sterile and fertile male offspring. Of the 91 wild males, 53 gave a sufficient number of sons (five or more), when mated with B10 females: of these, 23 wild males (set I) yielded only 'sterile' sons (mean testes weight below 85 mg,

cf. Table 1), 20 wild males (set II) sired only 'fertile' progeny (mean testes weight more than 110 mg, see Table 2) and 10 wild males (set III) displayed both types of son in their litters (Table 3).

Table 1. *Male wild mice – set I; mean testes weights of (B10 × W) male hybrids*

Male wild mouse		(B10 × W) hybrid male progeny		
			Testes weight*	
Locality	Coding no.	No. of males	Mean	S.E.
Zoo-3	152	24	57·8	2·8
	153	14	57·4	3·1
	155	17	51·7	1·5
Zoo-4	161	15	53·0	1·4
	202	17	51·8	1·1
Zoo-5	214	5	75·2	3·8
	215	7	70·6	2·5
	216	5	54·0	2·4
	224	13	75·1	2·1
	226	15	71·2	4·7
	227	6	63·2	2·8
	319	10	59·7	3·2
	320	11	62·9	5·8
	325	10	43·4	1·7
Zoo-OL	31	6	71·8	4·2
	42	27	70·1	3·4
	61	16	80·5	4·4
	62	11	75·6	4·9
	68	14	76·5	4·0
	96	5	60·8	4·8
	114	20	59·5	3·0
Suchdol	110	23	59·8	2·4
Jutland	3434	13	70·5	5·8

* Wet weight (mg) of paired testes.

Two conclusions can be drawn from the analysis of variation of the data shown in Table 1. (*a*) Mean testes weights of sterile offspring of wild males (set I) do not significantly differ at two localities (Zoo-3, Zoo-4). The locality Zoo-5 is heterogeneous in that it comprises three males (W216, W319 and W325) differing ($P < 0{\cdot}01$) from all other males of the locality in testes weights of their (B10 × W) offspring. The observation indicates that, even within one locality, the wild mice of set I are not uniform with respect to genes engaged in the hybrid sterility phenomenon. It may be worth while mentioning that at all Bohemian localities, except Zoo-OL, the trapping places were strictly limited areas not exceeding 9 m^2. (*b*) The significant differences in testes weights between sterile offspring of different wild males of set I indicate that the testes weight rather than fertility is under the more direct genetic control. Fig. 2 gives further evidence in favour of this statement. Two males of set III – W69 and W111 – sired progeny with bimodal dis-

tributions of testes weights which suggested primarily a single locus affecting the size of the testes. If, however, the fertility (OMU) were the sole criterion, then the resulting distribution of phenotypes would be more sophisticated, with the excess of fertile sons in the case of W69 and shortage of this phenotype in W111.

Table 2. *Male wild mice – set II; mean testes weight of (B10 × W) male hybrids*

Male wild mouse		(B10 × W) hybrid males		
			Testes weight	
Locality	Coding no.	No. of males	Mean	S.E.
Zoo-2	177	12	129·7	4·0
Zoo-4	171	15	119·4	2·0
Zoo-5	322	9	170·4	11·6
	323	8	173·1	6·6
	324	5	172·2	4·4
Zoo-OL	70	9	191·0	10·3
	78	12	150·8	6·0
	84	8	153·9	4·9
Botanická	76	8	169·6	8·3
	86	5	143·2	5·7
	88	8	201·6	8·3
	90	8	155·0	5·5
Kobylisy	82	15	170·8	5·0
Petrovice	208	19	152·1	6·7
	211	22	180·6	6·5
	212	15	174·4	5·7
Jutland	3428	6	210·3	8·1
G.G.I.	613	12	195·3	2·9
	625	11	241·8	8·9
	632	7	220·6	4·8

Table 3 shows that the proportion of 'low testes weight' and 'high testes weight' hybrids is nearly 1:1 in the progeny of all but two males of set III, which is consistent with the single-locus assumption. The two exceptional males, W64 and W79, sired an excess of 'high testes weight' hybrids, but both were found to be carriers of *t*-alleles. The significance of this finding will be discussed later.

It can be seen in Table 1 that all but two out of 23 wild males of set I were trapped at the localities of the Prague Zoological Garden. Nevertheless, all reasonably examined Zoo localities contained a mixture of wild mice of all three types (cf. Tables 1, 2 and 3). The samples from the 'non-Zoo' localities are too small to infer the ubiquity of this type of hybrid sterility phenomenon, but large enough to exclude a unique character of the Zoo populations in this respect. Five males outside Prague Zoo belong clearly to the sets I or III (Tables 1, 3).

Table 3. *Male wild mice – set III; mean testes weights of (B10 × W) male hybrids*

Male wild mouse		Sterile offspring			Fertile offspring		
			Testes weight			Testes weight	
Locality	Coding no.	No. of males	Mean	S.E.	No. of males	Mean	S.E.
Zoo-4	169	6	73·3	5·4	7	129·6	8·9
	173	7	47·4	1·3	7	121·4	4·9
	175	9	58·4	2·2	5	109·6	5·4
Zoo-OL	30	8	67·6	3·8	5	106·0	4·8
	64	7	77·1	6·7	15	161·9	10·5
	67	36	75·8	2·1	44	165·1	3·0
	69	25	75·1	3·2	23	163·7	4·4
Karlín	79	5	53·0	2·8	18	165·9	2·6
Kobylisy	81	9	51·7	2·3	13	132·0	3·1
Suchdol	111	84	46·7	0·6	72	101·0	2·2

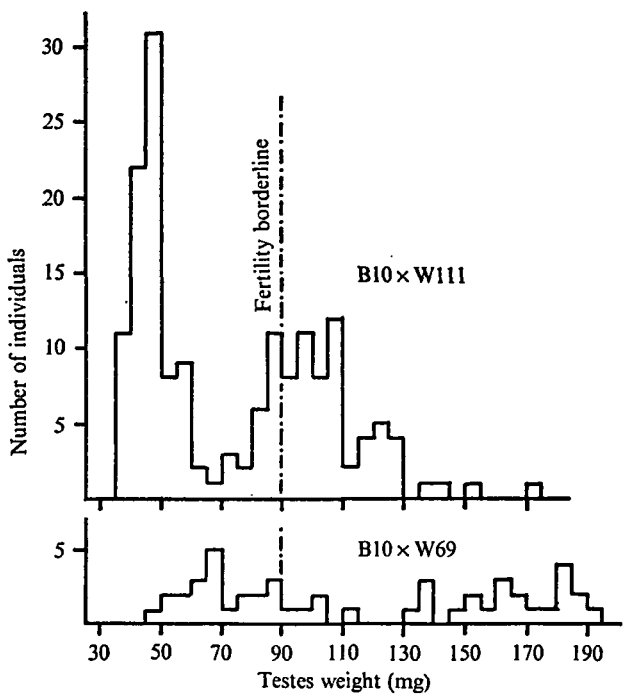

Fig. 2. Distribution of testes weights in 164 (B10 × W111) and 48 (B10 × W69) male hybrids. Bimodality of testes weight distributions is clearly expressed in both progeny, though it does not correspond to fertility borderline.

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(ii) *A proposal for nomenclature of the hybrid sterility genetic system*

Presentation of further data on the genetic control of hybrid sterility might be facilitated by introducing the symbols for postulated genes and their allelic forms (Table 4). The evidence on which the proposals are based will follow.

Table 4. *Proposal for nomenclature of hybrid sterility genetic system and fertility of various genotypes**

Allele symbol	Previous provisional designation†	Source of alleles	Fertility of genotypes‡			
			Hst-1^s	Hst-1^f	Hst^{ws}	Hst^{wf}
Hst-1^s	I-st	B10 strain	F	F	S	F
Hst-1^f	I-f	C3H strain	—	F	F	F
Hst^{ws}	W-st	Wild mice	—	—	F	F
Hst^{wf}	W-f	Wild mice	—	—	—	F

* The epistatic interaction of genes outside chromosome 17 in BC_1 crosses is not considered here.

† See Iványi *et al.* (1969).

‡ F = males and females fertile; S = males sterile, females fertile.

The symbol for genes involved in the *Hybrid sterility* genetic system would be *Hst* (*Hybrid sterility*). *Hst-1* would be a gene defined in inbred mouse strains, and located on chromosome 17. Chromosome 17 in B10 mice would carry *Hst-1^s* (sterility ensuring) allele; *Hst-1^f* (fertility ensuring) would be a symbol for the allele carried on chromosome 17 of C3H inbred strain origin.

Hst^w would be a provisional symbol for a gene located on chromosome 17 of wild mice. Wild mice of set I (Table 1), producing sterile sons with B10 strain mice, would be *Hst^{ws}/Hst^{ws}* homozygotes. *Hst^{wf}* would be an allele of *Hst^w* locus homozygous in wild mice of set II (Table 2). Wild males of set III (Table 3) thus would be *Hst^{ws}/Hst^{wf}* heterozygotes. If the allelism of *Hst-1* and *Hst^w* is later proved, the symbols can be changed to *Hst-1^{ws}* and *Hst-1^f*, respectively.

Table 4 shows an interaction character typical of hybrid sterility; all homozygous forms are fertile, only interaction *Hst-1^s/Hst^{ws}* ensures sterility.

(iii) *Single gene* (Hst-1) *responsible for different fertility of* (*B10* × *W*) *and* (*C3H* × *W*) *male hybrids*

The wild males of set I which produced only sterile sons with B10 females sired fertile progeny with females of the C3H inbred strain (Fig. 3). We have assumed that two allelic forms of a single gene may be responsible for the different testes weights of (B10 × W) and (C3H × W) male hybrids. The gene is located on chromosome 17 (linkage group IX) between the dominant factor *T* and the *H-2* genetic complex and we propose to designate it *Hybrid sterility-1*, *Hst-1*. The mapping is based on the cross where the wild males of set I are mated to (B10-*T* × C3H) short-tailed females. The dominant *T* allele (causing markedly shortened tail) is used as a marker of chromosome 17 of B10 origin. The testes weight values of

male progeny of this cross show a bimodal distribution without overlaps. Fig. 3 shows close linkage of *T* and *Hst-1* with recombination fraction 12/204, i.e. $5{\cdot}9 \pm 1{\cdot}65\,\%$.

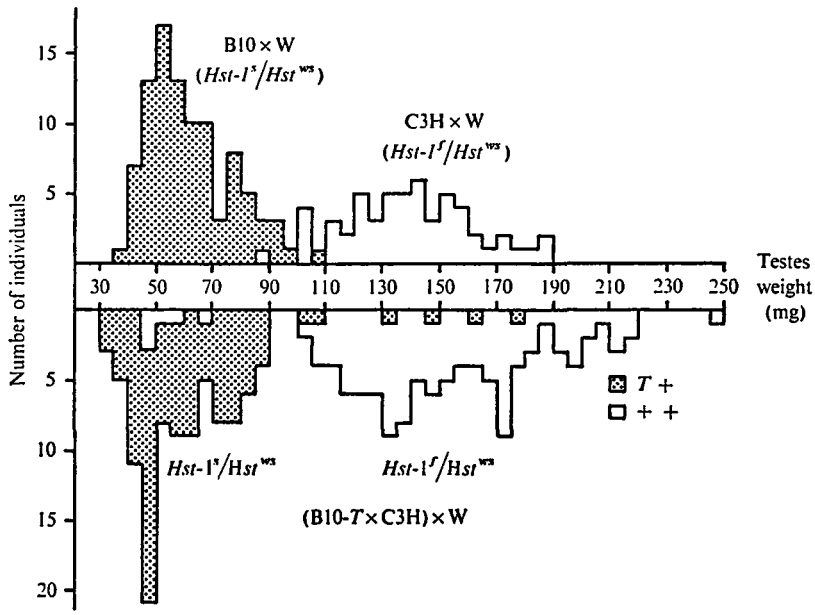

Fig. 3. Distributions of testes weights in (B10 × W), (C3H × W) and {(B10-*T* × C3H)-*T*/+ × W} hybrids. Within the progeny of individual W males the (B10 × W) and (C3H × W) sets do not overlap. The overlapping as seen in upper histogram may be considered artificial since it is caused by combining the progeny of ten W males of set I (cf. Table 1). The lower histogram shows the involvement of a single gene, *Hst-1*, with the major effect on the size of testes in {(B10-*T* × C3H)*T*/+ × W} hybrids. The non-random distribution of normal-tailed (+/+, empty columns) and short-tailed (*T*/+, shadowed columns) animals in respect to their testes weight, suggests the close linkage of *Hst-1* and dominant *T*.

Fig. 4 indicates that B10 and C3H inbred strains differ, with respect to the *Hybrid sterility* genetic system, at only the *Hst-1* locus without any apparent interference of the different genetic background of both strains. When mated to B10 females, two wild males (W161 and W202) gave sterile male progeny with an apparently identical distribution of testes weight values (Table 1). Both males were crossed with {(B10-*T* × C3H)*T*/+ ♀ × B10♂} short-tailed and normal-tailed BC_1 females. The genotype of short-tailed BC_1 females was $T/+\,Hst\text{-}1^s/Hst\text{-}1^s$, and normal-tailed females were $+/+\,Hst\text{-}1^r/Hst\text{-}1^s$. Fig. 4 shows that in accordance with the single-locus hypothesis, both sets of sterile males display a distribution of testes weight values not essentially different from the values obtained in F_1 hybrids. The same conclusion could be gained if the histogram in Fig. 3 was split up into its components composed of offspring of individual wild males.

The three-point test cross showed that the *Hst-1* gene was located between *T* and *H-2* (Table 5). Two types of cross were used, namely (B10-*T* × C3H) *T*/+ ♀ × W ♂

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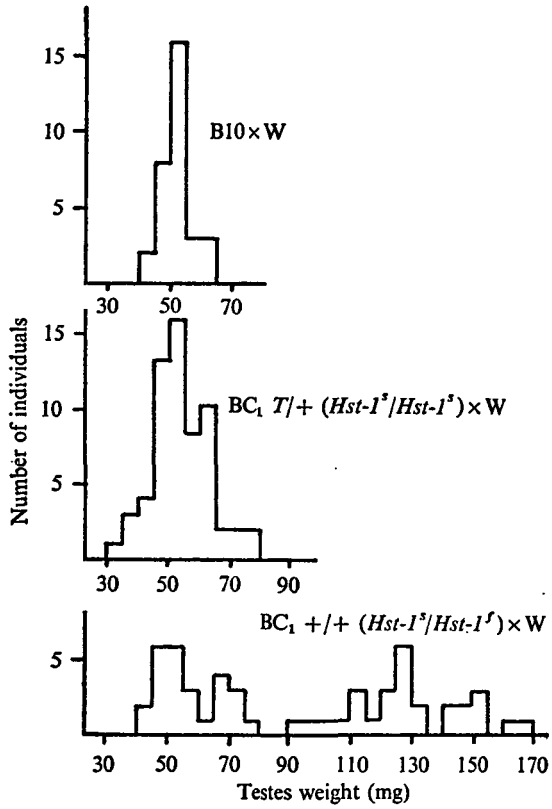

Fig. 4. Distribution of testes weights in (B10 × W) and {(B10-*T* × C3H)*T*/+ × B10} BC_1 × W hybrids. The distribution of testes weight values in all three sets of sterile male hybrids indicates no pronounced influence of B10 and C3H genetic backgrounds on the expressivity of *Hst-1* gene. See text for details.

Table 5. *Offspring of three-point test cross of the type T Hst-1^s H-2^b/+ Hst-1^f H-2^k*

Phenotype of offspring	No. of offspring		
	Expt I	Expt II	Total
T Hst-1^s H-2^b	15	20	35
+ Hst-1^f H-2^k	16	22	38
T Hst-1^f H-2^k	0	0	0
+ Hst-1^s H-2^b	3	1	4
T Hst-1^s H-2^k	4	3	7
+ Hst-1^f H-2^b	4	1	5
T Hst-1^f H-2^b	0	0	0
+ Hst-1^s H-2^k	0	0	0

	Recombinations		
		%	S.E.
T – Hst-1	4/89	4·5	2·20
Hst-1 – H-2	12/89	13·5	3·63
T – H-2	16/89	18·0	4·07

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(Table 4, Expt I), and {(B10-*T* × C3H)*T*/+ ♀ × B10♂}*T*/+ and +/+ BC_1♀ × W♂ (Table 4, Expt II). In the former test cross, each hybrid male was examined for *H-2* haplotype (*H-2*^{b} or *H-2*^{k}), testes weight and presence of short tail. In the latter cross, BC_1 females were checked for *H-2* and *T*, and then mated with wild males of set I in order to ascertain their *Hst-1* genotype. As shown in Table 5, both experiments indicated the order *T–Hst-1–H-2*.

(iv) *Location of a single gene in wild male mice (set III) responsible for different testes weight of (B10 × W) male hybrids on the chromosome 17*

The observation that the difference in fertility of (B10 × W) and (C3H × W) male hybrids is controlled by two allelic forms of a single gene, *Hst-1*, prompted us to study in more detail the wild males of set III whose male offspring with B10 females segregate with respect to testes weight and fertility.

Table 6. *Recombination between* Hst^w *and* H-2 *among the progeny of B10 × W* (Hst-1^s H-2^b/Hst-1^s H-2^b × Hst^{ws} H-2^{w1}/Hst^{wf} H-2^{w2}) *cross*

	Offspring						
Cross	*Hst*^{ws} *H-2*^{w1}	*Hst*^{wf} *H-2*^{w2}	*Hst*^{ws} *H-2*^{w2}	*Hst*^{wf} *H-2*^{w1}	Total	R.F. (%)	S.E. (%)
B10 × W67	35	39	4	3	81	8·6	3·12
B10 × W111	55	54	10	7	126	13·5	3·05

R.F. = recombination fraction.
S.E. = standard error.

H-2^{w1} defined by H-2.3 − 5 − in W67, and by H-2.16 + 41 + in W111.
H-2^{w2} defined by H-2.3 + 5 + in W67, and by H-2.16 − 41 − in W111.

All wild males of set III which were serotyped for H-2 antigens were found to be heterozygous for the *H-2* complex. Thus when crossing the wild males with females of the B10 inbred strain, the two *H-2* haplotypes were distinguished in hybrid progeny. The H-2 antigens therefore served as genetic markers of wild male chromosomes 17. Table 6 and Fig. 2 show that two allelic forms of a single gene, or a cluster of genes, associated with the *H-2* complex may be responsible for major differences in testes weight of the male hybrids studied. The recombination fraction between *H-2* and the postulated *Hst*^{w} gene is 7/81 (8·6 ± 3·12 %) and 17/126 (13·5 ± 3·05 %) in males W67 and W111, respectively. The difference is not significant.

As shown in section (ii), the *Hst-1* gene has been defined by means of two inbred strains, B10 and C3H. In other terms (B10 × C3H)F_1 hybrids are heterozygous (*Hst-1*^{s}/*Hst-1*^{f}) for a single gene responsible for sterility or fertility of {(B10 × C3H) × W} male hybrids. *Hst*^{w} gene heterozygous in wild males of set III has been located to the same linkage group as *Hst-1*, nevertheless there is no direct evidence as yet that this gene occupies the same locus, i.e. is identical with the *Hst-1* gene.

(v) *Relationship between* Hst-1 *gene and recessive* t *alleles of the* T-t *locus*

Two genetic systems have hitherto been recognized in mice which show polymorphism in natural populations and produce male-limited hybrid sterility. It was interesting to find that both loci, *T-t* and *Hst-1*, are located in the same part of chromosome 17.

Table 7. *Test for interaction of* Hst-1^s *and various* t *alleles among normal-tailed offspring of B10*(Hst-1^s/Hst-1^s) × T/t *cross*

	Tested *t* allele				
	t^{12}	t^6	t^{w1}	t^{w12}	t^{w8}
Testes weight (in mg) ± S.E.	241 ± 2·3	205 ± 3·1	238 ± 4·6	216 ± 6·15	244 ± 6·9
OMU*	6·3 ± 0·8	4·9 ± 0·9	7·0 ± 0·7	7·4 ± 0·7	8·0 ± 0·4

* OMU = criterion of fertility, see the text.

Most of the recessive *t* alleles extracted from wild mice exhibit crossing-over suppression over a region 6–10 cM long, distally from the dominant *T* (Dunn & Caspari, 1942; Lyon & Phillips, 1959; Forejt, 1972*b*). Since the *Hst-1* gene has been mapped inside the region controlled by the *t* alleles, the question arises whether the *Hst-1* is not a special form of a *t* allele without the *T* modifying effect. The data of Lyon & Meredith (1964*a*, *b*, *c*) on the segmental origin of *t* alleles seem to support the idea. The sterility effect can be separated from the *t* alleles by recombination, and the factor for sterility seems to map near the recessive mutation *tf* (Lyon & Meredith, 1964*b*; Forejt, 1971, unpublished). The distance between dominant *T* and *tf* is about 7 cM (Dunn, Bennett & Beasley, 1962) and essentially the same value has been estimated for the *T–Hst-1* interval. The close intimacy of *Hst-1* and *T-t* on the chromosome 17 together with the segregation of Hst^{ws} and *t* alleles in wild mice, appears as a reasonable indication to test the possible identity of both loci. For these purposes the following property of the *t* alleles was exploited: sterility is known to be achieved in males displaying two different *t* alleles provided that at least one of them is a recessive lethal (Dunn, 1964). Consequently if the *Hst-1*^{s} and/or Hst^{ws} were separated *t*-sterility factors, they should interact with various recessive lethal *t* alleles with resulting male sterility.

Three lines of evidence have been obtained disproving the identity of *Hst-1* (Hst^{ws}) and *t*-sterility factor. (*a*) Five recessive lethal *t* alleles (t^6, t^{12}, t^{w1}, t^{w12} and t^{w8}) belonging to four different complementation groups (Dunn & Bennett, 1971; Dunn, 1956) have been tested for interaction with *Hst-1*^{s}. Both the testes weight and OMU values were estimated in individual (B10 × *T*/*t*) normal-tailed male hybrids. Table 7 shows that all tested *Hst-1*^{s}/*t* combinations were fully fertile with appropriate testes weight values. (*b*) The t^{12} recessive lethal allele was combined with Hst^{ws} genetic factor. Six normal-tailed male offspring of this genotype from cross T/t^{12} × W96 were found to be fertile. Hst^{ws} genetic factor even did not inter-

fere with the high transmission ratio typical of the t^{12} allele (Table 8). A change of transmission ratio of t^{12} would be expected if the *Hst*^{ws} belonged to the *T-t* locus (Bennett & Dunn, 1971). (*c*) *Hst*^{ws}/*t* genotype was observed in males W64 and W79. In both cases *Hst*^{ws} was in repulsion to the *t* allele, and fertility of these males did not differ from other wild males studied.

It may be concluded that *Hst* and *T-t* are two different genetic systems; *Hst-1* (*Hst*^{ws}) and *T-t* do not interact in any known character (*T*/*t* interaction, t^x/t^x lethality, t^x/t^y sterility or change of transmission ratio). It will be shown later that hybrid sterility is controlled by more than one gene, while the *T-t* system behaves as a single gene or a cluster of closely linked genes. *Hst-1*^{s} and *Hst*^{ws} thus seem not to be identical with the *t* sterility factor.

Table 8. *Test for interference of* Hst^{ws} *with male transmission ratio of* t^{12} *allele*

	Offspring		
Cross	T/t^{12} (tailless)	T/Hst^{ws} (short-tailed)	$+/t^{12}$, $+/Hst^{ws}$ (normal-tailed)
♀$T/+$ × ♂t^{12}/Hst^{ws}	47	2	51

Transmission ratio of t^{12} – 47/49 = 96 %.

(vi) *Distribution of* Hst-1 *alleles in various inbred strains*

Wild males producing only sterile male progeny with B10 females were used as test males for preparing hybrid offspring with females of several inbred strains. We have found that five inbred strains, B10, A, BALB/c, DBA/1 and AKR/J, yielded sterile male hybrids, and five other strains, C3H, CBA/J, P/J, I/St and F/St, produced fertile hybrids. The simplest explanation might be the sharing of *Hst-1*^{s} by the former group of inbred strains, whereas the latter group would share in common the *Hst-1*^{f} allele. In fact, the possibility cannot be excluded that sterility of some of these hybrids is controlled by a gene different from *Hst-1*, or that in some combinations other than (B10 × C3H), more than one gene of the *Hst* system would segregate. So far, only linkage tests identifying the *Hst-1* gene by means of B10 and C3H inbred strains have been carried out.

Some additional data are available for the A inbred strain which gives sterile hybrids with significantly lower testes weights in comparison to appropriate B10 strain hybrids. The preliminary results of an experiment where (B10-*T* × A) short-tailed females were crossed with wild males of set I suggested the polygenic control of this inter-strain difference. The non-significant difference between mean testes weights of short-tailed hybrids and their normal-tailed sibs from this cross (60·8 and 56·8 mg) provided no indication of the involvement of different *Hst-1*^{s} alleles in B10 and A inbred strains.

(vii) *On the genes outside chromosome 17 belonging to the* Hst *genetic system*

The *Hst-1* gene, as defined by B10 and C3H inbred strains, is not the only gene responsible for hybrid sterility. It seems that at least two other genes segregate

independently in the progeny of (B10 × W) and (A × W) female hybrids. The estimate is based on analysing the testes weight in {(B10 × W) × B10} and {(A × W) × A}BC_1 progeny. The experiments have not been completed as yet, nevertheless only 30 males out of 168 BC_1 males displayed testes weights below 95 mg.

(viii) *Cytogenetic examination of sterile male hybrids*

Histological observations of testes from sterile male hybrids were described in a previous paper (Iványi *et al.* 1969). Spermatogenesis breakdown at the stage of spermatogonia was the most frequent finding in the testes from sterile descendants of wild males.

Examination of meiotic and mitotic chromosomes of sterile hybrids did not reveal any gross chromosomal rearrangement which could be a sign of the chromosomal type of sterility (Plate 1). The study of chromosomes at first meiotic division was conducted in heavier 'sterile' testes (i.e. 80–90 mg) where the spermatogenesis arrest was shifted towards the stage of secondary spermatocytes. No multivalents were found at diakineseis and metaphase I stages, which would indicate translocation heterozygosity of sterile male hybrids. High frequency of cells with 2–6 univalents and *X*–*Y* dissociation were frequently observed in sterile male descendants of all wild males examined.

The detailed cytogenetic analysis of sterile male hybrids will be presented in a separate paper. However, it can be preliminarily concluded that there is no evidence for chromosomal rearrangements, i.e. the analysed phenomenon seems to be an example of genic rather than chromosomal type of hybrid sterility.

(ix) *Hormonal control of spermatogenesis in hybrid males*

Hormonal control of spermatogenesis comprises the action of testosterone and its derivatives on the differentiation of spermatogonia towards primary spermatocytes, and the effect of hypophyseal gonadotropins on the course of later stages of spermato- and spermiogenesis (Steinberger, 1971).

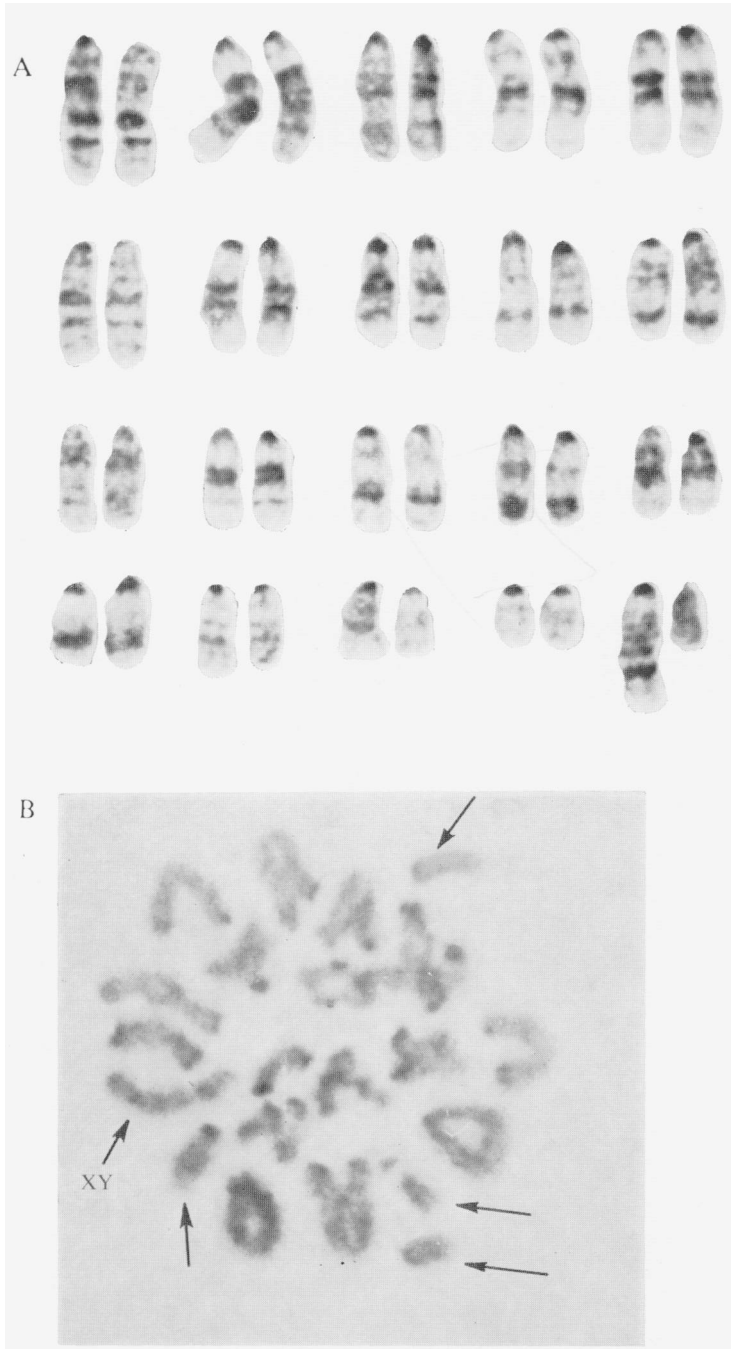
Table 9. *Seminal vesicle weights in sterile and fertile male progenies from (B10 × C3H) × W cross**

Fertility of male progeny	No. of males	Mean testes weight (mg) ± S.E.	Mean SW ± S.E.†
Sterile	36	61·3 ± 2·16	7·3 ± 0·27
Fertile	37	152·5 ± 3·92	7·5 ± 0·30

* W males of set I (see Table 1).

† $SW = \frac{\text{Seminal vesicle wet weight} \times 10^3}{\text{Body weight}}$.

Androgen activity of sterile males and their fertile brothers from the cross – (B10-*T* × C3H) × W – was judged from the relative weight of seminal vesicles (SW). The data from sterile and fertile hybrids show no relationship between spermatogenesis breakdown and androgen activity (Table 9). This is in accordance with the



(A) The G-banded karyotype of (B10 × W319) sterile male. The banding pattern does not reveal any chromosomal rearrangements. (B) Diakinesis from the same sterile male. Arrows indicate the univalents.

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direct estimation of testosterone concentration in blood plasma of a small sample of hybrid males (3 fertile, 5 sterile). The mean values were 3·2 μg/ml and 3·4 μg/ml for the sterile and fertile group, respectively. The levels are within physiological variations in mice (Hampl, Iványi & Stárka, 1971; Iványi *et al.* 1972).

The gonadotropin activity of hypophyseal homogenates from sterile and fertile male hybrids was estimated by the method of Wolfe (1971). Preliminary data based on testing four sterile and three fertile male hybrids showed no differences in gonadotropin activity between the two groups.

4. DISCUSSION

(i) *Significance of* Hst *genetic system in murine genome*

Dobzhansky (1951) defines hybrid sterility as a situation where two parental forms, each of which is fertile *inter se*, produce a hybrid which is sterile. According to the definition, the observed sterility of male hybrids between laboratory and wild mice could be regarded as hybrid sterility.

There are interesting similarities between hybrid sterility in *Drosophila* and *Mus musculus*. In both cases the sterility is associated with the reduced size of testes, the degree of spermatogenesis impairment being in reverse relationship to the weight of testes. In both species the first meiotic division displays variable numbers of univalents and the spermatogenesis usually does not proceed beyond the stage of primary spermatocytes. In the chapter dealing with genic sterility in the hybrids of *Drosophila pseudoobscura* × *Drosophila persimilis* Dobzhansky (1951) concludes that at least eight genes, scattered throughout the genome, control the degree of spermatogenesis impairment. The author further states that in *Drosophila* hybrids 'the fate of testis is determined by its own genetic constitution and not by the body in which it develops . . .'. The assumption, based on experiments with reciprocal testis transplantation, is compatible with our failure to detect some abnormalities in the hormonal control of spermatogenesis of the sterile murine hybrids.

The discussed sterility of *Drosophila* hybrids is a clear-cut example of the reproductive isolating mechanism operating between two distinct species. We do not know, however, how and why such a reproductive barrier developed between certain wild mice and mice of some laboratory inbred strains.

Mayr (1963) has suggested that the isolating mechanisms (including hybrid sterility) developed as mere by-products of genetic divergence resulting from geographical isolation. According to the alternate theory, natural selection in the zone of hybridization at 'species border' (Wallace, 1959) is a necessary condition for definite establishment of the isolating mechanisms.

It may be of interest in this context that all examined wild mice belonging to the sets I and III were classified as *Mus musculus musculus*, whereas the employed inbred strains were established about 60 years ago (Staats, 1966), probably from the *Mus musculus domesticus* subspecies. The Bohemian as well as Danish mice were extracted from localities very near, or in the zone of hybridization of the subspecies *M. m. musculus* and *M. m. domesticus* (Schwarz & Schwarz, 1943 –

quoted in Hunt & Selander, 1973). Thus, the observed hybrid sterility could be hypothetically a reflexion of the incipient reproductive isolation between the two subspecies. It should be noted, however, that Hunt & Selander (1973) failed to observe the assortive mating of *musculus* and *domesticus* subspecies in the zone of their hybridization on the Jutland Peninsula of Denmark. The authors suggested that both subspecies were not reproductively isolated, and explained the genetic isolation by reduced fitness of backcross generations resulting from breakdown of co-adapted parental gene complexes.

(ii) *The role of* Hst-1 *gene in hybrid sterility phenomenon*

Whatever is the function of *Hst* genetic system in natural mouse populations, the opportunity to identify the individual genes of the system deserves some interest. The exploitation, at least in part, of the genetically well-defined inbred strains may turn out as an actual advantage in the search for the mechanism of hybrid sterility.

From this viewpoint, the location of one gene, *Hst-1*, of the system on chromosome 17 may prove potentially useful since two inbred strains, B10 and C3H, share the same alleles at all *Hst* loci with a single exception, *Hst-1*. Thus, there seems to be no obstacle to producing congenic strains at the *Hst-1* locus, i.e. to transmitting the $Hst\text{-}1^f$ allele into the B10 genetic background and vice versa, to prepare the C3H.$Hst\text{-}1^s$ congenic strain.

The only firmly established fact concerning the other (non-*Hst-1*) genes of the system is that they do exist. The data from BC_1 populations indicate that besides *Hst-1*, 2–3 other major genes are involved. *Hst-1*, as well as Hst^w, seems to be unique in that it shows, unlike the other genes of the system, polymorphism in both inbred strains and natural populations, though this conclusion may be biased by the small samples examined.

The genes engaged in reproductive isolation are known to be fixed in homozygous state in Mendelian populations (Dobzhansky, 1951, 1972). *Hst-1* and Hst^w would then be an example of isolating gene under the influence of forced heterozygosity exerted by *t* alleles. Recessive *t* alleles, ubiquitous in natural populations, are mostly the recessive lethals. The suppression of recombination caused by *t* alleles extends over the *Hst-1* and Hst^{ws} loci, so that in wild mice possessing the *t* alleles Hst^{ws} cannot be fixed in homozygous state.

The complementation tests provided reasonable evidence for the non-identity of *Hst-1* (Hst^{ws}) and *t* alleles. The genetical evidence is in concordance with the phenotypical differences of *t* and *Hst* controlled sterility. While the *Hst* sterility is ensured by early spermatogenesis arrest, the spermatogenesis of t^x/t^y sterile males proceeds without apparent disturbances. Spermatozoa seem to be of normal appearance, and the cause of sterility consists in the inability of spermatozoa to reach the potential sites of fertilization in the female genital tract (Bennett & Dunn, 1967). Phenotypically similar but genetically clearly different is the spermatogenesis breakdown in males homozygous for semilethal *t* alleles (Johnston, 1968; Dunn & Bennett, 1971).

In the present state of ignorance concerning the function of the *Hst* genetic system in natural populations as well as the mechanism of hybrid sterility, any speculations dealing with the intriguing intimacy of *Hst-1* (Hst^w) and *T-t* on chromosome 17 are premature.

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REFERENCES

Bennett, D. (1964). Abnormalities associated with a chromosome region in the mouse. II. Embryological effects of lethal alleles in the t-region. *Science* **144**, 263–267.

Bennett, D. & Dunn, L. C. (1967). Studies of the effect of t-alleles in the house mouse on spermatozoa. I. Male sterility effects. *Journal of Reproduction and Fertility* **13**, 421–428.

Bennett, D. & Dunn, L. C. (1971). Transmission ratio distorting genes on chromosome IX and their interactions. In *Immunogenetics of H-2 System* (ed. A. Lengerová and M. Vojtíšková), pp. 90–103. Basel: Karger.

Burkholder, G. D. & Comings, D. E. (1972). Do the Giemsa-banding patterns of chromosomes change during embryonic development? *Experimental Cell Research* **75**, 268–271.

Cattanach, B. M. & Moseley, H. (1973). Nondisjunction and reduced fertility caused by the tobacco mouse metacentric chromosomes. *Cytogenetics and Cell Genetics* **12**, 264–287.

Dobzhansky, T. (1951). *Genetics and the Origin of Species*, 3rd ed. New York: Columbia University.

Dobzhansky, T. (1972). Species of Drosophila. *Science* **177**, 664–669.

Dunn, L. C. (1956). Analysis of a complex gene in the house mouse. *Cold Spring Harbor Symposia on Quantitative Biology* **21**, 187–195.

Dunn, L. C. (1964). Abnormalities associated with a chromosome region in the mouse. I. Transmission and population genetics of the t-region. *Science* **144**, 260–263.

Dunn, L. C., Bennett, D. & Beasley, A. B. (1962). Mutation and recombination in the vicinity of a complex gene. *Genetics* **47**, 285–303.

Dunn, L. C. & Bennett, D. (1971). Lethal alleles near locus T in house mouse populations on the Jutland Peninsula, Denmark. *Evolution* **25**, 451–453.

Dunn, L. C. & Caspari, E. (1945). A case of neighboring loci with similar effects. *Genetics* **30**, 543–568.

Forejt, J. (1972*a*). Giemsa-specific centromeric heterochromatin in three inbred mouse strains. *Folia biologica* **18**, 213–215.

Forejt, J. (1972*b*). Chiasmata and crossing-over in the male mouse (*Mus musculus*). Suppression of recombination and chiasma frequencies in the ninth linkage group. *Folia biologica* **18**, 161–170.

Forejt, J. (1973). Centromeric heterochromatin polymorphism in the house mouse. Evidence from inbred strains and natural populations. *Chromosoma* **43**, 187–201.

Gropp, A., Winking, H., Zech, L. & Muller, H. (1972). Robertsonian chromosomal variation and identification of metacentrio chromosomes in feral mice. *Chromosoma* **39**, 265–288.

Haldane, J. B. S. (1922). Sex ratio and unisexual sterility in hybrid animals. *Journal of Genetics* **12**, 101–109.

Hampl, R., Iványi, P. & Stárka, L. (1971). Testosterone and testosterone binding in murine plasma. *Steroidologia* **2**, 113–120.

Hunt, W. G. & Selander, R. K. (1973). Biochemical genetics of hybridization in European house mouse. *Heredity* **31**, 11–33.

Iványi, P., Hampl, R., Stárka, L. & Micková, M. (1972). Genetic association between H-2 gene testosterone metabolism in mice. *Nature, New Biology* **238**, 280–281.

Iványi, P. & Micková, M. (1971). Further studies on genetic factors in the ninth linkage group influencing reproductive performance in male mice. In *Immunogenetics of H-2 System* (ed. A. Lengerová and M. Vojtíšková), pp. 104–119. Basel: Karger.

IVÁNYI, P., VOJTÍŠKOVÁ, M., DÉMANT, P. & MICKOVÁ, M. (1969). Genetic factors in the ninth linkage group influencing reproductive performance in male mice. *Folia biologica* **15**, 401–421.

JOHNSTON, P. G. (1968). Male sterility in mice homozygous for the t^{w2} allele. *Australian Journal of Biological Sciences* **21**, 947–951.

LYON, M. F. & MEREDITH, R. (1964*a*). Investigations of the nature of *t*-alleles in the mouse. I. Genetic analysis of a series of mutants derived from a lethal allele. *Heredity* **19**, 301–312.

LYON, M. F. & MEREDITH, R. (1964*b*). Investigations of the nature of *t*-alleles in the mouse. II. Genetic analysis of an unusual mutant allele and its derivatives. *Heredity* **19**, 313–325.

LYON, M. F. & MEREDITH, R. (1964*c*). Investigations of the nature of *t*-alleles in the mouse. III. Short tests of some further mutant alleles. *Heredity* **19**, 327–330.

LYON, M. F. & PHILLIPS, R. J. S. (1959). Crossing-over in mice heterozygous for *t* alleles. *Heredity* **13**, 23–32.

MAYR, E. (1963). *Animal Species and Evolution*. Cambridge: Harvard University Press.

MICKOVÁ, M. & IVÁNYI, P. (1971). Histocompatibility antigens in the wild house mouse. In *Immunogenetics of H-2 System* (ed. A. Lengerová and M. Vojtíšková), pp. 20–34. Basel: Karger.

SEABRIGHT, M. (1972). The use of proteolytic enzymes for the mapping of structural rearrangements in the chromosomes of man. *Chromosoma* **36**, 204–210.

SELANDER, R. K., HUNT, W. G. & YANG, S. H. (1969). Protein polymorphism and genic heterozygosity in two European subspecies of the house mouse. *Evolution* **23**, 379–390.

STAATS, J. (1966). The laboratory mouse. In *Biology of the Laboratory Mouse* (ed. E. L. Green), pp. 1–11. New York: McGraw-Hill.

STEINBERGER, E. (1971). Hormonal control of mammalian spermatogenesis. *Physiological Review* **51**, 1–22.

STIMPFLING, J. H. (1961). The use of PVP as developing agent in mouse hemagglutination test. *Transplantation Bulletin* **27**, 109–110.

WALLACE, B. (1959). The influence of genetic systems on geographical distribution. *Cold Spring Harbor Symposia on Quantitative Biology* **24**, 193–204.

WOLFE, G. H. (1971). Genetic influence on gonadotropic activity in mice. *Biology of Reproduction* **4**, 161–173.