PERSPECTIVES

Maintaining evolvability

JAMES F. CROW*

Genetics Laboratory, University of Wisconsin, Madison, WI 53706, USA

Abstract

Although molecular methods, such as QTL mapping, have revealed a number of loci with large effects, it is still likely that the bulk of quantitative variability is due to multiple factors, each with small effect. Typically, these have a large additive component. Conventional wisdom argues that selection, natural or artificial, uses up additive variance and thus depletes its supply. Over time, the variance should be reduced, and at equilibrium be near zero. This is especially expected for fitness and traits highly correlated with it. Yet, populations typically have a great deal of additive variance, and do not seem to run out of genetic variability even after many generations of directional selection. Long-term selection experiments show that populations continue to retain seemingly undiminished additive variance despite large changes in the mean value. I propose that there are several reasons for this. (i) The environment is continually changing so that what was formerly most fit no longer is. (ii) There is an input of genetic variance from mutation, and sometimes from migration. (iii) As intermediate-frequency alleles increase in frequency towards one, producing less variance (as $p \rightarrow 1$, $p(1-p) \rightarrow 0$), others that were originally near zero become more common and increase the variance. Thus, a roughly constant variance is maintained. (iv) There is always selection for fitness and for characters closely related to it. To the extent that the trait is heritable, later generations inherit a disproportionate number of genes acting additively on the trait, thus increasing genetic variance. For these reasons a selected population retains its ability to evolve. Of course, genes with large effect are also important. Conspicuous examples are the small number of loci that changed teosinte to maize, and major phylogenetic changes in the animal kingdom. The relative importance of these along with duplications, chromosome rearrangements, horizontal transmission and polyploidy is yet to be determined. It is likely that only a case-by-case analysis will provide the answers. Despite the difficulties that complex interactions cause for evolution in Mendelian populations, such populations nevertheless evolve very well. Long-lasting species must have evolved mechanisms for coping with such problems. Since such difficulties do not arise in asexual populations, a comparison of epistatic patterns in closely related sexual and asexual species might provide some important insights.

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Introduction

Recent years have seen a surge of interest and emphasis on epistasis, particularly from developmental biologists, evo–devo enthusiasts, and human geneticists. As a typical example, see Carlborg and Haley (2008): '*Epistasis: too often neglected in complex trait studies*'. Epistasis has been recognized since the earliest days of Mendelism, and is especially conspicuous when the component genes have large effects. It has long been a standard tool for geneticists interested in using mutant genes to work out developmental pathways. It is also an important component of Wright's shifting balance theory (Wright 1965).

Another outcome of recent studies is the finding of more and more genes with large effects, most strikingly in macro-evolutionary studies (Carroll 2006). Since dominance and epistasis are greater for such loci, this also would argue for the importance of gene interactions. Epistasis is often enhanced between inbred strains. An extreme example that shows the effects of inbreeding, genes of very large effect, and strong antagonistic epistasis is provided by mice that have a chromosome from one inbred strain inserted into another (Shao *et al*. 2008).

As techniques improve, molecular geneticists are increasingly finding multiple, complex developmental and biochemical pathways. Networks and redundancy are common, all of which seem to shout 'epistasis'. It is clear, however, that although epistasis is ubiquitous, it cannot be too

*E-mail: jfcrow@wisc.edu.

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extreme. If every selected gene carried a large amount of epistatic baggage, these side effects could swamp the main one and evolution would grind to a halt. Despite this, evolution continues to advance. This is not surprising in asexual species where the whole genomic complex is inherited as a unit, but is a puzzle with Mendelian segregation and recombination. I think the continued evolutionary progress of sexual species implies that those species that have survived a long evolutionary past have found ways of evolving, despite epistasis, as well as ways of minimizing its retarding effects. Some mechanisms are obvious (e.g. modular development) but most are yet to be discovered. Perhaps some light on this question could be revealed by comparison of complex interactions in closely related sexual and asexual species.

Evolution of quantitative traits

A large and important part, perhaps the most important part, of evolution is quantitative. In addition to its obvious importance in animal and plant breeding, it is reasonable that much of evolutionary change consists of making continual adjustments to the deteriorating effects of an ever-changing environment. Most such changes are likely quantitative; at least that is the assumption of this article.

Despite countless examples of dominance and epistasis, animal and plant breeders have found that almost any trait responds to selection. This applies, of course, to traits with some variability; there is nothing to be gained by trying to select for two-headed dogs. Almost every population, except those that are highly inbred, has considerable genetic variation of which a substantial component is additive, i.e. it responds to selection. Breeders have generally ignored or minimized epistasis and usually without any loss of predictive value. The strong evidence for large additive variance of quantitative traits has long been realized and has recently been summarized by Bill Hill and his colleagues (Hill *et al*. 2008): "Data and theory point to mainly additive genetic variance for complex traits."

Human height has been regarded as a paradigmatic quantitative trait since the earliest days of genetics. That it is consistent with a large number of roughly additive loci was apparent in the masterful analysis by Fisher (1918). The large number of additive genes that are involved was documented in detail in three recent genome-wide association studies. These studies identified a total of 54 loci, which collectively account for about nine per cent of the population variance (Visscher 2008). Since only a small fraction of the loci were common to two or more of the studies, the great majority of loci must have not yet been identified. Considering this, and noting that the trait is 80% heritable, something like $54 \times (80/9) = 480$ loci would be needed to account for all the genetic variance. This is of course a minimum estimate, since only mutations with an effect larger than about 0.3% of the variance would have passed the stringent tests for inclusion in the study. There was little evidence for either dominance or epistasis. Fisher (1918) postulated dominance to account for the greater correlation of sibs than of parents and children, but this can as well or better be accounted for by the greater environmental similarity of sibs.

The longest continuous selection experiment was the Illinois corn project (Dudley 2007). It began at the end of the 19th century, and by 2005 selection for high and low oil content and for high and low protein of maize kernels had continued for 106 and 105 generations, respectively. The striking result is that progress continued with no obvious plateau or reduction in selectable variance for both high oil and protein content. The change was remarkable; the difference between the high and low oil lines was 32 times the standard deviation of the original population. This large change occurred despite the fact that only a dozen ears were saved each generation. Selection for low concentrations did slow down, but for the obvious reason that one cannot have less than zero content. Analysis of variance showed that the differences were mainly additive, with only slight effects of dominance and epistasis. The continued effectiveness of selection, along with various biometrical analyses, all suggested multiple factors, and this was recently confirmed by QTL analysis (Laurie *et al*. 2004; Hill 2005). There is not the slightest evidence that the population has run out of genetic variability.

Similar results have been reported for selection experiments in mice and *Drosophila*. Sometimes a plateau is reached, but this is not due to depletion of genetic variability, as is evident from the effectiveness of reverse selection. The cause of the plateau in selection response is usually some genetic complication (e.g. a balanced lethal system) or incompatibility of further advance in the trait and fitness due to trade-offs among fitness components. For example, the selected strains sometimes have had lowered fertility.

Although one cannot rule out the possibility that some of the effect in such studies showing long-term responses to selection without severe depletion of additive variance was due to mutations that occurred during the selection process, there is no doubt that an enormous amount of potential variation was concealed in the original population and preserved, despite there being only a dozen parents each generation.

The received wisdom

Natural selection depends on additive variance, and in the process of selection additive variance is expected to be depleted. As a consequence, the theory says that after many generations of directional selection, the additive genetic variance will approach exhaustion. In particular, this should be true for fitness, which has been continuously under directional selection for untold generations. To quote Alan Robertson (1955): "I have taken it as axiomatic that in a population at equilibrium there will be no additive variance in reproductive fitness". Yet, populations do continue to respond to selection, if not for fitness, then at least for characters correlated with fitness. In the corn experiment, oil and

protein content in the two selected strains must have become essentially fitness itself, since there was no other conscious selection and very little opportunity for natural selection.

Maintaining additive variance

We have seen that additive variance persists, even with strong selection. Certainly this is true for most quantitative traits and very likely for fitness also. What preserves selectable variance? I think there are four primary causes.

(i) Changing environment

One major relevant factor is fluctuating environment. Fisher once said, in commenting on Wright's peaks and valleys metaphor, that a better one might be the undulating waves of an ocean. If the environment changes more rapidly than the time required to exhaust a substantial part of the variance, variance can be maintained. One continuously changing environmental factor is the evolution of competing species. Hamilton *et al*. (1990) have emphasized parasites as a particularly rapid-changing environmental component of most species. Because of their short life-cycles, parasites can evolve rapidly relative to their hosts. Rapidly evolving parasites create a new environment faster than the additive variance is exhausted in a stable environment. The only way the host can keep ahead is to use latent variability; mutation is too slow. This of course implies sexual reproduction and recombination (Hamilton *et al*. 1990; Crow 1992). The population has to continue changing to keep up. It is like Lewis Carroll's Alice on the treadmill; you have to run awfully fast to stay in the same place.

(ii) Mutation

Additive variation can also be introduced into a population by various means, of which mutation is one. Hill (1982) and others have suggested that mutation may have played a role in the Illinois corn experiment. There are also other sources of new additive variation, e.g. migrants. I suggest, however, that mutation is not the major factor. Most selection for polygenic traits utilizes variance that already exists. In any case, whether my view is correct or not, it should be possible soon, by studying individual loci in strains selected in opposite directions, to distinguish between those genes that were originally polymorphic and those that have originated by mutation during the selection process. It would be particularly fortunate for such studies if seed from the original strains had been preserved.

(iii) Compensating allele frequency changes

This mechanism is a consequence of allele frequency change. Since the genetic variance is proportional to $p(1-p)$, where p is the allele frequency, genes of intermediate frequencies (not too far from $\frac{1}{2}$) make the largest contribution to the variance. As the population is selected, favourable alleles increase. As their frequencies increase toward $p = 1$, the variance decreases, and is on the way to exhaustion; as $p \rightarrow 1$, $p(1-p) \rightarrow 0$. But at the same time, favourable alleles that were initially rare increase in frequency and move toward $p = \frac{1}{2}$, making increasing contributions to the variance. These two processes—the decrease in variance from common favourable alleles becoming more common (moving away from $\frac{1}{2}$ toward 1) and increase in variance from rare favourable alleles becoming more common (moving toward $\frac{1}{2}$)—would be expected to roughly balance, with the result that the variance stays about the same (Crow 1992). Something like this must have been happening in the Illinois corn experiment.

(iv) Selection for fitness

Individuals of high fitness contribute more than their fair share to the population next generation. A disproportionate number of genes in the current generation, thus, came from parents with more than average fitness. In other words, there is selection for fitness. If the higher fitness continues into further generations, it must have been heritable. Thus, traits with any appreciable heritability and which increase fitness will tend to increase under natural selection, and this process is cumulative. As a result, a standing population will have more additive variance for fitness and traits correlated with it than would be predicted from the study of new mutations. I believe this could be a major reason why natural populations respond so readily to selection for polygenetic traits. Mathematical modelling is needed to compare this effect with the various mechanisms for depletion of genetic variance.

Additivity and size of the gene effect

There is abundant evidence that the smaller the effect of a gene, the more nearly additive it is. This has been shown experimentally many times in *Drosophila* (e.g. Greenberg and Crow 1960; Temin *et al*. 1969). It is also expected on more general grounds. Many effects—not restricted to biology—are more nearly additive as they become smaller. For this reason, the first term of a Taylor series is often a good approximation for small differences in a wide variety of physical and biological situations. The infinitesimal model (Bulmer 1980) seems to be appropriate for most quantitative traits.

Recent molecular studies, such as QTL analysis, have repeatedly discovered genes with major effects. An example is in maize, where the differences between maize and teosinte depend mainly on half a dozen loci. But there is still a large role for multiple genes with small effects, including supplying the perfecting modifications of the major-gene traits.

The effect of epistasis on selection

Fisher (1930) was the first to show that selection can change a population despite strong interactions caused by dominance and epistasis. By following a principle, essentially equivalent to least squares, the additive variance pulls out the additive components of dominance and epistatic variance (Fisher

1941). Thus, although interactions slow the progress of selection, the process still works at a rate mainly determined by Fisher's '*Fundamental theorem of natural selection*'. Only the additive × additive and additive × additive × additive, etc. components contribute to changes of a trait under selection and this is only part, perhaps a small part, of the genetic variance (Cockerham 1954; Crow and Kimura 1970, pp. 124–129). For example, a two-locus model of complete dominance, complementary epistasis, and allele frequencies of $\frac{1}{2}$ shows only 0.064 of the variance due to additive × additive epistasis (Crow and Kimura 1970, pp. 129). The proportion is smaller if the dominant alleles are less frequent; for example if the dominant alleles had a frequency of 10%, the proportion would be less than 1 per cent. Hence, it is not surprising that ignoring epistasis has not materially weakened prediction formulae.

Quasi-linkage equilibrium

There is also reason to think that even additive parts of the epistatic variance may not make a significant contribution. Selection tends to induce linkage disequilibrium, even with weak linkage. Kimura was the first to show that, with loose linkage, the population rather quickly reaches a state where the linkage disequilibrium variance exactly cancels out the epistatic variance. For a two-locus model, the condition for this process to work is that the epistatic differences be small relative to the frequency of recombination between the loci (Kimura 1965; Crow and Kimura 1970, pp. 217–224). Thus, in many natural populations gene-frequency change by selection may well be determined entirely by additive variance. It is quite possible that animal and plant breeders, by ignoring epistasis, may have gotten better prediction equations than if they had tried to include a correction for epistasis.

Quasi-epistasis

It is likely that the greatest effect of epistasis in natural populations is not caused by interaction of gene products, but rather by the way selection acts on the population. Breeders have long known that the most efficient way to select is by truncation or rank-order selection: save all individuals with phenotypes beyond a certain threshold. By aggregating genes with related functions into groups, there is effectively a high degree of epistasis, despite the fact that the genes may well be acting additively. I will call this 'quasi-epistasis', since the epistasis is generated by the selection process rather than by gene interaction.

Quasi-epistasis provides a mechanism for effectively removing mutations or otherwise deleterious genes in clusters, hence greatly reducing the load due to deleterious mutations or gene substitution (Sved 1968; Kondrashov 1982). It is, of course, extremely unlikely that nature performs exact truncation, but the process does not have to be exact. A rough approximation to truncation is nearly as effective in eliminating harmful mutations (Crow and Kimura 1979).

To repeat, the point of interest is that the genes are acting roughly additively. Yet, approximate rank-order selection changes frequencies of many similar acting genes at once, thus generating the population equivalent of epistasis. Since every species experiences period of overcrowding, this may well be the most important kind of epistasis in nature.

In praise of additive polygenes

Particulate inheritance, multiple independent factors, additivity, and sexual reproduction provide a system in which a maximum of potential variability is concealed in a population with only a moderate amount of standing variation (Crow 1992). As the Illinois corn experiment demonstrates so spectacularly, several generations of selection can carry a population far beyond the extremes of its founders. It is clear that many quantitative traits approach this ideal. The infinitesimal model (Bulmer 1980) provides a useful analytical approach to this issue.

That such a system is good for evolution is clear. It is also likely, for reasons given here, that natural selection tends to preserve such systems. Natural selection not only selects traits, but preserves a system under which it can continue to be effective. Of course there are many other properties that have important evolutionary roles (genes of large effect; duplications; polyploidy), but they are not the subject of this essay. Evolution is a complicated subject and quantitative traits constitute only a part of it, albeit an important one.

Conclusion

Students of developmental genetics have long emphasized gene interactions as of great importance. Dominance and epistasis have been regarded as useful concepts for understanding development. Similar emphasis is found in the evo–devo community. In contrast, animal and plant breeders have tended to down-play epistasis. Formulae predicting the effect of selection, natural or artificial, typically ignore epistasis and also dominance.

Why this difference in viewpoint and emphasis? I think there are several reasons. The major one, I think, is that students of development often observe mutations with individually large effects and it is these that tend to show the greatest interactions. Breeders, for the most part, select for quantitative traits, likely to be caused by the cumulative effect of genes with individually small effects. These tend to show the least interaction. Further, the fact that selection acts on the additive component of the genetic variance contributes to this viewpoint. Moreover, I suspect that Kimura's principle of quasi-linkage equilibrium is more often applicable than has been traditionally assumed. A near-additive, polygenic system not only has optimum evolutionary properties, but as shown in this article, the system tends to be preserved.

Finally, there is a difference in mind-set. To the developmental biologist, gene interactions are interesting and often provide the means to a deeper understanding. In classical

genetics, epistasis often provided the clue to developmental pathways. To the breeder, epistatic effects are more of a nuisance, like noise, that interferes with the accurate prediction of breeding results. Both views are appropriate in their context.

Despite the difficulties that complex interactions pose for Mendelian species, they evolve very well. It is likely that those species that have survived a long evolutionary past have somehow evolved ways to maximize the effectiveness of natural selection while still preserving some of the virtues of complex interactions. What such mechanisms might be, we have little idea. Since the complication does not arise in asexual strains, some insights might be obtained from a comparison of closely related long-term sexual and asexual strains. Bdelloid rotifers and their sexual relatives could be a good starting point. For beginning to such studies, see Crow (1957) and Malmberg (1977), showing that selection for resistance produces greater epistasis when recombination is reduced or absent.

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