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Why the evolution of resistance to anthropogenic toxins normally involves major gene changes: the limits to natural selection

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Abstract

Standard population genetic theory suggests that adaptation should normally be achieved by the spread of many genes each of small effect (polygenes), and that adaptation by major genes should be unusual. Such models depend on consideration of the rates of acquisition of adaptation. In practice, adaptation to pollutants and anthropogenic toxins has most frequently been achieved by the spread of major genes. A simple model is developed to explain this discrepancy, in which the determining factor is not the rate of spread, but the maximum response achievable under the two contrasting models of polygenic or major gene inheritance. In the short term, for a given mean and genetic variance, characters in which the additive genetic variance is produced by the segregation of many genes of small effect at intermediate gene frequencies are unable to produce as large a response to directional selection as characters in which the variance is caused by genes of large effect at low frequency. If the 'target' for selection is a long way from the mean prior to selection (as it may well be for adaptation to novel anthropogenic stresses) then adaptation can only be achieved by species possessing major genes. The model is discussed with reference to the example of heavy metal tolerance in plants.

Introduction

In an influential paper, Lande (1983) considered the evolution of a quantitative character governed by both major genes and polygenic variation. He argued that adaptation by minor genes was much more probable than by the spread of major genes, and suggested that, for major gene evolution to be important, strong selection over several generations would normally be required. Even so, this is not a sufficient condition for the spread of a major mutation, and his models suggest that polygenic adaptation should be common even where selection is strong.

For many adaptations to natural features of the environment his model is undoubtedly appropriate, and polygenic adaptation has frequently been demonstrated (Lande, 1981; Coyne & Lande, 1985; Macnair & Cumbes 1989), though recently the quality of the empirical evidence for this position has been challenged (Orr & Coyne, 1991). However, for one class of adaptations, the development of resistance to toxic substances of anthropogenic origin, the evidence is that major gene evolution is overwhelmingly more common than polygenic inheritance. Resistance to insecticides (Wood, 1981), warfarin in rats, mice and humans (Greaves *et al.*, 1976; Wallace & MacSwiney, 1976; O'Reilly *et al.*, 1964), and industrial melanism in insects (Lees, 1981) have almost always shown major gene inheritance, and it is probable that heavy metal tolerance and herbicide resistance in plants is similarly inherited (Macnair, 1989; Snape *et al.*, 1987; Jacobs *et al.*, 1987). Mallet (1989), in discussing the evolution of insecticide resistance, remarked on how standard neo-Darwinian theory would predict that adaptation

should occur by the spread of many genes of small effect, and contrasted this prediction with the observed facts.

This paper considers a very simple explanation for this observation, in which it is not the strength of selection which determines the genetic architecture of the adaptive response *per se*, but the amount of phenotypic change required to achieve the adaptation.

The model

Consider a population suddenly faced with a major new adaptive challenge. This could either be because the population has migrated to a new environment, or, more usually, because an anthropogenic change in its current environment has occurred. The change is sufficiently large that most or all of the present population are very unfit in the new conditions. In order for the population to survive, it has to adapt to the new environmental conditions: the mean phenotype of the population has to move from its old value to a new value that at least allows some survival in the new environment.

The selection acting on the system is hard as opposed to soft selection. In a newly contaminated or colonised environment, there may be rather few inter- or intra-specific competitors, but individuals have to have a phenotype that can grow and reproduce in the prevailing conditions. Individuals adapted to the old environment have a very low (but not necessarily zero) probability of survival in the new conditions; as adaptation proceeds their probability increases. In practice, the mean fitness of a population will need to exceed some threshold value before a viable population can be established in the novel environment; a population with a lower mean fitness may be able to survive in the short term (tens of generations).

Let us assume that there are n genes of equal effect segregating in the original population that give some adaptation to the new environment. The genes are assumed to act additively, and have the following effects:

genotype:	BB	Bb	bb
mean phenotype:	$2a$	a	0
genotype frequency:	p^2	$2pq$	q^2

where a is the additive effect of B allele, and p and q are the frequencies of the B and b alleles, respectively ($p + q = 1$). The mean of the character in the old environment is $2nap$ and the genetic variance $2npqa^2$. Selection can only be effective (ie adaptation achieved) if the genetic variance is large enough. A high genetic variance is obtained when either p and q are roughly equal, or when a is large. For a given mean, i.e. for constant $2nap$, a high genetic variance can either be achieved by having many genes of small effect at intermediate frequencies, or a few genes of large effect at low frequency. These two possibilities represent the two contrasting models of adaptation by polygenes or major genes. Lande (1983) compared the two models in terms of the relative *rates* of adaptation. He did not consider the problem of how far a population can actually move under the two models. The maximum response (MR) to selection will occur when all the B type alleles have gone to fixation. At this point, the mean of the population will be $2na$. MR is thus given by:

$$\mathrm{MR} = 2na - 2nap = 2naq. \tag{1}$$

This response can be expressed as a function of the mean

$$2naq/2nap = q/p, \tag{2a}$$

or as a function of the standard deviation

$$2naq/\sqrt{2na^2pq} = 2nq/\sqrt{2npq}. \tag{2b}$$

Thus if p and q are roughly equal, i.e. for genes at initially intermediate frequencies, MR is rather small: by $2a$, the mean can approximately double, or by 2b MR is $\sqrt{2n}$ standard deviations. For genes at initially low frequency, however, the response can be much larger: when $p << q$, 2b reduces to approximately $\sqrt{2n/p}$, which is likely to be much larger than $\sqrt{2n}$, even when n is small. Whether or not a population *can* adapt will depend on the relationship between MR and the distance the mean of the population has to move to give the population some adaptation (i.e. sufficient for the population to be viable) to the novel environment. Let us call this distance x. If x is not substantially larger than $2nap$ (the mean of the original population), then polygenic adaptation is both possi-

ble and probable. Any population with a reasonable level of additive genetic variance for the character should be able to achieve adaptation. If, on the other hand, x is substantially larger than $2nap$, then polygenic adaptation will be unable to give a large enough response, and the population will be unable to achieve the necessary adaptation in this way. Adaptation will only be possible if genes exist at low frequency that have a sufficiently large effect to achieve the adaptation. In this case, it is probable that only some species, and indeed some populations of those species, will have the requisite genetic variance, because whether or not a particular population has a gene of large effect at low frequency will be dependent on historical and stochastic events particular to that population.

It might be argued that the distinction between the models is too extreme, and that polygenic adaptation using genes initially at low frequency is also possible. The point here is that for any adaptation to occur, the genetic variance must be of reasonable magnitude. Genes of small effect at low frequency contribute very little to the additive genetic variance, and while 2b suggests that genes at low frequency can move the population a long way relative to the standard deviation, only if the standard deviation is of any magnitude can this be significant.

There is some controversy as to the origin and maintenance of polygenic variation. The classical view (e.g. Mather, 1973) is that much of the variation is maintained by selection, and that many of the genes responsible will be at intermediate frequencies. The alternative view (Lande, 1975) is that most of the observed variation could be maintained by a balance between mutation and stabilizing selection. Though such selection will tend to remove variation, the selection on individual loci will be small, and so considerable variation could be maintained solely by recurrent mutation. Such a model would imply loci at low frequency, but as argued by Barton and Turelli (1989; Turelli, 1984) it is likely that such loci would have to have relatively large effects. There are various reasons for believing that the mutation/selection balance model may not be generally appropriate (Maynard Smith, 1989), but until we know a lot more about the nature of the genes causing quantitative variation, we will not know which of the two models is more commonly found in nature.

It is important to note that this model does not depend upon the presence of an adaptive valley between two alternative peaks. In such a situation, major genes will again tend to be found (Turner, 1978), but for a different reason. If there is an adaptive valley, then the movement from one peak to another has to be achieved in a single generation, and in practice this will only be possible with major genes. The reason for this is that with polygenic inheritance the probability of obtaining an extreme phenotype in any one generation is $(p^2)^n$: even with high values of p this value rapidly becomes less than the mutation rate as n increases, so that the relative probability that a major gene mutation may give adaptation increases. In the adaptive valley model, the continuous build up of polygenic adaptation is precluded, so major genes are favoured; in the model outlined above it is not precluded, but it can only work on pre-existing variation, which can become exhausted before sufficient adaptation has been achieved. In this case also major genes are favoured.

The model outline above is not quantitative, of course, in that there is no scale and no way in which the initial mean, MR or x can be estimated. Zero is defined as the individual which has all decreasing alleles and can also not be estimated. This model however provides a conceptual framework within which one can consider whether polygenic or major gene adaptation is more probable.

An application of the model: heavy metal tolerance in plants

Many higher plants have evolved the ability to grow on soils heavily contaminated with various toxic heavy metals, such as copper, zinc, lead, nickel or arsenic (Antonovics *et al.*, 1971; Macnair, 1987; Baker, 1989). Only some species appear to be able to evolve tolerance, and some species can evolve tolerance to one metal, but not to others. Within a species, only some populations have the ability to evolve tolerance to any particular metal (Macnair, 1987; Al-Hiyaly *et al.*, 1988). The ability to evolve tolerance has been related to the probability that populations from uncontaminated areas possess tolerant individuals at low frequency (Bradshaw, 1985; Gartside & McNeilly, 1974).

Whilst it is probable that an individual has to have a certain minimum level of tolerance to be able to survive in a fully contaminated mine soil, mines are very heterogeneous places, and it is likely that there will be microsites within a mine, and on the mine edge, that provide habitats for individuals with intermediate levels of tolerance. It has been postulated (Antonovics, 1968; Gartside & McNeilly, 1974) that tolerance evolves through the colonization of these areas of lesser contamination ('nursery' areas), with the population gradually acquiring an increasing level of tolerance as it colonizes areas with greater and greater toxicity. This represents the polygenic hypothesis.

Antonovics (1976) gives a value for the increase of tolerance between a pasture and mine soil population of 6.3 standard deviations for zinc tolerance in the grass *Anthoxanthum odoratum*. If we assume that the heritability of tolerance is 100% (so the phenotypic variance of the base population is an estimate of the additive genetic variance, a conservative assumption), then from equation 2b we can calculate the number of genes involved, if genes are of equal effect and of intermediate frequency, as about 20 (ie $MR = 6.3 = \sqrt{2n}$).

There are several reasons why this represents a very unrealistic scenario for the evolution of tolerance. First, with this number of genes, a considerable number of generations will be required within the nursery areas to achieve full adaptation. In practice, the population sizes within these nursery areas will be much smaller than that of the surrounding pasture. As Antonovics (1976) shows, a small marginal population will be easily overwhelmed by geneflow from a much larger normal population, which will greatly impede population divergence. It should be possible to identify these areas with intermediate populations, but in fact no such populations have been found. For instance, Wu *et al.* (1975) looked at the development of tolerance in the grounds of a copper refinery in Lancashire. They identified lawns of varying ages (i.e. representing differing stages in the colonization process). Whilst it was true that the mean tolerance of the populations on these lawns increased with age, this was caused not by the occurrence of individuals with greater and greater tolerance, but rather by the increasing elimination of individuals with lower tolerance. Second, the polygenic model suggests that the initial base population has an intermediate level of potential tolerance, and that it should be possible to select it for greatly reduced tolerance as well as increased tolerance. It is very difficult, however, to conceive of the phenotype of an organism that was six standard deviations *less* tolerant than a non-tolerant population: non-tolerant individuals are already extremely susceptible to elevated zinc concentrations. Finally, if there were 20 tolerance genes segregating in a population, it would be impossible to select individuals from a population in a single generation that show almost complete tolerance: yet it is now clear that for a population to be able to evolve tolerance, it is normally possible to select such individuals directly from the base population (Bradshaw, 1984; Gartside & McNeilly, 1974).

In contrast, all the features of the evolution of metal tolerance are consistent with its initial evolution being dependent upon the presence of a small number (one?) of genes of large effect, initially present at low frequency in the base population. The genetic analysis of this phenomenon has not yet been performed in enough examples to test the generality of this prediction: initial experiments seemed to suggest that the inheritance was polygenic (see Antonovics *et al.*, 1971), but detailed analysis of a number of cases now appears to support the major gene hypothesis (Macnair, 1983, 1989; Schat & ten Bookum, 1991).

Discussion

Lande (1983) argued that polygenic adaptation was more probable than major gene adaptation, except where very strong selection was involved, and a major gene was already present at moderate frequency, when a major gene might become established. He suggested, however, that strong selection was not a sufficient condition for the fixation of a major gene. However, his model assumes that the adaptation is capable of being achieved by either method, and that the determining factor is the relative rates by which this is achieved. He is thus only considering situations where the maximum response of genes at intermediate frequency is greater than the distance that the mean requires to go to achieve adaptation, and thus he does not envisage a situation where evolution by many

genes of small effect might be constrained by the available genetic variation. The model discussed here argues that where such constraints exist, only major genes can achieve the necessary adaptation. Bradshaw (1984) has recently argued that many adaptive responses may indeed be limited by the availability of appropriate variation, and has coined the term *genostasis* to describe the situation in which a population has evolved as far as it can, and further evolution is constrained by the lack of the necessary additive genetic variation.

An important feature of the model is the nature of the selection pressure envisaged. Major genes will be much more likely where hard selection operates so that individuals need to have a certain minimum phenotype to be viable in the prevailing novel environment. Where soft selection operates, so that individuals are selected that have the most extreme values of a character, polygenes can obviously be selected until they go to fixation. Subsequently further progress can be made by recombination or mutation generating new variation. Such selection can guarantee polygenic adaptation, but it is limited by the constraints described here. It cannot in the short term (i.e. a few tens of generations, where a response depends on pre-existing variation, not on new mutations or rare recombination) give adaptation further than $\sqrt{2n}$ standard deviations from the mean. For instance, in most cases of natural evolution of insecticide resistance, major genes have been implicated. One apparent exception is in the case of *Drosophila melanogaster* (Crow, 1957). However, in this instance resistance was a result of stepwise artificial selection, which is almost invariably operated by soft selection. Interestingly, Crow (1957) noted that *D. melanogaster* had not developed the extremely high levels of resistance attained by houseflies (which evolved resistance very quickly in the field). Thus artificial selection had produced a change in phenotype by polygenic adaptation, but it had not produced as extreme a phenotype as had been achieved by the spread of major genes under natural selection.

It is probable that many of the challenges that are made to species by anthropogenic pollutants lead to hard selection, and, because the agents are qualitatively different to many other natural stresses, that the adaptive 'target' is quite a long way away from the mean in an unpolluted environment. In the case of toxins, such as pesticides and heavy metals, the nature of the selection and the requirement for a population to evolve a certain minimum resistance is obvious. However, other anthropogenic changes can exert just as strong selection. It is commonplace for man's activities to render species locally extinct, and they may be only able to reinvade once they have evolved some adaptation. For instance, Kettlewell (1973) reports the case of the Rosy Minor moth, *Miana literosa*. This moth was made extinct in Sheffield during the industrialization of the city, but recolonized Sheffield once it had evolved a melanic form. While some natural environmental stresses may also exert strong hard selection and lead to adaptation by a few genes of large effect (Parsons, 1987), in general it is probable that for most natural adaptive challenges, such as variation in normal features of the environment or competition (both intra- and inter-specific), it is both unlikely that the selection will be of this form, or that the adaptive 'target' will be so far away from the original mean. In these cases polygenic adaptation will be more common.

Another important feature of the model is that we are only considering short term changes. The model envisages adaptation caused by a rapid change in the environment, to which a population must adapt or go locally extinct (or, fail to colonize). Adaptation must depend on pre-existing variation. Obviously, if a population is subjected to long term directional selection, particularly if the selection pressure is essentially soft-selection, then there will be the opportunity for new mutations to arise which can move the population far beyond the limits discussed in this model. However, for this to be possible, the population must still be able to persist in reasonable numbers before the new mutation arises, and therefore is not appropriate to this situation.

This model obviously has similarities to models developed for the evolution of mimicry (e.g. Turner, 1978; Sheppard *et al.*, 1985). In these models, a major gene gives the initial similarity of mimic to model, a similarity that is later enhanced by (polygenic) modifiers. In earlier less formal presentations of the ideas developed here I have implied that they are essentially the same (e.g. Macnair, 1981). However, I believe that this formalization of the model has shown them to

depend on different processes. In mimicry, a major gene has to be involved because stabilizing selection on pattern generated by the behaviour of predators means that there is an adaptive valley between the two phenotypes, mimic and model, and this valley can only be crossed by a major gene mutation. In the model developed here, however, there is no need for an adaptive valley. It is perfectly possible for fitness to be a monotonically increasing function of phenotype: indeed, given the heterogeneity of environments subject to pollution or application of agents such as insecticides, it is almost inevitable that fitness will follow such a function. The important point, however, is that until the phenotype has reached some threshold value, the probability of survival and reproduction of a large enough group of individuals to form a viable population is not high enough: a major gene is required to give adaptation where that threshold is more than a few standard deviations away from the existing mean.

Finally, Lande (1983) and others have argued that the evolution of adaptation by major genes is relatively improbable because major genes are frequently associated with deleterious pleiotropic effects. Obviously if a major gene is lethal or nearly lethal as a homozygote then it cannot become fixed by natural selection, and such genes could not be involved in the processes discussed above. However, for a gene with a mild deleterious effect in a normal environment but giving a major effect in a novel environment, it would be easy for the advantage gained by adaptation to outweigh the pleiotropic disadvantages. To judge the importance of these pleiotropic effects, we need to know about the distributions of adaptive *vis a vis* negative side-effects in samples of natural mutations. Orr and Coyne (1991) have recently reviewed the evidence both that major genes are intrinsically deleterious and that they are rarely found in adaptation, and have found the data on these points inadequate to make any generalizations either way. Obviously further research is required in order to resolve this problem.

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