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A semilethal *t*-haplotype in the Orkney Islands

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SUMMARY

Breeding tests of wild house mice, trapped from an isolated population from Sanday in the Orkney Islands, have demonstrated the presence of a semilethal *t*-haplotype designated t^{w106}. Microscopic examination of sperm and testes from a sterile male obtained from this population revealed the histological characteristics typical for homozygotes for semilethal *t*-haplotypes. This report is the first description of the recovery of a *t*-haplotype from an island population of wild mice.

1. INTRODUCTION

Wild populations of mice are routinely found to be polymorphic for recessive lethal or semilethal factors associated with the *T*/*t*-complex on chromosome 17. Such recessive factors are readily detected in the laboratory because they interact with the well studied marker *T* to produce a distinctive phenotype, taillessness (Bennett, 1978). In addition to their deleterious effects on embryonic development, all wild *t*-haplotypes share several bizarre characteristics. In heterozygotes, crossing-over, and therefore genetic recombination, is restricted between the *t*-haplotype and its normal homologue to about one one-hundredth of normal in the 14 centimorgan distance between the loci of *T* and *H-2*. Thus, that region of abnormal chromosome, and to a lesser extent the same region of wild-type chromosomes, is inherited *en bloc* as a 'super-gene' (Snell, 1968) in wild polymorphic populations.

Furthermore, males heterozygous for all wild *t*-haplotypes transmit their abnormal chromosome to almost all their progeny. On the other hand, males homozygous for semilethal haplotypes are essentially aspermic and therefore sterile, although their endocrinology and mating behaviour is normal. The phenomenon of transmission ratio distortion in heterozygotes is no doubt an important factor in maintaining these recessive deleterious factors in wild populations in the face of natural selection. However, even though the advantage lent by transmission ratio distortion is an important one, it appears from computer-generated models for the behaviour of genes in wild populations, that it is not sufficient to assure the indefinite survival of a *t*-haplotype in any small population. In statistical analysis of such artificial populations, Lewontin (1968) found that no matter how high

their transmission ratio, *t*-haplotypes tended eventually to be lost from individual populations as wild type chromosomes became fixed. Interestingly, according to the computer models, semilethal haplotypes with high transmission ratios are much more evanescent than lethal ones, since the existence of homozygous sterile but sexually active males provides very strong negative pressure. The apparently paradoxical findings that *t*-haplotypes do not persist indefinitely in small artificial populations, yet are polymorphic in natural populations, can be reconciled by postulating some degree of migration between different populations. It is obvious that migratory males heterozygous for *t*-haplotypes would be very effective in introducing the mutant chromosome into new populations (Berry & Jakobson, 1974). In this context it is interesting to note that of wild populations of mice that have been extensively studied (more than 20 animals tested) only two have been free of *t*-haplotypes, and one of these was an island population with presumably restricted migration (Dunn, Beasley & Tinker, 1960).

We report here that three more isolated populations from Taunton in Southern England, and from Stenness and Sanday in the Orkney Islands north of Great Britain (latitude 59° N) have been sampled. Two proved, in small sample sizes of 6 and 4 to be negative, while the third sample, from Sanday, provided a semilethal *t*-haplotype designated t^{w106}. One of the males from this population was sterile; histological study of sperm and testis showed him to be a typical t/t homozygote.

2. MATERIAL AND METHODS

Genetic Tests

Mice were caught by hand when oat ricks were broken down for threshing; selected mature males were air-expressed to New York for genetic tests. Wild males were caged individually with 3–5 $T/+$ females. Litters were scored at birth for tail phenotype: normal tail = $+/+$ or $+/t$; short tail = $T/+$; and tailless = t/T. Tailless animals produced by Sanday males were crossed inter-se to analyse viability, and with $+/+$ females to determine transmission ratio.

Histology

The sterile male was killed by cervical dislocation, and the testes fixed in buffered glutaraldehyde followed by buffered osmium tetroxide and embedded in araldite (Dooher & Bennett, 1973). Sections 1 μm thick were prepared and stained with alkaline toluidine blue for study in the light microscope. Photomicrographs were taken on a Zeiss Photomicroscope II using Panatomic X film. To examine fresh spermatozoa, vasa deferentia were excised and contents gently expressed into Dulbecco's phosphate buffered saline containing five per cent fetal calf serum.

3. RESULTS

Table 1 shows that of six males from Sanday tested by $T/+$ females, two gave progeny consisting entirely of normal-tailed and tailless offspring, thus demonstrating both the presence of a *t*-haplotype (designated t^{w106}) and its high trans-

Table 1. *Results of breeding tests of wild males by Brachy* (T/+) *females*

		Offspring X $T/+$		
Source	No.	Normal Tailed	Brachy	Tailless
Sanday	1	26	0	27
	2	62	0	48
	3	10	9	0
	4	0	0	0
	5	26	5	(8)
	6	14	8	(2)
Stenness	1	20	8	0
	2	6	11	0
	3	10	13	0
	4	3	6	0
	5	8	7	0
	6	6	8	0
Taunton	1	7	7	0
	2	5	7	0
	3	21	20	(6)
	4	15	17	(3)

Table 2. *Breeding tests of tailless* T/t^{w106} *males derived from* $+/t^{w106}$ *wild Sanday males*

		Offspring		
Males	Mating	Normal Tailed (t^{w106}/t^{w106})	Brachy ($T/+$)	Tailless (T/t)
Sons of wild no. 1	× sibs	15	—	74
Sons of wild no. 2	× sibs	3	—	1
Total		18	0	75
		Normal Tailed ($+/t^{w106}$)	Brachy ($T/+$)	
Sons of wild no. 1	× normal +/+	166	51	
Sons of wild no. 2	× normal +/+	62	12	
Total		228	63	

mission through males. Another male produced only normal and short-tailed (Brachy) offspring, in approximately equal numbers, and thus did not appear to be heterozygous for a *t*-haplotype. Two other males produced small numbers of tailless offspring, but also relatively large numbers of Brachys. Since previous

evidence (Dunn & Morgan, 1953) showed that wild populations frequently possess 'minus modifiers' of the T mutation that lead to phenotypic tailless overlaps of the $T/+$ genotype, and since the high transmission ratio typically associated with wild t-haplotypes was not evident, we assumed these two males to be wild-type homozygotes. Progeny tests of five of their tailless sons by T/t females resulted in some Brachy ($T/+$) offspring, thus confirming that these tailless mice carried a '+' allele of T, and therefore were $T/+$ phenotypic overlaps.

Also shown in Table 1 are data for males trapped at Stenness and Taunton, in which no evidence for t-haplotypes was obtained, although the Taunton population again contained minus modifiers for T.

Table 2 gives breeding data for the tailless animals derived from each of the two t-heterozygotes. Inter-se matings produce both normal and tailless progeny, showing that t^{w106}/t^{w106} homozygotes are viable. Tests of T/t^{106} males by $+/+$ females give a transmission ratio of 78 %, substantially lower than the ratio from the original wild males, but still far in excess of the mendelian 50 %. These figures can be used to calculate the viability of t^{w106} homozygotes as follows: with a 78 % transmission ratio for t^{w106}, matings between T/t^{w106} parents should produce 11 % T/T, 50 % T/t^{w106} and 39 % t^{w106}/T^{w106} conceptuses. Since the T/T embryos die at birth we expect 0·39/0·89 or 44 % of offspring to be normal tailed t^{w106} homozygotes. Thus since only 18/93 or 19 % of normal tailed progeny were seen at birth, the t^{w106}/t^{w106} genotype has a viability only about one-half of normal.

Since t^{w106} is clearly a semilethal t-haplotype, it seemed likely that the original wild male that failed to breed was a homozygote. Observations on his reproductive tract showed that the vasa deferentia contained very few spermatozoa (less than 1×10^6/ml); a majority of cells were headless tails and spermatozoa with abnormally shaped heads. None had normal motility, although a few displayed sporadic, uncoordinated tail movements which failed to produce forward progression.

Inspection of 1 μm thick plastic sections of seminiferous tubules revealed that most tubules possessed abnormal histological features resembling abnormalities observed in known homozygotes for semilethal t-haplotypes (Dooher & Bennett, 1974). In about 10 % of tubules only Sertoli cells, spermatogonia, and a few spermatocytes were present (Plate I, Fig. 1). In virtually every tubule examined spermatids at stages of differentiation later than stage 13 were severely reduced in numbers and displayed abnormal morphology as well (Plate I, Fig. 2, 3). Nuclei frequently showed deep invaginations posterior to the acrosome (Plate I, Fig. 2). Acrosomes often contained pale staining vacuoles or atypical protrusions (Plate I, Fig. 2). In numerous late spermatids the nucleus was flanked by an abnormally broad expanse of cytoplasm which, in normal spermatids, consists of a narrow sheath filled with the microtubules of the manchette (Plate I, Fig. 3). Most seminiferous tubules contained the apparent remains of late spermatids including nuclei and tail fragments (Plate I, Fig. 4). Dense inclusions resembling nuclei of late spermatids were also observed near the basement membrane, evidently lodged within the cytoplasm of Sertoli cells (Plate I, Fig. 4).

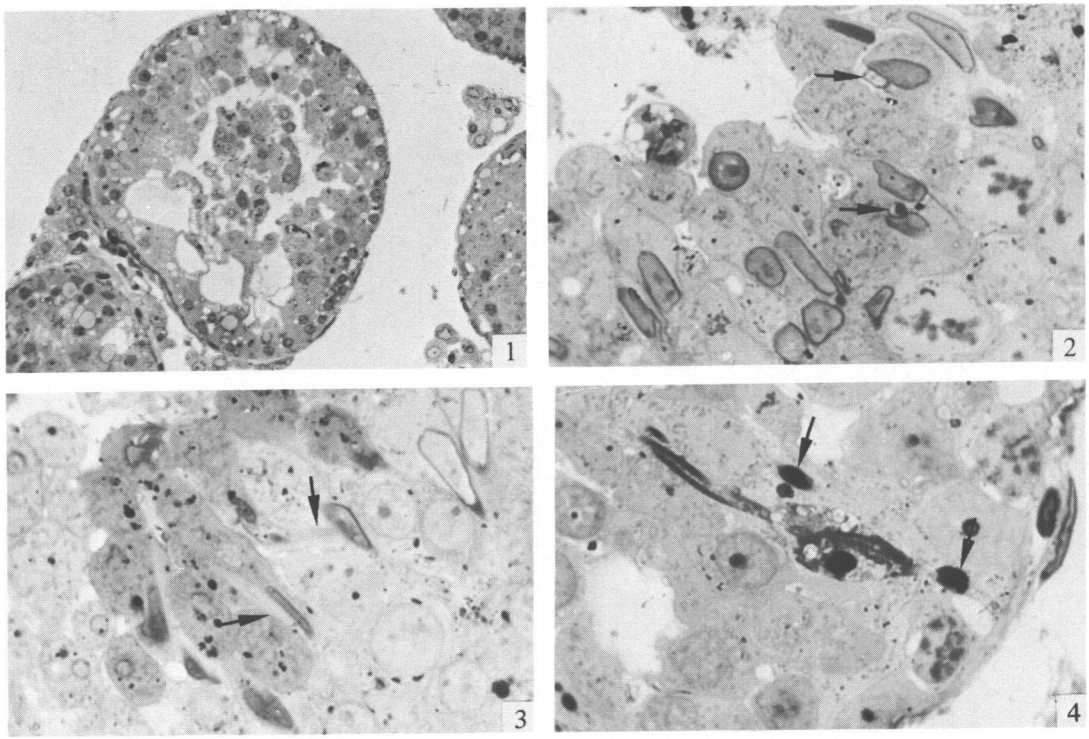

Fig. 1. Low power micrograph of degenerate seminiferous tubules from the sterile mouse trapped on Sanday, putative genotype t^{w106}/t^{w106}. At this magnification it is clear that the tubule is deficient in spermatids; Sertoli cells, spermatogonia and primary spermatocytes constitute the majority of cells. × 50.

Fig. 2. Stage 13 spermatids from the mutant, as well as having nuclei of abnormal shape, frequently contain acrosomes with large vacuoles (arrowed). × 650.

Fig. 3. Portion of a seminiferous tubule from the putative homozygote for t^{w106}. Although densely staining nuclei of late spermatids occupy the normal adluminal portion of the seminiferous epithelium, many of them are irregular in shape. Abnormally broad expanses of perinuclear cytoplasm, probably containing microtubules of the manchette are visible in some of the cells (arrowed). × 650.

Fig. 4. Micrograph of basal region of a typical seminiferous tubule from the mutant. A large, heterogeneous inclusion appears to contain remains of phagocytized spermatids including tails. A few densely staining bodies (arrowed), resembling nuclei of late spermatids, are also visible embedded in cytoplasm of Sertoli cells. × 650.

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4. DISCUSSION

The genetic data presented here clearly demonstrate the presence of a semilethal *t*-haplotype, t^{w106}, in Sanday, Orkney Islands. This is the first description of a wild haplotype in Great Britain, although other *t*-mutations have been detected there in laboratory stocks of mice (Carter & Phillips, 1950; Bateman, 1960). The Sanday *t*-haplotype is the first to be described in any island population of mice, and is therefore of special interest. This statement has to be qualified by the observation that very little *t*-testing has been carried out on island-caught mice, but it is pertinent to note that island mice are almost always much less variable than mainland populations because of the small number of animals usually involved in their establishment (Berry & Peters, 1977). Notwithstanding, the proportion of islands with a *t*-allele would be expected to be approximately the same as the frequency of an allele in a mainland population.

The sterile male trapped at Sanday appears to be a typical homozygote for a semilethal *t*-haplotype. The animal's aspermia is a feature shared by other homozygotes for semilethal *t*-haplotypes such as t^{w2} (Bennett & Dunn, 1967), whereas sterile mice that carry two different haplotypes produce large numbers of spermatozoa (Dooher & Bennett, 1977). Aspermia results from phagocytosis by Sertoli cells of late spermatids within the seminiferous epithelium in t^{w2} homozygotes (Dooher & Bennett, 1974) and, evidently, in the mouse studied here as well.

The morphological abnormalities of late spermatids described here also resemble abnormalities previously described in testes of t^{w2}/t^{w2} males (Dooher & Bennett, 1974); the spermatids also show acrosomes with unusual projections, as well as distortions of the nucleus posterior to the acrosome. Perhaps the most striking abnormality of late spermatids from t^{w2}/t^{w2} homozygotes is visible in the electron microscope and consists of a massive overproliferation of microtubules within the perinuclear manchette (Dooher & Bennett, 1974). The extensive cytoplasmic sheathes that flank the nuclei of late spermatids in the mouse described here almost certainly contain similarly disorganized microtubular arrays, although we did not examine them with the electron microscope.

Although these observations do not prove that this individual was homozygous for a semilethal *t*-haplotype, alternative explanations are unlikely. Clearly the observed aspermia rules out the possibility that the animal carried two different *t*-factors. Moreover, other mutations in the mouse which produce clear-cut syndromes of male sterility are associated with pleiotrophic effects ranging from specific coat color such as seen in homozygotes for sterility producing mutations at the pink locus (Johnson & Hunt, 1971) to skeletal deformities in hop-sterile homozygotes (Hunt & Johnson, 1971) or neurological disorders (quaking: Bennett *et al.* 1971).

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