The genetics and development of fused and supernumerary molars in the rice rat

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SUMMARY

Analysis of breeding records suggests that the occurrence of fused and supernumerary molars in the rice rat, associated with lower body weight than normal and with reduced fertility, is dependent on a single autosomal recessive gene subject to background modification.

Molar fusion, which is preceded by stripping of the external enamel epithelium from the interdental lamina, may involve the first two molars, or all three molars of the normal series in either jaw.

The supernumerary is a posterior tooth, developing after the three molars of the normal series in either jaw. Supernumerary development usually occurs with fusion of the molars of the normal series, but occasionally a supernumerary may be present in the absence of fusion.

Other examples of association between fusion, subsequent to separation of the external enamel epithelium from the interdental lamina, and supernumerary tooth development are cited. This association suggests that there may be a single common attribute of the dental lamina predisposing to epithelial stripping and to laminal hyperactivity.

INTRODUCTION

The occurrence of fused and supernumerary molars among members of a colony of rice rats (Oryzomys palustris), and the relationship between this trait and body weight, have been reported briefly elsewhere (Griffiths & Shaw, 1961; Shaw, Griffiths & Osterholtz, 1963). Originally, a few affected individuals were found sporadically with what appeared to be fusion of the first and second molars; and in some animals a supernumerary tooth was present, apparently posterior to the third molar. Subsequently, selection for animals with abnormal teeth rapidly resulted in a strain with a high incidence of fused and supernumerary molars. The fused and supernumerary molar trait was associated with lower body weight than normal, and breeding experience indicates that fertility in the fused molar strain is reduced. In other respects fused and supernumerary molar animals are outwardly normal.

Comparable but not identical abnormalities are found in the mouse mutants

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tabby (Ta), crinkled (cr) and downless (dl) (Grüneberg, 1965, 1966; Sofaer, 1969), and by a selected line of Lakeland Terriers (Hitchin & Morris, 1966). In the mutant mice, supernumerary teeth sometimes develop anterior to the first molar, and occasionally fusion between the supernumerary and first molar occurs. In the dog, fusion of the deciduous or permanent incisors has been observed, with or without a related supernumerary tooth.

The present paper is concerned with an analysis of the genetics of the condition in the rice rat; and with a consideration of the development of the abnormal dentition based on observations in adult animals and on direct examination of embryological material. The relationship of the abnormal dental condition in the rice rat to those in the mouse and dog is also discussed.

![Fig. 1. Diagrams of occlusal views of rice rat upper right molars: (a) normal; (b)–(d) from the fused molar and supernumerary molar strain.](image)

**MATERIALS AND METHODS**

The normal rice rat dentition is composed of one incisor and three molars in each quadrant. Fig. 1(a) illustrates the occlusal view of the three upper right molars in a normal animal. In abnormal animals molar fusion may occur with or without a supernumerary being present, and conversely, but infrequently, a supernumerary may be present without molar fusion.

Examination of several hundred abnormal animals suggests that the supernumerary tooth is nearly always situated posterior to the third molar and is the last tooth to develop. Fig. 1(b) shows a case where fusion of the first and second molars has apparently taken place. In such cases the composite tooth is nearly always slightly shorter than the combined length of the normal first and second
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molars, but some compensation often seems to have occurred through enlargement of the presumed third molar. Fig. 1(c) shows a similar case with an additional tooth situated posteriorly. In this kind of case the combined length of the composite tooth and presumed third molar is usually less than in case (b) where no supernumerary is present. Fig. 1(d) shows a case where all three molars of the normal series appear to have fused, and there is an additional tooth situated posteriorly. Similar cases without the supernumerary tooth have been observed frequently. Very occasionally (about 1% of the time) the additional tooth is not situated posterior to the third molar but lingual to, and in contact with, the second and third molars. Similar situations occur in the lower jaw.

It should be mentioned here that the composite teeth shown in illustrations (b)–(d) of Fig. 1 describe the maximum degree of maintenance of normal size and morphology of the component molars that was observed. In many cases reduction in size was greater, and in some cases, where reduction was extreme, it was not possible to identify all cusps of the component molars in the composite tooth.

For the genetic analysis, the distributions of offspring produced by mating phenotypically different combinations of animals were studied from the mating records kept over the 5-year period from 1963 to 1967 at the Harvard School of Dental Medicine. Each animal was classified according to the number of quadrants affected by either fusion or a supernumerary, or both. Throughout these discussions an ‘affected’ animal is one in whom 1–4 quadrants were affected. The affected and non-affected conditions are abbreviated as A and NA respectively.

The embryological material consisted of 23 animals, from both the fused strain and a normal control strain, ranging in age from an estimated 4–5 days before birth to 5 days after birth. These 23 animals were serially sectioned at 8 μm in the sagittal plane and processed by routine histological procedures.

RESULTS AND DISCUSSION

1. Genetics

Table 1 shows the distribution of progeny of different kinds of mating according to the number of quadrants affected. The distribution clearly shows that the condition is inherited, but simple single gene inheritance could not apply since A x A matings produced some NA progeny and since NA x NA matings also produced some A progeny. Further examination of A x A, A x NA and NA x NA matings, subdivided by sex, provided no evidence of any sex-linked effect. The two possible extreme hypotheses that could account for the inheritance of the condition are therefore (1) the segregation of two alleles at a single autosomal locus, with incomplete penetrance in abnormal homozygotes, and (2) multifactorial inheritance.

Multifactorial control would classify the condition as a quasi-continuous
character, dependent on some underlying continuous scale but expressed only above a certain threshold. A feature of such characters is the positive relationship between the proportion of affected individuals in a given population and the mean degree of expression of the character among those individuals who are affected (Grüneberg, 1952). Table 2 shows rank correlations between the percentage of A and the mean number of quadrants involved among A progeny, for different kinds of mating. The matings are divided into two groups, one in which at least one parent was NA, and the other in which both parents were A.

Table 1. The number of quadrants affected and the percentage of affected individuals among progeny of three different kinds of mating

<table>
<thead>
<tr>
<th>Mating</th>
<th>No. of matings</th>
<th>No. of progeny</th>
<th>Quadrants affected</th>
<th>Total</th>
<th>A (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A × A</td>
<td>185</td>
<td>24</td>
<td>54</td>
<td>1209</td>
<td>94-8</td>
</tr>
<tr>
<td>A × NA</td>
<td>24</td>
<td>1050</td>
<td>15</td>
<td>1146</td>
<td>43-2</td>
</tr>
<tr>
<td>NA × NA</td>
<td>54</td>
<td>185</td>
<td>128</td>
<td>213</td>
<td>8-4</td>
</tr>
</tbody>
</table>

There is no correlation in the first group, but a very high and significant correlation in the second. This suggests that the underlying variation among progeny of A × A matings is continuous, but that this continuity does not extend below the threshold when NA parents are involved. The implication is, then, that there is a relatively discrete difference between A individuals and at least some NA individuals. The fact that matings in which at least one parent was NA, particularly the 0 × 0 matings, produced A progeny with a relatively high mean number of quadrants affected suggests that there may have been segregation of a major recessive factor contributing to the abnormal dental condition.

The left half of Fig. 2 illustrates distributions of families of different kinds of mating according to the percentage of affected individuals they contained, each family comprising the total progeny (ranging from 2 to 47) of a single pair. There is overwhelmingly only one kind of family among A × A matings, but possibly three among A × NA matings, and probably more than one among NA × NA matings. This pattern is compatible with the hypothesis of a single autosomal recessive gene with incomplete penetrance in homozygotes, as shown below.

Under the single autosomal recessive hypothesis all A individuals must be considered homozygous, say ff; whereas NA individuals could have any one of the three genotypes: + +, f + or ff. There would then be only one kind of A × A mating, producing somewhat less than 100% A offspring (ff × ff); three kinds of A × NA mating, producing no A offspring (ff × + +), somewhat less than 50% A (ff × f +), and somewhat less than 100% A (ff × ff); and four kinds of NA × NA mating, producing no A offspring (+ + × + +; + + × f +, + + × ff), somewhat
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less than 25\% A (f+ × f+), somewhat less than 50\% A (f+ × ff), and somewhat less than 100\% A (ff×ff).

Having obtained this pattern of family distributions the mating records were re-examined to try to establish the genotypes, according to the single autosomal recessive hypothesis, of NA parents of A × NA matings, and of both parents of those NA × NA matings that produced some A offspring. Non-affected individuals whose parents were both affected were considered homozygous mutant (ff), those whose ancestors were consistently NA over several generations were considered homozygous normal (+ +), and those with one presumed ff parent and one presumed + + parent were considered heterozygous (f+). The results of this re-examination are shown in Table 3. The ‘probable’ heterozygotes were offspring of parents who were both presumed to be heterozygous themselves.

The right half of Fig. 2 illustrates distributions of progeny of the three different kinds of A × NA mating, and of the three different kinds of NA × NA mating that could produce affected offspring. Of the A × NA matings, none classified as ff× + + produced any A offspring. (Of the two A × NA families that produced between 1 and 20\% A progeny, shown in the left half of Fig. 2, one was classified as ff×f+ and the other as ff×ff.) The distribution of offspring of A × NA matings classified as ff×f+ strongly suggests segregation into a non-affected group and a group with a relatively high degree of expressivity, with rather less than half of the total affected. Offspring of A × NA matings

Table 2. Rank correlations between the percentage of affected progeny and the mean number of quadrants involved among affected progeny, for matings in which at least one parent was non-affected, and for matings in which both parents were affected

<table>
<thead>
<tr>
<th>Matings by quadrants affected</th>
<th>No. of progeny</th>
<th>A (%)</th>
<th>Mean no. of quadrants involved among A progeny</th>
<th>Rank correlations between % A and mean of quadrants involved</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 × 0</td>
<td>1146</td>
<td>8</td>
<td>2.6</td>
<td>Spearman = -0.10, Kendall = 0</td>
</tr>
<tr>
<td>0 × 1</td>
<td>11</td>
<td>18</td>
<td>1.5</td>
<td></td>
</tr>
<tr>
<td>0 × 2</td>
<td>83</td>
<td>43</td>
<td>2.0</td>
<td></td>
</tr>
<tr>
<td>0 × 3</td>
<td>51</td>
<td>61</td>
<td>2.4</td>
<td></td>
</tr>
<tr>
<td>0 × 4</td>
<td>232</td>
<td>39</td>
<td>2.8</td>
<td></td>
</tr>
<tr>
<td>1 × 1</td>
<td>83</td>
<td>70</td>
<td>1.7</td>
<td></td>
</tr>
<tr>
<td>1 × 2</td>
<td>58</td>
<td>71</td>
<td>2.0</td>
<td></td>
</tr>
<tr>
<td>1 × 3</td>
<td>22</td>
<td>55</td>
<td>1.6</td>
<td></td>
</tr>
<tr>
<td>1 × 4</td>
<td>41</td>
<td>90</td>
<td>2.4</td>
<td></td>
</tr>
<tr>
<td>2 × 2</td>
<td>262</td>
<td>92</td>
<td>2.3</td>
<td>Spearman = 0.95 (P &lt; 0.01), Kendall = 0.87 (P &lt; 0.01)</td>
</tr>
<tr>
<td>2 × 3</td>
<td>91</td>
<td>96</td>
<td>2.5</td>
<td></td>
</tr>
<tr>
<td>2 × 4</td>
<td>146</td>
<td>95</td>
<td>2.9</td>
<td></td>
</tr>
<tr>
<td>3 × 3</td>
<td>83</td>
<td>95</td>
<td>3.1</td>
<td></td>
</tr>
<tr>
<td>3 × 4</td>
<td>263</td>
<td>97</td>
<td>3.3</td>
<td></td>
</tr>
<tr>
<td>4 × 4</td>
<td>1131</td>
<td>99</td>
<td>3.8</td>
<td></td>
</tr>
</tbody>
</table>
classified as $ff \times ff$ showed no evidence of any such bimodality, and had a mean degree of effect somewhat lower than the presumed $ff$ homozygous progeny of the $ff \times f+$ matings. This difference of expressivity between the two groups of presumed homozygotes could be accounted for by a difference of genetic background, since the homozygous parent of the group with high expressivity was always affected, whereas one homozygous parent of the group with low expressivity was always non-affected.

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**Fig. 2.** Distribution of families of phenotypically different kinds of mating according to the percentage of affected individuals the families contained (left); and distributions of progeny of genotypically different kinds of mating, based on the single autosomal recessive hypothesis, according to the number of quadrants affected (right).

The distribution of progeny of $NA \times NA$ matings classified as $f+ \times f+$ was again strongly suggestive of segregation, with rather less than one quarter of the total affected, and with relatively high expressivity among the affected progeny. Evidence for segregation among the progeny of $NA \times NA$ matings classified as
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ff × f+ was not so convincing, possibly due again to a background effect imposed by a non-affected homozygous ff parent. The progeny of NA × NA matings classified as ff × ff showed no evidence of bimodality, and expressivity was rather lower than among offspring of A × NA matings classified as ff × ff. This also could be due to background, since in the group with lower expressivity both ff parents were non-affected.

Table 3. Presumed genotypes of non-affected parents, derived from an examination of their ancestry, according to the single autosomal recessive hypothesis

<table>
<thead>
<tr>
<th>NA parent of A × NA matings</th>
<th>NA × NA matings that produced some A offspring</th>
</tr>
</thead>
<tbody>
<tr>
<td>+ +</td>
<td>f+ × f+</td>
</tr>
<tr>
<td></td>
<td>ff × f+</td>
</tr>
<tr>
<td></td>
<td>ff × ff</td>
</tr>
<tr>
<td>Definite</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td>10</td>
<td>6</td>
</tr>
<tr>
<td>Probable</td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>4</td>
<td>3</td>
</tr>
<tr>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

The existence of a heritable background effect among presumed ff homozygotes was tested for by calculating the heritability of the number of quadrants affected from the regression of the mean of offspring on mid-parent value for the 10 different A × A matings listed in Table 2. The result, h² = 0.94, indicates that a very high proportion of the variation of expression among presumed ff homozygotes was due to additive genetic effects.

Thus, the observations are all compatible with the hypothesis of a single autosomal recessive gene with variable penetrance in homozygotes, with penetrance being largely dependent on genetic background.

It should be mentioned here that the apparently rather low numbers of individuals in the 1-quadrant and 3-quadrant classes of the distributions in the right half of Fig. 2 are probably due to relative developmental instability of the asymmetrical condition, since when two quadrants were affected they were most frequently in the same jaw. The relative stability of the 1-, 2- and 3-quadrants affected conditions can in fact be calculated if certain assumptions are made. The first requirement is a basically homogeneous group of individuals which can be taken as being normally distributed on some underlying continuous scale immediately related to the development of the phenotype. Different ranges on this underlying scale are assumed to correspond to different grades of phenotype (each grade being a different number of quadrants affected), and these different ranges are assumed to be separated by constant thresholds. The relative sizes of the intervals between the thresholds can then be derived from the frequencies of the different grades of phenotype (Falconer, 1964, 1965; Rendel, 1967). Calculation of the relative sizes of the 1-, 2- and 3-quadrant threshold intervals from the distribution of progeny of all A × A matings, on the assumption of normality of this distribution, showed that the 2-quadrant interval was twice as large as either the 1- or the 3-quadrant interval.
2. Development

At approximately 4 days before birth early morphodifferentiation of first and second molars, and the rudiment of the third molar were observed in both normal and fused molar animals (Fig. 3 A, B). In fused molar animals the external enamel epithelium between adjacent tooth germs was generally well separated from the underlying dental lamina, resulting in continuity of the stellate reticulum of all three developing teeth (Fig. 3 B). Normal animals showed a slight tendency towards separation (Fig. 3 A), but in no case was it observed to be as extreme as in the most abnormal fused molar animal.

By one day after birth hard tissue formation had begun in both first and second molars (Fig. 3C), and in fused molar animals fusion had been completed (Fig. 3 E, G). Cases were observed in which fusion of the first and second molars had taken place (Fig. 3 E), and in these cases the third molar germ was developmentally more advanced than normal. In normal animals invagination of the third molar germ was only just beginning, whereas in the abnormal animals a well-defined bell had already been formed (Fig. 3 E). Other cases were observed with a large composite germ in which there was a greater antero-posterior range of histodifferentiation than that seen in fused first and second molars. The antero-posterior length of these composite teeth (Fig. 3 G) was similar to the sum of the lengths of the fused first and second molar and advanced third molar of the type illustrated in Fig. 3 E. It was concluded that these composite teeth had developed from the rudiments of all three molars of the normal series. In one such case, the rudiment of an additional tooth was observed posteriorly (Fig. 3 G, inset).

At 5 days after birth normal third molars had reached the late bell stage (Fig. 3 D). In abnormal animals in which the third molar had remained separate, differentiation of the third molar was again more advanced than normal, and in some cases an extension of dental lamina distal to the third molar showed signs of early odontogenic activity (Fig. 3 F). Previous work (Grewal, 1962; Sofaer, 1969) suggests that such supernumerary buds may or may not be destined to form additional teeth. In the one abnormal animal of this age examined in which all three molars of the normal series had apparently fused, there was an additional germ, distal to the composite tooth, which had reached the early bell stage (Fig. 3 H).

Lower jaw findings were similar.

The mechanism of molar fusion in the rice rat seems to be basically the same as that proposed by Hitchin & Morris (1966) to account for fusion of the developing incisors of the dog. Following stripping of the external enamel epithelium from the interdental lamina, which was thought to be due to rapid growth of adjacent tooth germs, the internal enamel epithelium of adjacent germs would be free to come into contact and to fuse. The reason for epithelial stripping in the rice rat is not clear. However, once separation between the external enamel epithelium and the interdental lamina has occurred, it is easy
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Fig. 3. \(m_1\), \(m_2\), \(m_3\), upper first, second and third molars; \(s\), supernumerary.

(A) Control first and second molars, and the rudiment of the third molar, at an estimated 3–4 days before birth. Light arrows indicate points of relationship between the external enamel epithelium and underlying dental lamina. Heavy arrow indicates the normal anterior extension of dental lamina.

(B) First and second molars, and the rudiment of the third molar, at an estimated 4–5 days before birth, in an animal from the abnormal strain. Light arrows indicate points of separation of the external enamel epithelium from the underlying dental lamina.

(C) Control first and second molars at one day after birth.

(D) Control third molar at 5 days after birth.

(E) Fused first and second molars and developmentally advanced third molar, of an animal from the abnormal strain at one day after birth.

(F) Developmentally advanced third molar, and rudiment of a potential supernumerary tooth (indicated by arrow), of an animal from the abnormal strain at 5 days after birth.

(G) Fused first, second and third molars, and the rudiment of a supernumerary tooth (inset), of the same animal from the abnormal strain at one day after birth.

(H) Supernumerary tooth germ from a case as in (G) but at 5 days after birth.
to understand how the case illustrated in Fig. 3B could develop into that illustrated in Fig. 3G.

Despite the similarities between the rice rat and the tabby mouse, the origin of the supernumerary molar in the two animals is not the same. In the tabby mouse the supernumerary develops anterior to but later than the first molar, resulting in adult cases in which the most anterior molar (the supernumerary) is smaller than its neighbour. In no animal of the several hundred abnormal adult rice rats examined was the most anterior molar smaller than the tooth immediately posterior to it. Furthermore, histological examination failed to reveal evidence of proliferation of the normal anterior extension of dental lamina (illustrated in Fig. 3A). In addition, there was direct evidence for the development of an additional tooth both posterior to a third molar that had remained separate (Fig. 3F), and posterior to a composite tooth thought to be derived from rudiments of all three molars of the normal series (Fig. 3G, H). Therefore, as suspected from an examination of adult material, the rice rat supernumerary appears to be a posterior tooth.

A further difference between the rice rat and the tabby mouse concerns the relationship between fusion and the supernumerary. In the tabby mouse, fusion was always found to be secondary to supernumerary formation, and fusion always involved the supernumerary. As already mentioned, fusion may occur in the rice rat without a supernumerary being present, and conversely, a supernumerary may be present in the absence of fusion. Similarly, in the dog, fusion was not found to be dependent on the presence of a supernumerary tooth.

In the tabby mouse it was suggested that the supernumerary represented an attempt to compensate for small size of the developing first molar, caused by a suppressive influence at a particular stage of development. Subsequent relaxation of the suppressive influence was thought to result in rapid growth of the supernumerary and adjacent first molar in a restricted space, predisposing to stripping of the external enamel epithelium from the interdental lamina, and to fusion. In the rice rat, epithelial stripping apparently occurs without undue crowding of the developing teeth (Fig. 3B), and the supernumerary develops only after fusion has taken place. The supernumerary tooth in the rice rat may arise in some cases in response to a smaller than normal combined anteroposterior length of the molars of the normal series, consequent to fusion. On the other hand, since supernumeraries occasionally occur in the absence of fusion, this could not be the only explanation for their development.

In conclusion then, it seemed reasonable to suspect that the different kinds of association between fusion and the development of supernumerary teeth discussed here reflect a single common attribute of the dental lamina predisposing to epithelial stripping and to laminal hyperactivity.

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