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# *EVIDENCE OF EVOLUTIONARY FORCES LEADING TO THE SPREAD OF LETHAL GENES IN WILD POPULATIONS OF HOUSE MICE**

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A population changes in an evolutionary sense when there is a shift prevailingly in one direction of the proportions of genotypes of which it is composed. It is for this reason that agencies tending to change these proportions, derived primarily from the relative frequencies of gene forms or alleles, are referred to as "evolutionary forces."

Genotypic diversity, the usual condition of all cross-fertilizing populations in nature, tends to remain constant unless the gene frequencies are altered by one or more of these evolutionary forces. For any one pair of alleles, such as $A$ and $a$, with frequencies $q$ and $1 - q$, the constant or equilibrium frequencies of genotypes under random mating have long been known to be of the form $q^2AA + 2q(1 - q)Aa + (1 - q)^2aa = 1$.

Agencies hitherto recognized as leading to alteration of the equilibrium frequencies are (1) mutation pressure, changes from $A$ to $a$ and $a$ to $A$ at unequal rates; (2) natural selection, that is, unequal fitness or adaptive values of the several genotypes, such as lethality or lesser fertility of $AA$, $Aa$, or $aa$, relative to the others; (3) random fluctuation of gene frequencies in small sections of the population (random genetic drift); (4) differential migration of genotypes into or out of the population.

Equality of the genotypes in all these respects is of course the condition for maintenance of constant proportions.

The main purpose of this report is to present some new facts indicating that a fifth evolutionary force is primarily responsible for the maintenance of high frequencies of certain lethal genes in populations of wild house mice and to examine the effect of this force on equilibrium frequencies of such lethals. This fifth force I refer to as segregation ratio, since it alters the basic Mendelian proportion of functional gametes produced by heterozygotes $Aa$. In all the above equilibrium com-

putations this ratio is assumed to be 0.5; actually, in the cases I shall present, it averages in one sex (males) 0.95 to 0.05, in favor of the gametes transmitting the lethal, leading to an enormous advantage of a gene against which complete selection in homozygous condition is exercised, since all such homozygotes are eliminated before birth. The gene is thus enabled to spread against what might be called "evolutionary gravity."

The facts are as follows. Many, probably most, wild populations of the house mouse—*Mus musculus*—in the United States, living either near dwellings or in nature, contain animals heterozygous for variant alleles at one locus *T*. Such heterozygotes are detected by bringing wild mice into the laboratory and mating them with animals containing a dominant marker allele *T* which by itself produces a short tail. Wild mice containing a recessive at this locus produce in such crosses a unique class of offspring—tailless—which, when bred together, give rise to a true-breeding tailless line of genotype $T/t^w$, $t^w$ being a recessive lethal allele of *T* (from a wild heterozygote) which interacts with *T* to produce taillessness. In a few cases $t^w$ alleles have proved to form viable homozygotes $t^wt^w$, which, however, are male-sterile, so these, too, have an adaptive value of zero in an evolutionary sense.

All tailless lines with a lethal *t*-allele maintain themselves as balanced lethal systems with litter sizes reduced by death of two classes of lethal homozygotes to half the normal size.[1] Seventeen such balanced lethal lines have been studied, each originating from a single heterozygote from a wild population. Data from fourteen such lines are shown in Table 1. The second and seventh lines represent the be-

TABLE 1

BALANCED LINES MAINTAINED BY MATINGS OF TAILLESS BY TAILLESS ($T/t \times T/t$)
(Each Allele Derived from a Single Wild Heterozygote $+/t$)

| ALLELE | POPULATION | OFFSPRING | | LITTER SIZE |
|---|---|---|---|---|
| | | Tailless | Normal | |
| $t^{w1}$ | N.Y. 1 | 399 | 13* | 3.3 |
| $t^{w2}$ | N.Y. 1 | 355 | 235 | 5.7 |
| $t^{w3}$ | Conn. 1 | 438 | 5* | 3.5 |
| $t^{w5}$ | N.Y. 2 | 605 | 2 | 3.6 |
| $t^{w6}$ | Fla. 2 | 345 | .. | 3.2 |
| $t^{w7}$ | Tex. 1 | 75 | 71 | 4.9 |
| $t^{w11}$ | Conn. 2 | 521 | .. | 3.6 |
| $t^{w12}$ | Calif. 1 | 418 | 1* | 3.9 |
| $t^{w13}$ | Tex. 2 | 161 | .. | 3.6 |
| $t^{w14}$ | Vt. 1 | 183 | 1 | 3.7 |
| $t^{w15}$ | Kan. 2 | 92 | 1 | 3.7 |
| $t^{w16}$ | Vt. 2 | 71 | 1 | 3.9 |
| $t^{w17}$ | Ariz. 1 | 59 | .. | 3.0 |
| $t^{w18}$ | From $t^{w11}$ | 92 | .. | 4.0 |

* Dead at birth; microcephalic.

havior of viable alleles; the other twelve lines, lethal alleles. The exceptional normal-tailed offspring in the first, third, and twelfth lines, which were dead at birth, represent survivors of the lethal homozygotes which die between the eleventh day and birth. The other exceptions are probably mutants in each of which a lethal $t^w$ allele—early lethals in these lines—has mutated to a different $t^w$ allele. This has proved to be the explanation in all cases analyzed. This, then, is the usual behavior of balanced lethal lines containing variant alleles at locus *T*, occurring in different wild populations.

We have now to ask why such alleles, most of them lethal, are maintained in wild populations. Lethals should be eliminated by natural selection. What preserves these lethals in high frequency?

Our first answer came from testing the ratios in which the two alleles + and $t^w$ or $T$ and $t^w$ are transmitted to the next generation. The transmission ratio in females proved to be normal, i.e., 0.5 of each allele. Male heterozygotes, however, transmit the variant $t$ allele to a great majority of offspring. The clearest proof of this is given in Table 2. Individual males $T/t^w$ were tested by normal females known to be of genotype +/+. From 90 to 99 per cent of the offspring were of genotype $+/t^w$ (phenotypically normal); from 10 to 1 per cent were of genotype $+/T$ (phenotypically short-tailed). Similar ratios were obtained from $+/t^w$ males.

TABLE 2

RESULTS OF TESTING SEGREGATION RATIOS OF TAILLESS MALES WITH ALLELES FROM DIFFERENT WILD POPULATIONS

(Progenies from $T/t^w$ ♂ × +/+ ♀ ♀)

| ALLELE | FROM POPULATION | OFFSPRING: Normal $+/t^w$ | OFFSPRING: Brachy $+/T$ | TOTAL | RATIO $t^w$ TOTAL |
|---|---|---|---|---|---|
| $t^{w1}$ | N.Y. 1 | 325 | 38 | 363 | 0.89 |
| $t^{w2}$ | N.Y. 1 | 319 | 15 | 334 | .95 |
| $t^{w3}$ | Conn. 1 | 383 | 3 | 386 | .99 |
| $t^{w4}$ | Wis. 3 | 187 | 5 | 192 | .97 |
| $t^{w5}$ | N.Y. 2 | 479 | 30 | 509 | .94 |
| $t^{w6}$ | Fla. 2 | 230 | 4 | 234 | .98 |
| $t^{w7}$ | Tex. 1 | 214 | 16 | 230 | .93 |
| $t^{w11}$ | Conn. 2 | 518 | 19 | 537 | .96 |
| $t^{w12}$ | Calif. 1 | 393 | 18 | 411 | .96 |
| $t^{w13}$ | Tex. 2 | 502 | 11 | 513 | .98 |
| $t^{w14}$ | Vt. 1 | 392 | 20 | 412 | .95 |
| $t^{w15}$ | Kan. 2 | 391 | 40 | 431 | .91 |
| $t^{w16}$ | Vt. 2 | 107 | 6 | 113 | 0.95 |

One of the striking features of these tests is the similarity of the transmission ratio in all alleles derived from the wild, which, from evidence I cannot cite here in detail, we know to contain a variety of different, nonidentical alleles. From previously published work[2] we know that different alleles, arising in laboratory stocks and not exposed to selection in nature, show in males a variety of departures from the usual segregation ratio in both directions. Two salient facts in which wild populations agree are therefore (1) presence of lethals at this locus and (2) asymmetrical transmission ratios always favoring the lethal alleles.

We turn now to examine the theoretical consequences of this latter peculiarity. A preliminary account was given in 1953.[3] By the construction of mathematical models (due in the first instance to Prout[3] and corrected and extended by David Bruck[4] in our laboratory) it is found that values of the segregation ratio exist sufficient not only to compensate for the decrement in gene frequency of $t$ due to death of lethal homozygotes but also to bring the frequency of $t$ to its theoretical limit of 50 per cent, that is, to the condition in which the whole population, at equilibrium, is heterozygous for a lethal. Bruck's[4] Figure 1 (p. 156) shows the relation at equilibrium between $m$, the segregation ratio in males, and gene frequency, $t$, under the conditions of (1) absence of mutation pressure, (2) random mating in an infinite closed population, (3) complete selection against lethal homo-

zygotes and no selection for or against heterozygotes. It is obvious from these relations, as it was a priori, that the frequency of $t$ is a function of $m$. The frequency of $+/t$, before selection against $tt$ (death of lethal homozygotes), reaches a curious maximum above 50 per cent, at about 56 per cent, at a value of $m = 0.96$, which is close to the observed average value of $m$ for the thirteen wild alleles tested. The biological meaning of this is not evident, and it may be coincidence. The frequencies of $+/t$ computed *after* selection reach a maximum at 100 per cent, the highest incidence attainable by a heterozygote for a lethal. The effect of the segregation ratio advantage by itself is sufficient to effect fixation, in the above sense, of a lethal in a wild population in which such an allele has occurred by mutation or has been introduced by migration.

We have begun experimental tests of the degree to which one of the assumptions on which the above equilibrium computations rest corresponds to conditions found in nature. Is it true that homozygous normals ($+/+$) and heterozygotes ($+/t$) are equivalent in fitness, i.e., have equal survival value? Starting with a population of wild mice in which the proportions of $+/+$ and $+/t$ are equal at birth, we find that at the time of major reproductive activity (from the end of the second to the end of the sixth month of life) such a population actually consists of about two-thirds $+/t$ to one-third $+/+$. The experimental figures are at present 42 $+/t$, 20 $+/+$, the deviation from equality being significant at the 0.05 level. Most of the deficiency of $+/+$ is probably due to early differential mortality. In a previous test[5] we found more sterile males among the $+/+$ class. The indications are that $+/t$ is superior in early life in viability and fertility. We have still to determine the total net contributions of the two genotypes to the next generation. If in this also the heterozygotes are superior, we shall conclude that the assumption of adaptive equality of the two surviving genotypes is invalid and shall have to correct for this in computing gene frequencies at equilibrium. Since it is probable that $t$ alleles are favored both by transmission ratio and by natural selection, both tending to increase the frequency and spread of lethal alleles, we shall have to seek for other agencies tending to restrain them and keep the populations in balance. Our attention is now turning to one of the other assumptions on which our present equilibrium computations are based, namely, unlimited population size. If the effective breeding sizes of populations in nature are very small, then we shall have inbreeding, with consequent increase in relative frequency of homozygotes. Since one homozygous class is lethal, this would tend to decrease the frequency of such alleles. The theoretical model for this has not yet been worked out, but a priori one would not expect the intensity of continuous inbreeding which would be required to produce effects comparable in magnitude to the positive effects of transmission ratio or of selection favoring heterozygotes.

We have also some preliminary data[6] indicating that mutation from $+$ to $t$ is not sufficiently frequent to generate a significant pressure toward increase of frequency of such alleles. The fourth parameter, differential migration, has not been measured at all.

*Discussion.*—In summary then, we may say that, of the evolutionary forces identified as acting to change the frequency of alleles at this one locus in nature, the newly recognized force—segregation ratio—is quantitatively the most important, with heterozygote advantage probably playing a secondary role but not

yet quantitatively evaluated. This leads to the paradoxical situation that, in this species, genes leading to death and sterility are not only maintained but encouraged. This looks so much like race suicide that it is difficult for me to think that it will be found frequently.

In one other case, in *Drosophila*, studied by Sturtevant and Dobzhansky[7] in 1936, a meiotic irregularity in spermatogenesis was found to be responsible for the transmission of a sex-linked mutation "sex-ratio" to the great majority of offspring. In males carrying this mutation the X-chromosome divides twice at meiosis, while the Y-chromosome is usually eliminated, so that nearly all sperm carry an X-chromosome with the mutation and thus produce only XX daughters, with occasionally an XO son. The great excess of mutant sperm should lead to a rapid spread toward fixation of the mutant in the population, which would then consist almost entirely of females and thus bring itself to an end. However, in this case, as Wallace[8] has shown, females homozygous for the mutant and males carrying it are less fit than animals lacking the mutant, so that its frequency in a confined population declines when in competition with the normal allele. Race suicide is thus prevented by selection against the mutant which prevents its spread. In the case I have described, however, the spread of the lethal is facilitated both by segregation ratio and by heterozygote advantage. I can only conclude that populations in which such mutants are present—and they seem to occur in most—must adjust themselves to the lethals by means at present unknown. Search for these means may reveal new facts about the structure of wild mouse populations.

The present case was discovered because of the extreme inequality in the transmission ratio in one sex. Meiotic irregularities connected with other complex loci in man and other forms might well result in changes of lesser degree in transmission ratio and would escape notice unless deliberately sought for. Only after this has been done can the importance of this new evolutionary force be evaluated.

*Summary.*—An evolutionary force, additional to those usually considered effective in altering gene frequencies (mutation, selection, random genetic drift and migration), is found to play the dominant role in determining the frequency of lethal alleles at locus *T* in populations of wild house mice. The new force, *male segregation ratio*, changes the normal ratio from 0.5 to an average of 0.96 in favor of the mutant allele. Thirteen alleles from twelve different wild populations from different parts of the United States all show similar segregation ratios. This advantage favors the maintenance and spread of lethal alleles. In addition, preliminary evidence indicates that animals heterozygous for such alleles have a selective advantage over those of normal genotype during early reproductive life. The effects of these facts on equilibrium values of such alleles in wild populations are discussed.

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# *COMBINATORIAL PROBLEMS IN THE THEORY OF GRAPHS. IV*

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1. *Introduction.*—In the previous paper of this series[1] we derived asymptotic expressions for the numbers of star trees of a large number of points which are built out of stars from a given collection of types of star. These expressions were obtained from the functional equations satisfied by the counting series, as derived in Papers I and II. The only essential restriction in the derivation was the restriction that the collection of types of star be *finite*. With this restriction we could show that the counting series was convergent and that the singularities on the circle of convergence were all branch points of order 2. From this knowledge of the analytic behavior of the counting series, the asymptotic expressions for the coefficients could then be obtained, using standard methods.

The question arises whether the restriction to a finite collection of types of star is necessary. If *no* restriction is required, then our results would also be valid for the general connected graph of $p$ points. In the next section we will review some results of Riddell and Polya[2] on the asymptotic numbers of graphs, connected graphs, and stars of $p$ points and $k$ lines, from which it is apparent that the counting series for the general connected graph of $p$ points is always divergent. Clearly, then, *some* restriction on the collection of types of star is necessary for the convergence of the counting series. In Section 3 we will show that the results of Paper III are still valid for an infinite collection of types of star, if only each star in the collection has no more than a fixed number of independent cycles.[3] This restriction on the collection of types of star is surely not necessary for the validity of the results of Paper III. Whether necessary conditions can be formulated is an open question.

2. *The Asymptotic Results of Riddell and Polya.*—Riddell has investigated the asymptotic behavior of the numbers $C(p, k)$ and $S(p, k)$ of connected graphs and of stars with $p$ labeled points and $k$ lines. His result may be summarized by the following statement: For large $p$ and $k$ the overwhelming majority of all graphs are stars. More precisely, from equation (I, 18) and from equations (I, 20) and (I, 23) Riddell obtains the expansions

$$C(p, k) \sim \binom{{}^1/_2 p(p-1)}{k} - p\binom{{}^1/_2(p-1)(p-2)}{k} + \ldots, \tag{1}$$

$$S(p, k) \sim \binom{{}^1/_2 p(p-1)}{k} - p\binom{{}^1/_2(p-1)(p-2)}{k} - p(p-1)\binom{{}^1/_2(p-1)(p-2)}{k-1} + \ldots. \tag{2}$$