GENETIC LOAD

Contents

1. GENETIC LOAD

1.1. **What is it?**

The concept of genetic load comes from Haldane (1949) [4] and Muller (1950) [14]. It attempts to put some objectivity into the idea that populations have a mixture of genotypes, some of which are 'better' than others. To the extent that there is a 'best' genotype, any genotypes other than the best will be part of the genetic load in the population.

Genetic load is most easily understood in terms of one type of load, which is termed 'mutation load'. Certain genotypes, such as recessive

diseases that lead to infant death or disability, are unequivocally deleterious. These diseases constitute a real genetic load in the population. There are also various other types of genetic load. Two of these are 'balanced load' and 'substitutional load', which will be defined later.

1.2. **The genetic load paradox and my part in its downfall.** I'll go into more detailed calculations shortly. But the essence of the argument is as follows. All individuals contain tens of thousands of genes. It is likely that natural selection affects many of these, and therefore that there is a load associated with each such gene. Most of these genes do different things. Therefore, as a first approximation, it can be assumed that the loads from different genes cumulate. The net result is that populations as a whole must sustain large burdens, or genetic loads. Equivalently, any given individual ought to have a rather low probability of surviving.

Experience tells us that most individuals in a population are quite capable of surviving. This can be taken as showing that there can't be much natural selection affecting the genome. Alternatively there may be something wrong with the argument.

When I started as a postdoc in 1965, the genetic load paradox was just being formalised. I was fortunate in coming to Stanford University where Walter Bodmer, my main supervisor, and Ed Reed, a human geneticist at UC Berkeley, were doing calculations on how this paradox could be resolved. We ended up publishing a paper in 1967 [17] that put forward these arguments. This section shows how I managed to muscle in as senior author on the paper.

The account will be in terms of the balanced load, since that was the principal way in which the paradox arose. The balanced load comes from the case where the heterozygote is better than either of the homozygotes. The classic example of this is sickle cell anaemia in populations subject to malaria, where the heterozygote is protected against malaria compared to the 'normal' homozygote, while the sickle cell homozygote lacks oxygen transporting ability. The heterozygote is then the 'best' genotype, and the 'load' in the population arises from the fact that a maximum of 50% of genotypes can be heterozygous, and the rest must be the less fit homozygotes. This may seem a rather contrived example, and I need to start by explaining why the case of heterozygote advantage is, or was, thought to be an important class of model.

Lewontin and Hubby published the main argument in two papers in 1966 [6], [11]. [ASIDE: I think that we in fact had preprints of the papers. Being part of an inner circle at Stanford gave us a real advantage compared to those who only found out about the results when the papers came out.] Lewontin and Hubby were the first to show using gel electrophoresis that there were cryptic differences amongst normal enzymes. About 30% of enzymes they looked at were found to have Fast- and Slow-running variants. Later, following Kreitman (1983) [10], it became clear that at the DNA level there were many more differences than apparent at the protein level. The huge amount of variability in human populations revealed by Hapmap would have been hard to imagine at the time.

So what was important about the electrophoresis findings? According to Lewontin, who summarised many of the arguments in his book [**?**] a few years later, it meant that there are many more polymorphisms than had been previously recognised. Nobody knew how many loci there were in any organism, but it had to be a large number, and the number of polymorphic loci was estimated to be 30% of that large number, and so very substantial.

According to the prevailing orthodoxy at the time, each such polymorphism had to be maintained by selection. Following Fisher [**?**], nobody gave much thought to the possibility that selectively neutral polymorphisms could persist in populations. Indeed Fisher went further in claiming that no two variants at a locus could be selectively equivalent. Fishers ideas came from a time long before the polynucleotide nature of the gene was known, and it seems clear now that he was wrong in thinking that functionally neutral substitutions were impossible. However the idea of selectively neutral polymorphisms being maintained over long periods of time is probably still questionable, although I dont know of a good reference for this.

In 1966, although other models existed, the most widely discussed model for maintaining polymorphisms was the model of heterozygote advantage. Nowadays, knowing a lot about the complex distribution of variants in many populations of many loci, it seems hard to get excited about a model that predicts a polymorphism involving two variants. Perhaps it is still useful as a starting point in discussions about genetic load.

So the quandary as envisaged by Lewontin and Hubby was the following: Imagine that each of the polymorphic loci has a heterozygote that is 1% better than the two homozygotes (its simpler to talk about

a symmetric model in which the two homozygotes are equivalent, although the actual calculations below dont assume this). What does that say about the mean fitness of the population compared to the optimum?

1.3. **Some calculations.**

Say there are 25,000 loci this is based on a recent estimate for humans [15] calculations at the time usually assumed that there were many more loci. The number of polymorphic loci is 30% of 25,000, or 7500. Ill round this up to 8000 just to simplify the arithmetic.

The average individual in the population would have about half of 8000 loci heterozygous, compared to the optimum genotype with all loci heterozygous. What is the cumulative effect of having these 4000 homozygous loci?

Assuming that the loci act independently, and the homozygous genotype at each locus has a fitness of 0.99 compared to the heterozygote, the cumulative effect of these 4000 homozygous loci would be

$$(0.99)^{4000}$$

or approximately 3.8 x 10^{-18}.

Even if all homozygotes were only at a 0.1% rather than 1% disadvantage, the average individual would have a fitness of $(0.999)^{4000}$ which is around 2% of the optimum.

Note that the absolute fitness values in this calculation are arbitrary. What matters is the differential between the genotypes. So the above calculations are consistent with several possibilities, depending on whether the most fit individual has a fitness of unity (line [1] in the table below), the least fit has unity (line [2]) or the median individual has unity (line [3]).

homozygous loci	0	4000	8000
heterozygous loci	8000	4000	0
Fitness:			
[1]	1	3.5 x 10^{-18}	1.2 x 10^{-35}
[2]	8.3 x 10^{34}	2.9 x 10^{17}	1
[3]	2.9 x 10^{17}	1	3.5 x 10^{-18}

These calculations with their impossibly high numbers seemed to imply that there could not be so many selectively balanced polymorphisms. Either most individuals in the population would have an almost zero

fitness or alternatively there would need to be individuals with impossibly high fitnesses.

1.4. **An attempt to solve the problem.**
I recall sitting and staring at the problem of high genetic loads for about two weeks [probably in between experiments - I was trying to become a bacterial geneticist at the time working on transformation in *Bacillus subtilis*]. After about two weeks of deep thought I came up with the following:

(a) Consider a population of individuals with the above spectrum of fitness values. How much difference in fitness would there actually be between individuals in such a population.

(b) What's the best measure of this variability of fitness values?

(c) Why not the variance!!?

So I sat down to calculate the variance in fitness values, which turned out to be quite easy. Suppose there are three genotypes, and their frequencies (assuming random mating) and selective values are as follows:

	$\underline{A_1A_1}$	$\underline{A_1A_2}$	$\underline{A_2A_2}$
Frequency	p^2	$2pq$	q^2
Selective value	1 - s_1	1	1 - s_2

If there are N such loci, each with the same set of frequencies and selective values, and the selective values combine multiplicatively to give overall fitnesses, then the variance in fitness values is (I believe some details are in [17]):

$$[1 + \frac{s_1^2 s_2^2}{(s_1 + s_2 - s_1 s_2)^2}]^N - 1$$

As noted above, the absolute values are arbitrary. The above calculation assumes that the mean fitness of the population is unity. This is very similar to assuming that the median individual has a fitness of unity as in scenario [3] above. The numerical comparison is between $(0.995)^{4000}$ versus $(0.99)^{8000}$. It also assumes that the loci are at equilibrium, so that p is equal to $s_2/(s_1+s_2)$ and $q = 1-p = s_1/(s_1+s_2)$.

1.5. **Numerical values.**
Substituting $s_1 = s_2 = 0.01$ and N = 8000, the variance comes to 0.223!!!!.

In other words, in spite of the fact that the most fit individual in the population has a fitness that is hugely greater than the mean fitness, the variance is some quite small number. I recall that this was the moment when things suddenly seemed to fall into place, but I had to go back to the calculation several times to make sure that there wasn't a mistake.

1.6. **What is going on?**
The obvious answer to this apparent quandary is the frequencies in the population. There may be some individuals that have very high fitness, but they are awfully rare. In fact they are essentially non-existent.

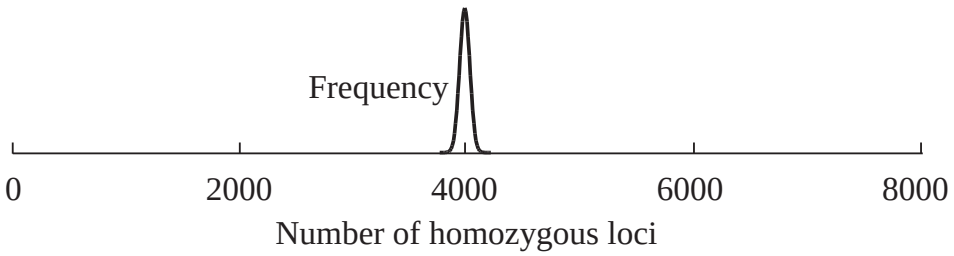
It is easy to do some calculations using the above numbers. The mean number of heterozygous loci is Np. As argued above, this is equal to 4000.

The variance can be calculated from the binomial, assuming that loci are independent, as Npq. This is equal to 2000.

The standard deviation (sd) is the square root of this number, which is about 45. The normal approximation to the binomial tells us that 3.29 x sd gives the 99.9% limits. So 99.9% of the population is expected to have a number of homozygous loci between

4000 - 3.29 x 45 and 4000 + 3.29 x 45

or 3853 and 4147 .



The frequency distribution, drawn approximately to scale, is shown above. Most of the population clusters close to the mean. The idea of

an optimum individual ever existing, ie. one having zero homozygous loci, is impossible in the absence of some currently unknown genetical trickery.

1.7. **Why the genetic load paradox is based on a fallacy.**
What is it about the traditional genetic load argument that forces this non-existent genotype to have a fitness of 2.9 x 10^{17} as in scenario [3] above. Who cares what is the fitness of this non-existent genotype? What if there was some overall limit to the fitness so that above a certain point the fitness doesn't get higher as the number of heterozygous loci increases? It was essentially this model that Ed Reed and Walter Bodmer were discussing, although they were considering a rather extreme version of the model.

It seems clear that it is possible to write down a model that satisfies the conditions of having 1% heterozygote advantage at 4000 loci and yet in which there is a reasonable upper limit to the fitness. Individuals with a fitness of 10, for example, would have about 231 extra heterozygous loci ($1.01^{231} = 9.96$). Extending the above calculation a little, the probability of even such a moderately heterozygous individual existing has s.n.d. = 231 / 45 = 5.13, P = 0.00000014. So putting an upper limit of 10 on the fitness makes essentially no difference to the selective values (marginal selective values) at all 8000 individual loci.

1.8. **Are limits to the fitness realistic?**
It seemed clear to us that not only did this model of an upper limit to the fitness solve the genetic load paradox in theory, but it also provided a more realistic model of the way that natural selection works, compared to the traditional multiplicative model. This is a more complex, and subjective, problem than just doing a bit of algebra and arithmetic to set up the model. Looking at the convoluted way we put the argument in our paper [3], I'm not sure that it is worth reading our paper on this point. First of all, two other papers, [9], [10], by Jack King and Roger Milkman, appeared in the same issue of Genetics as ours. [It may seem surprising that three different sets of authors would solve the same problem at exactly the same time. The reason, as stated previously, was probably that Lewontin set out the problem so starkly that it set everyone thinking about it. Crow [2] wrote a Genetics Perspectives article in which he described the papers as triplets, although I don't think they were as identical as all that]

King and Milkman solved the same problem as us, but in an entirely different way. Both said, essentially: what if natural selection acts

by truncating the worst (ie. in this case the most homozygous) individuals in the population? The obvious analogy here is with artificial selection. Both showed that this led immediately to the same sort of model that we had put forward, in which there is a substantial selective heterozygous advantage at each locus, but there is a limit to the optimum fitness. In this viability model the upper limit arises simply because an individual can't have a survival probability greater than one.

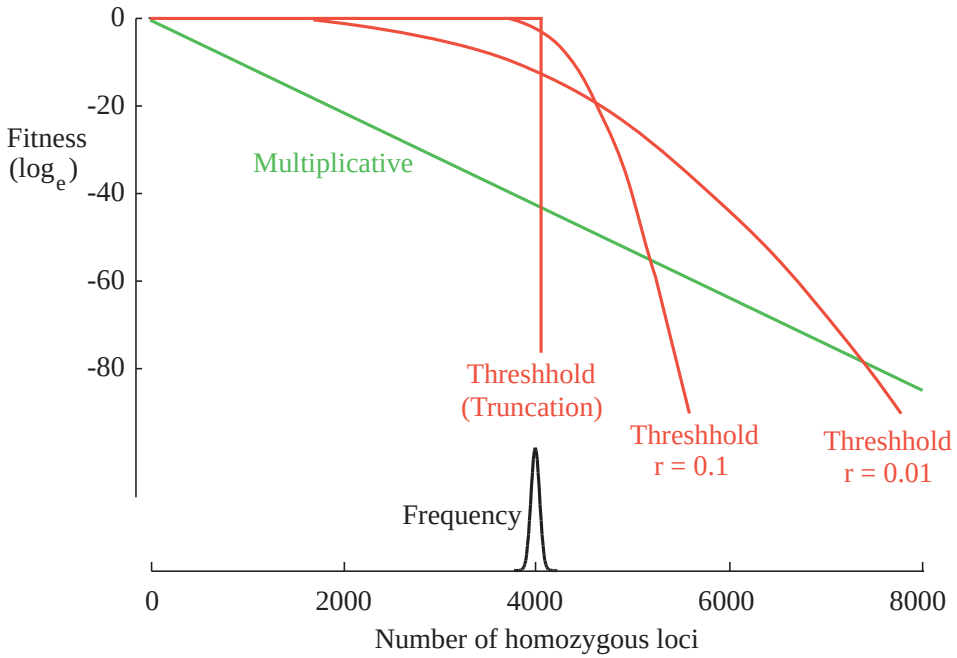
1.9. **Truncation (threshold / rank order) selection is the most efficient form of selection.**
An essential feature of the truncation model, that I'm not sure that either King or Milkman realised, was pointed out by John Maynard Smith [12]. If one wants to get the maximum effect at individual loci for a given death rate in the population, then it is impossible to do better than by selecting against the most homozygous individuals. This is exactly what the truncation model does. This model gives an upper limit to the marginal selective values at individual loci for a given death rate or genetic load in the population.

The numbers are rather surprising. In the multiplicative model given above, the proportion of the population surviving in order to maintain the 8,000 polymorphisms each with 1% selection is $(0.995)^{8000} = 2.6 \text{ x } 10^{-17}$. If, however, selection acts in the most efficient way, the number of survivors is around 73%! Amazingly, 1% selection differential can be maintained at all 8000 loci with a death rate of 27%, rather than 99.9999..%.

All of this doesnt make the truncation selection model a realistic model, as argued by Crow [3] and others. King to some extent anticipated such an argument by allowing chance to come into the model, albeit in a rather oblique way. The scale on which he truncated had a contribution from genotype as well as chance. Chance could in fact contribute 99% of the variance and a substantial marginal selective value could still be maintained, although not as striking as the above numbers.

The diagram below shows the results of some of the truncation selection models, together with the frequency distribution shown previously:

Four possible fitness relationships are pictured, plotted on a logarithmic scale. The first is the multiplicative model (green), which is linear on a logarithmic scale. The second is the basic truncation model. This is not easily plotted in the current diagram, since individuals to the left



of the cut-off point have a fitness of 1 (0 on a logarithmic scale) and individuals to the right have fitness of zero (-infinity on a logarithmic scale). The other two diagrams are given by King's (1967) model in which there is a threshold to which the genotype contributes 10% and 1% respectively. Essentially these curves are the cumulative normal, or probit, distribution. These relationships will be examined further in the section below entitled 'A more general model'.

1.10. **The independence assumption.**
All arguments of this section assume that herozygotes at different loci occur independently. Anyone looking at the section on linkage disequilibrium (LD) of this PIFFLE will find that I spend a great deal of time showing that this assumption is can't be true for finite populations. I have to admit here that the number of polymorphisms that can be maintained would not be as favourable if one takes this LD into account.

I don't know whether anyone has done the calculations on this point. It requires assumptions on N, the population size and c, the recombination frequency. If there has been no such calculation, it is probably an acknowledgement of the fact that nobody takes the genetic load paradox seriously any more.

1.11. **The truncation selection model and 'soft selection'.** Independently of the three papers that came out in Genetics, although a little later, Wallace[20] was arguing about different ways in which natural selection can occur, and coined the terms 'soft selection' and 'hard selection'. Although Wallace didn't come up with the arithmetic to back it up, he argued that soft selection would solve the genetic load paradox. There is nothing 'soft' sounding about truncation selection, but the essence of the soft selection model is that the genotype doesn't 'determine' death rates. Any genotype is capable of survival given the right conditions, and the death rate is imposed by ecological rather than genetical factors.

So, roughly speaking, the soft selection model corresponds to the truncation model, or the upper limit model, while hard selection corresponds to the traditional multiplicative model. We [17] had more or less the same distinction in mind, and considered various terms such as 'indeterminate' vs. 'determinate' selection, 'non-cumulative' vs. 'cumulative' selection, before giving up and just talking about 'Model 1' and 'Model 2'. We didn't have had the imagination to come up with Wallace's terms 'soft' and 'hard' selection. [Wallace wrote later that his inspiration came from the economic terms 'soft currency' and 'hard currency'. A soft currency is one that is unacceptable to the outside world whereas within the country of its use everything proceeds without problem. A slightly strange analogy but one that has a certain appeal].

It should also be noted that there is a close analogy between the terms 'soft' and 'hard' selection and the ecological terms 'density-dependent' and 'density-independent' population control. The soft selection model is essentially based on the premise of density-dependent control. There are only enough resources for a certain number of individuals to survive, and the death rate is set not by the number of homozygous loci but by the excess in fertility.

2. INBREEDING AND GENETIC LOAD

Our calculations on the balanced genetic load seemed to us to have important implications for inbreeding. Inbreeding systematically increases the number of homozygous loci, and takes the genotypes well outside of the normal range. This ought to have selective consequences. At the time, and perhaps still now, this could best be studied in Drosophila. It led me down a long path of studying the effects of

inbreeding in Drosophila, as described in another section of this PIFFLE.

Although the prediction of high inbreeding effects seems a fairly obvious one, it went against the prevailing orthodoxy of the time. For reasons that are difficult to explain here, a low level of inbreeding depression, as measured by the so-called B/A ratio, was taken to indicate heterozygote advantage and a high value to indicate that the polymorphisms were maintained by a mutation-selection balance. This led to a down-playing of the selective consequences of inbreeding under the heterozygous advantage model. One result of our paper was to re-focus attention on the inbreeding consequences of the heterozygous advantage model.

2.1. **BUT - a possible way out of the inbreeding prediction.**

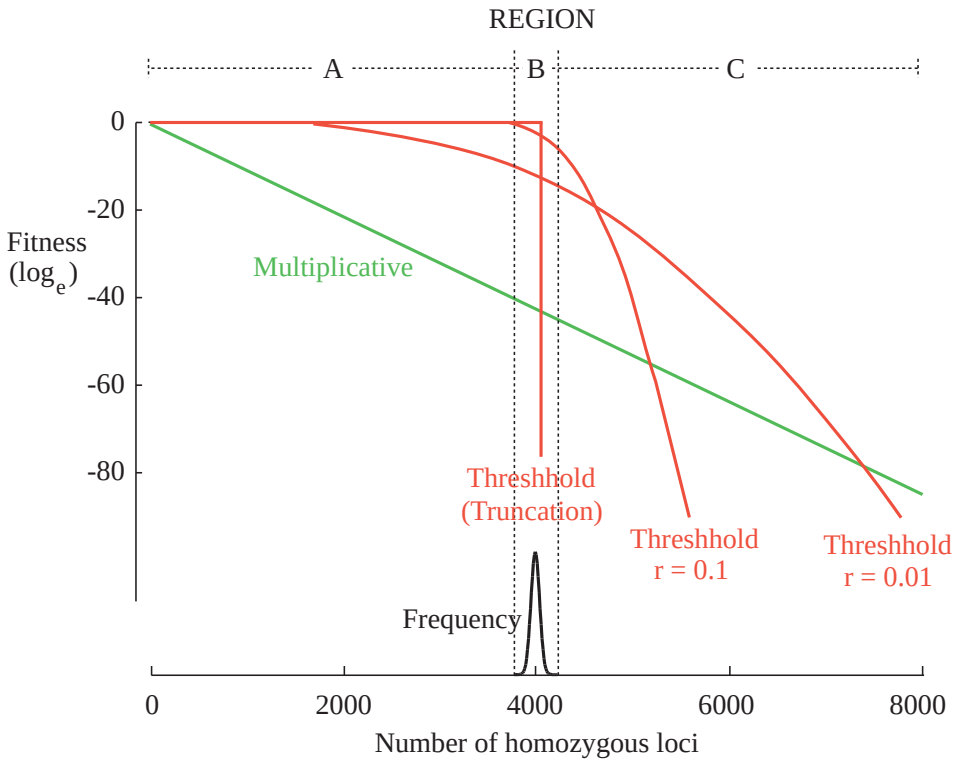
We [17] did find one further qualification to the argument. The multiplicative model predicts very high selective values for extremely heterozygous individuals, and equally low selective values for extremely homozygous individuals as produced by inbreeding. The truncation model gets around the problem of the highly heterozygous individuals, but actually predicts even lower selective values for the highly homozygous genotypes.

We queried whether this is really a necessary consequence of the soft selection model. Under this model, individuals are all capable of surviving, Even very homozygous individuals may, by chance, find resources that enable them to survive, So the high inbreeding prediction is not a 100% firm one under the soft selection model. It shows, contrary to what is sometimes assumed, that truncation selection does not necessarily accurately model soft selection.

3. A MORE GENERAL MODEL

Both Crow and Kimura [3] [8] and I [18] attempted to generalise the idea of a relationship between genotype and fitness. The argument as applied to the heterozygote advantage model is as follows. I have also published a related version dealing with mutation load [19].

The basic figure is an augmented version of the one shown previously (see below). It is convenient to divide the figure into three regions. Region B contains the bulk, 99.9%, of the population. Region A is the



region containing high fitness, but unlikely, genotypes. Region C contains the low fitness genotypes, which are similarly of low frequency in an outbred population, but can easily be produced by inbreeding.

3.1. **Calculating the marginal selective value.**

Each of the above functions relating fitness to genotype may be written in the form $w(x)$, where w, the fitness, is a function of x, the number of homozygous loci. To see the consequences of different fitness functions it is necessary to calculate what selection is generated at each individual locus, the so-called 'marginal selective value'.

In a genotype with x homozygous loci, the selective disadvantage of an extra homozygous locus is equal to $w(x+1)-w(x)$. To convert this to a selective value, it is necessary to normalise by dividing by the fitness. The selection coefficient, s, which is negative in this case, is equal to $[w(x+1)-w(x)]/w(x)$.

Taking into account the total frequency with which genotypes of x homozygous loci are expected to occur, $f(x)$, the total selective disadvantage of an extra homozygous locus is equal to:

$$\sum_{x=0}^{n} f(x)\frac{w(x+1)-w(x)}{w(x)}$$

It is convenient, as has been done in the above diagram, to depict the fitness functions as continuous functions rather than as discrete functions which they actually are. Passing to a continuous function, the selective disadvantage may be written as:

$$\int_0^n f(x)\frac{w'(x)}{w(x)}dx$$

or as:

$$\int_0^n f(x)\frac{d}{dx}ln[w(x)]dx$$

So if fitness is plotted on a log scale, as in the above diagram, the selective value is given by the weighted slope of the fitness function. In essence, it is the slope of the function in the region of the population mean that determines the selective intensity at each locus.

The above graphs do look as though they have the same average slope in the region of the population mean. Of course this is far from obvious for the pure truncation model where the slope is infinite for an infinitely small region and then zero outside - the calculation breaks down for this extreme case. But for the other three cases the slopes are similar, and equal to the selective value per locus, as shown in the above calculation.

The key difference between the multiplicative model and the truncation / threshold models is not the slope in the region of the mean, which has to be the same in all cases for a given marginal selective value. Rather it is what goes on in region A, the region of the non-existent genotypes. The multiplicative model insists that the slope in this region will be the same as in region B. This is an untestable proposition, and also an unlikely one unless one believes in hard selection for loci with reasonably small selective values.

For the threshold model, on the other hand, the slope starts to decrease exactly in the region of the population. This may seem like cheating in order to derive the best possible result, until one recognises that the

selective values are, in fact, determined by the interaction between individuals in the population. In the region where there are no individuals there is no scope for any selective value changes.

3.2. **Conclusions on the genotype - fitness relationship.**

The above calculations show that for a model in which there is a certain level of selection, as measured by the marginal selective values at each locus, there must be a particular slope in the region of the population mean. Outside of this region, anything goes. In region A, for example, the fitness could actually fall. The soft selection model predicts that the slope is zero in this region, while the hard selection model predicts that the slope is the same as in region B. Either is possible, although the soft selection model has always seemed to me much the more realistic.

It is also conceivable, if unlikely, that the fitness could start to rise again in region C. All the models given here predict that the slope is either constant or decreasing. As argued in the section above 'BUT - ..', this is only because the truncation model, as implemented here, is not necessarily a good model for soft selection. It might be worth trying to set up a more accurate model for soft selection in terms of resource utilisation or competition between individuals. But this would need to be rather specific for particular organisms, and also typically would differ for females and males. I'm confident, nevertheless, that this would have the desired properties of a low upper limit to the fitness and a non-zero lower limit at the other end of the scale.

4. THE SUBSTITUTIONAL LOAD - OR HALDANE'S DILEMMA

As mentioned in the introductory section, Haldane applied the same arguments for mutational load to the case of genes being substituted by natural selection [5]. If a gene has a 1% selective advantage over its ancestral allele, it will gradually be substituted in the population. This is the essence of Darwinian selection at the level of the gene.

What Haldane did was to sum the total load over all stages of the substitution of the gene. Consider the stage where the gene frequency is 20%, so that the unfavourable gene with a selective disadvantage s = 1% has a frequency of 80%. This assumes a haploid model, although diploidy can be taken into account. The implied load, or death rate if one is talking hard selection, is 80% of 1%, or 0.008. But this load goes on for many hundreds of generations, the time needed to substitute the

new gene. Haldane estimated that over the lifetime of the substitution, the total load is around 30, an astonishingly high figure to substitute one rather minor gene. Furthermore this figure is independent of the selective value s. Large values of s contribute more per generation, but the substitution requires correspondingly less generations.

Given all the different types of load in the population, Haldane felt that only 10% could be assigned to the substitutional load. If this is the case, and loads can be summed between different substitutions, this implies that on average there can be only one selective substitution per 300 generations.

The arguments involved in Haldane's limit attracted a lot of criticism. Much of this is summarised in the title of the paper by Brues (1964) [1] "The cost of evolution versus the cost of not evolving". The substitution is not really a cost - rather in the long run it avoids a cost. Nevertheless despite arguments such as this relating to what is and what isn't a 'cost', there was at the time no formal rejection of Haldane's calculations regarding possible rates of evolution.

It was obvious in 1967 that the arguments that applied to balanced load also apply to substitution load, ie that one can't necessarily just sum the loads from different loci. This seemed sufficiently clear that I felt reluctant to write a separate paper devoted to the Haldane limit. However I did some calculations based on King's truncation / threshold / probit model, and these gave such a simple end result that I did manage to get them published [16].

The main result from the calculations is that selective rates of substitution up to or greater than one per generation are possible. In theory, any number of favourable substitutions could occur simultaneously. The only restriction is that the more such substitutions occur, the lower the selective advantage s, and thus the slower the substitutions. In practice, it seemed unlikely that there really were such large numbers of new mutations available to be substituted.

Perhaps it is being overly pretentious, but I regarded, and still regard, Haldane's Dillema, as having been refuted by the arguments of [16], and also [12]. It can no longer be claimed that there is a limit of anything like one substitution per 300 generations. This limit is only true if natural selection acts independently on each mutation, a mode of action that seems patently false, especially for favourable mutations. Nevertheless Kimura, when introducing his arguments on neutral evolution [7], cited the avoidance of cost as a major benefit of the theory.

Kimura, and others, are probably right that the majority of substitutions are not positively selected, but I remain unconvinced by the cost argument to justify this assertion.

4.1. **The creationist position.**

Some controversy persists up to the present, as can be seen by typing 'Haldane's dilemma' into Google. This is primarily due to the unceasing efforts of one person, Walter ReMine, to rekindle the argument, primarily in 'Creation Research' journals and online, after being rebuffed in his efforts to publish in regular evolution journals. He often cites a rather ill-advised statement by GC Williams in 1992: "In my opinion the [Haldane's Dilemma] problem was never solved, by Wallace or anyone else." I wonder if Williams is among those who have not read my paper.

ReMine knows about my paper, because we have corresponded about it. His only reference to it that I know is the following: 'Likewise, Sved's version of truncation selection (1968) is often cited as a solution, yet evolutionary geneticists do not otherwise embrace it.' Again I acknowledge that many papers have been written that do not refer to mine, but I'm not sure that the lack of 'embrace' is enough to refute it.

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