THE SHELTERING OF LETHALS

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FROM the time of Muller's brilliant demonstration of balanced systems of lethal factors, a number of writers have taken the view that enforced heterozygosis by itself is sufficient to explain the accumulation of lethals in the sheltered chromosome. Thus it has been proposed that the genetic inertness of the Y-chromosome in *Drosophila* has arisen by the gradual accumulation of mutations having an inactivating effect on portions of this chromosome, it being supposed that such mutations would be sheltered by the mechanism of sex-linkage from the counter-selection by which mutations of this kind would, in other cases, be kept in check. The finding in *Drosophila* of a corresponding inert region in the X-chromosome is clearly at variance with this view, as also is the possibility of obtaining homozygotes for factors such as those producing the short-style in heterostyled plants, which are maintained constantly heterozygous, so long as only legitimate fertilizations are used, and which would therefore be expected to be accompanied by closely linked lethals. The following investigation shows that, by itself, enforced heterozygosis gives no such shelter to lethal mutations as would explain their accumulation from this cause alone.

Consider an animal or a plant invariably propagated by the union of members of two classes, one heterozygous, Aa, for a determinate factor, while the other is homozygous, aa. The chromosomes bearing the genes A and a will be supposed initially to be, in other respects, similar. The introduction of a lethal mutation in the same chromosome makes possible three types of organisms of the heterozygous class, namely AL/aL, Al/aL and AL/al, which we shall suppose to occur in the population with frequencies u, v, w, where

$$u + v + w = P.$$

In the homozygous class there are, in addition, two possible types aL/aL and aL/al, which we shall suppose to occur with frequencies x and y, where

$$x + y = Q,$$

and

$$P + Q = 1.$$

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The presence of the lethal in the population will be supposed to be maintained by a mutation rate, μ, affecting all gametes bearing *L*. Homozygotes *ll* are, of course, inviable. The relative frequencies of the possible types of gamete, taking account of the incidence of mutation, from these five types of organism may best be shown in Table 1.

TABLE 1

TYPE OF GAMETE

Parent organism	AL	Al	aL	al
AL/aL	$u(1-\mu)$	$u\mu$	$u(1-\mu)$	$u\mu$
Al /aL	———	v	$v(1-\mu)$	$v\mu$
AL/al	$w(1-\mu)$	$w\mu$	———	w
aL/aL	———	———	$2x(1-\mu)$	$2x\mu$
aL/al	———	———	$y(1-\mu)$	$y(1+\mu)$

It will be noticed that the entries in the first three lines of the table have a total $2P$, while those in the last two lines have a total $2Q$; but these factors will not require separate consideration.

Any condition of equilibrium of the system may be recognized by making the frequencies of the zygotic types proportional to products of the gametic types which produce them. If this is so we shall have the equations:

$$(1) \quad ku = (u+w)(2x+y)(1-\mu)^2$$

$$(2) \quad kv = \{(u+w)\mu + v\}(2x+y)(1-\mu)$$

$$(3) \quad kw = (u+w)\{2x\mu + y(1+\mu)\}(1-\mu)$$

$$(4) \quad kx = (u+v)(2x+y)(1-\mu)^2$$

$$(5) \quad ky = (2x+y)\{(u+v)\mu + w\}(1-\mu) + (u+v)\{(2x+y)\mu + y\}(1-\mu).$$

In order to take account of all possibilities the steps of solution will be set out formally. By addition of equations (1) and (3) it appears that

$$(6) \qquad k(u+w) = 2Q(1-\mu)(u+w),$$

whence it follows that either

$$(6a) \qquad k = 2Q(1-\mu)$$

or

$$(6b) \qquad u+w = 0.$$

In the second solution, since negative frequencies are not to be considered, u and w are both zero; and, in fact, all gametes containing A also carry the lethal l. It is, no doubt, the possibility of this solution which has inclined writers who have discussed the subject to the belief that it would be brought about merely by the occurrence of lethals closely linked with the heterozygous factor. A natural population, however, would not by itself tend to this condition unless AL gametes, supposing some of these to exist, were at a selective disadvantage, when account is taken of their mutation rate, compared with Al gametes; whereas it is manifest that unless al gametes are very rare the AL gametes would be at a selective advantage, and, if initially in a small minority, would tend to increase up to some frequency of equilibrium which must be given by the first solution, (6a).

To complete this solution we may note that from equations (1) and (2)

$$(7) \qquad k(u+v) = (2x+y)(1-\mu)P,$$

whence

$$(8) \qquad 2Q(u+v) = P(2x+y).$$

Also, by doubling equation (4) and adding equation (5), it appears that

$$(9) \qquad \begin{aligned} k(2x+y) &= (2x+y)(1-\mu)P + 2(u+v)(1-\mu)Q \\ &= 2(2x+y)(1-\mu)P. \end{aligned}$$

Hence

$$2Q(1-\mu) = k = 2P(1-\mu),$$

so that the frequencies of the two classes of organisms represented by P and Q are equal, each being one half.

With the aid of equation (8) we may now write equation (4) in the form

$$2x = (1-\mu)(x+\tfrac{1}{2})^2,$$

leading to the solutions

$$x+\tfrac{1}{2} = \frac{1 \pm \sqrt{\mu}}{1-\mu};$$

writing

$$\mu = z^2,$$

this gives

$$x+\tfrac{1}{2} = \frac{1 \pm z}{1-z^2} = \frac{1}{1-z} \text{ or } \frac{1}{1+z},$$

of which the first value is greater than unity, while the second is less. Since $y = \frac{1}{2} - x$, and can not be negative, we must choose the lesser value and obtain

$$x = \frac{1-z}{2(1+z)}$$

$$y = \frac{z}{1-z}$$

and, by substitution,

$$u = \frac{1-z}{2(1+z)}$$

$$v = \frac{z}{2(1+z)}$$

$$w = \frac{z}{2(1+z)}$$

The two types of double heterozygotes having frequencies v and w are thus equally frequent, as though linkage did not exist, while heterozygotes for the lethal are equally frequent in Aa and in aa organisms. The enforced heterozygosis of A has had no influence whatever on the situation in which the lethal mutant is in equilibrium.

Unequal Mutation Rates

Since the effective reason for the rarity of Al gametes, when the frequencies are in equilibrium, lies in their elimination when united with al gametes, it is instructive also to examine the consequences of postulating different mutation rates for the lethal in chromosomes carrying A and a, respectively. For simplicity we shall now write ν for $1-\mu$ in the A-chromosome, and ν^1 in the a-chromosome. Equation (6b) is then unchanged, but in place of (7) and (8) we have

$$k(u+v) = (2x+y)P\nu^1,$$

and

$$2Q\nu(u+v) = P\nu^1(2x+y).$$

Further it appears that

$$k(2x+y) = (2x+y)P\nu^1 + 2(u+v)Q\nu^1,$$

which is now equivalent to

$$(2x+y)P\,\frac{\nu^1(\nu+\nu^1)}{\nu};$$

so that

$$\frac{P}{2\nu^2}=\frac{Q}{\nu^1(\nu+\nu^1)}=\frac{1}{2\nu^2+\nu\nu^1-\nu^{1^2}}$$

The two complementary classes of organisms are in this case nearly, but not exactly, equally frequent. Substituting in equation (1), it now appears that

$$2(\nu+\nu^1)^2x=\nu^1(2\nu^2+\nu\nu^1+\nu^{1^2})\,(x+Q)^2.$$

If the roots of this quadratic are real, one will be greater than Q, and therefore unacceptable, while the other is less, as in the previous case; but they are only real when the quantity

$$\nu+\nu^1-2\nu^{1^2}$$

is positive. This will always be the case when ν is equal to or greater than ν^1, or when mutation is less active in a chromosome containing A than in one containing a; but, in the opposite case, it is possible for there to be no real solution.

In place of the expression,

$$\nu+\nu^1-2\nu^{1^2},$$

we may write

$$2\mu^1(2-\mu^1)-\mu-\mu^1,$$

or

$$3\mu^1-2\mu^{1^2}-\mu.$$

Since μ^{1^2} will generally be very small compared with μ^1, the condition for the unreality of the equilibrium value may with a good approximation be stated in the form that μ shall equal or exceed $3\mu^1$, or that the mutation rate in chromosomes carrying A shall be three times as great as that in chromosomes carrying a. The severity of this requirement would fully explain why it is that lethals do not appear, in fact, to have accumulated in many known cases of enforced heterozygosis.

The Stability of the Solution with Five Genotypes

The algebraic reality of the solution with five genotypes is not, however, a sufficient condition for its stability. Whenever two solutions of the conditions of equilibrium exist, we may be sure that if one is stable, the other is unstable, and *vice versa*. Consequently it will be sufficient to examine the stability of the solution

with three genotypes in which u and w are both zero. The fundamental equations for this solution are

$$kv = v\ (2x + y)\ (1 - \mu^1)$$
$$kx = v\ (2x + y)\ (1 - \mu^1)^2$$
$$ky = (2x + y)\ v\mu^1\ (1 - \mu^1) + v\ \{(2x + y)\ \mu^1 + y\}\ (1 - \mu^1)$$

whence, since v can not be zero,

$$k = (2x + y)\ \nu^1,$$
$$x = v\nu^1,$$

or, eliminating v and k,

$$y\ (2x + y)\ \nu^1 = 2\ (2x + y)\ x\mu^1 + xy,$$

or

$$\nu^1\ y^2 + \{2x\ (\nu^1 - \mu^1) - x\}\ y - 4x^2\ \mu^1 = 0.$$

In the solution,

$$\frac{y}{x} = \frac{-1 + 4\mu^1 + \sqrt{1 + 8\mu^1}}{2\ (1 - \mu^1)}$$

we may with advantage put

$$(1 + 4z)^2 = 1 + 8\mu^1,$$

or,

$$\mu^1 = z\ (1 + 2z).$$

Then we find

$$\frac{y}{x} = \frac{4\ (1 + z)\ z}{(1 - 2z)\ (1 + z)} = \frac{4z}{1 - 2z},$$

and so,

$$\frac{y}{2\ (x + y)} = \frac{2z}{1 + 2z}.$$

Now the proportion of the gametes from aa organisms which carry the lethal is

$$\frac{y + \mu^1(2x + y)}{2(x + y)} = \frac{2z + \mu^1}{1 + 2z} = \frac{z(3 + 2z)}{1 + 2z}.$$

This must be the proportion of Al gametes eliminated in each generation. If now a sprinkling of AL chromosomes were introduced, they would suffer no elimination through lethality, but would be diminished only by mutations of L to l at the rate μ in each generation.

Consequently, unless the rate of mutation from L to l in the presence of A satisfies the relation

$$\mu \geqq \frac{z(3+2z)}{1+2z},$$

the AL gametes will increase in proportionate numbers, or, in other words, the solution in three genotypes will be unstable. The condition for the stability of the solution in five genotypes is, thus, when μ is small, approximately that μ shall be less than $3\ \mu^1$. At higher mutation rates the critical ratio is somewhat nearer to equality, but does not reach equality until μ and μ^1 are each raised to unity. If μ were only twice as great as μ^1 the mutation rates would have to be raised respectively to about 36% and 18% before the stability of the solution in five genotypes would be upset.

It appears, then, that obligatory heterozygosis will only afford shelter to a lethal, or other highly disadvantageous, mutation, sufficient to allow of its establishment, in exceedingly exceptional circumstances:

(i) At ordinary mutation rates, when the mutation rate in the sheltered chromosome is almost treble that at the same locus elsewhere.

(ii) For more nearly equal mutation rates, when the rate mutation is so great that a large proportion of all the parent genes present in the species mutate in each generation.

The accumulation of lethals in such sheltered situations is therefore not likely to have occurred in natural populations.

Sheltering by Tetraploidy

Many species exhibit recessive forms depending on duplicate or triplicate factors in which only organisms homozygous for two or more completely recessive factors are to be distinguished. It is generally believed that many of these cases originated in polyploidy. It is true that, in tetraploids of recent origin, simplex organisms, containing only one of the four possible dominant genes, are usually distinguishable from those that contain two or more, so that in these cases the typical situation of duplicate factors has not been produced by tetraploidy. However, a moderate amount of further modification in the direction of more complete recessiveness would often be sufficient to reproduce the typical case. In consequence, the effects of incomplete dominance in the simplex condition can only be regarded as evidence against the origin of duplicate factors by tetraploidy on the view, upheld by

Sewell Wright, that dominance relationships are not, in fact, modified by the selective actions available, which are, however, known to tend in that direction.

The formation of a tetraploid race, by duplicating every locus, must supply the condition for the establishment of an immense number of pairs of duplicate factors. If the tetraploid originated through hybridization of two nearly related species, the duplicated chromosomes would already be differentiated in respect of those loci which had suffered an evolutionary modification during the process by which these species had become differentiated. Unless a considerable proportion of the germinal material is usually modified by such a process, the proportion of duplicated loci must still be large. In the course of time, the number may be reduced by the extinction of the parent gene in one of the duplicate loci, and the establishment, in its place, of one of its mutant allelomorphs. It is therefore worth considering in what circumstances a lethal or otherwise deleterious gene will be able to establish itself in one of a pair of duplicate loci, under the shelter afforded by the other.

Let us suppose that inheritance is strictly diploid and that a lethal gene l appears in both loci, with mutation rate μ in each generation. Selective elimination will only take place when the double homozygotes $l_1l_1l_2l_2$ are produced. The normal genes L_1 and L_2 do not occur among the organisms eliminated; the only wastage to which their numbers are subject is that due to mutation. Consequently, the proportional frequency of both will decrease when the proportion of double homozygotes is less than μ; both will increase when it exceeds μ; and both will be in equilibrium when it is equal to μ, irrespective of the frequency of self-fertilization. Formally, therefore, and apart from chance fluctuations, the proportion of mutant genes in the two loci may be equal, or unequal to any extent, without upsetting the equilibrium between mutation and extinction.

Difference of Mutation Rates

The fact that equilibrium is possible, whatever be the ratio of mutant genes in the two loci, shows that if the mutation rates in the two loci were different, no equilibrium would be possible, short of the extinction of the parent genes in the locus with the higher mutation rate. The rate of elimination will now lie between the two mutation rates, so that one mutant gene tends constantly to

increase, and the other to decrease. The rate of increase is thus always less than the difference between the mutation rates. The proportion of normal genes at the locus with the higher mutation rate will decrease less rapidly than the function

$$e^{-(\mu_1-\mu_2)\,T},$$

where T is the number of generations. Although this supplies an under-estimate of the time required, it is correct in its order of magnitude. After a lapse of time,

$$T=\frac{4}{\mu_1-\mu_2}$$

the parent gene will certainly be somewhat rare in the locus with the higher mutation rate. A difference of 4 per cent., in a mutation rate of one in 1,000,000, would bring this about in 100,000,000 generations. Mutation rates which were much larger and more widely unequal would of course act more quickly.

Since the opportunities for the establishment of a deleterious mutant in place of the parent gene at one of a pair of duplicate loci seem somewhat slight, attention may be given to the effects of chance at the initiation of the tetraploid form. If this originated from a single individual containing the duplicated contents of two gametes, then the race so formed would, from the first, be homozygous for any lethal factors which these gametes might contain. With cross-breeding species, a gamete chosen at random is not very infrequently the bearer of a deleterious recessive. For a lethal having a mutation rate of one in 1,000,000, the proportion of such gametes would be one in 1,000, so that this fraction of the duplicated germ-plasm may be expected to be differentiated from the start. The fraction would, however, be smaller with frequently self-fertilized organisms.

It appears, therefore, as far as deleterious mutations are concerned, that we ought to expect species which originated in tetraploidy, or members of groups of species having such an origin, to contain duplicates of almost every locus at which genetic effects can be observed. The typical appearance of duplicate factors will be conserved whenever the original parent genes have been both unchanged, or when the same gene substitution has taken place in both.

With respect to differentiation brought about by advantageous

gene substitutions, the position is, in many respects, fundamentally different. These must certainly originate with mutation rates so excessively low that their occurrence at one locus does not imply their simultaneous occurrence at the duplicate locus, even when the probability of such occurrence is strictly equal. Moreover, when, at one locus, such a favorable mutation has established itself, there is little reason to suppose that its occurrence at the second locus also will produce further advantage. Consequently, it is not improbable that such mutations will establish themselves, subsequently to the formation of the tetraploid condition, in one only of the two duplicate loci.

The differentiation of loci found in tetraploid species can, therefore, only to a minute extent be accounted for by the accumulation of lethal or deleterious mutations under the shelter afforded by the other locus. It must for the most part be due to the progressive changes which have taken place in two stages:

(a) Evolutionary differentiation by allied specific forms, prior to the formation of a tetraploid by hybridization, will have already introduced differences at a number of loci. Those who believe, with the writer, that species formation is usually accompanied by gene substitutions at a large number of different loci, each substitution having exerted, in most cases, only small morphological influence, will consider that the proportion of loci so affected may, in some cases, be large. If, on the contrary, species were formed, as some have thought, by the occurrence of only a few gene substitutions, then the vast majority of the loci in allotetraploids must be initially identical.

(b) Subsequently to the establishment of tetraploidy, it appears that further differentiation may proceed by progressive modifications (favorable mutations), but scarcely to any appreciable extent by regressive changes.