STUDIES OF THE GENETIC VARIABILITY IN POPULATIONS OF WILD HOUSE MICE. II. ANALYSIS OF EIGHT ADDITIONAL ALLELES AT LOCUS T*

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RECENT work has shown that many North American populations of *Mus musculus* are polymorphic for variant alleles at one locus (DUNN 1955). The methods used for detecting and isolating seven of these alleles have been described by DUNN and SUCKLING 1956. The method essentially is to bring wild mice into the laboratory and to testcross them individually with mice of a domestic stock carrying a dominant marker allele *T*. Wild animals heterozygous for a mutant allele at this locus ($+/t^w$) reveal their genotype by producing tailless offspring T/t^w which, when inbred, produce balanced stocks. When the wild allele (t^w) is lethal, such matings produce only tailless offspring in small litters which have been depleted by the death of two classes of lethal zygotes T/T and t^w/t^w . Each allele to be tested is derived from a single heterozygote taken from a wild population.

A fact of special interest is that each of the seven "wild" alleles previously studied is transmitted by male heterozygotes to a great majority of the offspring. The average "male segregation ratio" of the above seven t^w alleles was found to be about 94 percent. Such a male transmission ratio should favor the spread of t^w alleles even when these are lethal in homozygotes (DUNN 1953), and unless the spread is opposed by other evolutionary forces, such as selection against heterozygotes $+/t^w$, we should expect to find high frequencies of heterozygotes in those wild populations in which a t^w allele is present.

Additional wild populations in Connecticut, California, Texas, Vermont, Kansas, and Arizona have now been sampled, eight additional lethal alleles have been identified, and their male segregation ratios determined. The results, reported in this paper, are in general agreement with those from the first seven alleles. In addition cross-tests of alleles from different populations have been made and the lethal alleles classified into groups, crosses between which produce viable compounds such as t^{wn}/t^{wz} .

OBSERVATIONS

Population Connecticut 2

Collecting was begun June 2, 1955 in the grain room of the new horse barn of the College of Agriculture, University of Connecticut at Storrs, about a half mile from

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TABLE 1
Testcrosses of wild mice from population Connecticut 2 by Brachy

Parents	Offspring				
	Normal	Brachy	Tailless	Total	Litters
5 wild ♀ +/ <i>t^{w11}</i>	81	37	33	151	20
2 wild ♀	14	12		26	4
13 wild*	182	150		332	51
1 wild ♀ +/ <i>t^{w11}</i> *	2	2	4	8	1

* Members of two litters found in the feed room, fostered by domestic mothers and testcrossed with Brachys.

TABLE 2
*Results of inter-se matings of tailless offspring from four wild +/*t^w* females from population Connecticut 2*

Wild ancestor	Offspring		Remarks
	Tailless	Litters	
147 +/ <i>t^{w11}</i>	44	14	16 with stump tails*
148 +/ <i>t^{w11}</i>	149	44	
149 +/ <i>t^{w11}</i>	16	4	
150 +/ <i>t^{w11}</i>	173	46	
Intercrosses†	84	20	
Total	466	128	Litter size 3.6

* Two with tails of half normal length, tested and found to be *T/t^w*.

† Tailless descendants of 150 × tailless from 147 and 148 and from 147 × 148 and 150.

the campus. The barn is a cement building, relatively isolated. After the first four females had been tested and proved to carry a *t* allele, PROFESSOR LANDAUER of Storrs kindly continued the collecting, sending in single animals as trapped and two litters found in the grain room, which were fostered on domestic mothers in the laboratory. The results of testcrossing the captured animals by domestic Brachy females are given in table 1. The frequency of heterozygotes amongst individually trapped mice (excluding members of litters) gives a minimum estimate of five out of eight. The average litter size of the wild females mated with Brachy males was 7.4. Twenty-two litters born in the laboratory from matings of wild females by wild males yielded 134 offspring, an average litter size of 6.1.

Extraction of alleles

Tailless offspring from testcrosses of each of the first four heterozygous females were tested in sibling matings. The results are shown in table 2. Tailless descendants of each wild female gave only tailless offspring with the exception of one pair mating from ♀ 150 to be described later. When it was apparent from this balanced lethal behavior that each wild female transmitted a lethal *t* allele, intercrosses were made between tailless descendants of 147, 148, and 150. These also produced only tailless offspring, indicating that the lethal alleles in each of these wild females were probably

the same. This allele was designated as t^{w11} . Litters from tailless matings averaged 3.6 offspring at birth compared with 7.4 per wild female in the testcrosses. This is consonant with the expectation of 50 percent reduction in litter size from tailless matings due to segregation of two lethals, T and t^{w11} .

One mating gave an exceptional result. This was a mating of tailless $F_1 \sigma 772 \times$ tailless $F_1 \phi 773$, siblings from testcross of $\phi 150$ wild $+/t^w$ by Brachy. This mating yielded three litters consisting of eight tailless and six normal-tailed offspring, the result to be expected if 772 and 773 contained different t alleles. $\sigma 772$ was tested by known T/t^{w11} females, and gave 29 offspring, all tailless. $\phi 773$ tested by a known T/t^{w11} male gave seven tailless and two normal. This was interpreted to mean that $\sigma 772$ was T/t^{w11} while $\phi 773$ contained a different allele which was designated t^{w18} . The latter allele was extracted and found to be a new and different lethal. It appears then that $\phi 150$ transmitted two different mutant alleles, t^{w11} to the majority of offspring, t^{w18} to at least one ($\phi 773$). It is assumed that $\phi 150$ was $+/t^{w11}$, and that a mutation to t^{w18} preceded the formation of at least one gamete. Such mutations have been found previously in balanced lethal stocks but this was the first identified from $+/t$ and the first case in which it could be shown that the mutation had occurred in a female. If we had sampled this population *after* the occurrence of this mutation we might have found within it two different t alleles, a condition which we have not yet observed.

Two inbred stocks of T/t^{w11} have been established, one derived from $\phi 150$ wild, and one from $\phi 148$ wild.

One inbred line of T/t^{w18} has been established. This has given 151 tailless (litter size 3.3) indicating the presence of two lethals.

Population California 1

On June 23, 1955 we received from DR. K. W. DE OME, Cancer Research Laboratory, Oakland, California, a shipment of mice descended from two pregnant wild females captured one in San Mateo and one in Marin County, California, and subsequently inbred in confinement. Twenty-five of these produced offspring from testcrosses by Brachy as shown in table 3. One female ($\# 259$) descended from a female trapped in a salt marsh at Corte Madera, Marin County, produced tailless offspring. All others (17 descended from the Marin County female and three descended from the San Mateo County female) gave only normals and Brachys. The wild females gave an average litter size of 5.2. From the information available it is impossible to

TABLE 3
Testcrosses of wild mice from population California 1 $\times$ Brachy

Parents	Offspring				
	Normal	Brachy	Tailless	Total	Litters
13 wild $\sigma \sigma$	221	210	—	431	53 (8.1)
11 wild $\phi \phi$	72	69	—	141	27 (5.2)
1 wild $\phi +/t^{w12}$ ($\phi 259$)	4	8	7	19	3

TABLE 4
Testcrosses of wild mice from population Texas 2 by Brachy

Parents	Offspring				
	Normal	Brachy	Tailless	Total	Litters
1 wild ♂	76	3	59	138	21
2 wild ♀ ♀	23	8	13	44	7

estimate the frequency of heterozygotes in the original wild population sample. If the allele isolated from the one female was derived from the wild ancestor and had not occurred by mutation after the population was confined, then the one animal taken from the wild was heterozygous, indicating a high frequency of the allele.

Matings among the tailless descendants of ♀ 259 have given 418 tailless and one exceptional animal with a normal tail, dead at birth with microcephaly. This may have been a mutant or a homozygote of the *t* allele from this population which is obviously a lethal, designated t^{w12} . The average litter size of the litters from matings between tailless animals was 3.9, indicating effect of two lethals *T* and t^{w12} .

Population Texas 2

In a garage in El Paso, Texas, MARY ANN DANN trapped several wild house mice during the spring of 1955 and in July sent us three specimens all of which proved to be heterozygous for a *t* allele which we designated t^{w13} . We have no information on the size of the population from which they were taken, but since the only three animals tested were heterozygous, the allele may have been common. The testcross data are given in table 4.

Inbred tailless lines were established from descendants of one male and one female. Each gave only tailless offspring, and crosses between the two lines gave only tailless, indicating probably the same lethal in each of the wild heterozygotes. A total of 161 tailless offspring was recorded from the tailless matings with an average litter size of 3.6.

Population Vermont 1

This population was found by DR. M. T. M. RIZKI and MISS HANNAH CROASDALE of Dartmouth College in a group of buildings on the Metcalf farm on Dutton Hill, Norwich, Vermont, and collections were begun in the autumn of 1952. Subsequently, in 1955, collecting was resumed with the cooperation of FRED E. and ABBIE METCALF and HARRIET TINKER. The ecological situation on this farm will be described in more detail later. At present we report the source of the animals tested as different rooms and buildings, since some of these by reason of separation by predators (cats) may be separate populations. The testcross data are in table 5. Out of 28 animals tested, two proved to be heterozygous, and tailless lines were established from each. The allele from the first of these was designated t^{w14} . Matings between tailless *T/t^{w14}* animals produced 183 tailless and one exception with a normal tail, with an average litter size of 3.7. The t^{w14} allele is evidently a lethal, and the normal exception may be a mutant.

TABLE 5
Testcrosses of wild mice from population Vermont 1 by Brachy

Parents	Location	Offspring				
		Normal	Brachy	Tailless	Total	Litters
7 wild ♂♂	Horse barn	109	74	—	183	22
1 wild ♀	Horse barn	1	4	—	5	1
1 wild ♂	Kitchen	24	20	—	44	6
3 wild ♂♂	Grain room	57	80	—	137	19
3 wild ♀♀	Grain room	24	30	—	54	11
1 wild ♂	Cow barn	13	10	—	23	2
1 wild ♀	Cow barn	7	12	—	19	4
3 wild ♂♂	North cow barn	69	83	—	152	20
3 wild ♂♂	Hen house	68	46	—	114	16
1 wild ♂ +/ <i>t</i> ^{w14}	Hen house	12	2	11	25	2
3 wild ♀♀	Hen house	29	24	—	53	10
1 wild ♀ +/ <i>t</i> ^{w16}	Wagon shed	21	13	7	41	9

TABLE 6
Testcrosses of wild mice from population Kansas 1 by Brachy

Parents	Offspring				
	Normal	Brachy	Tailless	Total	Litters
8 wild ♂♂	128	112	—	240	33
1 wild ♂ +/ <i>t</i> ^{w15}	4	—	5	9	1
6 wild ♀♀	32	19	—	51	8

The second allele found was designated *t*^{w15}. Matings between tailless animals from this line have given 115 tailless (litter size 3.9) and one normal exception. The *t*^{w16} allele is also probably a lethal, and as discussed in a later section may not be different from *t*^{w14}. If this proves to be so, and if we consider the Metcalf farm as one population, then the allele frequency is two out of 28 tested, the lowest frequency yet found in any population. However, the question remains open until a population study is made.

Population Kansas 1

PROFESSOR J. A. WEIR of the University of Kansas sent us two samples of mice from a feral population in the State Game Preserve eight miles west of Lawrence, Kansas, one in January 1953 and one in November 1955. The testcross data on these are in table 6.

From tailless descendants of the single heterozygote a tailless line has been established which has given 60 tailless offspring with an average litter size of 3.5. Apparently a lethal designated as *t*^{w15} has been isolated. But one tailless male which also obtained his *t* allele from the original wild ancestor behaves in an exceptional manner, producing normal and tailless offspring when tested by six different *T/t*^{w15} females, as though he contained a *t* allele different from *t*^{w15}. This case is being studied further.

TABLE 7
Testcrosses of wild mice from population Arizona 1 by Brachy

Parents	Offspring				
	Normal	Brachy	Tailless	Total	Litters
1 wild ♂	67	66	—	133	19
2 wild ♀ ♀	15	16	—	31	6
1 wild ♀ + t^{w17}	28	11	14	50	7

TABLE 8
Segregation ratios of wild heterozygotes

Parents	Offspring			
	Normal +/+ & +/ t^w	Brachy $T/+$	Tailless T/t^w	Total
6 wild ♀ ♀ +/ t^{w11} × $T/+$	83	39	37	159
1 wild ♀ +/ t^{w12} × $T/+$	4	8	7	19
2 wild ♀ ♀ +/ t^{w12} × $T/+$	23	8	13	44
1 wild ♀ +/ t^{w16} × $T/+$	21	13	7	41
1 wild ♀ +/ t^{w17} × $T/+$	28	11	11	50
Totals	159	79	75	313
Expected (normal ratio)	156.5	78.25	78.25	313
1 wild ♂ +/ t^{w13} × $T/+$	76	3	59	138
1 wild ♂ +/ t^{w14} × $T/+$	12	2	11	25
1 wild ♂ +/ t^{w15} × $T/+$	4	—	5	9
Totals	92	5	75	172
Expected (normal ratio)	86	43	43	172

Population Arizona 1

In February 1956 four wild mice were trapped in the studio of Mr. Ted De Grazia on the outskirts of Tucson, Arizona. The testcross data are in table 7. From the tailless offspring of the single wild heterozygote (+/ t^{w17}) a tailless line has been derived which has given 114 tailless with an average litter size of 3.0.

Male segregation ratio

The segregation ratios of the t^w alleles were estimated first from wild heterozygotes testcrossed by Brachy. The pertinent data are brought together in table 8. The data from female heterozygotes agree with the normal expectation and indicate a gametic frequency of .5+ and .5 t^w . The data from male heterozygotes clearly do not agree with this but indicate a segregation ratio of .06 and .94 t^w , very close to the ratio obtained from wild male heterozygotes carrying any one of the first seven alleles (DUNN and SUCKLING 1956).

Subsequently, samples of five tailless males (T/t^w) from each of the tailless lines were testcrossed with normal (+/+) females derived as segregates from our inbred Brachy stock. These gave the results shown in table 9. For comparison females from tailless lines were testcrossed with normal (+/+) males from the Brachy stock

TABLE 9
Results of mating tailless (T/t^w) males individually with normal $+/+$ females

Male No.	Offspring		Totals	
	Normal ($+/t^w$)	Brachy ($+/T$)	Normal	Brachy
641 T/t^{w11}	107	8		
791 T/t^{w11}	103	1		
1129 T/t^{w11}	110	2		
1130 T/t^{w11}	107	6		
1303 T/t^{w11}	91	2	518	19
651 T/t^{w12}	90	5		
652 T/t^{w12}	98	3		
1154 T/t^{w12}	77	7		
1155 T/t^{w12}	54	2		
2210 T/t^{w12}	74	1	393	18
1133 T/t^{w13}	101	7		
1415 T/t^{w13}	93	1		
1482 T/t^{w13}	97	—		
1490 T/t^{w13}	114	—		
2179 T/t^{w13}	97	3	502	11
2159 T/t^{w14}	94	2		
2185 T/t^{w14}	112	5		
2207 T/t^{w14}	96	10		
2158 T/t^{w14}	90	3	392	20
1804 T/t^{w15}	96	14*		
1805 T/t^{w15}	60	3		
2540 T/t^{w15}	82	3		
2542 T/t^{w15}	83	9		
2645 T/t^{w15}	90	11	391	40
1912 T/t^{w16}	68	5		
2495 T/t^{w16}	15	1		
2498 T/t^{w16}	24	—	107	6
Total			2303	114
Percent			96.1	3.9

* One tailless = $T/+$ by progeny test.

(table 10). The results were striking and consistent. Whereas females T/t^w gave normal segregation ratios, males uniformly gave great excesses of normal offspring which when progeny tested proved to be $+/t^w$. The average segregation ratio from T/t^w males can be estimated from the data as .04 T and .96 t^w .

In tests made for another purpose wild males $+/t^{w11}$ derived from matings of wild $+/t^{w11}$ females with wild $+/+$ siblings were testcrossed with Brachy females. These gave 492 normal, 29 Brachy, and 421 tailless, an estimated segregation ratio of .06 and .94 t^{w11} .

TABLE 10

Results of mating tailless females (T/t^w) individually with normal ($+/+$) males (combined results)

	Offspring	
	Normal $+/t^w$	Brachy $T/+$
37 ♀ $T/t^{w1} \cdot T/t^{w12}$	187	184

There seems little doubt that in wild male heterozygotes and in T/t^w males derived from wild heterozygotes all t^w alleles segregate in high ratios. There may be some variation amongst alleles—a study of these variances is under way—but the essential fact is the similarity of all wild heterozygotes and their immediate male heterozygous descendants. This is in striking contrast to the results obtained from male heterozygotes carrying t alleles which have arisen under observation in the laboratory or which have been maintained for many generations in the laboratory. Of these domestic alleles three (t^0 , t^1 , t^{12}) give high male ratios from .6 to .9 t ; three (t^3 , t^7 , t^8) give normal ratios of about .5 t ; while three (t^4 , t^9 , t^{13}) give low ratios of .35 to .45 t . One difference between the domestic and wild populations is that the latter have been subjected to selective forces which operate on wild populations; the former have for some generations been removed from these conditions. This suggests that the relative uniformity of the segregation ratio of different alleles in wild populations is in part at least a result of natural selection. This hypothesis will be tested in future experiments.

Differences between t alleles from different wild populations

Two classes of alleles from different wild populations are readily differentiated: those which are viable when homozygous, and those which are lethal. Of the first seven alleles tested (DUNN and SUCKLING 1956) five were lethal and two viable. In the present sample of eight, all are lethal.

A test is available which when positive is competent to indicate that lethal alleles derived from different wild heterozygotes are different. The test depends on the viability at birth of some of the compounds containing t alleles derived from balanced lethal lines. Thus tailless animals from the inbred line T/t^0 mated to tailless from the line T/t^1 (of independent origin) produce some viable normal-tailed animals which can be shown to be t^0/t^1 (DUNN and GLUECKSOHN-SCHOENHEIMER 1943). This complementary interaction in compounds from which both balanced lethal lines can be recovered, is taken to indicate that the alleles, such as t^0 and t^1 , are different.

There is no comparable test by which two lethal alleles of independent origin can be proved to be the same. If crosses between two balanced tailless lines, such as T/t^{w5} and T/t^{w6} yield only tailless offspring at birth, it may mean that the compound t^{w5}/t^{w6} is lethal before birth. Difference in such a case could be proved only by identifying the compounds during embryogeny or by demonstrating differences between the two kinds of homozygotes, and such methods have not yet been applied routinely.

Lethal alleles may therefore be classified into those which give viable normal-tailed progeny in compounds and those which have not given such progeny at birth. The

TABLE 11

Results of cross-testing tailless animals from different balanced lethal lines. Number of tailless progeny is in Roman (above); normal progeny in italics (below); in **bold face** are given the number of tailless progeny when no exceptions have been found in fifty or more offspring

	T/t^{w1}	T/t^{w3}	T/t^{w5}	T/t^{w6}	T/t^{w11}	T/t^{w12}	T/t^{w13}	T/t^{w14}	T/t^{w15}	T/t^{w16}	T/t^{w17}
T/t^{w1}		91	17 20	36 17	4 4	73	20 9	17 16	13 8		6 6
T/t^{w3}			9 8	19 13	6 9	116	7 1	5 4	12 17		7 2
T/t^{w5}				121	40	9 7	36	60	11		16
T/t^{w6}					34	45 15	24	13		21	15
T/t^{w11}						15 14	52	51	29	31	55
T/t^{w12}							36 25	10 11	9 6	15 7	12 4
T/t^{w13}								33	7		56
T/t^{w14}									8	53	

data are at present incomplete, since because of the infertility frequently found in tailless animals cross-tests must be made in large numbers and over a long period. But the data available now (shown in table 11) are adequate to reveal one new and interesting point: the tendency of lethals from different populations to fall into groups. Within each group, alleles have not formed compounds viable at birth; whereas alleles belonging to different groups do form viable compounds with normal tails.

Thus it is clear from the table that t^{w1} , t^{w3} , and t^{w12} fall in the same group (group 2). In intercrosses between lines carrying any of these alleles only tailless young were found at birth. The number of these (280) makes it highly probable that compounds viable at birth do not occur. Moreover the behavior of these lines in crosses with members of other lines is similar and consistent. Thus t^{w1} , t^{w3} , and t^{w12} all form viable compounds with t^{w5} , t^{w6} , t^{w11} , t^{w13} , t^{w14} , t^{w15} , and t^{w17} . Preliminary evidence indicates that t^{w1} , t^{w3} , and t^{w12} form similar homozygotes which die in the period between 11 days of gestation and birth. All are retarded in development, have normal tails, and have microcephaly and other head abnormalities. Similar normal-tailed animals with microcephaly have been found dead at birth in tailless lines T/t^{w1} , T/t^{w3} and T/t^{w12} and only in these. These alleles are thus very similar and may be identical.

Similarly t^{w5} and t^{w6} belong to the same group (group 3) showing no compounds at birth in 121 test progeny, both give viable compounds with t^{w1} , t^{w3} , and t^{w12} , and only with these alleles. To the same group also belong t^{w11} , t^{w13} , t^{w14} , t^{w16} and t^{w17} .

The t^{w15} allele appears to belong to group 3, since it gives viable compounds with group 2, none with group 3. Of t^{w18} it can be said at present that it does not belong to group 3. The viable alleles (t^{w2} , t^{w7} , t^{w8}) are assigned to group 1.

A preliminary classification of alleles would be as follows: Group 1, t^{w2} (New York City), t^{w7} (Austin, Texas), t^{w8} (Rumford, Va.); Group 2, t^{w1} (New York City), t^{w3} (Mystic, Conn.), t^{w12} (Marin County, Cal.); Group 3, t^{w5} (New York City), t^{w6} (Sarasota, Fla.), t^{w11} (Storrs, Conn.), t^{w13} (El Paso, Texas); t^{w14} (Norwich, Vermont), t^{w15} (Lawrence, Kansas); t^{w16} (Norwich, Vermont), and t^{w17} (Tucson, Arizona; Group unknown, t^{w18} (mutant in stock from Storrs, Conn.).

No obvious geographical relationships appear to connect members of the same group or to differentiate members of different groups.

Two relationships are of special interest. t^{w14} and t^{w16} were isolated each from a heterozygote taken in adjacent buildings on the same farm. To date crosses between these two lines have given no viable compounds at birth, and the alleles may be identical, perhaps drawn from the same population. The allele t^{w18} arose as a mutant in a wild female shown to be $+/t^{w11}$. This indicates a possible mechanism by which two different alleles might be present in the same or neighboring populations.

Other alleles

Three other alleles have been found but are not reported here. ANDREW BEASLEY has isolated a viable allele, t^{w8} , from collections made in Rumford, Virginia, and this will be reported separately by him. A wild mouse trapped in Tiemann Place, New York City by DR. DOROTHEA BENNETT proved to be a heterozygote, and a line containing this allele was isolated as T/t^{w9} . Subsequently doubt arose whether the animal from which the allele was isolated was in fact the animal brought in by DR. BENNETT, and the line was discarded. The designation t^{w10} was reserved for an allele isolated by TUTIKAWA (1954) from a population near Missima-Sen, Japan. This and several other alleles found in Japan are being studied further by TUTIKAWA. There is no information at present on their relations to each other or to alleles from American populations.

Several other wild populations in the United States have been sampled and have as yet yielded no additional heterozygotes. Except for one sample of 28 animals tested from Great Gull Island in Long Island Sound, the samples tested are too small to constitute evidence against the occurrence of t alleles in them. The feral population on the uninhabited and isolated Great Gull Island is at present the only adequate sample which has yielded no heterozygote. The negative data will be reported later.

DISCUSSION

Questions of primary interest on which this report may shed some light concern: (1) the frequency of heterozygotes in wild populations; (2) the ratio of lethals and viable t alleles found in the wild; (3) whether a single wild population contains more than one allele; (4) the distribution of "male segregation ratio abnormality" in the different wild and laboratory populations; (5) identity or difference of alleles in different populations.

1. Concerning the first point our information is still inadequate. There are two chief

difficulties in reaching estimates of heterozygote frequency. One is lack of essential ecological information on what constitutes a population in either a geographical or genetic sense. The other is our failure to get adequate progeny from many wild females which frequently produce only one or two test litters in the laboratory, so that they often cannot be classified decisively as to genotype. In addition, many wild females do not breed at all in the laboratory, and if (as suggested by the preliminary evidence of DUNN and SUCKLING 1955) the genotype $+/t^w$ is more likely to be fertile than $+/+$, then the frequencies of $+/+$ and $+/t^w$ amongst those that do breed may give an overestimate of $+/t^w$ frequency. The data on the seven alleles of this report which were found directly in wild heterozygotes indicate that 13 heterozygotes were found out of 29 trapped animals tested. If we assume, pending further study, that animals trapped in the same station belong to the same population, then nearly half the members of these populations are heterozygotes. This is similar to the estimate reached by LEVINE and DUNN (1956) for one large enclosed wild population which had been bred for several years in the laboratory. Perhaps the best statement at present is that heterozygotes in wild populations are more likely to be common than to be rare.

2. At present, 13 lethal alleles have been isolated from wild heterozygotes (alleles $w1$, 3, 4, 5, 6, 11, 12, 13, 14, 15, 16, 17, and 18) and only three viable alleles ($w2$, 7, and 8) which are male-sterile. This ratio is quite different from that found in domestic populations maintained in the laboratory. Of the latter six are lethal (0, 1, 2, 4, 9, 12, 14) and five are viable (3, 7, 8, 11, and 13) and male-fertile. The difference may be due to wild mice heterozygous for a lethal being favored by natural selection in the wild, but not in the laboratory, a point which is being studied. The sterility of males homozygous for viable wild alleles lowers the adaptive values of such alleles and allows them to be treated in respect to their evolutionary fate, like the lethals.

3. As to whether more than one different allele occurs in any one wild population, the evidence is at present negative but not decisive. What appears to be the same allele was found in all five wild heterozygotes of population Connecticut 2, and in all three from Texas 2; other alleles were found in single wild heterozygotes. In the first series of alleles (DUNN and SUCKLING 1956) four heterozygotes of Texas 1 appeared to have the same allele; t^{w1} and t^{w2} were found in an enclosed population originating from two wild sources; others were from single wild heterozygotes. If, as is probable, new wild alleles arise by mutation in populations containing an antecedent *t* allele (as in the case of t^{w18} reported here), then occasionally two different alleles should be found in the same population.

4. The "male segregation ratio" abnormality is apparently a common property of all *t* alleles, both lethals and viables, found in wild populations, in this as in the previously reported sample of alleles from the wild. Whether this property is inherent in the locus or whether it is conferred upon it by the rest of the genotype cannot be decided at present. The fact that this property is not common to alleles arising by mutation in laboratory stocks not exposed to selection in nature suggests that the latter alternative is more probable. A useful hypothesis at present is to suppose that mutation to a *t* allele predisposes the locus to influences of other genes upon the male segregation ratio.

5. The comparison of alleles from different wild heterozygotes is obviously in a preliminary state. It is at any rate clear that the present sample of eight lethals includes several nonidentical alleles from different populations. Amongst all wild alleles, a minimum of three groups of nonidentical alleles exists; one group of three viables and at least two groups of lethals. It is not proved that the members of the same group are identical. It is not excluded that all 15 reported in this and the previous paper in this series are nonidentical. When to these are added the 11 studied from laboratory populations and the first described allele *T*, the potential complexity of this locus becomes evident.

It is probable that the structure of a locus which includes many sites of mutation is causally related to its function in development and its role in the evolutionary differentiation of wild mouse populations. It is to the elucidation of the relationship of these three aspects of this locus that future work is to be directed.

SUMMARY

1. Eight additional lethal alleles at locus *T* are described, each derived from a wild heterozygote in one of six different wild populations.

2. The frequency of heterozygotes appears to be high in most wild populations, possibly as high as 50 percent.

3. In two of the populations in which several heterozygotes were found, the same allele was isolated from each heterozygote of the same population. One heterozygote was found in each of two subpopulations on the same farm. These two alleles have not been shown to be different. One wild heterozygote from another population transmitted two different alleles; probably one was a new mutant, giving rise to the possibility that more than one allele may occur in the same population, although the rule at present is to find but one type of variant allele per population.

4. All wild *t* alleles show the "male segregation ratio peculiarity" by which male heterozygotes transmit the *t* allele to a great majority (about 96 percent) of the offspring. This may be due to the effects of natural selection on factors favoring high transmission ratios.

5. *t* alleles from the wild fall into at least three groups, one with three viable alleles, and two groups of lethals. Lethals within the same group have not formed viable compounds when combined; lethals belonging to different groups do form viable compounds by complementary interaction and are thus shown to be nonidentical.

6. Since 29 alleles have now been detected at this locus, many of which are nonidentical, the locus is assumed to contain many sites of mutation and to have great potential complexity.

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LITERATURE CITED

- DUNN, L. C., 1953 Variations in the segregation ratio as causes of variations in gene frequency. *Acta Genet. et Stat. Med.* **4**: 139-151.
- 1955 Widespread distribution of mutant alleles (*t*-alleles) in populations of wild house mice. *Nature* **176**: 1275-1276.
- DUNN, L. C., AND JOAN SUCKLING, 1956 Studies of the genetic variability in wild populations of house mice. I. Analysis of seven alleles at locus *T*. *Genetics* **41**: 344-352.
- DUNN, L. C., AND S. GLUECKSOHN-SCHOENHEIMER, 1943 Tests for recombination amongst three lethal mutations in the house mouse. *Genetics* **28**: 29-40.
- DUNN, L. C., AND JOAN SUCKLING, 1955 A preliminary comparison of the fertilities of wild house mice with and without a mutant at locus *T*. *Am. Naturalist* **89**: 231-233.
- LEVINE, L., AND L. C. DUNN, 1956 The frequency of *t^w* alleles in a confined population of wild house mice. *Proc. Soc. Exp. Biol. Med.* **92**: 308-310.