LETHAL ALLELES IN A WILD POPULATION OF

MUS MUSCULUS (HOUSE MICE)

by

Andrew Bowie Beasley

Thesis submitted to the Graduate Faculty of the
Virginia Polytechnic Institute
in candidacy for the degree of
MASTER OF SCIENCE
in
ZOLOGY

APPROVED:

E. C. Randolph
Director of Graduate Studies

J. D. Wilson
Head of Department

J. E. Miller
Dean of the School of Applied
Science and Business Administration

Major Professor

May 14, 1956
Blacksburg, Virginia
CONTENTS

I.  INTRODUCTION  
    Page 4

II. MATERIALS  
    8

III. PROCEDURES AND RESULTS  
    9

IV. DISCUSSION AND ANALYSIS OF RESULTS  
    14

V. CONCLUSIONS  
    17

VI. SUMMARY  
    18

VII. ACKNOWLEDGEMENTS  
    19

VIII. REFERENCES  
    20

IX. VITA  
    21
<table>
<thead>
<tr>
<th>Table</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Results of Mating Brachyury Females (T/+)&lt;br&gt;with Wild Males (+/?).</td>
<td>10</td>
</tr>
<tr>
<td>2</td>
<td>Results of Mating Tailless Females (T/t&lt;sup&gt;W&lt;/sup&gt;)&lt;br&gt;with Tailless Males (T/t&lt;sup&gt;W&lt;/sup&gt;).</td>
<td>11</td>
</tr>
<tr>
<td>3</td>
<td>Results of Mating Normal Tail Females (+/+)&lt;br&gt;with a Tailless Male (T/t&lt;sup&gt;W&lt;/sup&gt;).</td>
<td>12</td>
</tr>
</tbody>
</table>
I

INTRODUCTION

Some of the goals of genetics are to acquire information as to changes (mutations) that occur in genetic material, to study the influence of these mutations on organisms and to observe the fate of the mutation in a species. These desired goals are often difficult and time consuming to reach in man and many economically important mammals, because it is necessary to evaluate several generations and observe large numbers of offspring before valid conclusions can be made. These factors have caused most population geneticists to conduct experiments with various species of the genus *Drosophila*. In this study, *Mus musculus* (the house mouse) was used because it satisfies these requirements and it has the advantage of being a mammal. Thus, acquired information can be more accurately correlated to other important mammals that are not good laboratory animals.

The primary objectives of this investigation were to sample a wild population of *Mus musculus* in Virginia and to report on the nature of the genetic material located at a specific point on the ninth chromosomal linkage group in this species. This point is referred to as the "t" locus because of the phenotypic influence of the genetic material there upon the tail of the mouse. The normal allele at this locus is indicated by the symbol "+". Mutations at the locus are designated by the upper-case
letter "T" if the mutation is dominant to the normal (+) or by the lowercase letter "t" if the mutation is recessive to the normal. It has been reported that many different recessive mutations occur at this locus (Dunn and Glueckshon-Wealsch, 1953). These mutations are referred to in general as "tX", each specific one being named "t" with a numerical superscript, that is, tX equals t1, t2, t3 and so forth.

In addition to its polymorphism, this locus is of particular interest because reports of other investigators (Dunn and Morgan, 1953b; Dunn, with Appendix by T. Prodt, 1953) indicate that (1) combinations of the mutations in the homozygous form, i.e., T/T, t1/t1, etc. are generally lethal; (2) combinations of different mutations (heterozygotes), i.e., T/t1, T/t2, t1/t2, etc. are viable and "T" in combination with any of the recessive mutations (tX) result in tailless animals with the genotype T/tX but combinations of the recessive mutations result in normal tail animals; and (3) the heterozygous males do not produce gametes in a 1:1 ratio as would be expected according to Mendel's first law of genetics.

The first mutation at this locus was discovered and reported in 1927 (Dobrowolskaia-Zavedtskaia, 1927). It produced a reduction in the tail to 1/2-7/8 of the normal length. The phenotype was named "Brachyury" and the gene symbol "T" was applied to it. A strain of mice with this mutation has been maintained since that time. Crosses of Brachyury females (T/+) with Brachyury males (T/+) indicated that the T/T genotype was lethal and exerted its influence during embryonic development. The progeny obtained from these crosses were of two classes, Brachyury and normal, in the ratio of 2(T/+) : 1(+/+). Embryonic investigations showed that about
1/4 of the embryos, presumably those of the genotype T/T, died during the 10th day of embryonic development as a result of influences on the notochord and allantois (Chesley and Dunn, 1936; and Dunn and Gluecksohn-Schoenheimer, 1939). A second mutation at this locus was detected when a tailless offspring appeared in the Brachyury lines, and genetic analysis showed it to be of the genotype T/t. Further analysis showed that the genotype t/t was also lethal, but it differed from the first mutation in that it was lethal at a different period of embryonic development and the gene was recessive to the normal (Chesley and Dunn, 1936; Dunn and Fleucksohn-Schoenheimer, 1939). Since the tailless animal was viable, a balanced lethal line was established. The principle of the balanced lethal line is illustrated by the following diagram:

```
Tailless Female (T/t) X Tailless Male (T/t)
```

```
<table>
<thead>
<tr>
<th>T/T</th>
<th>T/t</th>
<th>t/t</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lethal</td>
<td>Viable</td>
<td>Lethal</td>
</tr>
<tr>
<td></td>
<td>Tailess</td>
<td></td>
</tr>
</tbody>
</table>
```

Ratios: 1 : 2 : 1

In the maintenance of this balanced lethal line, 11 exceptions to the expected tailless progeny were obtained from a total progeny count of 3462 (Dunn and Gluecksohn-Waelsch, 1953). The exceptions had normal tails. When seven of the animals were analyzed, it was found that they were of the genotype t/t\(x\). Detailed analysis showed that in each case, the exception was a new and different mutation, i.e., \(t^x\) equals \(t^2\), \(t^3\), \(t^4\), etc.

Such a high mutation rate at this locus led investigators (Dunn and Morgan, 1953a) to consider the possibility that this was a feature peculiar to the strain of mice used in the investigations. To test the hypothesis, 10
wild male mice from a confined population were mated with Brachyury females (T/+) of this strain. The intent of this experiment was to determine the mutation rate at this locus in the wild males, but this could not be accomplished, since five of the eight males that produced offspring were apparently of the genotype +/t^W (Dunn and Morgan, 1953a). This high frequency of heterozygous animals from the wild population changes the plan of the experiment to determining the extent of the "t" alleles in different wild populations.

At the beginning of the present investigation, ten wild populations had been tested in the United States (Dunn, 1954). Seven of these populations were found to include mice heterozygous at the "t" locus and the other three populations were negative. Five of the wild alleles were lethal and two were viable when homozygous.

All of the mutants from wild populations have been designated "t^W", the specific alleles being named t^W1, t^W2, etc.
II

MATERIALS

The materials used in this study were wild mice, a laboratory strain of tester mice, and mice cages with receptacles that gave the animals a constant supply of food and water. The wild house mice were captured on November 18, 1954, December 27, 1954, and January 22, 1955 in a grain barn on the farm of Andrew Beasley near Runford, Virginia. The laboratory strain of tester mice, Brachyury (T/4), was obtained from Dr. L. C. Dunn of Columbia University, New York. Mice with normal tails with the genotype +/+ were also used in this investigation. These animals were obtained from litters produced by Brachyury pair-matings, i.e., T/+ (Brachyury) x T/+ (Brachyury) produces 1/4 T/T (lethal); 2/4 T/+ (Brachyury); 1/4 +/- (normal).
The captured wild male mice were segregated and mated individually to females of the Brachyury tester strain, and the wild females were placed in a single cage with a tester male. If a wild animal were heterozygous at the "t" locus, the first generation of progeny, the $F_1$, would be partially indicative of this fact. The principle of the test cross is illustrated by the following diagram:

\[
\text{Brachyury (T/+)} \times \text{Normal Wild (+/?)}
\]

<table>
<thead>
<tr>
<th>Genotypes:</th>
<th>T/+</th>
<th>T/?</th>
<th>+/+</th>
<th>+/?</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ratios:</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
</tbody>
</table>

If the wild animal tested were homozygous +/+ (t equals +), the progeny would be of two classes, Brachyury (T/+) and normal (+/+). If the animal were heterozygous +/t\(^W\) (t equals t\(^W\)), the progeny would be of four genotypes and three phenotypes, Brachyury (T/+), Tailless (T/t\(^W\)), and normal (+/+ and +/t\(^W\)). Of the 11 mice captured, five were females and six were males. One of the males and two of the females died in captivity, and the remaining females did not produce any progeny. Wild females rarely breed in captivity unless their oestrus cycles are stimulated by exercise and at the beginning of this experiment the proper type of activity wheels were not available to give the females exercise. The remaining five wild males did produce and the results are shown in Table 1.
Table 1

<p>| Animal Number of  | Progeny |     |     |</p>
<table>
<thead>
<tr>
<th>Wild Male</th>
<th>Brachyury</th>
<th>Tailless</th>
<th>Normal</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>9</td>
<td>15</td>
<td>2</td>
</tr>
<tr>
<td>2</td>
<td>3</td>
<td>14</td>
<td>13</td>
</tr>
<tr>
<td>6</td>
<td>11</td>
<td></td>
<td>10</td>
</tr>
<tr>
<td>10</td>
<td>12</td>
<td></td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>6</td>
<td></td>
<td>2</td>
</tr>
<tr>
<td>Totals</td>
<td>41</td>
<td>15</td>
<td>34 110</td>
</tr>
</tbody>
</table>

The results shown in Table 1 indicate that of the five males tested, four (animals number 1, 6, 10 and 11) were probably of the homozygous normal genotype (+/+), and one (animal number 2) was heterozygous (+/tW).

To further test the nature of the genetic material located at the "t" locus of the heterozygous wild male, the tailless progeny were allowed to reach maturity and were pair-mated. If the pair-mating produced only tailless offspring, it could be concluded that the tW mutation was lethal to the organism before birth. If, however, both tailless and normal tail progeny were obtained, it could be concluded that the homosygote for this mutation was not lethal. The principle of the crosses is given in the following diagram:

```
Tailless Female (T/tW)  X  Tailless Male (T/tW)
```

```
T/T  T/tW  tW/tW
Lethal  Tailless  Normal Tail if Viable
```

Thus, if the homosygote tW/tW is lethal, only tailless offspring are expected; but if the homosygote is viable both tailless and normal offspring would appear. The results of the pair-matings are given in Table 2.
Table 2
Results of Mating Tailless Females (t^W/t) with Tailless Males (T/t^W)

<table>
<thead>
<tr>
<th>Progeny</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Tailless</td>
<td>25</td>
</tr>
<tr>
<td>Normal</td>
<td>12</td>
</tr>
</tbody>
</table>

These results indicate that the mutant "t^W" allele carried by the heterozygous wild male in the Virginia population was not lethal in the homozygous condition.

Data concerning the ratios of gametes produced by a male heterozygous for the "t" locus were obtained by mating a tailless male (T/t^W) with homozygous normal tail females (+/+), obtained from Brachyury pair-matings. Only one heterozygous male was tested since a limited number of the tester females (+/+) was available. From such a cross the ratio of the T:t^W gametes produced by the heterozygous male is indicated by the phenotypes of the resulting progeny, as illustrated by the following diagram:

Normal Tail Female (+/+) X Tailless Male (T/t^W)

\[ \text{Brachyury (T/+)} \rightarrow \text{Normal Tail (+/t^W)} \]

Ratio Expected: 1 1 1

Thus, if there is any disturbance in the expected 1:1:1 ratio of gametes produced by the heterozygous male, it could be detected by an appropriate statistical test. No reciprocal crosses were made to test the segregation ratios in heterozygous females because the time required to accumulate such data from a female is much greater than from a male. A male can sire many more offspring than a female can produce in a like period of time. The results of the ratio test are given in Table 3.
Table 3
Results of Mating Normal Tail Females (+/−) with a Tailless Male (T/tW).

<table>
<thead>
<tr>
<th>Tester Female</th>
<th>Progeny</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Brachyury</td>
<td>Normal</td>
</tr>
<tr>
<td>1</td>
<td>1</td>
<td>4</td>
</tr>
<tr>
<td>2</td>
<td>1</td>
<td>9</td>
</tr>
<tr>
<td>3</td>
<td></td>
<td>6</td>
</tr>
<tr>
<td>4</td>
<td>1</td>
<td>9</td>
</tr>
<tr>
<td>5</td>
<td></td>
<td>8</td>
</tr>
<tr>
<td>6</td>
<td></td>
<td>8</td>
</tr>
<tr>
<td>7</td>
<td></td>
<td>7</td>
</tr>
<tr>
<td>8</td>
<td></td>
<td>4</td>
</tr>
<tr>
<td>9</td>
<td></td>
<td>9</td>
</tr>
<tr>
<td>10</td>
<td>1</td>
<td>6</td>
</tr>
<tr>
<td>Totals</td>
<td>3</td>
<td>70</td>
</tr>
</tbody>
</table>

These data represent a highly significant deviation from the expected 1:1 ratio.

In studying this mutation further, several preliminary observations beyond the scope of this thesis may be mentioned:

A. From the tailless by tailless matings, the normal tail homozygous animals (tW/tW) were allowed to reach maturity and were mated to animals of known fertility. No progeny were obtained from four females over a period of three months and no progeny were obtained from three males in individual mating cages with five, four and three mates over a period of two months.

B. A tailless male (T/tW) was mated to tailless females (T/tW) from a balanced lethal line and only two tailless offspring were
obtained. Both progeny were stillborn.

C. A tailless male (T/t\textsuperscript{w}) was mated to tailless females with the genotype T/t\textsuperscript{w7}(t\textsuperscript{w7} being another viable wild "t" allele).

Both tailless and normal tail progeny were obtained.
IV

DISCUSSION AND ANALYSIS OF RESULTS

The results from five tested wild male house mice taken from a Virginia population demonstrate that one of the animals was heterozygous (+/t^W) at the "t" locus. This fact adds to the findings of other investigators (Dunn, 1954) that mutant alleles at this locus are present in many widely distributed populations of house mice. This is the eighth allele to be found at the "t" locus in a population of wild house mice and it is named t^W8. This is not meant to imply that t^W8 is different from the two other viable alleles (t^W1 and t^W7) since tests have not been completed to prove it to be different.

The recessive mutation discovered in the Virginia population is different from five of the seven previously discovered wild mutations at the "t" locus in that it is viable in the homozygous condition. The sterility of the homozygotes indicate however, that the mutation is selected against, and in the last analysis, this is equivalent to its being lethal. The question then arises as to what factors are responsible for maintaining the genes in a wild population in apparent high frequencies.

Geneticists have considered only four factors as being responsible for any change in the gene frequencies of a population. These factors are mutation, selection, migration, and chance variation. Unequal production of gametes by an organism has never been considered as a factor to cause a change in gene frequency since such considerations would represent contradictions to Mendel's basic law of genetics.
The results of this experiment suggest that there is an unequal segregation in the gametes from male animals heterozygous at the "t" locus. The numbers of the three classes of progeny obtained from the wild heterozygous male, 3 Brachyury; 15 tailless; 14 normal tail, give an early indication of a disturbance of gamete production by a heterozygote. The theoretical expectation is that of the 32 progeny obtained, eight would be Brachyury, eight would be tailless, and 16 would be normal tail (see diagram on page 9 above). Using the $X^2$ test, the results obtained are considered significant since the chance probability of such an occurrence is less than .01. There is a deficiency in the number of Brachyury and an excess in the number of tailless animals. The ratio of Brachyury to normal tail progeny obtained from mating the normal tail females (+/+) with the tailless male (T/t^wG) as shown in Table 3 adds to this conclusion. The observed results of 3 Brachyury; 70 normal tail progeny clearly demonstrate that the normal tail progeny are in excess. A 1:1 ratio was expected in this case and the chance probability of such an unusual occurrence by chance is less than 0.0001 ($X^2$ test). In each case the t^wG alleles are significantly in excess of the theoretical expectations. In the first cross the t^wG gametes are greater than the + gametes and in the second case the t^wG gametes are greater in number than the T gametes. Similar segregation ratio deviations for other "t" alleles have been reported and the equilibrium frequencies for such alleles under certain conditions have been calculated (Prout, 1953).

The results from the pair-matings of the tailless animals as given in Table 2 would indicate that there is no deviation from the normal segregation and it could be expected that there would be some indication of
abnormal segregation since the genotypes of the tailless males (T/t<sup>w3</sup>) is identical with that of the animal used in the segregation ratio test. Two possibilities can be advanced to explain these results: 1) partial lethality of the t<sup>w3</sup>/t<sup>w3</sup> which exerts its influence before birth and 2) chance variation. The first possibility is considered to be the probable answer since the litters from the tailless pair matings were small (approximate average litter size was five progeny); yet, if the segregation in the tailless male is approximately 5 gametes:95 t<sup>w3</sup> gametes the results can be explained by a slight chance deviation in the tailless females. The $X^2$ value for the results in Table 2 is 3.90 if the segregation in females is considered to be 1:1 and 95:5 in males and at the 5% significant level the $X^2$ value is 3.84.

An interesting hypothesis has been made to explain this abnormal segregation (Dunn and Gluecksohn-Schoenheimer, 1939). Dunn and Gluecksohn-Schoenheimer interpreted their findings of abnormal segregation as being due to an effect of the "t" allele on segregation to cause additional equational divisions in spermatogenesis.

This segregation ratio disturbance of gametes may also give an answer as to the reason for the unexpected high frequency of some deleterious alleles found in human populations (Dunn, 1953). For example, the frequency of the sickling and of the thallassemia alleles is greater than the theoretical equilibrium values (Heel, 1951a and 1951b; Silvestroni, 1950).
CONCLUSIONS

Experiments with a sample of Mus musculus (house mice) taken from a population in a grain barn near Rumford, Virginia, lead to the conclusions that this population contains a mutant allele at the "t" locus on the ninth chromosomal linkage group.

This mutant is recessive to the normal chromosomal material at this locus. Heterozygous $+/t^N$ animals appear normal and $T/t^N$ animals are tailless.

The discovered mutation is the eighth such allele found in wild populations of Mus musculus. This allele differs from five of the seven previously reported alleles in that it is viable in the homozygous condition ($t^w^3/t^w^3$).

A very significant deviation from the normal segregation ratio of testcross progeny of heterozygous male animals is demonstrated. In each case the $t^w^3$ gametes appear to function in excess of expectations.
Tests made on a sample of wild *Mus musculus* (house mice), taken from a Virginia population, show that the population contains an allele that is not considered a normal allele at the "t" locus on the ninth chromosomal linkage group. The presence of this mutant allele was demonstrated by crossing the wild animals with a tester strain of known genetic constitution. These findings add to the conclusions of other investigators that such alleles are present in many wild populations of house mice.

Data are presented to show that the newly discovered allele is recessive to the normal genetic material. This mutation in combination with "T", the first mutation discovered at this locus in 1927, produces tailless animals (*T/t^{WS}*). This mutation is designated *t^{WS}* since it is the eighth such allele to be found in wild populations. It appears from the data that there is an unequal segregation of gametes from males heterozygous at this locus (*+t^W* and *T/t^W*). The *t^W* gametes appear to be in excess of the theoretical expectations.

No explanation can be given to satisfactorily explain these aberrant segregation ratios. If they are caused by non-Mendelian segregation, i.e., unequal formation of gametes by the heterozygote, geneticists would have to consider it as a factor that would change the gene frequency in a population. If these unusual findings are caused by abnormal chromosomal conditions it may be that information about the mechanisms involved can be obtained from cytological investigations of the gonads.
The writer is indebted to Dr. Max Levitan for stimulating an interest in genetics, for continuous assistance, for guidance and for advice that has made this study possible; to Dr. I. D. Wilson for his guidance and encouragement in the study of a biological science; to Dr. I. C. Dunn of Columbia University, New York, for presenting an opportunity to explore unknowns in genetics by his interest, advice and supplying of materials necessary for this study; to Dr. Darwin E. Morby for his assistance and suggestions on this report; and to the Virginia Polytechnic Institute for providing facilities during the experiment.
REFERENCES


IX
VITA

I was born on September 7, 1931 near the former Post Office of Upper Zion, Caroline County, Virginia. I began my formal education in the public schools of this county at the age of six. In 1942, I moved with my family to King William County near Dumfords, Virginia. In 1948, I was graduated from King William High School and enrolled at the Virginia Polytechnic Institute. There, I took a course of study as outlined by the Biology Curriculum and was graduated with a Bachelor of Science degree in Biology in 1952. In August 1952, I was called to active duty in the armed services. I performed duties with the Air Force at several locations in the United States and in Korea. Returning from Korea in 1954, I was released from active duty with the rank of First Lieutenant, United States Air Force Reserve. In September 1954, I again enrolled at the Virginia Polytechnic Institute to study for the Master of Science degree in Zoology. At the present, I am Research Assistant to Dr. L. C. Dunn at the Nevis Biological Station of Columbia University and I am studying toward the degree of Doctor of Philosophy at Columbia University.

Andrew B. Beasley

May 14, 1956