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Induction of recessive lethal mutations in the *T/t–H-2* region of the mouse genome by a point mutagen[1]

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Summary

The *T/t–H-2* region on mouse chromosome 17 is known from complex natural variants ('*t*-haplotypes') to contain numerous genes, including some affecting the immune system and the development of the embryo. Rapid progress in the isolation of recombinant DNA clones for this 50 megabasepair region is generating the material for its complete molecular anatomy. A crucial step in revealing the biological functions controlled by the region is to obtain mutants in which genes are inactivated individually. We have used a pair of inbred mouse strains and a series of classical breeding schemes that permit the detection of recessive lethal and detrimental mutations in the *T/t–H-2* region.

In this initial phase of our study, 280 gametes mutagenized in the male germ line by ethylnitrosourea (ENU) have yielded eleven independent pre-natal recessive lethal mutations. Four have been mapped against *T* mutations and have been shown to complement one another in all pairwise combinations.

1. Introduction

Natural polymorphisms in the mouse include numerous *t* haplotypes with pleiotropic genetic effects (Gluecksohn-Waelsch & Erickson, 1970; Bennett, 1975; Sherman & Wudl, 1977). These include recessive defects in spermatogenesis and embryogenesis, distortion of Mendelian segregation in the male, as well as the defining characteristic of *t* haplotypes (*tct*): lack of a tail in compound heterozygotes with the brachyury mutation, *T*. Certain serologically-defined antigens expressed on embryos have been associated with *t* haplotypes. Finally, cloned DNA sequences elicited by microdissection of karyotypically-marked metaphase chromosomes have been mapped to the region of the *T/t* complex (Fox *et al.* 1985). How can one unambiguously relate any of the several characters associated with each *t* haplotype to an individual gene in the *t* haplotype?

The highly polymorphic major histocompatibility complex, *H-2*, is linked to the *t* region. The fine structure of the *H-2* locus is being resolved by methods of molecular cloning (Steinmetz & Hood, 1983). Some information on the natural functions of the genes in this complex comes from the study of effects of allotypic variation. However, one cannot fully evaluate the natural roles of these genes without mutant alleles that have lost function, particularly null alleles. For example, deletion of a portion of one of the class II histocompatibility genes (encoding antigen Eα) permits a rigorous demonstration of the role of this gene in the immune system of the mouse. Animals carrying the deletion cannot mount an immune response to certain synthetic antigens. Introduction of a cloned Eα gene to create a composite genome restores the missing immunoresponsiveness to the 'transgenic' mouse (Le Meur, *et al.* 1985; Yamamura, *et al.* 1985).

It has been shown that treatment of males with ENU efficiently induces germline mutations detectable in the specific-locus test (Russell, *et al.* 1979) and in the electromorph screen (Johnson & Lewis, 1981). More recently, Bode (1984) and Justice & Bode (1986) have induced mutations in genes controlling visible characters in the *T/t–H-2* region: new alleles at the quaking (*qk*), tufted (*tf*) and *hst* loci have been induced in both normal and in *t*-chromatin.

We have initiated a fundamental approach to understanding the individual functions of the vital genes in the *T/t–H-2* region by seeking to induce recessive lethal mutations with the presumptive point mutagen, ENU (Vogel & Natarjan, 1979; Popp *et al.* 1983; Ehling & Neuhäuser-Klaus, 1984). A classical method

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to detect recessive lethal mutations in diploid organisms is to follow an inbreeding pedigree emerging from a gamete that carries a recessive visible marker mutation in the region of interest (Muller & Altenberg, 1919; Snell, 1935). Failure to recover homozygotes for the visible marker implies that a recessive lethal or detrimental mutation lies in the region. The region scanned can be increased by the use of crossover-suppressors.

It is not yet known whether recessive lethal mutations can be efficiently induced in the *T/t–H-2* region by a point mutagen. The lethality of many natural *t* haplotypes may involve multiple additive elements (see Artzt, McCormick & Bennett, 1982; Silver, 1986). In addition, lethality may come from changes in repetitive DNA or from genetic rearrangements within *t* chromatin (Lyon, *et al.* 1979; Silver & Artzt, 1981; Shin, *et al.* 1983). Therefore, point mutations would identify those single genes, with strong effect, in the region and would present a simplification for interpreting the relationship between genotype and phenotype.

In this first report from our laboratories, we show that one can efficiently induce, detect and propagate recessive lethal mutations lying at any of numerous loci in the *T/t–H-2* region.

2. Materials and Methods

Mice. The BTBR strain*, homozygous for tufted (*tf*) and segregating brachyury (*T*), and the tailless strain*, BTt, carrying t^{w73} and *T-tf* were inbred in one of our laboratories (J.-L. G.), as were tufted mice carrying the deletion T^{Orl}. Tufted males carrying the deletion T^{hp} were kindly supplied by Dr L. Silver. The tufted phenotype was scored in weekly screenings starting at weaning and, in case of doubt, extending to 8 weeks, t^{w73} was scored either by its interaction with *T* to produce taillessness (Bennett, 1975), or by microcytotoxicity assay (Murphy & Shreffler, 1975) using a BTBR anti-BTBR + $/t^{w73}$ serum prepared in our laboratory. Since t^{w73} is the only *t* haplotype employed in these experiments, we shall refer to it simply as *t* in this text.

Mutagen handling. Ethylnitrosourea ($C_2H_5N(NO)CONH_2$), referred to below as ENU, was obtained commercially from three sources: Pfaltz and Bauer, Stamford, CT 06902; Radian Chemical Corp., Austin, TX 78759; and Sigma Chemical Corp., St Louis, MO 63178. ENU was kept stable by a trace of acetic acid and stored in a desiccator at −20 °C. In our early experiments, ENU was dissolved in dimethylsulphoxide at a nominal concentration of 200 mg/ml. Nine volumes of buffer (0·1 M dibasic sodium phosphate, 0·05 M citrate, pH 5·0) were added to reduce the concentration to 20 mg/ml ENU. More recently we have dissolved ENU to approximately 100 mg/ml in 95% ethanol. The precise concentration is determined in a stoppered cuvette from the absorbance near 398 nm of a 50-fold dilution of this stock solution. The peak absorbance for 1 mg/ml is 0·94 in ethanol (Lawley & Shah, 1972) and 0·72 in aqueous buffer. The stock solution is then diluted to 10 mg/ml by adding phosphate-citrate buffer, pH 5·0. Animals can be injected with up to 1 ml of such solutions without excessive intoxication from the 10% ethanol. At pH 5, ethylnitrosourea is stable for many hours at room temperature. Open samples of ENU are handled in a high-flow chemical hood. All spills and excess mutagen are rapidly inactivated with alkaline sodium thiosulphate (0·1 M-NaOH; 20%, w/v $Na_2S_2O_3$). Treated animals are kept in the chemical hood for 1 day. These precautions permit safe handling of this potent mutagen/carcinogen.

Mutagenesis. Males, at 6–12 weeks, were mutagenized, in sets of twelve, by intra-peritoneal injection of ENU at 150 or at 200 mg/kg body weight. About 4 weeks post treatment, these mice enter a sterile period. Typically, 10–30 weeks later six to ten of these males have recovered fertility.

3. Results

Several sets of normal-tailed BTBR adult males were mutagenized with ENU as described in Methods. Recovered animals were mated with BTt females carrying genetic markers (*T* and *t*) on chromosome 17 (see map, Fig. 1). Each first generation (Gen-1) mouse resulting from these matings is the founder of a family carrying a mutagenized paternal gamete, designated *. Families are inbred by mating the founder with BTt mice and backcrossing progeny to the founder parent. At this inbreeding generation one will recognize families carrying a lethal mutation on chromosome 17: these will fail to yield normal-tailed Tufted offspring which would have been homozygous for the lethal mutation (see Fig. 2).

The use of *t* in this breeding protocol serves two

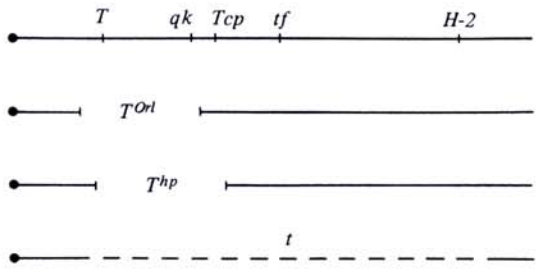

Fig. 1. Genetic Map of the *T–H-2* Region of Chromosome 17. *T*, brachyury: dominant mutation resulting in short-tailed heterozygotes, homozygous lethal; T^{hp}, deletion spanning *T*, *qk* and *Tcp-1*; T^{Orl}, deletion spanning *T* and *qk*; *qk*, quaking: recessive mutation resulting in trembling of homozygotes; *tf*, tufted: recessive mutation resulting in waves of hairloss, seen as bands of baldness starting at 4–8 weeks; *Tcp*, *t*-complex protein: formerly testicular cell protein. *H-2*, the major histocompatibility locus; *t*, *t* complex.

* Stocks obtained from D. Bennett and further inbred, over 30 generations in the case of BTBR, and over 17 generations in the case of BTt.

Table 1. *Evidence for* T-*linked recessive lethal mutations*

Cross: $\frac{T+}{+\ l_i} \times \frac{T+}{+\ l_i}$

Lethal	Total progeny	Normal tailed		
		Expected	Observed	Percent
1	389	130	5	1±0·5
2	552	184	2	0·4±0·3
3	244	81	18	7±2
4	207	69	8	4±1

Short-tailed (*T*-bearing) mice heterozygous for the lethal mutation (l_i) were intercrossed. Since *T* is a homozygous lethal mutation, one third of the progeny are expected to be normal-tailed. This number is presented in the column titled 'Expected'. The observed normal-tailed progeny (scored at weaning) are presumptive recombinants, and the frequency of such recombinants is a measure of the distance between *T* and the lethal mutation.

(l_i). In the absence of any lethal mutation, one would expect one third of the progeny to be normal-tailed, due to the homozygous lethality of the *T* mutation (see Fig. 3B). Normal-tailed offspring are presumptive recombinants between *T* and l_i. The distance between *T* and l_i is therefore proportional to the percent normal-tailed progeny. It can be seen that *l*(17)-1^{Wis} and *l*(17)-2^{Pas} are tightly linked to *T* (about 1% recombination) while the other mutations are further from this marker.

Localization of these mutations to deletion intervals can be achieved by using the known deletions T^{hp} and T^{Orl} (Johnson, 1974; Erickson, Lewis & Slusser, 1978; Alton *et al.* 1980; Babiarz, Garrisi & Bennett, 1982). T^{hp}, the largest known deletion in the *T–H-2* region, spans the loci brachyury (*T*), quaking (*qk*) and *t* complex protein-1 (*Tcp-1*) in the proximal (3–5 cM) portion of chromosome 17. T^{Orl} is a smaller deletion, extending from *T* through *qk* but not into *Tcp-1*.

The two lethal mutations tightly linked to *T*, *l*(17)-1^{Wis} and *l*(17)-2^{Pas}, were tested for viability in combination with T^{hp} and T^{Orl}. Short-tailed females, heterozygous for a lethal mutation in coupling with *tf*, were mated to T^{hp} males that were homozygous for *tf*. From such crosses, short-tailed progeny were scored for the Tufted character. If the lethal mutation is viable in combination with the deletion, half of the short-tailed mice should be Tufted. The absence of this class indicates that the lethal mutation is inviable in combination with T^{hp}. In a parallel set of matings, females carrying a lethal mutation and heterozygous for *tf* were mated to *tf*/*tf* males carrying T^{Orl}. Table 2 summarizes the results of these crosses. It can be seen that *l*(17)-1^{Wis} females produced 2 Tufted among 19 short-tailed progeny when mated to T^{hp} males, and only 2 Tufted out of 33 short-tailed progeny when mated to T^{Orl} males. All of these Tufted mice have been progeny tested and shown to be *T-tf* recombinants not carrying the lethal mutation. Thus, it appears that *l*(17)-1^{Wis} maps within both deletions. In contrast, *l*(17)-2^{Pas} produced 10 Tufted among 25 short-tailed progeny sired by a T^{hp} male, and 7 Tufted among 27 short-tailed sired by T^{Orl}. Thus, *l*(17)-2^{Pas}, unlike *l*(17)-1^{Wis}, is viable in combination with these deletions. Since *l*(17)-2^{Pas} is close to *T*, we conclude that it lies proximal to this marker.

Complementation tests between the four lethal mutations shows that they lie in separate genes. Table 3 presents the results of pairwise intercrosses between mice doubly heterozygous for *T* and each *l* mutation. It can be seen that all combinations yield the expected number of normal-tailed progeny.

Table 2. *Deletion Mapping of* l(17)-1^{Wis} *and* l(17)-2^{Pas} *using* T^{hp} *and* T^{Orl}

Cross: $\frac{(+l_i)\ tf}{(T+)+} \times \frac{T^{del}\ tf}{(++)\ tf}$ $T^{del} = T^{hp}$ or T^{Orl}

		Short tailed					
		TF		Non TF		Normal tailed	
	Total progeny	Expected	Observed	Expected	Observed	Expected	Observed
l(17)-1 × T^{hp}	36	12	2[a]	12	17	12	17
l(17)-2 × T^{hp}	35	12	10[b]	12	15	12	10
l(17)-1 × T^{Orl}	63	21	2[a]	21	31	21	34
l(17)-2 × T^{Orl}	40	13	7	13	20	13	13

[a] All have been progeny tested and shown to be recombinants NOT carrying the lethal mutation.
[b] Five have been progeny tested for T^{hp}: 2 appear to be *T* – *tf* recombinants not carrying T^{hp}, and 3 have been shown to carry T^{hp}.
Non-Tufted, short-tailed (*T*-bearing) females heterozygous for the lethal mutation (l_i) were mated to Tufted (TF) males carrying T^{del} (either T^{hp} or T^{Orl}). Progeny were scored for tail type and Tufted character at weaning and again at 8 weeks. *T* is lethal in combination with both deletions. Therefore, if both lethal mutations map outside these deletions, we expect one third normal tailed progeny and equal numbers of TF and Non TF among the short-tailed offspring.

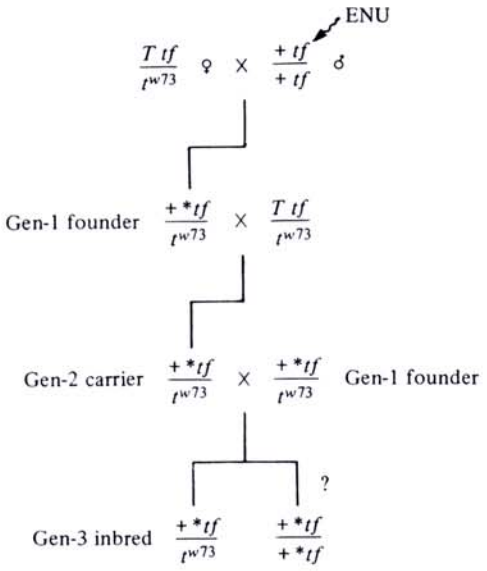

Fig. 2. Detection of recessive lethal mutations in mutagenized (*) *T/t–H-2* region of the mouse genome.

major functions. First, as a genetic marker, it allows one to distinguish the mutagenized copy of chromosome 17 from that brought in from the non-treated parent. Second, as a suppressor of recombination in the *T/t–H-2* region (Klein & Hammerberg, 1977), it increases the distance over which this portion of the genome is scanned for recessive lethal mutations.

There are, however, two drawbacks resulting from the properties of the *t* haplotypes. First, males heterozygous for *t* preferentially transmit *t*-bearing gametes to their offspring (Bennett, 1975). This segregation distortion is about 90% for t^{w73} on the BTBR genetic background in our laboratories. Therefore, as shown in Fig. 2, when $*(+tf)/t$ mice are intercrossed to produce the homozygous mutants, only occasional offspring would be expected to be Tufted, even without any lethal mutations in the region. Therefore, about 100 progeny must be generated before the absence of the Tufted class is significant ($P \leqslant 0{\cdot}01$).

This difficulty can be partially circumvented by modifying the breeding protocol as shown in Fig. 3. In this scheme, segregation distortion is bypassed by crossing Gen-1 $*(+tf)/t$ females to short-tailed BTBR ($Ttf/+$) males to yield short-tailed sons (and daughters) carrying the mutagenized chromosome 17. Now, at the backcross generation, 50% of the normal-tailed offspring will be Tufted, unless a lethal mutation is present (see Fig. 3A). In this modified protocol we have eliminated segregation distortion but permitted free recombination in the region of interest in the Gen-2 male.

The second drawback, present in both breeding protocols, is that the recessive lethal factors carried by ***T*** and t^{w73} prevent us from isolating induced lethal mutations which fall into these two particular complementation groups.

Among 280 families examined so far, we have positively identified four pedigrees carrying prenatal lethal mutations on chromosome 17: designated *l*(17)-1 through *l*(17)-4. [An additional seven families in this sample appear to be carriers of prenatal or early postnatal lethal mutations on chromosome 17 (A.S. in progress).] Table 1 presents the results of intercrossing short-tailed mice (*T*), carrying each lethal mutation

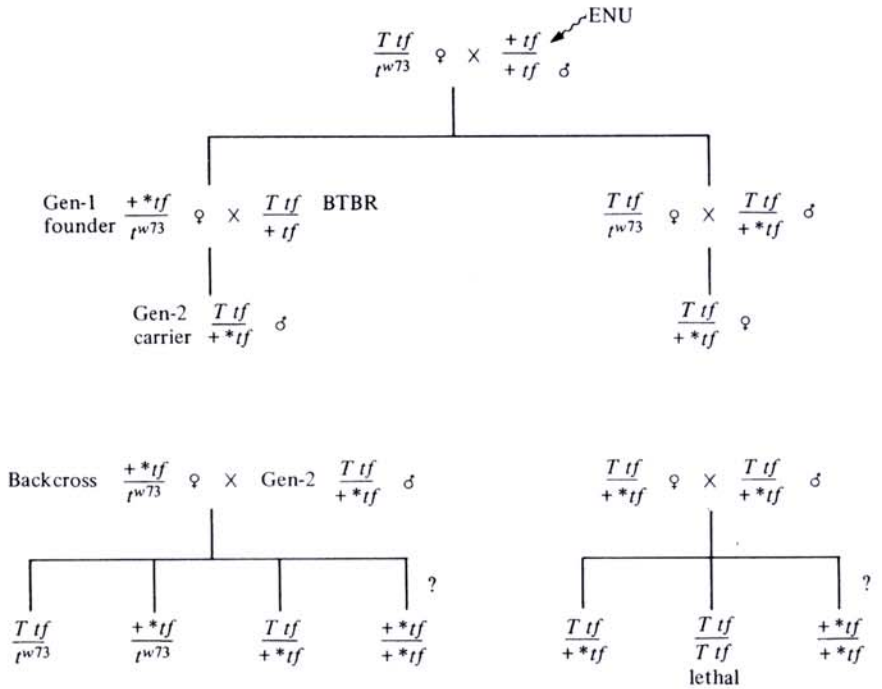

Fig. 3. Bypass of segregation distortion in the detection of recessive lethal mutations in a mutagenized (*) *T/t–H-2* region of the mouse genome.

several non-allelic lethals arising from males whose sibs establish multiple lethal-free families. The complementing mutations 1, 3 and 4 from the Wisconsin set all emerged from a single mutagenized male, who also sired seven lethal-free families. We have noted that his five brothers each sired only lethal free families – a total of eleven.

Additional evidence that the mutations recovered in this program are indeed mutagen-induced comes from the observed visible mutations. Although the BTBR and BTt strains are maintained by strict brother-sister inbreeding, none of the mutant phenotypes reported have occurred in the absence of mutagen.

The phenotypic expression of the four induced lethal mutations we have studied is apparently fully recessive: we have been unable to detect a heterozygous phenotype for viability, fertility, or visible characters (see Dove, 1984). We intend to pursue this question further, however, to look for subtle heterozygous expression.

The action of ENU is thought to yield primarily point mutations rather than deletions (Vogel & Natarjan, 1979; Popp *et al.* 1983). Antigen-gain mutation is consistent with base-substitution or insertion mutagenesis rather than with deletion formation (Bailey & Kohn, 1965). We have observed a background level of 1 per 230 antigen-gain histocompatibility phenodeviants among F1 offspring of normal-tailed BTBR males. This frequency increases to 3 per 130 among F1 offspring of ENU-treated males. Although only one of these phenodeviants has been tested for heritability (LLJ, data not given), these results are taken to confirm that ENU is not a deletion mutagen.Thus, it is plausible that we are dealing largely with induced lethal point mutations. However, ultimately this must be demonstrated in a molecular analysis (see below).

It is important to ascertain whether any genes in the *T/t–H-2* region encode cell-surface molecules (Bennett, 1980). Access to this information may be provided if mutant gene products either lose a polymorphic antigen or gain antigenicity. If mutant animals are coisogenic with an inbred strain, either case can be uncovered by exchange grafts or immunizations between heterozygous mutant and homozygous wild-type F1 animals. Our present study, however, involves two different inbred starting strains, BTBR and BTt. Here, one can detect antigen-gain mutations expressed by lethal/+ heterozygotes if they are antigenic in F1 (BTBR × BTt) recipients. We envisage a breeding scheme that is fully coisogenic when an induced *tct* mutation (Bode, 1984) becomes available on an inbred background such as BTBR.

We calculate that one can scan the *T/t–H-2* region at an efficiency of at least 85% without blocking recombination (see Appendix A). In the course of completely testing mutagenized gametes for both lethal and visible mutations in the *T/t–H-2* region, one will also detect recessive visible mutations lying at unlinked loci. Consider a Gen-1 male carrying a recessive mutation unlinked to the *tf* marker. If he is mated to three of his daughters, and at least six progeny are scored from each mating, then it can be shown that at least one affected homozygote will arise with a probability of about 0·8 (See Appendix B). Thus, in our goal to fully test a large number of gametes in the *T/t–H-2* region, the rest of the genome will be tested for recessive visible mutations at about 80% efficiency. Since the entire genome is approximately 100-fold larger than the *T/t–H-2* region, it is clearly fruitful to devise sensitive tests for phenodeviants arising in these mutagenized pedigrees.

The high efficiency with which ENU induces point mutations is a necessary component of a program of saturation point mutagenesis involving a pedigree analysis of mutagenized gametes. The link from mutations to molecules will most probably require a step of recombinant DNA analysis. Here, point mutations do not give immediate access to DNA clones for the affected region of the genome, in contrast to retroviral insertion mutations (Schnieke, Harbers & Jaenisch, 1983).

With retroviral insertion mutagenesis, however, there is reason to believe that the frequency of mutation per locus is at least an order of magnitude lower than that for ENU. If one can achieve one new integration per genome per generation (Jenkins & Copeland, 1985; van der Putten *et al.* 1985), and if the integration must be within 30 kb of the gene of interest to permit tranposon-tagged cloning, then useful retroviral insertions will arise randomly at a rate in the order of 10^{-5} per locus. Therefore, the number of gametes that would have to be analyzed in pedigrees to saturate the *T/t–H-2* region is prohibitively large.

The building of links between mutations and molecules may become feasible by coupling saturation of a region both with mutations, as envisaged here for the *T/t–H-2* region, and with clones of this same region (see Fox *et al.* 1985; Steinmetz & Hood, 1983). The member of a set of recombinant DNA clones that corresponds to the wild type allele of an ENU-induced mutation can in principle be deduced by showing that when the clone is introduced in expressed form into the genome of a transgenic mouse (Storb, *et al.* 1984; Chada, *et al.* 1985), it can suppress the lethal mutation.

These considerations indicate that the saturation mutagenesis of a region of the mouse genome by ENU can productively combine with saturation DNA cloning to link the molecular anatomy of the region to its physiological genetics.

5. Appendix A

The efficiency with which the region near *T* is scanned for induced recessive lethals may be calculated as follows, for the breeding scheme, $*/T \times */T$, to produce Gen-3 progeny. Without a *T*-linked lethal mutation, the ratio of normal-tailed to total viable progeny

Table 3. *Complementation between lethal mutations*

		Normal tailed		
		Expected		
Cross	Total progeny	Without complementation	With complementation	Observed
1 × 2	29	< 1	10	7
1 × 3	14	< 1	5	6
1 × 4	43	1	14	16
3 × 2	30	1	10	9
4 × 2	9	< 1	3	6
4 × 3	19	1	6	5

Crosses were carried out as indicated in Table 1. In the column labelled 'Cross', the lethal mutation *l(17)-1*, *-3* or *-4* carried by the female parent is indicated by the first number. The second number indicates the lethal mutation carried by the male parent.

The mutations *l*(17)-1^{Wis} and *l*(17)-2^{Pas} are being examined prenatally to determine the stage at which embryonic death occurs. Preliminary experiments indicate that *l*(17)-1^{Wis} homozygotes die about day 8 of gestation, and *l*(17)-2^{Pas}homozygotes die even earlier.

In addition to the recessive lethal mutations described above, a number of visible mutations unlinked to *tf* have also been detected among the 280 families examined. Two dominant mutations have been found: one affects pigmentation, and the other results in hair-loss. Several independent recessive mutations affect the coat in colour, texture or density. In addition, mutations affecting body size, coordination and skull anatomy have been observed. These will be described in detail separately.

4. Discussion

The fundamental goal of our work is to create a full set of point mutant alleles, each of which has lost function for a gene in the *T/t–H-2* region in the mouse genome. Our initial results indicate that this is both feasible and efficient on the scale of a standard mouse genetics facility.

Single mutations will not have a strong phenotypic effect if they lie in genes that are included in families with redundant function. Even the simple haploid eukaryote, *Saccharomyces cerevisiae*, has shown a number of cases of such functional duplication: the two-member histone H2B family (Wallis Rykowski & Grunstein, 1983) and the two-member Ras-homolog gene family (Tatchell, *et al.* 1984 and Kataoka, *et al.* 1984). Thus, in saturating a region with recessive mutations, one is concentrating on the single-copy genes. In testing 280 ENU-mutagenized gametes, we have characterized four, and found seven additional, independent recessive lethal mutations in the *T/t–H-2* region representing about 1% of the total genome. If ENU induces forward mutations at a frequency of 1/2000 per locus at the doses employed (Russell, *et al.* 1982; Ehling & Neuhäuser-Klaus, 1984; Bode, 1984), and if these mutations are all in separate genes, this incidence is consistent with the presence of approximately 80 single-copy essential genes in the region [(2000/no. gametes tested) × no. lethals]. The total number of essential genes in the mouse has been estimated at 5–10 × 10^3 by Carter (1957) and Lyon (1959); thus, we conclude that the *T/t–H-2* region is a typical target for recessive lethal mutations. If a mutagenized gamete carries too many recessive lethal mutations distributed over the genome, then progeny in an inbreeding generation will have a high probability of homozygosity for one or another of those mutations ('extinction'). The estimate of number of vital genes and induced mutation rate per gene given here would imply that each mutagenized gamete carries in the order of 4 ± 2 recessive lethal mutations. Our experience is consistent with the expectation for this mutational load – we find at most 20% reduction in litter size in a backcross generation of a pedigree initiated by a mutagenized gamete.

Are the observed recessive lethal mutations induced by ENU, or could they have a spontaneous origin? Lüning & Searle (1971) have evaluated several sets of data on the frequency of recessive lethals in the mouse. They estimate an incidence of 1·5 × 10^{-3} (0–3·3 × 10^{-3} at 95% confidence). The breeding scheme in the work reported here recovers recessive lethal mutations in a region representing about one percent of the genome. Therefore, we estimate that approximately one gamete in 670 would carry a spontaneous recessive lethal in the region studied. This is more than an order of magnitude less than the observed incidence of about one in 30. [Here, we assume that we have identified a total of eleven lethal families: the four described plus the additional set of seven indicated on page 137 of the results section.] Further evidence against a spontaneous origin of the majority of our mutations emerges from the pattern in which they arise. If they were spontaneous and pre-existing in origin, one would expect them to recur in sibships. However, we find

would be 1/3. However, a lethal mutation c units from T changes this ratio to $2c-c^2/2+c^2$, which is nearly c. If n viable progeny are scored, and a criterion number, x, are found to be normal-tailed, one can estimate the probability of failing to find an induced lethal mutation c units from T. To arrive at this estimate, we sum the first x terms of a binomial distribution with parameter $2c-c^2/2+c^2$. For example, if $n = 18$, $c = 0{\cdot}15$ (approximately the distance from T to *H-2*), and a criterion of $x = 5$ (slightly less than 1/3 of 18) were chosen, the probability of erroneously concluding that there was no lethal mutation is approximately 0·027. Thus, the scheme is highly efficient at this stage, and is essentially limited only by the efficiency of retaining the induced mutation in the pedigree up to the stage at which the indicated mating is set up. Thus, if there is only a single opportunity for recombination between an induced mutation and T up to that stage (e.g. breeding scheme as in Fig. 3A), then the overall efficiency of recovery of lethals linked to T is essentially $1-c$.

In his discussion of the recovery of lethals lying near visible markers, Haldane (1956) used far more stringent criteria in his choice of x. In our experiment the probability of obtaining 4 or fewer normal-tailed progeny out of 18 is approximately 0·23, when there is no induced linked lethal. However, we find that our relaxed initial criterion does not create excessive effort following false leads – the BTBR stocks are excellent breeders and pedigrees in question are readily expanded.

6. Appendix B

The efficiency with which the entire genome is scanned for induced recessive visibles can be estimated as follows. Consider a Gen-1 male heterozygous for an induced mutation. The probability that a randomly chosen daughter will not inherit the mutation is 1/2. Then, the progeny of a mating between the carrier male and this daughter will all be nonmutant (probability = 1). The probability that a daughter inherits the mutation is also 1/2. Now, the probability that there will be no mutants among n progeny from these two carriers is $(3/4)^n$. Thus, the probability of obtaining no mutant progeny from the mating of a carrier male with a randomly chosen daughter is $1/2(1)+1/2(3/4)^n$. If the carrier male is mated to k randomly chosen daughters, the probability of obtaining no mutant progeny from any of the matings is $(1/2+1/2(3/4)^n)^k$. Thus, the 'efficiency of scanning' is $1-(1/2+1/2(3/4)^n)^k$. For $n = 6$ progeny per mating, and $k = 3$ daughters, this expression takes the value 0·80.

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