STUDIES OF THE GENETIC VARIABILITY IN WILD POPULATIONS OF HOUSE MICE. I. ANALYSIS OF SEVEN ALLELES AT LOCUS T[1]

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GENETICAL polymorphism, the occurrence of several genotypes in the same population, is now recognized as the normal state of the population structure of many crossbreeding species in nature. The maintenance of the genetic variance in a state of equilibrium, as in balanced polymorphism, has been ascribed in a number of cases to the superior fitness of heterozygotes. DOBZHANSKY, (1949) for example, has shown that most of the chromosomes of wild populations of several Drosophila species contain genes which are lethal or otherwise deleterious when homozygous.

Little has been known about such genetical conditions within wild populations of mammals, largely due to the difficulties of analyzing the genotypes of wild mammals in the laboratory. These difficulties have now been overcome with one species, the house mouse (*Mus musculus*), to a sufficient extent to permit rapid diagnosis of the conditions at one locus in animals taken from wild populations. The methods of doing this have been described (DUNN and MORGAN 1953), and the results of a preliminary survey (DUNN 1955) indicate that the majority of wild populations from several parts of the U.S. contain heterozygotes for different genetic variants at this locus.

In addition, two possible mechanisms contributing to the maintenance of heterozygosity in these populations have been suggested by the evidence. One is superior fertility of heterozygotes (DUNN and SUCKLING 1955); the other is a novel means, discovered in this material, by which gametes carrying the variant allele, are transmitted in higher than normal frequency by male heterozygotes. The latter, which we have called segregation ratio advantage (DUNN 1953) appears to be a component additional to those usually considered in explaining the equilibria characteristic of wild populations; namely, mutation rate, selection or fitness, migration and random drift. The genetical state of a wild population may thus be conceived as the balance reached through the interactions of these five elementary agencies with a particular environment. The wild mouse populations provide an opportunity of evaluating certain of these, particularly the segregation ratio effect at the one locus at which it has been identified. Estimation of the other parameters will be more difficult in mammals, because of the long generation interval and the slow development, but the latter, when aided by embryological methods, may turn out to be an advantage, since the components of fitness—such as fecundity, fertility, viability of embryos and of suckling young, growth, life-length and the like—may be studied separately, and the effects of individual genes upon them elucidated. For the mouse there is already much information of this sort (cf. GRÜNEBERG 1951).

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WILD MOUSE POPULATIONS

The house mouse is a commensal, its geographical distribution corresponding closely with human habitation. While its spread over both hemispheres has probably been due in the main to human agencies—shipping and the like—and while most populations are found in dwellings and places of food storage, there are nevertheless many free-living populations remote from habitations. These are presumably feral, originating from commensal populations and possibly in reproductive communication with them. Most of the populations studied up until now appear to be small—from a few individuals to a few hundred—and the small amount of data available indicates that each population is limited to a rather restricted home range (cf. BLAIR 1953). It is probable that the species now consists of a large number of small populations, each occupying its own ecological niche. The factors influencing reproductive isolation of separate populations are probably those which operate on mammalian populations generally, but in the case of the mouse, some are probably connected with the human occupation pattern. The populations can thus be conceived as "natural" ones only in a limited sense. The species can be treated as intermediate between such domesticated populations as have no known ancestral population in its natural state (e.g., chickens, dogs) and those living in independence of man. We shall refer to "wild" mouse populations to distinguish them from those bred in the laboratory or by fanciers, without implication as to their natural state. In collecting from wild populations we, of course, avoid the immediate neighborhood of populations of laboratory or domesticated mice, but there is no way of knowing, except from the study of the wild mice themselves, what their connections with domestic populations may have been.

METHODS OF STUDY

Mice are caught individually in traps (we have used the "Hav-a-Hart" from Albia Products Co., New York) baited with peanut butter. The point at which each mouse is caught is noted on the trap when collected, if out-of-doors by landmarks, if indoors by identification of building and room. In the laboratory matings are arranged (by inbreeding or testcrossing) in such a way that the "wild" genes of any descendant trace to a particular wild individual. Wild females are mated in pens with exercise wheels which tend to induce and maintain oestrus, although even with this treatment some wild females have remained in anoestrus. Wild males do not require this and breed well with domestic females in ordinary steel mouse boxes ($6'' \times 6'' \times 12''$) with wire mesh tops from which feed wells depend into the pens. Wild mice, like the laboratory stocks, are fed the usual ration of pellets (Rockland Farms) supplemented by green feed pellets (Full O' Pep), rolled oats and ground wheat with water from drip bottles. Litters from wild females are usually given to domestic females for fostering.

BREEDING ANALYSIS

Although the samples from most wild populations show evidence of being polymorphic in several noticeable characters (white belly-yellow belly-gray belly; white spotting on forehead, belly or tail; different densities of black pigment in the agouti

pattern) we have as yet studied only the variants at locus T. Each wild mouse is first testcrossed with mice of genotype $T/+$ from a standard laboratory stock of this mutant (Brachy = short tail). If tailless mice are born from this mating, these are then tested to determine whether they contain an allele of the series collectively known as t^w, which in compound with $T(T/t^w)$ produced taillessness, or whether they are $T/+$ with minus modifiers of tail-length. The latter have been found in several wild populations. Such tests are carried out by mating F_1 tailless from a wild parent (1) with each other (2) with tailless laboratory stocks known to be T/t. If the test crosses yield Brachy ($T/+$) offspring, the F_1 tailless is classified at $T/+$ and discarded. If only tailless or tailless and normal-tailed offspring are produced, the F_1 tailless is tentatively diagnosed as T/t^w. If the testcross by a domestic stock such as T/t^0 yields only tailless, then the t^w allele from the wild is diagnosed as either the same as the domestic test allele or lethal in compound with it (e.g. t^w/t^0). Only one such case has occurred in our tests. If inter-se matings of F_1 tailless yield only tailless offspring, while test matings with a domestic allele yield both tailless and normal, the allele from the wild is diagnosed as a lethal which forms a balanced lethal stock in compound with T; that is, $T/t^w \times T/t^w$ give as viable zygotes only T/t^w. If inter-se matings of F_1 tailless yield tailless *and* normal offspring the allele from the wild is diagnosed as viable, and the normals are then assumed to be t^w/t^w, an assumption which can be tested by mating them with $T/+$. If the assumption is correct this cross should yield only tailless (T/t^w) and normals ($+/t^w$) and never any Brachys ($T/+$). Inbred stocks, each containing a different wild allele $T/t^{w1\cdots n}$ are then set up and different balanced lethal lines are then cross-tested with each other. If the cross $T/t^{w1} \times T/t^{w5}$, for example, yields both tailless (T/t^{w1} and T/t^{w5}) and normal offspring the two t^w alleles are assumed to be different and the normals are assumed to be t^{w1}/t^{w5}, an assumption which can be tested by mating them with Brachy as outlined above. If the cross $T/t^{w5} \times T/t^{w6}$ (for example) yields only tailless, then the alleles are assumed to be either the same, or to be lethal in compound. The latter assumption can only be tested by identifying as dead zygotes during embryogeny those assumed to be t^{w5}/t^{w6}. Viable alleles cannot be distinguished directly as the same or different by the above methods. Here recourse must be had to another criterion. Male compounds containing a lethal and one viable allele, e.g. t^0/t^{w2} (t^0 lethal, t^{w2} viable) may be completely sterile or quasi-sterile, while with a different viable allele, t^7, the compound is fertile (DUNN 1952). This test is so laborious that it is resorted to only in cases of great importance. The result is that for practical purposes viable alleles from different populations cannot be differentiated, and the tests are routinely applied only to lethal alleles.

RESULTS

Preliminary results of the analysis of the first four alleles found in wild populations have been published (DUNN and MORGAN 1953). Of the four alleles identified three were lethal and one viable. One of the lethals could not be distinguished from the domestic allele t^0, and was not studied further. Additional data on the first three are reported here together with studies of three new alleles identified in three new and different wild populations. The basic data on the seven alleles are set out in table 1.

TABLE 1[1]

Results of breeding analysis of T-alleles identified in wild populations

Allele	Source	Testcross wild $+/t^w \times T/+$			Tailless × Tailless $T/t^w \times T/t^w$			Tailless ♂ × normal ($T/t^w \times +/+$)		Ratio t^w
		Normal	Brachy	Tailless	Tailless	Normal	Litter Size	Normal	Brachy	
t^{w1}	N.Y.1(1♂)	23	2	20	206	(10)*	2.90	262	49	.84
t^{w2}	N.Y.1(1♂)	80	3	54	355	235	5.67	319	15	.95
t^{w3}	Conn.1(1♀)	10	4	3	261	(3)†	3.77	379	4	.99
t^{w4}	Wisc.3(1♂)	16	1	10	19	—	4.75	187	5	.97
t^{w5}	N.Y.2(1♂)	5	1	2	368	1‡	3.77	446	30	.93
t^{w6}	Fla.2(1♂)	3	—	2	212	—	3.65	90	1	.99
t^{w7}	Tex.1(1♂)	24	5	17	75	71	4.86	212	15	.93

[1] We are indebted, for specimens collected and sent to us, to: DR. HOWARD SCHNEIDER (N.Y. 1); MR. W. C. MORGAN, SR. (Conn. 1); MR. CHARLES SOUTHWICK (Wisc. 3); MISS NAOMI FITCH (N.Y. 2); MR. SIDNEY HOLMAN (Fla. 2) and DR. W. FRANK BLAIR (Tex. 1).

* 10 dead at birth or stillborn, 9 with microcephaly.

† 3 stillborn, 1 with microcephaly.

‡ Viable exception, quasi-sterile male t^{w5}/t^x.

The results of testing the original wild heterozygote from which each allele was derived are shown in columns 3, 4 and 5. In all except one case this was a male, and the results show clearly the unequal ratio of offspring derived from the +gamete (15 Brachy) and from the t^w gamete (108 tailless). This is a constant feature of all male heterozygotes from wild populations and since it is usually apparent in the first litter, male heterozygotes will usually be diagnosed first and the new alleles extracted from them. The population samples tested were all small: 12 males in the case of N.Y. 1 of which seven were $+/t^w$ and five $+/+$; three females from Conn. 1 of which one was $+/t^w$; six males from Wisconsin (a composite sample from several sources near Madison) of which one male was from a population inbred in confinement from a wild source; this male was $+/t^w$; a single male from N.Y. 2 (W. 106th Street) which escaped after spending one night in the laboratory, during which time he mated with a Brachy female and proved to be $+/t^w$; 4 males from Florida 2 (Sarasota) of which one proved to be $+/t^w$; six males and four females from a Texas population (near Austin) of which three males and one female were $+/t^w$. In this case the alleles extracted from all four heterozygotes were tested and behaved alike, as viable when homozygous. We assumed all four to be the same allele and saved only that derived from the first male. One population (N.Y. 1) yielded two different alleles, one lethal and one viable; but this population was of composite origin, descended from a mixed sample from New York City and Philadelphia, Pa., which had been inbred for eight years in the laboratory of DR. HOWARD SCHNEIDER of the Rockefeller Institute for Medical Research before we tested it. Although this is the only case in which two different alleles were found in the same sample, the numbers of animals tested from other samples were too small to give any estimate of the likelihood that single populations contain more than a single allele. Larger samples from additional populations are now being tested with this important point in mind.

The data are also inadequate to give good estimates of the frequency of heterozygotes in wild populations. Combining the above data with those from five additional populations in which heterozygotes have recently been found leads to an estimate of about 50 percent as the average frequency of heterozygotes in such populations (DUNN 1955).

The results of inter-se matings of tailless offspring derived from single wild heterozygotes are shown in columns 6, 7 and 8. The alleles t^{w2} and t^{w7} are diagnosed as viable on the basis of the inter-se matings and on progeny tests of the normals both from such matings, which prove to be t^{w2}/t^{w2} and t^{w7}/t^{w7} respectively. Females of these genotypes produce only tailless (T/t^w) and normal t^w/t^w when tested by Brachy ($T/+$) and never give $T/+$ offspring. Males homozygous for either of these alleles are generally sterile although we have found one quasi-sterile male of genotype t^{w2}/t^{w2}.

Alleles t^{w1}, t^{w3}, t^{w4}, t^{w5} and t^{w6} are diagnosed as lethals on the behavior of the tailless lines T/t^w as balanced lethal systems. Matings of T/t^{w1} produced in the early generations 10 exceptional animals, all dead at birth or stillborn, nine of them abnormal with small heads and failure of development of the eyelids. This suggested that they might be t^{w1}/t^{w1} and that death of such homozygotes might occur near the time of parturition. No such exceptions have been noted in the last six generations and the point has not been investigated embryologically. Three similar exceptions occurred in the first two generations of T/t^{w3} matings but none since. Matings of T/t^{w5} produced a single exceptional normal male in the sixth generation. This male was quasi-sterile, probably of genotype t^{w5}/t^{wx}, although the new allele assumed to have arisen by mutation (cf. similar cases in DUNN and GLUECKSOHN-WAELSCH 1953) has not yet been isolated. Matings of T/t^{w6} have given only the results expected from a balanced lethal line.

Comparison of litter sizes confirms the above diagnosis. The average size of 301 litters from balanced lines containing lethal t^w alleles was 3.58. The average size of 134 litters from lines containing viable t^w alleles was 5.49. If we assume the former were reduced before birth by 50 percent (two lethals) and the latter by 25 percent (one lethal, T) then the unreduced litters in the first case would average 7.16, and in the second 7.32, i.e. comparable sizes.

Segregation ratio abnormality in wild heterozygotes

One of the most striking features of the results of testing wild populations is the very unusual outcome of all test crosses of $+/t^w$ males by $T/+$ females. The expected results are, of course, 50 percent normal ($+/+$ and $+/t^w$) 25 percent Brachy $T/+$, and 25 percent tailless T/t^w, and these results are obtained when $+/t^w$ *females* are tested by $T/+$. But when $+/t^w$ *males* are tested, the ratio of Brachy to tailless, which expresses the segregation ratio of $+$ to t^w gametes, is never 1 to 1. From tested male wild heterozygotes the ratio of Brachy to tailless was 16 to 108; that is, about 87 percent received the t^w allele.

That the ratio is a genotypic one, rather than due to some phenotypic disturbance, was shown in one case by testing all of the available normal offspring of one $+/t^{w2}$ male which had been mated to $T/+$ females. Out of 46 tested, 42 (91%) proved to

be $+/t^w$, showing that the abnormal ratio found between the Brachy and tailless classes also obtains within the phenotypically normal class.

The chief evidence on segregation ratios of alleles derived from the wild consists of the results of testcrosses of tailless males T/t^w by normal $+/+$ females from our standard inbred ($T/+$) stock (table 1, columns 9, 10 and 11). This cross produced two phenotypic classes, normal and Brachy. The former outnumber the latter by about 9 to 1. Large samples of the normal-tailed class have been progeny-tested and always prove to be $+/t^w$. We have, therefore, assumed that there is an abnormality either in the segregation mechanism by which T and t^w sperm are produced, or by which these two types of sperm function in fertilization to very different degrees. For the purposes of a first analysis we assume the former; namely, that the gametic ratio of t^w to T is abnormal, and that t^w sperm are produced in great excess. The ratio of t^w to T varies somewhat in different males (as deduced from progenies) but is always in excess of .8 and is generally over .9.

It is not clear from the present evidence whether variations in the ratios are determined by the properties of the individual alleles, or whether the ratios are affected by factors external to but acting on the t-alleles. These hypotheses can be tested first by comparing the ratio abnormalities of a series of different alleles when placed in the same isogenic background; second by comparing the ratios of the same allele in different genotypic backgrounds. These tests have not been made for the wild alleles. Partial studies of some domestic alleles give some indication that each allele has its characteristic ratio (DUNN and GLUECKSOHN-WAELSCH 1953) which, however, may be modified somewhat by selection. The fact that all alleles in wild populations give extremely aberrant ratios in favor of t^w suggests that other factors in the wild genotypes, which tend to maximize this ratio, have some selective advantage under wild conditions; and, thus, that the ratio abnormality itself or the great excess of heterozygotes to which it leads, is advantageous. It may be that effects of t-alleles on the meiotic mechanism itself, of which the ratio change is the expression, is the property responsive to natural selection.

In the wild populations, the ratio abnormalities actually found must confer an enormous advantage on the t^w alleles as compared to the normal alleles. This is sufficient to counterbalance the selection against the t^w lethals and the t^w viable alleles which are male sterile (PROUT 1953). The equilibrium values to be expected from different values of the ratio factor, when opposed by selection due to lethality, are being worked out.

Preliminary results (to be published elsewhere) indicate that for a segregation ratio advantage of about .8, the equilibrium value for t^w heterozygotes is near to 50 percent and this is about the average value found in samples from the first 12 wild populations tested (DUNN 1955). However, these computations have been based on random mating in large populations, whereas wild populations are probably small and hence inbred; and theory has still to be developed for such models.

The cause of this ratio abnormality is not known. One hypothesis being tested is that in spermatogonial stages of $+/t^w$ males, t^w induces a mutation or conversion of the $+$ allele to t^w, resulting in a mosaic gonad consisting of large sections t^w/t^w and smaller sections $+/t^w$. Other hypotheses are, of course, possible, but those upon which

some evidence has been obtained, such as post-reduction reduplication of t^w cells, have not seemed promising (DUNN and GLUECKSOHN-SCHOENHEIMER 1939). It must not be forgotten that some of the *t*-lethals may be connected with a chromosomal rearrangement and this may introduce irregularities into spermatogenesis. Too little is known of the cytogenetic situation in this chromosome to permit evaluation of this possibility.

Identity of lethals from different wild populations

A question of primary importance is whether different alleles are found in different wild populations and whether the wild alleles differ as a group or individually from any of the alleles which have arisen under observation in the domestic stocks of the laboratory which have been shown to be of mutative origin. Of the latter, eleven have been studied as follows: *T*, a lethal with dominant effect on the tail; t^0, t^1, t^4, t^9 and t^{12} are lethals shown to be different from each other, since they form viable compounds with each other; t^3, t^7, t^8 and t^{13} are viables of which t^3 is probably different from t^7 and t^8, the other relations being untested.

All seven of the wild alleles have been tested by t^0 and t^1. Of the lethals, only t^{w4} gives no evidence of being different from t^0 by the compound viability test. Fifty offspring from $T/t^0 \times T/t^{w4}$ were tailless; so the compound t^0/t^{w4} may be lethal. Of intercrosses among wild lethal alleles only t^{w5} and t^{w6} have failed to produce viable compounds at birth. Embryonic stages of intercross hybrids of $T/t^{w5} \times T/t^{w6}$ are now being examined. The cross $T/t^{w1} \times T/t^{w3}$ has not yielded sufficient offspring to permit a decision on these. At present it can be said that t^{w1} is different from t^{w2}, t^{w4}, t^{w5}, t^{w6} and t^{w7}; t^{w2} is different from all others except possibly t^{w7}, the other viable allele; t^{w3} is different from t^{w2}, t^{w4}, t^{w5} and t^{w7}; t^{w4} has been insufficiently tested and because of its similarity to t^0 has been discarded; t^{w5} is different from t^{w1}, t^{w2}, t^{w4} and t^{w7}; t^{w6} is different from t^{w1}, t^{w2} and t^{w7}.

It is clear that there is a variety of alleles in different wild populations and that most of these are different from at least two of the domestic alleles.

THE MANNER OF ORIGIN OF WILD t^w ALLELES

The seven alleles which were observed at origin in laboratory stocks all arose as exceptions from the balanced lethal line T/t^1; similar exceptions have been observed in the line T/t^0 but were either lost or were sterile (DUNN and GLUECKSOHN-WAELSCH 1953). A similar exception was found in line T/t^{w5}. This was a male, quasi-sterile as some other analysed exceptions have been and hence compatible with the hypothesis that it was t^{w5}/t^{wx}. Because of the sterility, no line has yet been derived from this exception so the hypothesis is untested. The fact that all new alleles observed at origin were from lines which already contained a *t*-allele, suggests that they arose by mutation either from a pre-existing *t*-allele, or were induced by the *t*-allele in the partner chromosome. The latter hypothesis is being tested in an experiment now in progress. No evidence of recombination in these balanced lethal lines has been found (DUNN and GLUECKSOHN-WAELSCH 1953).

If the origin of a new *t*-allele thus depends on the prior presence of a *t*-allele, how are we to explain the origin of the first one which induced the later ones, and how are

we to explain the multiplicity of different alleles in the wild populations? It is a noteworthy fact that no case of origin of a new *t*-allele has been observed in the Brachy ($T/+$) stock which has been inbred in the laboratory in large numbers since 1932. A few exceptional tailless animals have been found in this line but all of those tested have proved to be $T/+$, probably with modifiers at other loci. Mutation of either T or $+$ to t would be readily detected in this stock. If T and $+$ are relatively stable alleles, while the combination T/t is unstable, the cause of instability is probably connected with the nature and effects of *t*-alleles.

In the wild populations in which $+/t^w$ heterozygotes are found we may infer that t^w has arisen either by mutation of $+$, of which no instance has been found, or more probably that the t^w allele has arisen from an antecedent t^w in that population or in an ancestral one. Some chainwise connection between t^w alleles is thus suggested and with it a possible method for tracing relations between populations. This would only be valid if it could be shown that $+$ alleles from populations shown not to contain a t^w allele are in fact stable. This point is being tested by crossing such $+/+$ animals from wild populations by $T/+$, attempting to detect changes from $+$ to t^w. At the rate of mammalian reproduction, even in mice, this will require a long time.

SUMMARY

Evidence is presented on the breeding behavior of seven alleles t^{w1}–t^{w7} at locus T in the house mouse, each allele being derived from a single heterozygote caught in a wild population. Five of the alleles were lethal before birth, two formed viable homozygotes. All alleles produced a tailless phenotype when combined with the mutant T and none showed recombination with T.

Each allele tested showed normal segregation in T/t^w and $+/t^w$ females, but T/t^w and $+/t^w$ males always produced a great excess of offspring which inherited the t^w allele. This is attributed to an abnormality in the segregation mechanism leading to ratios of t^w sperm from .8 to .99, usually above .9. This gives the t^w alleles a great advantage and permits the lethals to be maintained in wild populations, in which up to 50 percent of individuals are heterozygotes. There may be in addition a selective advantage accompanying the heterozygote state.

Lethal alleles occurring in different wild populations were shown in several cases to be different alleles, compounds with different alleles such as t^{w3}/t^{w5} being viable. Several of them were also shown to be different from alleles previously found to have arisen by mutation in balanced lethal lines maintained in the laboratory (domestic alleles t^0–t^{13}). A considerable variety of allelic transformations at this locus is thus indicated.

The causes of the segregation ratio abnormality and the manner of mutation at this locus are unknown but it is suggested that they are connected, possibly through the induction of changes in the normal allele by an existing *t*-allele.

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