

## Additive effects of the genes *tabby* and *crinkled* on tooth size in the mouse

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### SUMMARY

The semi-dominant X-linked gene *tabby* (*Ta*) in the mouse and its recessive autosomal mimic *crinkled* (*cr*) produce the same mutant syndrome involving abnormalities of the hair, teeth and certain exocrine glands. Previous work has provided some indication of interaction between these loci in terms of vibrissa number. The results of the present study demonstrate that, for tooth size, mice doubly heterozygous for *tabby* and *crinkled* show a more extreme phenotype than either heterozygotes for *tabby* or *crinkled* alone.

### 1. INTRODUCTION

The semi-dominant X-linked gene *tabby* (*Ta*) in the mouse, its two recessive autosomal mimics *crinkled* (*cr*) and *downless* (*dl*), and its one dominant autosomal mimic *sleek* (*Slk*), appear to produce the same mutant syndrome involving abnormalities of the hair, teeth and certain exocrine glands (Falconer, Fraser & King, 1951; Falconer, 1953; Grüneberg, 1965, 1966*a, b*, 1971; Cattanaich, 1975; Sofaer, 1969*a, b*, 1977). *Crinkled* is located on chromosome 13, and both *downless* and *sleek* on chromosome 10. Linkage tests have shown *dl* and *Slk* to be less than 4.8 map units apart with 95% certainty, though allelism has not been demonstrated (Crocker, 1978).

The most general effect of the mutant genes on the dentition is to reduce tooth size, but, at least for the upper and lower first molars of *tabby* heterozygotes, there are three fairly distinct levels of reduction. The most severely reduced first molars are always found adjacent to abnormal additional teeth. On the other hand, minimally reduced and moderately reduced first molars do not occur with supernumerary teeth, though it seems likely that each moderately reduced first molar was associated with an unsuccessful attempt to form a supernumerary tooth during its early development (Sofaer, 1975).

Previous work has provided some indication of interaction between *Ta* and *cr* in terms of vibrissa number. However, the results were difficult to interpret in that there were instances of both enhancement and reduction of the normal mutant effect in doubly mutant animals (Kindred, 1967). The present report describes a study of molar tooth size in which mice doubly heterozygous for *tabby* and

crinkled were compared with tabby heterozygotes and crinkled heterozygotes, the object being to provide further evidence of interaction between these two loci.

## 2. MATERIAL AND METHOD

The tabby allele used was that originally designated  $Ta^c$  (Roberts, 1966). This can now be regarded as equivalent to the allele  $Ta^j$  of earlier and independent origin (Stevens, 1963). Tabby and crinkled were transferred to a largely common genetic background by crossing to the inbred strain  $JU/Fa$ . Four groups of mice were examined:  $JU/Fa$  animals, wild type for  $Ta$  and  $cr$ , and  $Ta/+$ ,  $+/cr$  and  $Ta/+ . +/cr$  mice with backgrounds produced by either two or three crosses to  $JU/Fa$ . Three of the groups were composed of around 25 animals each, but the fourth, the doubly heterozygous group, consisted of only nine animals because of poor fertility. In contrast to the  $Ta/+$  and  $Ta/+ . +/cr$  groups, the inbred and  $+/cr$  groups contained males and females in approximately equal numbers. The  $Ta/+$  and  $Ta/+ . +/cr$  animals were also all the progeny of  $Ta/Ta$  mothers, whereas mice of the  $+/cr$  group were the progeny of heterozygous mothers. However, these differences are unlikely to confound the results since previous work has estimated the proportion of the total variance of tooth size due to the difference between sexes at only about 1% (Bader, 1965), and no evidence of a difference in maternal effect on the mutant phenotype has been found between  $Ta/+$  and  $Ta/Ta$  mothers (Kindred, 1961) or between  $+/cr$  and  $cr/cr$  mothers (Sofaer, 1968).

All molar teeth of the normal series were removed from the skulls of four week old mice prepared by papain digestion. The teeth were measured using a projection microscope, a magnified silhouette ( $\times 83$ ) of each tooth being projected onto a graduated screen. The measurements made were the maximum antero-posterior diameters of the molar crowns parallel to the occlusal plane. Corresponding measurements from the right and left sides were pooled.

## 3. RESULTS

The data are presented as distributions of raw measurements in Fig. 1. There is a clear trend between groups common to measurements for all teeth. Compared with inbred controls, the  $+/cr$  group is most normal, which is consistent with the recessivity of  $cr$  for other characters, the  $Ta/+$  group less so, and the doubly heterozygous group distinctly most abnormal. However, there was an apparently anomalous finding for the lower first molar in that the effect of the mutant genes was to increase rather than reduce the size of this tooth.

Because there is reason to believe that the distributions, at least of first molar measurements, are not strictly homogeneous, statistical procedure was confined to the calculation of means and  $t$  values to test the significance of the difference between the mean of the double heterozygotes and that of the  $Ta/+$  group, the most abnormal single heterozygote group. Since, ideally, the  $t$  test requires normality of the distributions being tested, it must be regarded as only a rather gross indicator of significance in the present situation.

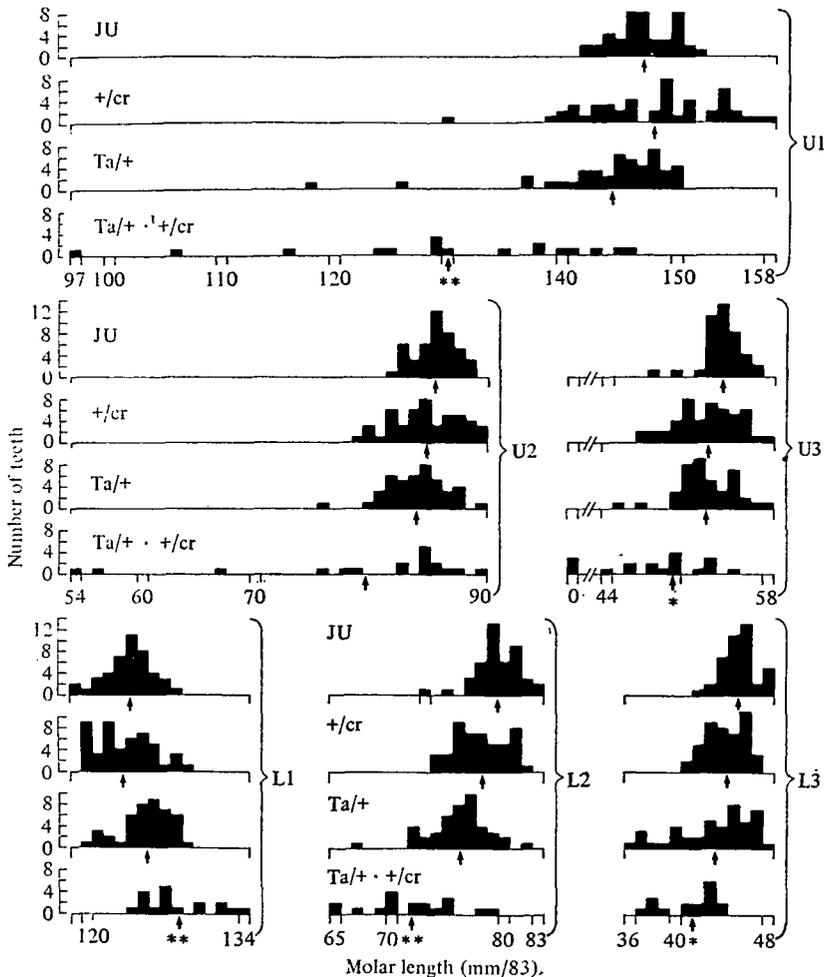


Fig. 1. Distributions of molar length (antero-posterior crown diameter) for the upper 1st, 2nd and 3rd molars (U1, U2, U3) and lower 1st, 2nd and 3rd molars (L1, L2, L3). Arrows show the means of the distributions. A single asterisk indicates a significant difference ( $P < 0.02$ ) and a double asterisk a highly significant difference ( $P < 0.001$ ) between double heterozygote and *tabby* heterozygote means for each tooth.

The three absent upper third molars in *Ta/+ · +/cr* mice (plotted against zero length) were not included in the calculation of the mean for this group.

#### 4. DISCUSSION

The discovery of mimic genes leads naturally to speculation over the functional relationship between them. Since homozygosity at any one locus alone is sufficient to produce the mutant phenotype, their functions are unlikely to be identical. The simplest explanation is that the wild type allele of each gene is responsible for one of a number of related steps, either in series or in parallel, towards the

formation of an end product or developmental state that is necessary for the production of the normal phenotype. Complete blockage at any point along an isolated pathway is expected to produce the same result. If blockage is incomplete, or if there are cross connexions with other pathways, differences between the genes may be detectable.

Such differences, although not apparent under normal conditions, may be disclosed in different ways: first, by changing genetic background, and second, by various environmental manipulations. Background changes have produced broadly similar modifications of the mutant phenotype for *Ta*, *cr* and *dl*, though certain phenotypic details showed minor differences of response (Sofaer, 1969*b*). A more definite difference between *Ta* and *dl* has been demonstrated with respect to the ability of embryonic tail tissue to produce hair follicles when cultured on the chick chorioallantoic membrane. Downless tails failed to produce follicles, which is in keeping with the adult phenotype of both downless and tabby mice, whereas tabby tails produced follicles at about 40% of the normal control level. Furthermore, dermis-epidermis recombination experiments have demonstrated clearly that the effects of both *dl* and *cr* are restricted to the epidermis, but have not provided similar evidence of a primary epidermal effect for *Ta* (Sofaer, 1974; Mayer, Miller & Green, 1977).

A third way of drawing inferences about the functional relationships between mimic genes is to compare the usual mutant phenotype with that shown by doubly mutant animals. Kindred (1967) investigated the effects on the coat and vibrissae of combinations between *Ta* and *cr*, the genetic backgrounds being those of lines selected for high and low total vibrissa number in tabby mice. Both *Ta* and *cr* normally reduce the number of vibrissae. However, the addition of a crinkled allele increased vibrissa number in both *Ta*/+ mice with the high selection line background and *Ta*/Y mice with the low selection line background. In other words, the addition of crinkled in these situations produced a phenotype closer to wild type. By contrast, when *cr* was added to *Ta*/Y on the high selection line background, the mutant effect was enhanced. The coat findings were inconclusive. Similar evidence of interaction between *Ta* and *cr* had already been found in other stocks by Fraser, Nay & Kindred (1959) but was then thought to be due to a background effect. These findings are difficult to interpret, though they do suggest the possibility of interaction between the two loci.

The size of molar tooth crowns is a valuable indicator of early developmental conditions since, once the crowns have been formed, they do not alter except as a result of occlusal wear. Such wear does not appreciably affect the dimensions normally studied in younger animals. The present results therefore show that on the *JU/Fa* genetic background, at least during the time when the molar crowns develop, the combination of heterozygosity at both the tabby and crinkled loci produces a more extreme mutant phenotype than heterozygosity at either locus alone. For all teeth except the lower first molar the change in phenotype was in the expected direction of reduction in tooth size. The paradoxical increase in size produced by the mutant alleles in the lower first molar was nevertheless consistent

with the findings for the other teeth in that the deviation from wild type was least in the *+cr* group, greater for *Ta/+* and greatest of all for *Ta/+ . +cr*.

The increase in size of lower first molars may be explained in terms of a difference of developmental timing between upper and lower first molars. The basis of such an explanation is as follows. The mutants appear to have their effect in two main phases characterized by partial suppression of epithelial growth and differentiation. These two phases are separated by a period, from 17 days of gestation to birth, during which there is no suppression. Early growth of the epithelium of the first molar occurs during the first suppression phase, with the majority of its later growth and differentiation occurring when suppression is no longer active. After suppression has been released there appears to be rapid compensatory increase in size, the amount of size increase probably depending on the state of epithelial differentiation. Indeed, in cases where supernumerary teeth develop, it is just this period when they reach a remarkably large size and advanced state of differentiation in a relatively short time (Sofaer, 1969*a*). A slight difference of degree in epithelial differentiation between upper and lower tooth germs at 17 days of gestation could perhaps allow more rapid compensatory increase in size in the lower first molar than the upper, sufficient in some cases actually to result in overcompensation. This phenomenon would not apply to the other molars since the second half of the growth and differentiation of the second molar germ, and the entire development of the third molar germ, occur during the second suppression phase.

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