A COMPARISON OF THE EFFECTS, IN COMPOUNDS, OF SEVEN GENETICALLY SIMILAR LETHAL *t* ALLELES FROM POPULATIONS OF WILD HOUSE MICE¹

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POPULATIONS of wild house mice in different parts of the United States are frequently polymorphic for variant alleles (*t* alleles) at the complex locus, *T*. One step in the analysis of any such polymorphism is to determine whether the variant alleles found in different populations are nonidentical and, thus, probably due to separate events, or whether they can be shown to be similar or identical and, thus, possibly traceable to a common origin.

In previous papers (BENNETT and DUNN 1958; BENNETT, DUNN and BADENHAUSEN 1959) we have described the methods by which lethal *t* alleles from different wild populations have been classified into two groups. Lethal alleles are assigned to different groups when some of the compounds between them are viable until birth. We have taken such evidence of complementary interaction as a criterion of nonidentity for the alleles concerned. The alleles in heterozygotes which when crossed fail to produce such compounds viable at birth are tentatively assigned to the same group. Since absence of compounds at birth is not a decisive criterion of identity of the alleles concerned, the homozygous effects of alleles assigned to the same group are then compared by embryological methods. Such comparisons have been made for four (including *t*^{w20}) group 2 lethals (BENNETT, BADENHAUSEN and DUNN 1959), and for five group 3 lethals (BENNETT and DUNN 1958). (Group 1 consists of indistinguishable viable alleles). The conclusions were that members of each group were indistinguishable from each other when judged by effects on homozygotes. Group 2 lethals were however clearly distinguishable from those of group 3.

Members of these two groups of lethals are unequally represented in the wild populations which we have sampled. We have found group 2 lethals in three populations (*t*^{w1}, New York 1; *t*^{w3}, Mystic, Conn.; *t*^{w12}, Marin County, California) and group 3 lethals in nine populations (*t*^{w5}, New York 2; *t*^{w6}, Sarasota, Florida; *t*^{w10}, Clark Fork River, Montana (collected by Miss VIRGINIA VINCENT and reported here for the first time, but not yet studied embryologically); *t*^{w11}, Storrs, Conn.; *t*^{w13}, El Paso, Texas; *t*^{w14}, Norwich, Vermont; *t*^{w15}, Lawrence, Kansas; *t*^{w16}, Norwich, Vermont; *t*^{w17}, Tucson, Arizona. Since *t*^{w14} and *t*^{w16} came from different stations on the same farm, they may have come from the same population. We may say then that group 3 lethals are found in nearly three

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times as many locations as group 2 lethals. This may be due to chance. It does, however, suggest caution in drawing conclusions as to similarity or identity among the alleles of a group so widely disseminated in different habitats throughout the United States.

Embryological examination revealed that the five alleles studied from this group were not entirely consistent in the degree of their effects. One of them, t^{106} , especially, seemed to have somewhat more severe and earlier effects in homozygotes than the other members of the group. We have, therefore, studied the many members of this group more thoroughly and extensively by methods which we consider to be more sensitive for disclosing differences between them. In essence, we sought for evidences of partial complementarity amongst the alleles of this group by examining the compound embryos in all of the combinations that could be produced by crosses between stocks containing eight lethal alleles belonging to this group, including the five previously studied in homozygous form.

MATERIALS AND METHODS

The eight alleles (omitting t^{110}) were carried in our laboratory as tailless balanced lethal lines of the type T/t^w , each of which had been derived from the wild population indicated by: (1) the initial crossing of the normal tailed ancestor carrying the t allele to an inbred Brachy laboratory stock, (2) two or three subsequent backcrosses of tailless animals derived from the first cross to the Brachy stock, and (3) three or more generations of inbreeding of the descendants of a pair of tailless animals derived from the last backcross.

To obtain for study embryos heterozygous for two different t^w alleles, crosses were made in every possible combination between tailless balanced lethal stocks. Our previous study on homozygotes had indicated that the presence of T/T embryos did not interfere with the identification of t^w/t^w homozygotes since T/T homozygotes are rarely found (because of the high proportion of t^w gametes produced by males) and died at a much later stage showing a different syndrome of abnormalities. Where possible at least six litters of embryos were obtained from each cross; they were timed by the conventional vaginal plug method, and pregnant females were sacrificed at appropriate times to give embryos (for study) which were between seven and ten days postfertilization. From our previous studies on homozygotes, we could expect that compound t^{107}/t^{108} embryos, if they were similar to the homozygotes, would already have detectable abnormalities at the beginning of this period and would have undergone maximum differentiation (and often the deterioration and death which always follows) by the end of it. All the embryos obtained were examined histologically; the details of the procedure followed are given in full in our previous paper (1958).

RESULTS

The first and rather broad test for the existence of differences among the alleles studied was carried out in the following way. As stated above, the embryos were obtained during a time period in which we expected to be able to

recover and identify the great majority of embryos abnormal because of genotypic constitution t^{wx}/t^{wn} , if the compound behaved in a similar way to t^{wx}/t^{wx} and t^{wn}/t^{wn} . If the t^{wx}/t^{wn} compound behaved differently, i.e., was more viable or more normal morphologically, we expected to detect it in one of two ways: (1) gross deviations from the morphological pattern typical of homozygotes, or (2) significant departures from the segregation ratio expected; most probably this would be manifest as a deficiency of embryos scored as "abnormal." Table 1 gives the segregation data for the individual crosses. The embryos listed as "typical abnormal" were all similar in every way to the homozygous abnormal embryos described previously (1958) and thus did not fulfill the criterion for morphological diversity. It is clear, also, that in spite of the rather small numbers of embryos obtained from some of the crosses, there are no significantly large deviations from the expected segregation of about 0.48 t^{wx}/t^{wn} :0.52 $+/+$ or T/T , a figure based on an estimated average segregation ratio in males heterozygous for these alleles of 0.95 t :0.05 T . It was immediately obvious then, that if a condition of partial complementarity did exist between any of these alleles, it was of too minor a nature to be detected by these relatively gross methods.

The material was therefore subjected to what we thought would be a more

TABLE 1

*Percentages of abnormal (t/t) embryos found in crosses among members of the t^{w5} group.
(Expected percent of "typical abnormal" $\approx$ 48 percent)*

	Number of litters	Number of embryos	Number of typical abnormal embryos	Percent of typical abnormal embryos
$t^{w5} \times t^{w6}$	7	51	23	45
$\times t^{w11}$	6	47	20	43
$\times t^{w13}$	6	57	27	47
$\times t^{w15}$	7	61	27	44
$\times t^{w16(14)}$	6	43	19	44
$\times t^{w17}$	6	46	20	43
$t^{w6} \times t^{w11}$	6	43	22	51
$\times t^{w13}$	3	9	6	67
$\times t^{w15}$	5	31	12	40
$\times t^{w16(14)}$	5	35	17	49
$\times t^{w17}$	6	36	15	43
$t^{w11} \times t^{w13}$	3	25	9	36
$\times t^{w15}$	5	38	16	42
$\times t^{w16(14)}$	4	35	16	46
$\times t^{w17}$	4	27	13	48
$t^{w13} \times t^{w15}$	4	35	17	49
$\times t^{w16(14)}$	4	34	18	53
$\times t^{w17}$	3	19	7	37
$t^{w15} \times t^{w16(14)}$	5	36	15	43
$\times t^{w17}$	6	40	19	48
$t^{w16(14)} \times t^{w17}$	6	52	25	48
Totals	107	800	353	..

delicate analysis, according to the following rationale. In our previous paper, the abnormal embryos were staged and divided into groups, not on the basis of their own morphology, but according to the developmental stage of their normal litter mates. This was necessary to permit us to form some idea of the temporal relationships between normal and abnormal development. For the purposes of this paper, however, now that we already have basic information concerning timing and morphology, and where we were interested in discriminating as delicately as possible among conceivably different types or degrees of abnormality, we staged the abnormal embryos only with reference to themselves. Accordingly, a series of stages of abnormal development was prepared, in such a way as to include as many discrete and identifiable stages as possible. This permitted the construction of a series of frequency distributions of the various stages of abnormal embryos found in each separate cross, comparable with one another, and in which differences of distribution of abnormal stages could be easily detected. A brief description of these stages will be sufficient here; a more complete exposition of the abnormal embryology is given in our previous paper on group 3 (BENNETT and DUNN 1958).

The stages used were the following: 0.—Embryo entirely resorbed. I.—Embryo exists as a nubbin of undifferentiated cells; it looks as if almost no differentiation had occurred, and as if none will. Capsule and ectoplacental area are small. II.—Embryo is a degenerate egg cylinder, with many pycnotic granules and often grossly crumpled; however, it is large and presumably will develop further. III.—Embryo is very small and lumplike, and shows little or no differentiation; it differs from Stage I only in that the capsule is larger, and the embryo looks as if growth had proceeded somewhat, before regression occurred. IV.—Embryo has infolding ectoderm, but it is small with little or no development of mesoderm; looks as if it will not develop much further. V.—Embryo is large and healthy looking, with infolding ectoderm and developing extra embryonic membranes. VI.—Degenerating lump of ectoderm with small extra-embryonic membranes is the only vestige of embryo. The whole capsule is small and looks as if development will shortly stop. VII.—Large and healthy extra-embryonic structures with a variable amount of surviving ectoderm on top. VIII.—Small and degenerate "extraembryonic organism." IX.—Large and healthy "extraembryonic organism." X.—Rudimentary embryo, with organization of tube or rosette-like structure in the embryonic ectoderm, with some mesoderm surrounding it; all associated with large extraembryonic structures. XI.—Most advanced differentiation. Possible neural folds, notochord, and somites.

Since in the first analysis no members of group 3 had been detected as clear-cut deviants (either in morphological pattern or in segregation) we decided to consider the alleles as a group and test one after another individually against the remainder of the group. In addition, we knew from observation that these lethal compounds developed slowly enough as to form a fairly homogeneous group at 7–8 days gestation, and also at 9–10 days.

With these ideas in mind, we prepared the histograms shown in Figure 1.

FIGURE 1.—Histograms indicate the distribution, according to stage, of lethal embryos at ages 7-8 days and 9-10 days, with each allele considered separately in compound with all other alleles studied.

If any one of these alleles had been different from the others, this difference should have been detectable in its cross with all the other alleles by a distribution of abnormal stages which was clearly different from any of the others. Inspection of Figure 1 indicates, however, that the differences in the frequency distribution of abnormal stages between crosses at 7–8 days and at 9–10 days are not large enough to give any substantial hint that the effects of any one of these alleles is different from any of the others; the only one that might be thought to be deviant is t^{w6} .

Still another method of comparison involved assigning to each stage its own number, as a "weight," since the stages had been made up as a series indicating progressively increasing life span and/or differentiation. This made it possible then to prepare an "index of abnormality" for each of the crosses. These data are presented in Table 2. Again it is evident that all the alleles behave in much the same way in compounds. For comparison with the indices of abnormality, the average litter size for all this material is presented in the last column. The allele t^{w6} deviates somewhat from the others in two ways. It has the lowest index of "abnormality stage" indicating slightly earlier attainment of the lethal syndrome on the part of any compounds in which it enters; and litters of which a t^{w6} heterozygote was one of the parents were smaller than those produced by other heterozygotes. Both of these differences may be reflections of the state of the t^{w6} balanced stock which, perhaps because of its residual heredity, has been inferior to the other stocks in its fertility and the viability of its progeny.

DISCUSSION

This detailed comparison of the effects on embryonic development of lethal alleles derived from seven different wild populations indicates that their effects in compound with each other do not differ significantly from their effects when homozygous. The differences shown at 7–8 days (Figure 1) can be interpreted as minor variations on the same theme, which among the survivors at 9–10 days show a similar and consistent pattern. We have, moreover, to consider that genetical tests had shown no evidence of complementary interaction amongst them, and that the syndrome which all exhibit is unique both in time and

TABLE 2

A comparison of the effects, in compound, of seven lethal t alleles each derived from a different population of wild house mice

Type of mating		Average abnormal index; 7–8 days	Average abnormal index; 9–10 days	Average litter size
t^{w5}	Mated by all other alleles	3.4	5.9	8.5
t^{w6}		2.6	5.0	6.5
t^{w11}		3.8	6.3	7.7
t^{w13}		4.6	6.5	7.8
t^{w15}		4.0	6.7	7.5
$t^{w16(14)}$		4.8	6.4	7.9
t^{w17}		3.6	5.6	7.3

manner of expression amongst the lethals so far examined in the house mouse. The alleles of this group have also been shown to share other genetical properties: all have male transmission ratios in excess of 0.9, and all appear to suppress recombination in the region to the right of the locus. We consider these resemblances to come as near to proof of identity as it is possible to reach with such material.

If these do represent occurrences of the same allele in widely separated populations, how are we to account for their disjunct distribution? Two main hypotheses suggest themselves: (1) all arose in one act of "mutation" whether this were to be considered as point mutation in the usual sense or as a rare recombination near this locus; (2) they represent recurrences of the same event. We have no direct evidence in favor of one or the other of these hypotheses. The second hypothesis, assuming repeated independent acts of mutation resulting in alleles with the same or similar properties, encounters two difficulties. One is that mutation from the wild type to a mutant condition of this locus ($+/+$ to $+/t$) must be a rare event since we have encountered no instance of it in large numbers (over 20,000) of gametes from $+/+$ or $T/+$ when tested in matings by $T/+$. The other is that since the only observed method of origin of new t alleles is a change in the properties of t alleles already present (T/t^n or $+/t^n \rightarrow t^x$; cf., DUNN 1956), such changes, if they were to account for the origins of the eight similar alleles described here, would all have to have occurred in populations polymorphic for a t allele and in similar ways to produce indistinguishable lethal alleles. This would require multiple repetitions of the same event. We have observed two occurrences of such a repetition in the origins of t^{w20} from $+/t^{w15}$ and t^{w21} from T/t^{w5} . Both t^{w20} and t^{w21} are indistinguishable from the previously studied alleles of group 2, and repetition of events resulting in similar alleles is, thus, not to be excluded. On the other hand, out of 22 such events which we have analyzed, 14 have resulted in viable alleles and eight in lethals, only two of which are known to be duplicates. We should, therefore, have to assume some other agency favoring the retention of similar lethals in wild populations. Since all eight lethal alleles reported here are characterized by high transmission ratios from male heterozygotes (LEWONTIN and DUNN 1960), this may be the agency leading to the retention of duplicates. Since the second hypothesis is a possible one, we may compare it with the first hypothesis.

If we assume a single origin for the eight alleles, their similarities in effects on development, on male transmission ratio and on suppression of recombination would all be explained by their common origin. We should then have to explain their distribution in widely different populations. This could be due to migrants carrying the same allele to other populations. Migration rates in this species are not well known although, in general, the range of movement of individual mice is rather restricted (BLAIR 1953). But as a commensal its mobility is likely to be increased by travelling with man. It is therefore possible that the disjunct distribution of the same allele could be due to human agencies.

In the absence of decisive proof of either hypothesis, we may suspend judgment but with the remark that fewer difficulties are encountered by the hypothesis of common origin.

SUMMARY

Eight lethal alleles at locus *T* in the house mouse, each derived from a single heterozygote $+/t$ found in one of eight different North American populations, were compared by breeding tests and by embryological study. All possible compounds proved to be lethal at similar stages and to have similar patterns of abnormality between the seventh and tenth days of development when death occurred. No constant differences between compounds and homozygotes were found.

The essential disturbances in development, shared by the eight lethals, were those identified in a previous study of five of them as follows: pycnosis and faulty growth of the embryonic ectoderm as soon as it formed; the subsequent degeneration of all actual embryonic structures, and the preservation for a short time thereafter of nearly normal extraembryonic structures.

Since the alleles are indistinguishable by genetical and embryological methods, two hypotheses to explain their disjunct distribution in widely separated populations are examined *viz.*, (1) common origin in a single mutation followed by dispersal, possibly by human agencies; (2) repeated independent occurrences of the same mutation in different populations. Although no final decision is possible from present evidence, the first hypothesis encounters fewer difficulties than the second.

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