THE OCCURRENCE AND GENETIC BEHAVIOR OF DUPLICATE LOWER INCISORS IN THE MOUSE*

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A new trait in the mouse, involving the appearance of extra lower incisor teeth, seems worthy of note because of its morphological interest and its unusual genetic behavior. The trait, which may be designated as di (duplicate incisors), is characterized by the appearance on one or both sides of a supernumerary and somewhat aberrant tooth located close to the mandibular symphysis and directly behind or slightly medial to the corresponding normal tooth. The accompanying figures show duplicate teeth as they appear in a living animal and in a cleaned lower jaw. A brief description of the trait, a few circumstances surrounding its origin, and some preliminary breeding records can be reported at this time.

Normal lower incisors erupt at about the eleventh day of postnatal life and the accessories, with less regularity, a few days later, but their rudiments when present can be demonstrated in newborn and prenatal young. Each accessory has its own alveolus, which is so close to that of the normal tooth that the two may seem to emerge from the same socket. The duplicates are usually clavate at first and for a brief period may appear to be as large as, or even larger than, the normals. Later, they tend to lose their bulbous tips and become smaller by comparison and more uniform in diameter. A few are closely appressed to the normal teeth with their tips correspondingly beveled, but most of them become deflected and are ultimately broken off or displaced from their sockets. When the latter happens, bone of the alveolar process soon fills in the deficiency making it difficult or impossible to say whether or not an extra tooth has ever been present. But not all duplicates are lost, some of them, or their broken roots, remaining indefinitely. Such persistent remnants often force the normal teeth sufficiently out of alignment to cause serious malocclusion, an eventuality that can be avoided if need be by early extraction of the duplicates. Thus far, aside from indirect effects of malocclusion, possibly somewhat reduced vigor, and a rather high incidence of mammary tumors, no indication has been found of other physical or physiological peculiarities that might be attributed to di genes.

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In view of the increased interest in mutagenesis that has been engendered by the “atomic age,” it seems desirable in recording new traits to indicate in so far as possible the circumstances under which they arose. It has to be remembered, however, that apart from statistical probabilities it is still impossible to distinguish between mutations that have been induced and those that may have occurred spontaneously. In the present instance a deliberate attempt had been made to induce mutations and the \textit{di} trait is among those that appeared in course of the experiment.

Duplicate incisors were first noticed in two males descended from mice which in earlier generations had been treated with nitrogen mustard (HN$_2$). The first mouse so treated, a \textit{ruru} female from a general purpose stock, was given a barely sublethal injection shortly after mating and while the copulation plug was still present. She gave birth to three young whose descendants were interbred in various combinations to produce numerous lines of descent, some of which had heritable anomalies. All the members of one apparently normal family among these descendants were given injections of nitrogen mustard that would ordinarily have been lethal but which in this instance were accompanied by protective doses of cysteine. Most of these mice survived and their descendants were

\begin{figure}[h]
\centering
\includegraphics[width=0.5\textwidth]{image1.png}
\caption{Accessory incisors in a 20-day old female mouse. It may be noted that the extra teeth extend upward and outward from behind the normal ones. At 32 days the accessories had been broken off and were not apparent on superficial inspection, but by 67 days they were again prominent. When the mouse was killed and autopsied at 120 days the extra teeth were both present, but small and pointed at their tips.}
\end{figure}
designated as the “J line.” Crossed to long inbred C57Bl/6 mice this J stock produced vigorous hybrids. One $F_2$ female (No. 100) from such a cross was mated to her father ($F_1$) and observed to have a copulation plug on March 4, 1954. On March 15 she was injected intra-abdominally with 0.05 cc of a solution made by dissolving 10 mg of $\text{HN}_2$ in 20 cc of 0.75 percent NaCl in distilled water. The mouse showed no observable effects of the injection and on March 24 gave birth to nine young, four females and five males, all apparently normal. However, since we have found that similar treatment of females in comparable stages of pregnancy ordinarily results in abnormalities among the young (Danforth and Center 1954), it may be assumed that in this case also the fetuses were, in effect, treated in utero. Two males were discarded and the rest of the litter kept for further observation.

On June 8, 1954, one pair of these ‘treated-in-utero’ mice produced three young, two females and a male, which were retained as a breeding group. They in turn gave rise to a considerable number of offspring, but no significant anomalies were noticed until January 12, 1955, when among 13 adolescent young Dr. Elizabeth Center, to whom the writer is much indebted for cooperation in this study, found two males each with an extra lower incisor on one or both sides. This first observed appearance of the trait was thus in the second generation from young that had been treated in utero. The two males mated to three of their full or half sibs are ancestral to all di mice that have since appeared. It may be noted here that among the antecedents of these mice no individual had ever been treated more than once during its lifetime.
Laterality in expression of the trait

Since the \( di \) trait shows some variation in the form of its manifestation, most of the young have been recorded on the basis of their phenotypic appearance, \( B \) indicating those in which an extra tooth was clearly present on both sides, \( R \) those in which it could be detected on the right side only, \( L \) on the left side only, and \( O \) on neither side. All young classified as \( B \) unquestionably were such, but an occasional one in which a tooth had been pushed out of its socket or broken off beneath the gums may have been assigned to the wrong group. In an effort to avoid errors that might arise in this way, almost all animals not to be used for breeding were sacrificed before reaching six weeks of age and their jaws cleaned in a sodium hypochlorite solution (commercial “PUREX”), making it possible to confirm or correct the original diagnoses. This rather laborious procedure, involving the preparation of many hundred specimens, corrected a few oversights but served chiefly to check the accuracy of direct observations on living young. It should be mentioned, however, that a few extra teeth were mere spicules that could not have been detected in living animals and that embryological evidence indicates that an occasional accessory enamel organ fails to produce any recognizable tooth at all.

There is some indication that an extra tooth is more likely to be present on the right side than on the left. Among 129 jaws showing asymmetry, the extra tooth was larger on the right side, or present on that side only, in 74 cases, while the opposite was true in only 55 cases.

Breeding tests

Distribution of the trait and grades of its expression in progenies of inbred, outcrossed and extracted parents are shown in the table, where there are probably very few errors in classification of the young, but where an occasional parent listed as \( R, L, \) or \( O \) may have actually belonged to a grade higher than the one indicated. It may be mentioned at this point that after the breeding tests were well under way, and most of the data presented here had been accumulated, the colony fell victim to an epidemic which decimated some lines and curtailed the reproductive performance of others. This has left some obvious hiatuses which will need to be filled in when the surviving stock can be brought back to normal. In the meantime it seems desirable to present available data on the approximately 2000 offspring whose records are unimpaired.

A. Outcross matings. One of the original \( di \) males, and later inbred individuals of both sexes, served for outcrossing to four different lines which may be briefly characterized.

A-1. The “Swiss” referred to in the table were supplied by another department which had obtained albino stock from several sources, one of which specializes in the Webster strain. It is probable that more or less remote ancestors of some of these mice had been subjected to nitrogen mustard or other presumed carcinogenic agents.
TABLE 1

Distribution of the di trait among the offspring from various types of mating

<table>
<thead>
<tr>
<th>Matings</th>
<th>A. Outcrosses</th>
<th>Offspring</th>
<th>Matings</th>
<th>B. Inbred matings</th>
<th>Offspring</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>R</td>
<td>R</td>
<td>L</td>
<td>O</td>
<td>Totals</td>
</tr>
<tr>
<td>1. di × Swiss</td>
<td>F1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>27</td>
</tr>
<tr>
<td></td>
<td>F2</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>276</td>
</tr>
<tr>
<td></td>
<td>BC</td>
<td>10</td>
<td>10</td>
<td>11</td>
<td>282</td>
</tr>
<tr>
<td>2. di × C57Bl</td>
<td>F1</td>
<td>0</td>
<td>0</td>
<td>0</td>
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<td></td>
<td>F2</td>
<td>2</td>
<td>3</td>
<td>3</td>
<td>286</td>
</tr>
<tr>
<td></td>
<td>BC</td>
<td>11</td>
<td>6</td>
<td>7</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>R × R</td>
<td>26</td>
<td>12</td>
<td>10</td>
<td>17</td>
</tr>
<tr>
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<td>BC</td>
<td>11</td>
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<td>7</td>
<td>100</td>
</tr>
<tr>
<td>3. di × P-Jax</td>
<td>F1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td>F2</td>
<td>0</td>
<td>0</td>
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<td>2</td>
<td>0</td>
<td>53</td>
</tr>
<tr>
<td></td>
<td>R × R</td>
<td>26</td>
<td>12</td>
<td>10</td>
<td>17</td>
</tr>
<tr>
<td>4. di × “100”</td>
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<td>0</td>
<td>1</td>
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</tr>
<tr>
<td></td>
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<td>3</td>
<td>0</td>
<td>48</td>
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<td></td>
<td>BC</td>
<td>23</td>
<td>18</td>
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<td>435</td>
</tr>
<tr>
<td></td>
<td>R × O</td>
<td>2</td>
<td>1</td>
<td>0</td>
<td>16</td>
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<td>F1</td>
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<td>1</td>
<td>2</td>
<td>7</td>
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<tr>
<td></td>
<td>BC</td>
<td>27</td>
<td>24</td>
<td>22</td>
<td>1377</td>
</tr>
</tbody>
</table>

B. duplicate incisors on both sides; R, a duplicate on the right side only; L, duplicate on the left side only; O, no duplicate on either side; D, at least one duplicate, but record as to side incomplete. C-1 shows offspring of R mice derived from di × Swiss, backcrossed to di; C-2 and ff. give results from mating some of these young.

A-2. C57Bl/6 mice were obtained from the Roscoe B. Jackson Memorial Laboratory and were from at least the 42nd generation of brother-sister matings, or from one or two later generations in which matings may have been between parent and offspring. One subdivision of the group, however, was derived from the mating of a di female to a C57 male whose grandmother had received an injection of nitrogen mustard during pregnancy. This subgroup accounts for 79 of the 294 young in F2. One of these was di, the other 78 were normal.

A-3. P-Jax, a strain homozygous for a number of recessive genes, was also obtained from the Jackson laboratory, where over 50 generations of brother-sister matings had been recorded.

A-4. Mice designated as “100” were all descended exclusively from the surviving seven young produced by female No. 100 following her treatment with nitrogen mustard. None of these came directly from the di subline, but one of the progenitors of that line may have been ancestral to some of those in this group.
Section A of the table shows the observed frequencies of $B$, $R$, $L$, and $O$ individuals among the young that could be examined in the $F_1$, $F_2$ and $BC_1$ generations from crosses A-1, A-2 and A-3, and the $F_1$ and $F_2$ generations form the A-4 cross. Data from $F_3$, $BC_2$, and other less critical matings are not included, but they appear to be in essential agreement with the figures presented. Summarizing these figures (A-5), and counting all $B$, $R$ and $L$ mice as $di$, it appears that in $F_1$ there was a total of 301 young, of which three (all from A-4) were $di$, giving an over-all incidence of about one percent. In $F_2$ there were 655 young of which 11, or approximately 1.7 percent were $di$, and in $BC$, 494 young with 59, or about 12 percent $di$.

B. *The inbred line.* All the mice in this series were descended exclusively from one or both of the original $di$ males mated to one or more of their three sibs. Section B of the table shows the types of matings that were made and characteristics of the young that were produced. In this section, $D$ indicates that the animals in question were definitely $di$ but with the exact grade unrecorded. Any $D$ mouse may have been $B$, $R$ or $L$. A summary of the figures shows that of 368 young from $di \times di$ matings, 325, or approximately 88 percent were $di$, while of 74 young from parents one of which was $di$ the other $O$, but of $di$ ancestry, 47 percent were $di$. There were also 23 young from parents both of which had been classified as $O$, and among them 16 were $di$. A few individual matings selected from these series sufficed to show that any type of parental combination may at times produce $B$, $R$, $L$ or $O$ offspring.

C. *Progenies from matings of extracted $di$ parents.* A few extracted $di$ mice were interbred and distribution of the trait among their young roughly paralleled that in the inbred line. The group selected for the table came from three $R$ young born at the same time but to different parents. It may be noted that among their descendants all grades of the trait appeared.

**Interpretation of the findings**

A. *Data from outcross matings.* The figures are sufficient to show that the $di$ trait is hereditary, and that it is not an ordinary dominant. Its first observed appearance, however, coincided exactly with what might have been anticipated if a single recessive mutation had occurred in the germinal tissues of one of the embryos treated *in utero*. If such a mutation did in fact occur, the low frequencies in the $F_2$ and $BC$ generations could conceivably be due either to reduced viability of homozygous recessives or to some inequality in the number or functional potency of germ cells carrying alternative alleles. That such alleles are not always distributed impartially to developing germ cells might be inferred from studies on "affinity" (Michie 1953, 1955; Wallace 1953) and the observations of Dunn (1957) and several of his associates who have found what appears to be an unaccountably high incidence of sperm cells carrying specific lethals in a number of strains of wild mice. The alternative to some such hypothesis is that the $di$ trait must be attributed to the effects of more than one independent gene.

A $BC/F_2$ index is useful in testing such postulates. The index, representing the
ratio between the incidence of a trait in BC and in F₂, derives its value from the
fact that, with reference to each particular gene, an F₁ individual can produce
two kinds of germ cells while a homozygous recessive can produce only one. This
circumstance gives rise to the expectation that, in the absence of complicating
factors, the incidence of a recessive trait in BC will always be the square root of
its incidence in F₂, irrespective of the number of contributing genes that may be
involved. By means of this index it is possible to utilize simultaneously the res-
ults from two critical types of mating in testing agreement between observed
and expected figures.

In the present instance, if the trait is a semilethal in the sense that a dis-
proportionate number of recessives are lost between zygote formation and the
time when they can be counted, the fact should be reflected in the BC/F₂ index.
Since the 'normal' incidence of a recessive is 1/(3 + 1) in F₂ and 1/(1 + 1) in BC,
the BC/F₂ index is ordinarily (3 + 1)/(1 + 1), but if a relatively smaller pro-
portion, n, of the recessives survive, the index becomes converted to (3 + n) /
(1 + n), a fraction whose value must always lie between 2, when n = 1 (all reces-
sives surviving) and 3 when n = 0 (no recessives surviving). In other words, what-
ever the extent of postzygotic lethality due to any one gene the BC/F₂ index must
always fall between 2 and 3 unless entirely extraneous factors are involved. The
di figures yield an index of approximately 7, (i.e., 0.12/0.017) which makes it ex-
tremely unlikely that any one gene can account for the shortage of di individuals.

Even though an explanation of the numerical deficiencies must apparently be
sought in pre- rather than postzygotic stages, the BC/F₂ relation may still be of
significance. If germ cells with alternative alleles are produced, or function, in
unequal numbers, p may be allowed to stand for the relative number or potency
of those carrying the dominant allele and q for those with its recessive counter-
part. Using this notation, the expected incidence of a simple recessive in F₂ is
\[\frac{q}{p+q}\]^2, and in BC \[\frac{q}{p+q}\]. On the basis of the total figures for F₂ (A-5),
where 0.017 may be presumed to correspond to \[\frac{q}{p+q}\]^2, the value of q in
terms of p is about 0.129. For BC it is about 0.119, and the weighted average is
0.125+. This value of q leads to an expectation of 10 di individuals instead of the
observed 11 in F₂ and 61 instead of 59 in BC, an agreement as close as could be
expected if the hypothesis were known to be correct.

Finally, if instead of a single mutant gene with reduced effectiveness, the trait
is presumed to be due to multiple recessives of the usual sort, the BC/F₂ index
should be 2^a, and the calculated index of 7+ is so close to 2^a as to suggest that
three pairs of alleles might be involved. This would lead to essentially the same
expectations as the prior assumption, and it must be concluded that in this case
the data from outcross matings are not competent to differentiate between ex-
pectations based on a single recessive gene with \(\frac{1}{8}\) the potency of its normal
allele and those based on the necessary presence of three contributing but inde-
pendent normal recessives. Except for the circumstances surrounding the first
appearance of the trait, there would have been little hesitancy in accepting the
second alternative.
B. Data from the inbred line. Figures from the inbred line indicate that when both parents have di ancestry di young are to be expected, and that the grades of expression in the young are related to those in the parents. As indicated above, combining the data from matings B-1 to B-4, where both parents in each case were di, gives a total of 368 young of which 325 (88 ± 1 percent) were di, while matings B-5 and B-6 in which one parent was di, the other O but of di ancestry, show 74 young of which 35 (47 ± 4 percent) were di. In the small B-7 group, some O × O matings are recorded as having produced di young, a seeming inconsistency that may be due to erroneous classification of one of the parents.

That a bilateral expression of the trait mirrors some kind of cumulative genic effect is suggested by the relative frequencies indicated under B-8 in the table, where a progressive decrease in incidence is shown from 84 percent when both parents were B to 43 percent when each parent showed the trait on one side only. On the whole, the various data from inbred matings supplement the evidence that multiple genes are involved in production of the di trait.

C. Data from matings of extracted individuals. Among the young from the extracted group, occurrence of the di trait roughly paralleled its occurrence in the inbred line, which seems to indicate that the essential genes are capable of independent segregation, or at least are not closely linked.

DISCUSSION

In view of the conditions under which the di trait was first observed, the possibility that it could be accounted for by a single gene mutation has called for special consideration. The BC/F₂ index effectually rules out any likelihood that a single mutant gene could be semilethal to an extent that would account for the observed figures, and as between a single recessive with reduced efficiency and multiple recessives with normal potencies, the evidence on the whole favors the latter, with indications that the number of critical genes is probably three. Nevertheless there is an appreciable margin of deviations from expectations calculated on either of the assumptions. Such deviations, commonly attributed to incomplete penetrance and expressivity (cf. Landauer 1955), vary with the type of mating. The B × B matings indicate a penetrance of 93 percent, and it is clear that at least part of the missing seven percent can be accounted for by failure of some of the accessory enamel organs to produce teeth. Postulating a fourth effective gene would throw the expected and observed figures far out of line. In view of these considerations, the best inference that can be drawn from the available data would seem to be that the di trait is dependent on three separate complementary genes.

With the preponderance of evidence indicating a three-gene combination, the problem arises as to how such a complex could have come about. Even though our knowledge of the action of chemical mutagens is meager (Auerbach 1949), it would seem unlikely that a single treatment could cause simultaneous changes in three, even functionally related, genes. A more plausible inference is that two of the necessary genes were already in the germplasm when the final di mutation
occurred. Such genes might have come from either the C57 or the J ancestry, and the figures from A-2 and A-4 afford some, but very slight, support for such a view. Whether the final di mutation was spontaneous or induced can not be answered with certainty, although the manner of its first appearance makes induction seem probable, as do some of the mutations observed by Falconer, Slizynski and Auerbach (1952) following treatment with nitrogen mustard. That the mutation happened to occur in a milieu which permitted it to be expressed is possibly no more than a coincidence. That this milieu, however, is not merely a kind of generalized background but a matter of only two, or at most three, specific genes seems to be clearly indicated.

Whatever other functions they may have, the alleles of these background genes tend to submerge the effects of the new mutation and in that sense contribute to a kind of "genetic homeostasis" (Lerner 1954). In such a case as the present one, phenotypic stability could depend on concurrent action of both dominant and recessive genes, and figures in section B of the table suggest that, in the presence of the other two, at least one of the contributing genes may act as a semidominant. As yet no independent pleiotropic effect of any one of these three genes has been detected, and this makes it impossible at present to differentiate between them. The possibility that some undetermined form of chromosomal aberration may account for deficiencies in appearance of the trait is not supported by evidence thus far available.

Behavior of the di trait provides an interesting sidelight on the possible fallibility of conventional tests designed to detect mutagenic effects. Falconer (1949) has presented a valuable discussion of the number of suspected gametes that can be adequately tested and the reliability of tests when a possible recessive is involved, but in cases such as presented by the di complex the results of such tests might easily be misleading. When one of our di males was outcrossed to unrelated stocks, 189 F1, F2 and BC1 young were raised to the proper age and examined before the first di individual appeared among them. If female sibs of this original male had not been available for further tests it is probable that the trait would have been regarded as sporadic with no hereditary background. It is not likely that behavior of the di trait is unique and, if it is not, it becomes probable that in mice and other mammals many traits that are ordinarily regarded as sporadic do in fact have a definite and analyzable, even though complex, genetic background.

**SUMMARY**

A new trait, di (duplicate incisors), appeared in the second generation from mice treated in utero by injecting the pregnant mother with nitrogen mustard. When di mice were outcrossed, frequency of the trait was found to be low in the F2 and BC generations; 1.7 and 12 percent respectively. A BC/F2 index shows that the low incidence can not be accounted for on the assumption of a single semilethal mutant gene, but that it could be explained by postulating a gene which reduces the zygote-forming capacity of any germ cell which happens to carry it. The same figures could be explained equally well by assuming that
instead of one defective gene there are three normal recessives all of which must be present to insure appearance of the trait. Results from the inbred matings favor the second alternative and provide some evidence that one or more of the three genes may act as a semidominant when the other two are homozygous. A small part of the numerical deficiency is due to an unexplained failure of certain accessory enamel organs to produce teeth.

The sudden appearance of a trait apparently dependent on three different genes presents problems which at present can not be answered. It is conceivable, but extremely unlikely, that three mutations occurred simultaneously in the same embryo. It is hardly more likely that complementary mutations occurred in the two embryos which were to become ancestral to the di line. Instead, it seems probable that two of the mutations had already occurred, either spontaneously or as the result of treatment in earlier generations, and remained latent until the third one brought all of them to light. With three separate genes, none of which can as yet be individually identified, the group as a whole will have to be referred to simply as the di complex until such time as one of the genes becomes revealed through some distinctive effect of its own.

Experience with the di complex serves to emphasize the possible inadequacy of tests for mutagenic effects when such tests are predicated on the assumption that only simple monofactorial traits need be looked for. In an animal with the genetic complexity of the mouse it is probable that there are many latent genes whose presence would never be detected in the absence of an additional potentiating mutation. Whenever the number of genes necessary for the expression of a trait reaches three or more the genetic situation becomes difficult to analyze. Individually ineffective hidden genes may presumably accumulate in appreciable numbers and, in infrequent combinations, be responsible for a considerable part of the unexplained manifestations which are usually regarded as sporadic.

LITERATURE CITED