The molars of the tabby mouse, and a test of the ‘single-active X-chromosome’ hypothesis

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The sex-linked gene for tabby (Ta) in the mouse, in addition to its effects on fur and skin, has a characteristic dental syndrome (Grüneberg, 1965). Some tabby heterozygotes have a spectacular mixture of molar teeth, some of which are normal, others which are tabby, and some which combine features of both. This prima facie evidence in favour of the single-active X-chromosome hypothesis of Lyon (1961) can be turned into a more exacting quantitative test of that concept. An autosomal mimic of tabby, called crinkled (cr; linkage group 14), with the same dental syndrome, offers opportunities for further tests of the hypothesis. It will be seen that, contrary to first impressions, the quantitative study reveals a situation which is not in agreement with the Lyon hypothesis.

The molars of tabby hemizygotes and homozygotes

With the exception of 29 animals obtained from Dr D. S. Falconer (Edinburgh), all the material was bred in this laboratory. Both groups of animals were of genetically heterogeneous backgrounds; as they did not differ appreciably from each other, they have been combined. Altogether there are 41 Ta♀♀ and 13 Ta/Ta ♀♂ which have been pooled as their dental anomalies are alike; and 121 Ta/+ ♀♀. As it is essential to distinguish the effects of tabby from those of the genetic background, 42 normal litter-mates (40 ♂♂, 2 ♀♀) have been included in this investigation. These proved to be entirely free of the peculiarities which will be described below in Ta/+ ♀♀. The remarkable uniformity of dental morphology in the normal mice and the wide gap which separates it from the anomalies of the tabby molars is the solid basis on which rests the interpretation of the tabby heterozygotes.

It will be necessary to describe the anomalies of tabby molars in somewhat more detail than previously (Grüneberg, 1965). All the molars are reduced in size (Fig. 1). As there is a minimum size below which a tooth germ will regress without having formed hard substances (Grüneberg, 1951; Grewal, 1962), the reduction in size leads to the absence of many third molars.

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The first upper molar (m1) has more erect cusps and a narrow neck which gives the crown a bulbous appearance. Buccally, cusps B1 and B3 are regularly absent. The situation on the lingual aspect is more variable; 48 out of 108 teeth had a shallow separation between cusps L1 and L2; in nine these cusps touched and were separated by a groove only, and in five they were represented by a single cusp; in a further 22 teeth, L1 or L2 or both were reduced to a varying extent; the tooth shown in Fig. 1 has a reduction of L1. The root is usually single though surface mouldings generally still indicate its composite

Fig. 1. Left normal (a and b) and tabby (a' and b') molars. In the normal specimen, a key to the cusps of the first and second molars is given. In the triserial upper molars (a) the central row of cusps is designated as 1, 2 and 3 respectively; the buccal cusps as B1, B2, etc., and the lingual ones as L1 and L2. In the biserial lower molars (b), there is no central row, except for cusp 4 at the rear end of m1 and m2. In the upper molars (a and a'), the buccal aspect is on the left and the lingual aspect on the right; in the lower molars (b and b'), the situation is reversed. Arrows in this and subsequent figures point forward. All illustrations are camera lucida drawings. (a) and (b) are from a Ta/+ with normal phenotype, (a') and (b') from a Ta brother of that animal.
Molars of the tabby mouse

nature; in 11/108 teeth, the posterior root was completely or largely free; this variant tended to occur symmetrically.

(2) The second upper molar (m2), like m1, has a rather bulbous crown with a narrow neck; cusp B2 is finger-like and erect and B3 regularly absent. B1 is increased in size and becomes continuous with L1; these two cusps thereby form a kind of ‘rampart’ in front of cusp 2, which normally forms part of the anterior surface of the crown. Lingually, L1 and L2 are reduced to a variable extent. All 108 teeth had a single root which corresponds mainly or entirely to the lingual root of the normal m2. In normal m2, the anterior buccal root is sometimes more or less fused with the lingual one, as in Fig. 1.

(3) The first lower molar (m1) is regularly reduced both anteriorly and posteriorly to a greater or lesser extent. A medium degree of reduction is shown in Fig. 1 and more extreme forms in Grüneberg (1965, figs. 15, 16). Cusp L1 is regularly and B1 often absent, with corresponding reduction of the anterior root; strongly reduced m1 have but a single root. Posteriorly, cusp 4 is regularly absent, and B3 and L3 coalesce into a single cusp.

(4) The overall size of the second lower molar (m2; see also Fig. 8 a, e) depends on that of m1: where m1 is fairly large, m2 is small; but when m1 is much reduced, m2 tends to increase in size and may become larger than normal. Cusps B1 and 4 are regularly absent. As in m1, B3 and L3 coalesce to form a single cusp, and the same usually happens anteriorly with B2 and L2. The roots are compressed from side to side rather than from front to back as in the normal. Depending on the size of the tooth, the roots may be far apart like the legs of a striding man, or they may fuse to a varying extent.

Thirty out of 54 tabbies lacked one or more third molar; altogether 58 m3 and five m3 were absent (44.4 and 4.6 % respectively); there was an obvious correlation between right and left and ‘bunching’ within litters. Absence of third molars in tabbies thus behaves like that in the inbred strain CBA (Grüneberg, 1951), but is more extreme. None of the 42 normal litter-mates of tabbies lacked third molars.

THE MOLARS OF TABBY HETEROZYGOTES

(1) Mosaic dentition

The aim of this paper was to use the dentition of the Ta/ + ♀ as a test system for the ‘inactive-X-chromosome’ hypothesis of Lyon (1961) or, as it has been rather more appropriately called by Russell (1964), the ‘single-active X-chromosome’ hypothesis. The first such heterozygote examined (Fig. 2) seemed to settle the question in favour of that hypothesis, and among the 121 Ta/ + ♀ collected, many are similar or represent variants of the same theme. There is a peculiar mixture of teeth. The right m1 (no. 4) is perfectly normal; the accessory cusp between B2 and B3 is a common minor variant which has nothing to do with tabby. By contrast, the left m1 (no. 3) is unmistakably a tabby tooth with a
small bulbous crown and erect cusps; B1 and B3 are absent and L1 and L2 represented by a single cusp; there is a single composite root. Two other teeth are typically tabby, namely the left m1 (no. 10) with a greatly reduced crown and a single root, and the right m2 (no. 8) which conforms to the tabby pattern in every respect. The other teeth combine features of normal and tabby. The left m2 (no. 2), as seen from the buccal aspect, looks very much like a tabby tooth (prominent and erect B2, absence of B3 and the increased B1 which together with L1 forms a ‘rampart’ in front of the central cusp 2). But as the lingual side

is quite normal and the posterior buccal root separate, the tooth is ‘mixed’. Similarly, the right m2 (no. 5) has a single root like a tabby tooth, and B3 is absent; but B1 is small as in a normal tooth and there is no rampart in front of cusp 2; also, there are no abnormalities on the lingual side. In the mandible, the right m1 (no. 9), what there is of it, is essentially normal, except that cusp L1 is absent and the anterior root is correspondingly reduced. The second tooth on the left (no. 11) is larger than a normal m2 (perhaps because it follows a very small m1); B1 and B2 are more widely separated from each other than normal, whereas in a tabby m2 (no. 8), B1 is completely missing; posteriorly, the tooth has typical tabby features (absence of cusp 4 and coalescence of B3 and L3). The right m3 is absent, which suggests that it was reduced and hence of tabby pattern; the left m3 (no. 12) is larger than normal and its crown reminiscent of a tabby m2; the root is single. The upper third molars are difficult to interpret; the left one (no. 1) is rather larger, the right one (no. 6) smaller than normal and not far from the minimum size for that tooth.

According to the single-active X-chromosome hypothesis, patches manifesting either one or the other of the two alleles should be present side by side
Molars of the tabby mouse

like the black and orange blotches in a tortoiseshell cat, provided, of course, that this mosaic pattern is not blurred or extinguished by the existence of diffusible substances. Figure 2 illustrates without ambiguity the autonomous development of the normal and tabby molar patterns which can be recognized side by side in a single tooth. Indeed, the patchwork of normal, tabby and mixed teeth in that mouse is exactly what one would expect on the Lyon hypothesis. However, whereas qualitative agreement with that hypothesis is obvious, it does not necessarily follow that a quantitative analysis of a larger sample of tabby heterozygotes might not reveal features at variance with that hypothesis.

In the course of the analysis, unexpected complexities were revealed which had to be cleared up before a quantitative treatment could be attempted. These are discussed in the following section and will necessitate a good deal of re-interpretation of the dentition just discussed.

(2) Twin teeth in tabby heterozygotes

Occasionally, tabby heterozygotes have four rather than three molars in a row. In such cases, either m₁ or m₂ is represented by two teeth. In a total of 121 Ta/+ ♀♀, there were eight instances of overt twinning of m₁ and three of m₂ (Figs. 3, 5). In addition, there are instances of incomplete twinning (Figs. 4, 6).

Fig. 3. Twinning of the right m₁ in Ta/+ ♀♀. Buccal aspects on the left, lingual on the right. In (a), the crowns of the twin teeth are separate, but the root (accidentally broken) was common to both. In (b) and (c), the twins are completely separate. Note that in (b), the crown pattern of the posterior twin is indistinguishable from that of a tabby m₂, and that in (a) and (c) it approaches that pattern.
The anterior twin tends to be the smaller of the two. In Fig. 3(c) the anterior tooth is essentially a single cusp; the crown of the posterior twin resembles a tabby m\textsuperscript{1} in that it has three central cusps and lacks B1 and B3; however, both B2 and L1 are duplicated. In Fig. 3(b) the two teeth are more nearly alike in size; the anterior twin resembles a tabby m\textsuperscript{1} and the posterior twin a tabby m\textsuperscript{2}. Evidently, a derivative of the first tooth germ can have the morphology of m\textsuperscript{2}. The important fact that the homology of a tooth cannot be deduced unambiguously from the morphology of its crown alone will have to be taken into account in the interpretation of other more complex situations to be described below.

A stage intermediate between complete and incomplete twinning of m\textsuperscript{1} is shown in Fig. 3(a). The crowns of the twins are completely separate and covered by smooth enamel throughout, but they were originally connected by a common root. In this particular case, the twins were followed by m\textsuperscript{2}, but m\textsuperscript{3} was absent; none the less, the interpretation was not in doubt.

Cases of incomplete twinning of m\textsuperscript{1}, with about five roots each, are shown in Fig. 4(c, d). In d, there are four rather than three central cusps, B2 is duplicated and L1 triplicated. The situation in c is slightly less extreme, but there is no doubt that this is an incomplete twin tooth. Almost the only marginal case is b. It seems that the two major buccal cusps with the nodule between them represent a duplication or beginning triplication of B2 similar to the situation in c, and the broadened lingual root also suggests incomplete twinning; but the interpretation of this particular tooth is not beyond doubt.

The two crowns of twin teeth together tend to represent more material than the crown of a normal m\textsuperscript{1}, and the same is patently true for the incomplete twins c and d (Fig. 4). One gets the impression that there is a maximal size for m\textsuperscript{1}; if that is exceeded, the tooth germ tends to break up into two. However, in the finished tooth, we cannot distinguish between size at the time of twinning and subsequent growth.

Overt twinning of m\textsubscript{1} (with four molars in a row) is shown in Fig. 5(b), where the anterior twin is essentially a single cusp; the posterior twin has the morphology of a normal m\textsubscript{1} except that it lacks L1 and has a correspondingly reduced anterior root. One gets the impression that L1 has made itself independent. In a, the anterior twin is larger with three cusps, and the posterior twin is smaller; it is morphologically scarcely distinguishable from a normal m\textsubscript{2} and again demonstrates the fact that the morphology of the crown is no safe guide as to the homology of the tooth in the series.

A single but striking example of incomplete twinning of m\textsubscript{1} has been encountered (Fig. 6). The tooth is much longer than normal; the extra material is all in front (extra lingual cusp; B1 lengthened). The massive anterior root shows a suggestion of beginning splitting in its surface moulding: if the split had actually occurred, the resulting twins would have closely resembled those in Fig. 5(a).
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The situation is sometimes complicated by the fact that small tooth germs may regress without having formed hard substances (Grüneberg, 1951; Grewal, 1962) and this, presumably, is not confined to third molars. In Fig. 7 the abnormal mouse shows overt twinning on the left (right in drawing) and has three teeth on the right. In the absence of other information, one might be inclined to identify these teeth as m1, m2 and m3 respectively, with reduction of m1 and compensating increase in the following teeth. Comparison between the two sides suggests that the first two teeth on the right are, in fact, twins and thus jointly correspond to m1 (like the overt twins in Fig. 5a), and that, in turn, m3 is absent. Similarly, in the first Ta/+ ♀ (Fig. 2), it now appears probable that teeth 10 and 11 are twins, and that m3 is absent, like its fellow on the other side.
Not rarely, there are three teeth which are clearly equivalent to $m_1$, $m_2$ and $m_3$ respectively: but $m_1$ lacks $L1$ like the overt posterior twin in Fig. 5(b). In such instances, it is difficult to avoid the conclusion that the truncated $m_1$ is, in fact, the survivor of a twin pair of which the anterior partner was too small to calcify. An example, again in the first $Ta/+$, is the right $m_1$ (Fig. 2, no. 9); if so, twinning in that mouse occurred in both mandibles; on one side both twins survived, on the other only the bigger one. Indeed, perhaps the upper molars nos. 2 and 3 are also a twin pair, in which case tooth no. 1 would be $m^2$, with $m^3$ absent.

Fig. 5 Twinning of $m_1$ in $Ta/+$ $♀$. (a) Right; (b) left $m_1$. In (a) buccal aspect on left, in (b) on right. In both cases (as in Fig. 3 (b) and (c)) the twin teeth were followed by second and third molars.

Fig. 6. Incomplete twinning of $m_1$, together with normal tooth for comparison. Buccal views on top, lingual on bottom. Both teeth have a bifid cusp 4, a variant apparently unconnected with $Ta$. The incomplete twin was followed by $m_2$ and $m_3$.

Obviously, in tabby heterozygotes there are numerous instances of concealed twinning which, in the aggregate, considerably outnumber the overt cases. In the upper jaw, a continuous scale of intergradations leads from obvious to only plausible or merely possible cases; in the mandible, the situation tends to be clearer. Whereas at present not all cases of twinning can thus be enumerated with confidence, we can ask the question, can all anomalies of the molars in tabby heterozygotes be explained in terms of twinning? This question can be answered in the negative. The abnormal $m_2$ in Text-fig. 8(b–d) were in each case preceded by a perfectly normal $m_1$. As no similar anomalies have been observed
in the normal litter mates of tabbies, they must be ascribed to the tabby gene, and as the preceding $m_1$ is normal, the anomalies cannot be explained in terms of twinning.

Fig. 7. Buccal aspects of the lower molars of a $Ta/+ \delta$ with normal teeth (top) and a $Ta/+ \delta$ with overt twinning on the left (right in drawing); on that side, the first two teeth jointly represent $m_1$. The same evidently is the case on the other side where the anterior putative twin is larger and the posterior is smaller, and where $m_2$ is absent. The two animals are litter mates.

Fig. 8. Left $m_3$ of $Ta/+ \delta$ (a–d) and of a $Ta/Ta \delta$ (e). Buccal views on top, posterior views below. (a) Normal; (b) shallow separation of B1 and B2, and cusp 4 slightly reduced; (c) sharp separation of B1 and B2 (though B1 slightly reduced), and cusp 4 missing, but B3 and L3 still separated by a depression; (d) tooth approaching tabby phenotype (absence of B1 and 4; B3 and L3 no longer separated by depression though posterior surface not quite smooth. Note that roots are essentially as in the normal). (e) Tabby tooth of usual type; separation of B2 and L2 more nearly like that in (d) occasionally occurs in tabbies.
EVIDENCE FOR TWINNING IN TABBY AND THE CRINKLED HOMOZYGOTE

In view of the frequent occurrence of twinning in tabby heterozygotes, it is peculiar that no case of overt twinning (four teeth in a row, or convincing incomplete twins) has been observed in 54 tabbies or in 58 crinkled (cr/cr) mice which have the same dental syndrome. However, there is some evidence for concealed twinning, at least in crinkled. In three animals, one of which is shown in Fig. 9(b), there are only two teeth on one side which are evidently m₁ and m₂, m₃ being absent (the only alternative would be to regard

![Fig. 9. Evidence for the occurrence of twinning in cr/cr mice. (a) Normal (+/cr ☺); (b–e) cr/cr mice (♀, ♂, ♂, ♀). Explanation in the text.](image-url)
Molars of the tabby mouse

these teeth as $m_2$ and $m_3$ respectively, with $m_1$ absent: this lacks all morphological plausibility, and there is no other evidence at all that in these mutants $m_1$ may be absent altogether. Now, if the two teeth on one side are $m_1$ and $m_3$, the same evidently applies to their fellows on the other side, and the small tooth preceding $m_1$ can only be its twin; perhaps, as indicated by the question mark, a twin too small to calcify was originally present on the other side. Other cases similarly interpreted are shown in (c-e), and they include close counterparts to instances of concealed twinning observed in $Ta/+ \varphi\varphi$.

Table 1. The third mandibular tooth in tabby and crinkled mice

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<th>Gene</th>
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<td>23</td>
<td>59</td>
<td>6</td>
<td>22</td>
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Additional indirect evidence for concealed twinning comes from the behaviour of the third mandibular tooth where such is present (Table 1). Both in tabby and in crinkled, absence of $m_3$ only occurs where $m_1 > m_2$ (as in a normal mouse). Where a third molar is present, it is usually of normal size or small, and its crown pattern tends to be simplified (posterior cusp $L3$ rudimentary or absent, as in Fig. 1(b'), and in Fig. 9(d)). However, where $m_1 < m_2$ (as in Fig. 9(b, c)), the third tooth is often large and has a conspicuous posterior cusp (sometimes bifid, as in Fig. 16(c) in Gruneberg, 1965). This can be interpreted in terms of competition: where $m_1$ is small, $m_3$ may benefit, and that was the line taken in 1965. Now, an alternative interpretation appears more probable, namely that often, if not always, the small anterior tooth is, in reality, an anterior twin rather than the whole $m_1$; if so, the third tooth is homologous to $m_2$ which, in both mutants, has a large posterior cusp not unlike $L3$ in a normal $m_3$. Regarded as $m_2$, the third tooth is usually small; this may again be interpreted in terms of competition: the twin pair has left too little for $m_2$ and nothing for $m_3$.

A more sensitive way to discover concealed twinning is in material sectioned prior to the regression of minute tooth germs. In a normal mouse, at the age of
4–5 days after birth, m\textsuperscript{1} is in an advanced and m\textsuperscript{2} in an early stage of enamel and dentine formation; m\textsuperscript{3} is in the cap stage and thus far from calcification or regression whichever may be its ultimate fate. The heads of a litter of six Ta ♀♀ and one Ta/+ ♀ were serially sectioned in the sagittal plane. None of the six Ta ♀♀ showed any signs of twinning in the upper molars. By contrast, the Ta/+ ♀ had four tooth germs on one side; the first two were about equally far advanced and clearly a twin pair; the first was larger than the second twin, and if the last tooth germ (= m\textsuperscript{3}) had regressed as seems probable, the twin nature of the first teeth would not have been in the least obvious. The third tooth germ (m\textsuperscript{2}) was smaller than a normal m\textsuperscript{2} though larger than a normal m\textsuperscript{3}, and it had just started to form dentine.

Clearly the question of whether twinning occurs in tabby mice requires detailed embryological studies which will be carried out in another laboratory.

STATISTICAL ANALYSIS

The majority of the 121 tabby heterozygotes fall into two distinct groups, 42 normals and 52 striking ‘mosaics’ which can be recognized at first glance. The remaining 27 animals show only minor signs of the tabby phenotype which are usually confined to the second molars and which could easily be missed. However, as similar stigmata were absent in the 42 normal litter-mates examined, they are evidently what they appear to be, i.e. minor manifestations of tabby in the heterozygote. Adding these marginally affected individuals to the clear ‘mosaics’, there are 79 phenotypically recognized animals, or 65%.

The analysis may start with the first molars whose identity is never in doubt.

Table 2. Involvement of m\textsuperscript{1} and m\textsubscript{1} in 121 Ta/+ ♀♀

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In Table 2, N stands for 'no abnormality discovered' and A for 'tabby type to a greater or lesser extent'; the latter class includes a small number of marginally affected teeth.

The two sides are affected about equally (right 57, left 67 times; \( \chi^2 = 0.81 \)), and the same applies to upper and lower molars (\( m^1, m_1 \) 69, 55 times; \( \chi^2 = 1.58 \)). It is thus legitimate to pool all first molars, and to ask the question, do the first molars vary independently of each other? If so, the frequency of mice with 0, 1, 2, 3 and 4 affected molars should be binomially distributed. In Table 3, for sake of convenience, the expectation has been calculated on the basis of the binomial \((3/4 + 1/4)^4\) which is very close to the observed values for N and A, viz. 360:124. There is a striking excess of animals with 0 and with 3 or 4 teeth affected, and a corresponding deficiency in the remaining classes. The discrepancy is so great as to be beyond the need of significance tests. Table 3 thus substantiates the statement made at the beginning of this section, that \( Ta/ + \) fall in the main into two fairly distinct groups, the normals and the 'mosaics'.

There is a highly significant correlation between right and left both for \( m^1 \) and for \( m_1 \) (\( \chi^2 = 30.3 \) and \( 31.1 \), respectively).

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</tbody>
</table>

The overall incidence of 'A' type first molars in \( Ta/ + \) is 124 out of 484, or 25.6%. Many of these are by no means 'pure' tabby teeth; the majority are of mixed phenotype and a few are only marginally affected. For instance, in most of the overt and concealed twins of \( m_1 \), the larger posterior twin, what there is of it, is morphologically perfectly normal. The value of 25.6% is thus an upper limit, and the tabby contribution to the phenotype of the first molars is probably less than 20%.

The analysis of the second molars is complicated by the phenomenon of twinning. Where a row starts either with a normal first molar or with a twin pair, the identity of the second molar is not in doubt. But where it is uncertain whether the first tooth is a member of a twin pair, the next tooth may be either its posterior twin or a second molar. No satisfactory criteria for distinguishing one from the other have been found for the upper molars though it is quite clear that both types occur. The situation is more favourable in the lower molars which may be classified as follows:

(a) Overt twins. A small tooth with a single root precedes a definite two-rooted \( m_1 \) which lacks L1 and often also B1. Twice cusp 4 of the posterior twin was reduced; in the rest the posterior twin was normal.

(b) Overt incomplete twin (Fig. 6).
(c) Concealed twins. As in (a), but anterior twin missing; posterior twin normal as far as it goes (as in Fig. 2, no. 9).

(d) Concealed twins (other probable cases). Four times posterior twin normal as in (c); in the rest cusp 4 reduced.

(e) Teeth reduced both anteriorly and posteriorly as in tabby and probably not twins.

(f) Marginal cases with fusions between B1 and L1, except one with cusp 4 'a little reduced'.

Thirty and probably 39 out of 55 'A' type m₁ (a–d) thus involve twinning, and eight of the marginal cases (f) may be rudimentary forms of it. The identity of m₂ is in no real doubt except in (e) and possibly in (d), i.e. at most in 16/242 cases. Accepting the above classification, the m₂ in Table 4 have been subdivided into two groups according to whether they are preceded by a normal or by an 'A' type m₁. An abnormal m₁ is nearly always followed by an abnormal m₂ though the abnormality is sometimes only slight (like a shallow separation between B₁ and B₂; or a reduction of cusp 4). By contrast, a normal m₁ is usually followed by a normal m₂, and where there is an abnormality of the second molar it tends to be slight rather than marked. Somewhat surprisingly, the overall involvement of m₂ (114/242 or 47 %) is much higher than that of m₁.

Table 4. Involvement of m₂ in 121 Ta⁺⁺

<table>
<thead>
<tr>
<th>Second lower molar</th>
<th>Normal</th>
<th>Marginally abnormal</th>
<th>Clearly abnormal</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>m₁ normal</td>
<td>127</td>
<td>38</td>
<td>22</td>
<td>187</td>
</tr>
<tr>
<td>m₁ abnormal</td>
<td>*1</td>
<td>10</td>
<td>44</td>
<td>55</td>
</tr>
<tr>
<td>Total</td>
<td>128</td>
<td>48</td>
<td>66</td>
<td>242</td>
</tr>
</tbody>
</table>

* The corresponding m₁ was marginally affected (category f).

Table 2 includes 83 Ta⁺⁺ with symmetrically normal m₁. In 50 of these, neither m₂ was affected, in ten the left one, in seven the right one, and in 16 both m₂. There is thus a highly significant correlation between the two sides (χ²₁ = 21·63).

Absence of m₃ in Ta⁺⁺ occurred in 36/242 teeth or 14·9 % (13 right, 11 left, 6 bilateral). In 29 cases there were only two teeth in the row; in the rest, the first two (out of three) elements in the row were unmistakable twins. In 33 out of the 36 cases the missing tooth was preceded by an abnormal m₂ and, in 18 of these, m₁ was also abnormal. In these 33 cases, absence of m₃ may thus be regarded as part of a larger anomaly. One of the remaining cases had massive maxillary, but no other mandibular anomalies; another had mild anomalies of m₁ and m₂ on the contralateral side, but m₁ and m₂ of the affected side were quite normal. In both cases the involvement of m₃ seems to be independent of that of the rest of the dentition. The last
case occurred in an otherwise completely normal dentition and may represent a 'mosaic' for a single tooth, unless it is a sporadic case of absence of m3.

The tabby-type abnormalities of the lower molars spread in an anteroposterior direction. Thus, an abnormality of m1 is nearly always accompanied by an abnormality of m2, and m3 is rarely absent unless m2 is also involved. But the process may start either with m1, or with m2, or with m3. Thus, there are 55 abnormal first molars which are nearly all followed by abnormal second molars. But there are another 60 abnormal second molars which follow on normal first molars, and there are three missing third molars following on normal m1 and m2.

The upper molars are less suitable for similar studies, as the identity of m2 is often in doubt, except when it follows on twins or a normal m1. The m2 following twins is usually so small that it is difficult to distinguish specific tabby features from the results of small size; essentially normal as well as mixed teeth occur, but there are too many doubtful cases to make an attempt at an enumeration worth while. The large majority of normal m1 are followed by normal m2, far more than in the corresponding mandibular teeth. A few m2 following normal m1 show tabby features, however, such as a rampart in front of cusp 2, or a reduction of B3; again, enumeration is difficult; in this case mainly as it is uncertain how much importance to attach to various incomplete fusions of roots up to almost typically tabby type roots.

We now have to examine the distribution of tabby features in individual mixed molars. In 40 out of 55 abnormal m1, the tabby phenotype was confined to the anterior end of the tooth; in 14 teeth both ends were affected, as in tabby. In only one marginal case was the anomaly confined to the rear end of the tooth, cusp 4 being 'a little reduced'. Evidently, in m1 the tabby phenotype spreads from the front backwards. To what extent this may be a consequence of twinning is a moot point.

This is even more uncertain in the upper molars. Overt twinning starts with cases where the anterior twin is a small conical tooth, and where the posterior twin resembles an ordinary tabby m1; if the anterior twin fails to survive, the posterior twin would be mistaken for the whole m1. Where twins are more nearly alike in size, the anterior twin tends to approach m1 and the posterior twin m2 in crown morphology, both tabby type. If m3 is absent, the anterior twin will be mistaken for m1 and the posterior one for m2 (indeed, the possibility must be considered that in tabby itself, 'm1' and 'm2' are in fact sometimes, often, or always a twin pair, with m3 regularly absent whenever twinning takes place). To reduce the effect of twinning, all overt twins and all instances where m1 < m2 have been omitted from the following analysis. Of 31 m1 with typical tabby-type crowns (i.e. absence of both B1 and B3, with or without involvement of L1 and L2), five had all roots fused as usual in tabby; nine had two roots, the free one being generally the posterior one; and 17 had three free roots though these tended to be less splayed than in a normal m1. Ten
crowns were mixed in that either B1 or B3 were involved, but not both; nine of these had three separate roots, one two roots. Six crown had vague hints of tabby type (somewhat bulbous shape with rather erect cusps, but otherwise an essentially normal cusp pattern) and all of these had three separate roots. Finally, there was one specimen with a normal crown, but with fusion between the anterior and the lingual root as commonly found in tabbies.

![Image of dental crowns showing different root configurations.]

Fig. 10. Complete twinning of left m\(^1\) (top) and incomplete twinning of right m\(^1\) (bottom) in a +/cr \(\delta\). Buccal views top left and bottom right. The posterior twin resembles in type a crinkled m\(^2\) except that a rudimentary cusp 1 is interpolated between B1 and L1.

The 31 m\(^3\) crowns with typical tabby morphology undoubtedly include some twins, possibly a large number. Whether the process of twinning itself tends to produce the tabby morphology of m\(^1\) and m\(^2\) is a moot point. In the lower molars this does not seem to be the case, as the posterior twin is usually morphologically indistinguishable from the corresponding region of a normal m\(_1\).

THE MOLARS OF CRINKLED HETEROZYGOTES

Altogether, 58 crinkled homozygotes (cr/cr) and 87 +/cr heterozygotes were examined. The range of molar variation of cr/cr is the same as in tabby, and quantitative differences (as in Table 1) are presumably due to the genetic background.

The phenotype of +/cr is similar to that of Ta/+ in kind, but differs from it considerably in degree. Heterozygous manifestation is almost confined to the upper jaw, with a heavy preponderance of m\(^1\) over everything else. 38 out of 87 mice had normal m\(^1\); the remaining 49 had one or both m\(^1\) affected; the two sides were involved to about the same extent, and there was a significant correlation between right and left \((\chi^2 = 12.88)\). Altogether, 71/174 teeth are affected, or 40.8%; \(\delta\delta\) were affected rather more often than \(\varphi\varphi\), but the difference was not significant.
The most striking manifestation includes twinning which is similar in every respect to that in $Ta/+$ (Figs. 10, 11). It includes one overt case with four teeth in a row (Fig. 10) and two or possibly three concealed cases with absence of m₃; the least certain of these is shown in Fig. 11(b). In addition, there are four instances of incomplete twinning the interpretation of which is not in doubt, and about seven cases of rudimentary twinning which includes several dubious cases. Twinning in $Ta/+\text{ and in } +/cr$ is unmistakably the same phenomenon.

Fig. 11. Incomplete twinning of left m₁ in a $+/cr \delta$ (a). On the right side of the same mouse (b) there are two separate molars of 'mixed' phenotype (both with three separate roots; anterior tooth has smaller crown than normal with erect cusps and incomplete separation of L₁ and L₂; but it has a separate B₁ and a rudimentary B₃; posterior tooth with rampart in front of cusp 2, but no reduction of B₃, and with essentially normal lingual aspect). It is suspected that these two teeth are in reality twins jointly representing m₁, with m₃ absent.

About 20 m₁ show definite features of crinkled. Four or five are small bulbous teeth with reduction or absence of B₁ and B₃ and imperfect separation of L₁ and L₂; another 13 are similar, but rather larger and do not involve the lingual cusps; and two teeth show reduction of B₃ together with imperfect separation of L₁ and L₂. Some 37 teeth are only marginally affected; they include 21 with a somewhat bulbous crown, erect cusps and reduced B₃, and 11 with reduction of B₃, but without erect cusps; and five teeth with erect cusps, but no appreciable reduction in the cusp pattern. In none of these 57 teeth was there any abnormality of the roots.

The remainder of the molars are much less affected. In eight m₂, B₁ is increased and joins with L₁ to form a rampart in front of cusp 2 (as in the smaller tooth in Fig. 11(b), if that tooth is in fact an m₂ rather than a posterior twin); in most instances, this rampart is rudimentary. Reduction of m₃ together with absence of m₃ occurred in one case following on incomplete twinning; and there may be two or three additional cases of absence of m₃, depending on whether the diagnosis of concealed twinning is correct. In five mice symmetrically and once hemilaterally, cusp B₃ of m₃ was increased in size so that B₂ and B₃ touch
each other or nearly so; this is probably a variant which has nothing to do with
krinkled, and which cannot be detected in a cr/cr mouse. Finally, in one mouse,
cusp 4 of m_8 was reduced on both sides, and in one mouse, cusp L3 of m_3 was
absent or nearly so on both sides. The last two cases are probably minor
manifestations of krinkled in the heterozygote, their rarity notwithstanding.

DISCUSSION

The single-active X-chromosome hypothesis of Lyon (1961, 1962, 1963) is
based, in part, on the peculiar patchwork or mosaic appearance of hetero-
zygotes for a number of sex-linked coat colour genes in the mouse (and, simi-
larly, in the tortoiseshell cat). It is assumed that during development, one of the
two X-chromosomes of such a female is inactivated; once inactivation has
occurred, it is irreversible, and the sector of cells derived from such a cell will
manifest either one allele or the other. The mosaic is thus believed to differ from
an ordinary (autosomal) heterozygote (in which alleles are thought to interact
with each other in the same cell) in that in any one patch either one or the other
allele is active. As ex hypothesi inactivation involves the chromosome as a whole
and is independent of the genes carried in it, there is an equal chance that in a
heterozygote A/a the chromosome carrying A or that carrying a will be inacti-
vated. Hence, with some qualifications to be mentioned presently, A and a areas
should occupy, on an average, one half each of the region in which the genes can
manifest themselves. The chief qualification, of course, is autonomous develop-
ment of the alleles: a diffusible substance produced by one allele could blur or
extinguish the manifestation of the other across a border. Similarly, there
might be mechanical interactions between neighbouring patches: in a structure
like the crown shape of a molar this has to be seriously considered, and there
can be little doubt that this will limit the size of a contrasting patch which can be
recognized as such in a phenotypically mixed molar. The third qualification is
that the mitotic rate of a patch with A should not differ from that of a twin
patch with a. In a genetically heterogeneous strain, such an effect, if it should
occur, would also be influenced by other sex-linked genes which, in relation to
A/a, would be in coupling or repulsion at random and thus act as a buffer.

A second consequence of the random inactivation of whole X-chromosomes
is that the resulting patches should not form a pattern, i.e. regularities of distri-
bution whether detectable by eye or by statistical analysis. Again, there is a
qualification, namely that there might be regularities resulting from cell lineage.

The Lyon hypothesis was put forward, in part, to account for dosage com-
pensation in the X-chromosome, i.e. that the phenotypic effect of a gene in the
XX ♀ is generally the same as in the XY ♂: if in the female one only of the two
X-chromosomes is active in any one cell, effective gene dosage is alike in both
sexes. It is in keeping with this hypothesis that tabby stripes are present in the
sex-linked Ta/+ heterozygote, but absent from the autosomal +/cr heterozygote.
If stripes were present in both, one would have to conclude that, in Ta/+, they cannot legitimately be explained in terms of X-chromosome inactivation.

The three consequences of the Lyon hypothesis outlined above will now be tested in turn. Only one first molar out of four is involved in Ta/+ $\varnothing$, and the majority of these include both normal and tabby features. The large discrepancy from the 50 % expectation cannot plausibly be explained in terms of non-autonomous development, as normal and tabby features occur cheek by jowl in mixed teeth, including the small second molars. Indeed, if it were not for the obvious autonomy of development, this analysis could never have been attempted. However, if a tooth is predominantly of one kind, a small patch of the other kind will probably often be missed by inspection. Could this account for the apparent absence of more than one half of the expected tabby contribution? If a small tabby area will tend to be missed in an otherwise normal tooth, this could be so for (at least) three different reasons. The tabby area may occur in part of the tooth in which tabby and normal do not differ appreciably from each other; or, for mechanical reasons, the area may be too small to force adjacent larger areas into its own growth pattern; or, finally, there may be a chemical interaction by way of a diffusible substance. The first two of these possibilities should apply equally to small tabby areas in normal teeth, or to small normal areas in tabby teeth and should thus, in the aggregate, not affect the proportion of normal and tabby areas. If there should be diffusible substances which would make tabby areas phenotypically normal, such an effect must be fairly limited in view of the obvious autonomy of development in mixed teeth. Moreover, if we were to invoke this kind of explanation for the first molars, we would have to explain why the same thing does not happen even more strikingly in the smaller second molars. But it was shown that the percentage of m$_2$ involved was considerably higher than that in m$_1$ and m$_1$ (47 as compared with 26 %). For the same reason, a difference in the mitotic rate will not account for the discrepancy: if tabby slows down cell multiplication in the first molar, it should have the same effect in the second molar. We conclude that the deficiency of tabby in the molar phenotype is a real effect, and that it is at variance with the Lyon hypothesis.

It was shown (Table 3) that the first molars of Ta/+ $\varnothing$ do not vary independently of each other; there was a striking excess of animals which were either completely normal, or which had three or four of these teeth affected. There were strong right–left correlations both in m$_1$ and in m$_1$, and there was a correlation between upper and lower first molars. Similarly, it was shown that there is a significant right–left correlation between m$_2$ preceded by normal m$_1$. It was also shown (Table 4) that an abnormal m$_3$ is nearly always followed by an abnormal m$_2$, and that m$_3$ is rarely missing unless m$_2$ is abnormal. The involvement of the molars, whether it starts with m$_1$ or with m$_3$, thus tends to involve the teeth farther back. The involvement of the molars is thus far from random, but shows considerable regularities. Can this pattern be explained in terms of cell lineage? If nothing were known about the development of
vertebrates, one might consider the possibility of a common stem cell for all the molars, far forward in the embryo, in which the inactivation of one or the other of the X-chromosomes takes place. This would account for the correlations between first molars and the spreading of the tabby phenotype from the front backwards. But it would not account for the right-left correlation between $m_2$ preceded by normal $m_1$. Quite apart from this, the concept of a common stem cell for bilateral organs in a mammal is so patently incompatible with the known facts of vertebrate development that few embryologists would seriously consider it. We conclude that the non-random involvement of the molars cannot plausibly be explained in terms of cell lineage; and hence that it is at variance with the Lyon hypothesis.

The effects of $Ta$ and $cr$ in heterozygous condition are similar in kind but differ in degree. On its present genetic background, $+/cr$ mainly involved $m^1$, but here it showed the same range of phenotypes as $Ta/+\$ including overt twinning, complete and incomplete. There cannot be the shadow of a doubt that we are dealing with the same phenomenon in both genes. But we cannot invoke different explanations for the same event, chromosome inactivation for the sex-linked gene and conventional incomplete dominance for its autosomal mimic. Clearly, for the third time, the facts are at variance with the Lyon hypothesis.

By verbal argument, any single $Ta/+\$ 'mosaic' can be interpreted in terms of chromosome inactivation. But that interpretation breaks down completely when confronted with a quantitative test of a larger sample. Contrary to the impression created by the first few cases, I am thus driven to the conclusion that the behaviour of the $Ta/+\$ molars cannot be accounted for in terms of that hypothesis. But, if that is accepted, it follows by exclusion that we are dealing with incomplete dominance or co-dominance; i.e. both alleles are active in the same cells, and physiological conditions at multicellular level determine the outcome by their interplay of forces. Immediately, all difficulties disappear. There is no reason why normal and tabby areas should be equal, nor is there any objection to the presence of a pattern, or to similarities between the $Ta/+\$ and the $+/cr$ heterozygotes. One wonders whether other sex-linked 'mosaics' which have been deemed to conform to the Lyon hypothesis would stand up any better to a quantitative test. However, it is not intended, in this paper, to discuss that hypothesis in all its implications. But whatever its ultimate fate, account will have to be taken of the discordant behaviour of the tabby molars.

**SUMMARY**

The sex-linked gene for tabby in the mouse is responsible for a characteristic dental syndrome. The dentition of tabby heterozygotes often includes normal, tabby and mixed molars which, in individual mice, creates the impression of a random mosaic. However, a statistical analysis of a larger sample reveals the existence of a characteristic pattern, and the involvement of the various molars
Molars of the tabby mouse is far from random. The tabby contribution to the molar phenotype falls short of that expected on the Lyon hypothesis, particularly in the first molars. Moreover, an essentially similar heterozygous manifestation also occurs in an autosomal mimic of tabby, crinkled. It is concluded that the behaviour of the tabby molars cannot be accounted for as the result of chromosome inactivation at the cellular level; but that it is easily understood as the result of ordinary semi-dominance or co-dominance with both alleles active in all the relevant cells and physiological control of the outcome of their interaction at a multicellular level.

Both the tabby and the crinkled heterozygotes sometimes show overt twinning of the first molars and more often signs of concealed twinning. No cases of overt twinning have so far been observed either in Ta $\delta\delta$ and Ta/Ta $\varphi\varphi$ or in cr/cr mice, but there is some evidence for the occurrence of concealed twinning, at least in crinkled.

ZUSAMMENFASSUNG

Die Molaren der tabby Maus, ein Prüfstein der Hypothese, dass nur ein einziges X-Chromosom wirksam ist


Sowohl bei Heterozygoten für tabby als auch für crinkled kommt zuweilen offenkundige und öfter versteckte Zwillingsbildung der ersten Molaren vor. Bisher sind keine Fälle von offenkundiger Zwillingsbildung bei Ta $\delta\delta$ und Ta/Ta $\varphi\varphi$ oder bei cr/cr Mäusen zur Beobachtung gekommen; aber wenigstens bei crinkled bestehen Anzeichen für das Vorkommen versteckter Zwillingsbildung.

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