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MULTIPLE FITNESS PEAKS AND EPISTASIS

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ABSTRACT

The importance of genetic interactions in the evolutionary process has been debated for more than half a century. Genetic interactions such as underdominance and epistasis (the interaction among genetic loci in their effects on phenotypes or fitness) can play a special role in the evolutionary process because they can create multiple fitness optima (adaptive peaks) separated by fitness minima (adaptive valleys). The valleys prevent deterministic evolution from one peak to another. We review the evidence that genetic interaction is a common phenomenon in natural populations. Some studies give strong circumstantial evidence for multiple fitness peaks, although the mapping of epistatic interactions onto fitness surfaces remains incompletely explored, and absolute proof that multiple peaks exist can be shown to be empirically im-

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possible. We show that there are many reasons that epistatic polymorphism is very difficult to find, even when interactions are an extremely important part of the genetic system. When polymorphism results in the presence of multiple fitness peaks within a group of interbreeding populations, one fitness peak will quickly be nearly fixed within all interbreeding populations, but when epistatic or underdominant loci are nearly fixed, there will be no direct evidence of genetic interaction. Thus when complex landscapes are evolutionarily most important, evidence for alternative high fitness genetic combinations will be most ephemeral. Genetic interactions have been most clearly demonstrated in wide crosses within species and among closely related species. This evidence suggests that genetic interactions may play an important role in taxonomic diversification and species-level constraints. Population genetic analyses linked with new approaches in metabolic and molecular genetic research are likely to provide exciting new insights into the role of gene interactions in the evolutionary process.

INTRODUCTION

The ecological, developmental, and metabolic requirements of life pose problems to be answered by evolution. These problems most often have more than one genotypic or phenotypic solution; however, not all these solutions are necessarily equally fit. Some fit types may be separated from more fit forms by intermediates of lower fitness. With these "adaptive valleys" intervening, a species cannot evolve from one "fitness peak" to another by the deterministic process of natural selection. Significant controversy has surrounded the arguments concerning the mechanisms for adaptive change from one fitness peak to another, particularly those dealing with Sewall Wright's "shifting balance" process. Most of the controversy has centered around the relative roles of selection and drift in evolution and on the likelihood of population structures capable of producing peak shifts in nature. A more fundamental issue, however, is often not addressed. There is little consensus about the extent or importance of genetic interactions and about whether actual fitness surfaces have multiple adaptive peaks. In other words, it is an open question whether peak shift models are needed at all. In this review we assess the empirical and theoretical evidence on the need for a peak shift model; that is, we review the evidence that fitness functions have multiple peaks.

In this review we argue that multiple-peaked fitness functions exist in nature and that ruggedness may be a common feature of adaptive landscapes. We argue this from three perspectives: that genetic interactions among loci (i.e. epistasis) are common and important in the mapping between genotypes and fitness, that underdominance can sometimes create multiple peaks from interactions within loci, and that phenotypic fitness functions are also rugged with multiple peaks. After some introductory sections to clarify some of the concepts and terminology, this review consists of essentially three parts: The first two sections focus on the reasons to expect epistatic and dominance interactions that can cause complex landscapes, and also review some of the evidence that such interactions are in fact common. We briefly review the evidence that epistatic interactions affect phenotypic expression, then we concentrate on the genetic interactions that can be shown to affect fitness. We then discuss some ecological and quantitative genetic reasons to expect disruptive selection on hypothetical adaptive landscapes, and we review the evidence for these multiple peaks on a phenotypic scale. Finally we discuss in some detail the reasons that epistasis is extremely difficult to detect, such that the examples observed represent a very small fraction of the cases where epistasis and multiple peaks are important components of evolution.

This paper essentially makes two points. First that there is substantial evidence that adaptive landscapes are not smooth, but there are interactions within and between genetic loci in determining fitness. The evidence for this comes from a variety of studies, many of which are difficult to do (and therefore are rarely done,) and which are biased against finding evidence for these interactions, so the studies we have must represent the tip of the iceberg. Second, we show that proving these rugged landscapes have true multiple peaks is impossible, but we maintain that the fact that landscapes are rugged per se should motivate more studies of evolution on complex adaptive landscapes.

Some of the literature on epistatic interactions has been reviewed previously (1, 51, 88). Because of the limitations of space, we have not been able to cite all of the literature that bears on this broad topic; instead we have attempted to choose representative examples.

Genotypic Versus Phenotypic Fitness Functions

The adaptive landscape can be drawn at two distinct scales: mapping genotypes to fitness or mapping phenotypes to fitness. Ultimately what matters to evolution is the fitness of genotypes, of course, so landscapes must be uneven at the genotypic scale for genetic interactions to affect the course of evolution. Often, however, we know little about the genetic basis of phenotypes and more about the relationship between the phenotype and fitness. For traits determined by many loci that interact additively, complex phenotypic fitness functions imply complex genotypic fitness functions. This paper examines both scales of fitness function.

The Definition of Epistasis and of Multiple Peak Systems

Using Sewall Wright's familiar metaphor, the mean fitness of a population can be viewed as a function of the gene frequencies of the population (135, 136) if one is focusing on the genetic scale, or as a function of the frequencies of particular phenotypic classes (68, 69, 112) if one is focusing on the phenotypic scale. The shape of this mean fitness function, or adaptive landscape, to a large degree specifies the evolutionary direction of the population under the deterministic influence of natural selection. Natural selection can therefore be seen to drive populations to local peaks on the adaptive landscape. (Even if the adaptive landscape metaphor does not strictly hold, there are stable states, perhaps even strange attractors, analogous to adaptive peaks towards which populations will evolve—5). An adaptive landscape thus serves to visualize dynamical systems in which there are multiple stable equilibria.

As originally envisaged by Wright, these multiple adaptive peaks are generated by epistatic interactions between genes (135). The term epistasis actually has two distinct usages in the genetic literature (129). Epistasis was originally coined (8) to describe the interaction between genes in which the action of one gene was blocked by the action of another gene (epistasis literally means "standing above"—44). This is the standard usage of the term today in molecular and developmental biology. Other forms of interaction were also given names such as "cumulative" and "mutually supplementary" (77), but these descriptions seem to have fallen out of favor. Evolutionary geneticists tend to use epistasis to describe any form of gene interaction. This usage stems from Fisher (38), who described all genetic variance not attributable to additive and dominance effects as caused by "epistacy." The evolutionary genetics literature generally has continued to focus on the variance definition of epistasis (129), but we make a strict distinction between gene action, the statistical "effects" attributed to that action, and the genetic variance that arises from the multilocus composition of individuals within a population. As we show in the next section, there can be substantial epistatic gene action, but little manifestation of this in the form of epistatic genetic variance within a population. Indeed, one of the major conclusions of this paper is that this is a common state of natural populations.

Following the evolutionary genetic usage, we use epistasis to describe any form of gene interaction, but we focus on gene interactions that generate multiple fitness peaks. In the simplest two-locus case, multiple adaptive peaks can be generated when two mutually exclusive sets of alleles at each locus produce high fitness genotypes, but any mixing between the sets results in genotypes of lowered fitness (Table 1). For multiple peak epistasis, it is important to make a distinction between epistasis for an arbitrary character and epistasis for the particular character that is fitness. To a large extent, epistasis is often viewed as a nuisance that arises from measuring a character on a particular scale. Historically, quantitative geneticists have tried to eliminate epistatic variance via transformation, although it is not always clear how to proceed (98), and transformations may simply shift the context in which epistasis is observed (54). In this regard, fitness is unique because it does not

Table 1 A simple epistatic fitness function.

	AA	Aa	aa
BB	1 + i	1	1 − i
Bb	1	1	1
bb	1 − i	1	1 + i

exist on an arbitrary scale and should not be transformed (72). Thus, epistasis for fitness will always be epistasis and is not an artifact of scale.

In determining whether a particular fitness function generates a fitness landscape with multiple peaks, it is very important to distinguish between the fitness of individuals and the mean fitness of the population. Mean fitness functions are always less peaked than the individual fitness functions of which they are composed. If an individual fitness function has multiple peaks, but there is a lot of phenotypic variance in the population which is evolving on that landscape, the mean fitness function need not and often does not have multiple stable equilibria (this relationship is even more complex in the presence of frequency dependent selection and other factors) (31).

Epistatic Variance versus Epistatic Genetic Effects

There is an important distinction between epistatic genetic variance and the genetic effects due to epistasis. Particular alleles at different loci may interact strongly to produce radically different phenotypes relative to when they are paired with other alleles, but if these alleles are rare, then there will be very little epistatic variance. Epistatic variance is also a function of the allele frequencies (proportional to $p_A q_A p_B q_B$, where p_A and p_B are the allele frequencies at loci *A* and *B* and the *q*'s are equal to 1 −p), whereas the potential effect of an allelic combination depends only on the actual phenotype produced by that combination. Fitness functions are defined in terms of the fitness of allelic combinations; hence, the evolutionary potential of allelic combinations depends on these epistatic effects, rather than the epistatic variance.

A Simple Model of Gene Action

Many of the issues involved in the estimation of the potential for epistatic gene action can best be discussed with a simple model of gene interaction. The essential attribute of such a model for our purposes must be that it generates more than one fit genotype. Figure 1a presents a simple two-locus model with interaction for fitness for the loci. This sort of model closely parallels the kinds of fitness interactions we might expect from simple stabilizing selection or a canalized metabolic pathway. Notice that the mean fitness function has two peaks, at opposite corners of the distribution, with a valley between, which,

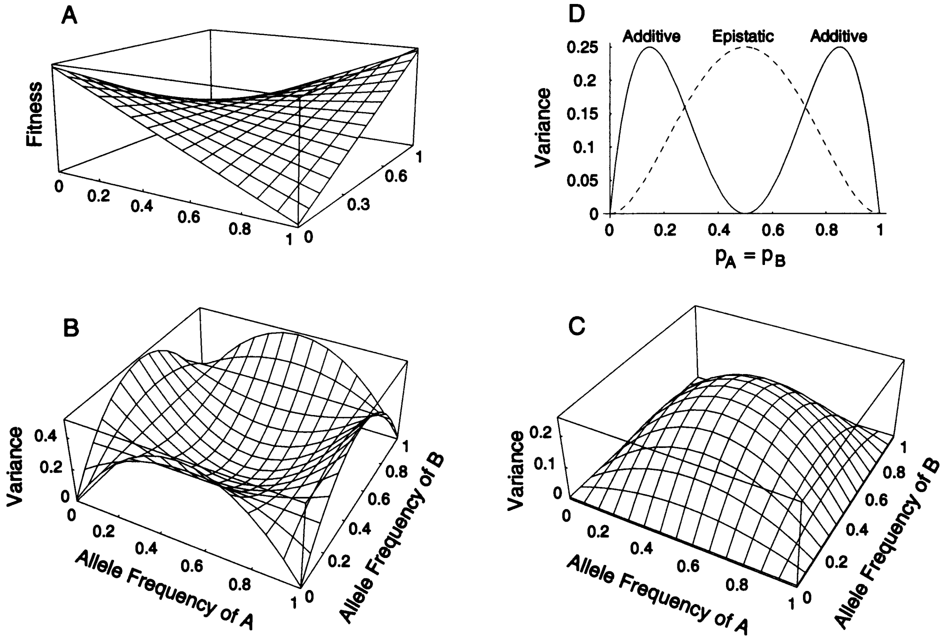

Figure 1 An epistatic fitness landscape and the variance components for fitness. The fitness function listed in Table 1 translates into the mean fitness landscape illustrated in *a*. The two horizontal axes represent the allele frequencies of A and B; the vertical axis represents the mean fitness of a population that has those allele frequencies. In *b*, the additive variance for fitness is represented as a function of these allele frequencies. Notice that the additive genetic variance is at a minimum at the saddle on the mean fitness landscape. Figure *c* represents the epistatic genetic variance as a function of the same allele frequencies. Epistatic variance reaches a maximum at the saddle point but increases very slowly near the fixation points. Near the stable equilibrium allele frequencies (near fixation for AB or ab), most of the genetic variance for fitness is additive, not epistatic, despite the fact that the interaction of genes in this system is entirely synergistic. This is shown in *d*, where the diagonal across gene frequency space connecting the two peaks is plotted (i.e. the x-axis represents the allele frequency $p_A = p_B$) against the variance components for fitness.

in this case, is deepest at $p_A = p_B = 0.5$. Populations at equilibrium will be near one or the other of these peaks. The gene action of these alleles is clearly epistatic for fitness. Yet if we examine the variance components for fitness with this model, we see that most of the genetic variance for fitness near the stable points is additive, not epistatic (see Figure 1b–d). In fact, the only point in genotype space with a large proportion of epistatic variance is near the saddle, at $p_A = p_B = 0.5$. This relationship can be formalized by remembering the least squares definition of additive variance—the additive variance for fitness components corresponds to the slope of the mean fitness function. The slope of the fitness function is 0 at maxima and minima, so their additive variance goes to zero. Any genetic variance at these points must therefore be nonadditive. In our example the stable points correspond to fixation points, which will always be the case without some form of overdominance. When equilibria are at fixed states, nonzero allele frequencies are expected by mutation-selection balance, which causes small allele frequencies precisely in the range where the ratio of epistatic to additive genetic variance is small. We therefore expect to see large amounts of additive variance, or in the case of overdominance, dominance variance, in natural systems even in systems with a large degree of epistatic gene action.

The Importance of Epistasis and Multiple Peak Systems

The critical problem posed by rugged fitness landscapes is: How do species pass through valleys to reach other, higher fitness peaks? Wright differed from Fisher in his answer to this question. Fisher thought the problem to be reasonably unimportant, thinking that there is always genetic variation for fitness available to a species, largely because the environment was constantly changing (in other words, that fitness landscapes are like the surface of a turbulent ocean). Wright, however, viewed the question of adaptive valleys as central to the study of evolution. In response to this difficulty, Wright created his famous Shifting Balance theory (135, 136), which hypothesizes that populations can move from one peak to another by random genetic drift caused by periods of small population size. Following this drift to the domain of attraction of a new peak, a population would be selected uphill to the new peak. The new peak would then be "exported" to other populations by differential migration and/or extinction. This theory has proven to be one of the more intriguing and controversial in the history of population genetics.

There are many other models of peak shifts that may also explain evolution between adaptive peaks. It is possible that macromutations, either in the sense of hopeful monsters (43) or merely in the sense of mutations of large effect (14), can cause the transition between peaks on the phenotypic fitness function. Similarly, change in the biotic or abiotic environment can cause selection pressures to change sufficiently to allow transitions (5, 63, 133), as can changes in the phenotypic variance of populations due to inbreeding (133) or in the genetic correlations among traits (100).

Whatever the mechanism, it is critical that we know the extent to which adaptive landscapes are peaked in nature, in order that we can know the importance of peaks shifts in general. If landscapes are rugged and stable through time, then many traditional models of evolution will need to be reevaluated. The response to selection and adaptation to local peaks, such as that described by Fisher's Fundamental Theorem of Natural Selection, depends on additive gene action (39, 91); if epistatic deviations from additivity are common, then the particular processes involved in transitions between peaks will have to play a more central role in evolutionary thought.

There are many other processes in evolutionary biology that have been shown to be significantly changed by the presence of epistasis for fitness. The evolution of sex (65), diploid life cycles (67), and mating systems (66) are all significantly influenced by interactions among loci. Inbreeding depression and heterosis (52, 54) as well as outbreeding depression (130) may be influenced by epistatic interactions among loci. The evolution of reproductive isolation (27) also depends critically on epistatic interactions. The importance of epistatic interactions and complex landscapes extends well beyond the initial interest motivated by Wright.

EPISTASIS IN THE MAPPING BETWEEN GENOTYPE AND FITNESS

An important way that genetic landscapes can have more than one adaptive peak is if there is significant interaction among alleles at different loci. For epistasis to act as a genetic constraint (such as in the shifting balance models), there have to be significant genetic interactions for fitness. In other words, genotypes must combine nonadditively to determine the reproductive success of individuals; for some purposes, the interactions have to be severe enough to cause multiple peaks on the adaptive landscape. This section reviews some of the evidence for epistatic interactions for fitness.

Direct Measures of Fitness Effects

Classical genetic analysis has determined that there is epistasis for fitness components (e.g. 123) or for strongly selected traits (e.g. 19, 20) in many cases. The tightly linked loci within messenger RNA are selected on their ability to create an appropriate secondary structure; this results in strong disequilibrium among species in predictable allele combinations (119). Genes also are known to interact strongly with sex-determining factors to determine fitness (104).

Studies of the phenotypic effects of particular proteins often indicate epistatic interactions. Examples come from examination of the phenotypic and fitness effects of electrophoretic variants of enzymes (e.g. 13, 86). In the tidepool copepod *Tigriopus californicus*, osmoregulation is accomplished by manipulation of cellular free amino acid content. Osmoregulation is differentially influenced by production of alanine and proline. Sharing of substrates between the Krebs cycle and the alanine and proline pathways means that genes for cellular energetics are epistatic with osmoregulatory genes (45). Alternative alleles for 6-phosphogluconate dehydrogenase in *E. coli* show fitness differences dependent on genetic backgrounds that differ in the presence or absence of an alternative pathway for the metabolism of 6-phosphogluconate. However, they showed no fitness differences in their "normal," i.e. coevolved, background (35).

Epistasis for Phenotypes

Many types of evidence indicate the existence of epistatic interactions in the mapping between genotype and phenotype. The fitness of organisms depends on the phenotypes, and therefore epistatic interactions for phenotypic traits imply the strong possibility of rugged adaptive landscapes. Epistatic interactions among loci have been investigated using a variety of techniques, ranging from classical genetic analyses to modern QTL mapping, from molecular biology to field ecology. Epistatic interactions have been observed directly, with the genotype and phenotype both known, and they have been inferred by the patterns of genetic variation present in populations. In fact, it was Wright's early investigations of guinea pig coloration patterns (138) that initially led him to consider the importance of epistasis in the evolutionary process (101).

VARIANCE COMPONENT ANALYSES As we have seen, it is possible to have pronounced epistatic interactions among alleles, without that trait expressing much epistatic variance at all. The opposite is not true, however; epistatic variance does imply the presence of epistatic interactions at the allelic level. The epistatic variance component is extremely difficult to measure (see below), but the presence of epistasis has been tested and found a number of times, particularly in agriculturally important species (41, 54, 102). Many other examples can be drawn from the literature. It is worth noting, however, that the proportion of variance explained by epistatic effects is normally rather small (< 30%) even when significant. Later we explain why this is expected even if there are large interactions among alleles.

QUANTITATIVE TRAIT LOCI Recent advances in the types of polymorphic genetic markers available to evolutionary geneticists and breeders have enabled the location of the major (and some of the minor) loci responsible for observed phenotypic variation in polygenic traits. Tanksley (122) and Cheverud & Routman (17) review the identification of quantitative trait loci (QTL) through the use of polymorphic DNA markers. These authors also review some of the advantages and drawbacks of estimating genetic interactions in this way. The general picture that emerges from the limited data on QTL for crop species is that few epistatic interactions are important for determining the phenotypes of interest.

Long et al (79) recently completed a study of a synthetic population formed by hybridizing lines selected for high- and low-bristle number in *Drosophila melanogaster*, one of the classical examples of an additive polygenic trait. They produced a highly saturated map of chromosome 3 and the X chromosome, with markers approximately every 4 cM. Thirteen of 60 tests for two-factor epistasis were significant; the probability of this occurring by chance is less than 0.00001. The epistatic effects were of the same order of magnitude as the average allelic effects of the QTL, as were the sex-by-QTL interactions. Thus, although the variation they detected behaved overall as though it were additive, when two-way interactions were examined, 20–25% of the loci showed important epistatic effects. Moreno shows similar results for bristle number mutations acting in concert (88).

THE GENETIC INTERACTIONS OF METABOLIC PATHWAYS The properties of metabolic pathways imply that both dominance and epistasis are inevitable consequences of the sequential processing of substrates and products in metabolic chains. Kacser & Burns (58) introduced the control coefficient to quantify the extent to which flux through the pathway depends on the catalytic properties of any one enzyme. The introduction of an allele that changes the kinetic properties of any rate-limiting enzyme in the pathway necessarily changes the control coefficients of all the enzymes in the pathway. Thus, the phenotypic effect of one locus is modified by the alleles present at another locus. In spite of the prevalence of such epistatic interactions, the epistatic variance in these systems is expected to be small (60).

If selection instead optimizes flux at an intermediate value, the fitness of alleles at a locus will depend on their relative kinetic properties, the control coefficient for that enzyme, and the flux rate through the pathway as a whole. As the flux rate for the pathway as a whole is moved above and below the optimum, the fitness effects of increases in velocity through any one enzyme will change from negative to positive, respectively (120). Metabolic pathways under selection for intermediate fluxes are therefore very much like any other trait undergoing optimizing selection: There is diffuse epistasis for fitness among the loci affecting the trait (see the section on stabilizing selection below).

DEVELOPMENTAL PROCESSES AND EPISTASIS Genes must interact via developmental pathways, in much the same way as genes interact in metabolic pathways. Decisions made early in development affect the phenotypic manifestation of genes expressed later. Homeotic mutants in both animals (see 88) and plants (see 22) give clear examples. The segregating alleles at loci polymorphic for homeotic mutations influence the phenotypic expression of whole suites of genes during organ development. There are many examples of gross and widespread epistatic interaction during development varying from the earliest events, i.e. meiosis (107) and mitosis (92), through embryogenesis (110), metamorphosis (105), final height (53), and reproduction (e.g. 22). See

(88) for a review of the literature on epistatic interactions in *Drosophila* development.

Mutation Accumulation

There is not sufficient data on the interactions of new mutations affecting fitness and phenotypes. Some evidence suggests that new mutations are likely to interact predominantly additively (11). Moreno (88) argues that mutations whose singular effect is small and additive can have large synergistic effects with other mutations. New mutations conferring T4 phage resistance in *Escherichia coli* have negative effects on fitness through other pathways but rapidly evolve compensatory epistatic modifiers (74). Similar results in other organisms (cited in 74) suggest that epistatic interactions can be both primitive and evolving traits of genes, but more studies are required.

Recombination Load

Recombination load is the loss in fitness because recombination breaks up associations between beneficial combinations of interacting alleles (16). If recombination load could be precisely measured, it would give the difference between the fitness at the highest peak on the landscape produced by existing genotypes and the mean fitness of the population as it is now. Unfortunately, this is not possible, but we can ascertain the fitness costs of a single generation of recombination, at least in organisms without recombination in one sex or in those like *D. melanogaster* for which we have many genetic markers. Dobzhansky and his co-workers first measured the loss of fitness in recombinant chromosomes, which have lower fitness than nonrecombinant controls (34, 115–117). Chromosomes derived from wild-caught male flies (which do not have recombination) have higher fitness than those derived from females from the same collections (which do recombine) (16, 89). The empirical literature on recombination load has been reviewed briefly in a theoretical context by Charlesworth & Barton (15). The absolute magnitude of this load, as measured by these experiments, is not great but strongly implicates epistatic interactions in determining fitness.

F_2 Breakdown

As we discuss further below, the nature of genetic variation within species is restrained by selection to be near adaptive peaks, and this is likely to constrain epistatic variance. If alleles are maintained in different populations or different species, however, this constraint is lifted, and we might begin to get a less biased perspective on the ruggedness of adaptive landscapes. Thus, one of the best available ways to investigate the importance of epistatic interactions for fitness is by measuring the fitness of the first and second generation offspring of hybrid crosses. If the second generation (the F_2) has an average fitness less than the average of the parents and the F_1, then this is referred to as F_2 breakdown, which can only be attributed to epistasis for fitness. Reductions in fitness in the F_1 (outbreeding depression) can also be attributed to epistasis or dominance and (in the absence of environmental differences) may imply that the adaptive landscape is uneven (see the later section on underdominance).

F_2 breakdown is frequently observed in intraspecific crosses between lines or distant populations (10, 49, 62, 126) (see reviews in 41, 139), particularly in crosses between more divergent lines or populations (41). Hybrid breakdown also occurs in interspecific crosses (46, 118). Loss of fitness, or diminishment of selected traits, has often been observed in crosses between selected lines and unselected controls, showing that genetic interactions can be important in evolution on a very short time scale (106).

Speciation Genetics

The genetic studies of the results of inter-specific crosses have revealed at least two relatively general patterns (30): genes responsible for the loss of fitness in hybrids between species are likely to be on the X-chromosomes (the "large X-effect"), and if only one sex is affected, that sex is likely to be the heterogametic sex (i.e. the sex with only one sex chromosome, like male mammals—this fitness pattern is called Haldane's Rule—47). That alleles on the X chromosomes are fit in their own species but fail in the genetic context of other species' genes (even in a hemizygous state) implies that epistasis between X-linked loci and the autosomes determine the fitness of hybrids (27, 33). For some species of *Drosophila,* even in a homozygous state, X-linked alleles in the presence of autosomes from another species can cause low fitness (94).

We would like to know whether this sort of epistasis implies multiple fitness peaks, but unfortunately this information is unavailable and indeed unknowable. We do not know the full fitness function for any of the speciation genes studied. We do know that the fitness of same-species homozygotes is high, and that the fitness of cross-species heterozygotes is low. However, we cannot know the fitness of all cross-species homozygotes, i.e. individuals homozygous for alleles from the same species at each locus, but with different loci having different sources. For the very reason that the phenotype being studied is loss of fitness and certain hybrids are inviable or sterile, all of these genotypes cannot be constructed. That being so, it is impossible to reconstruct the fitness functions.

Even if the fitness functions for a two-locus pair were measured, this still would not tell us if the fitness landscape had true valleys. It is always possible that the state of a third locus determines the precise contour of the two-locus landscape, and that some unexamined state of that third locus might allow deterministic evolution over the two-locus space. Furthermore, it is also possible that within one of the loci, there has been a sequential substitution of alleles. For example, let A_1, A_2, and A_3 represent alleles at a locus A, and B_1 and B_2 at locus B, where A_1 and B_1 are the ancestral alleles. If A_1 and A_2 (but not A_3) interact well with B_1, and A_2 and A_3 (but not A_1) interact well with B_2, then in an evolving species A_2 can be substituted first for A_1, and then to B_2, and then from A_2 to A_3. The population need never pass through an adaptive valley, but the derived species would be reproductively isolated from the ancestral species in a way that would implicate epistasis. It is impossible to demonstrate that an allele such as A_2 did not ever exist, and therefore we cannot show that a landscape is truly peaked. Similar problems haunt our studies of F_2 breakdown of among-population crosses, recombination load, and linkage disequilibrium (below).

Recent, more precise maps of isolating factors have shown that epistasis influences fitness at a very small chromosomal scale as well. A series of papers from Wu and colleagues have shown epistatic interactions in a variety of regions in crosses between *Drosophila simulans* and its close relatives, *D. mauritiana* and *D. sechellia* (12, 32, 96). Factors that seemed to indicate single locus effects, on more detailed investigation turned out to be closely linked sets of loci that interact epistatically to affect fitness in male hybrids (12, 96); in each case, two closely linked factors were required to get the deleterious effects. These kinds of interactions are of the type described in the previous paragraph: Interaction with a third locus determines the fitness consequences of a two-way interaction. Isolating mechanisms in *Mimulus* also show epistatic effects (82).

Stabilizing Selection and the Adaptive Landscape

A presumably common form of selection on quantitative characters is stabilizing (or optimizing) selection, where selection favors intermediate trait values in preference to either extreme. Stabilizing selection on polygenic traits, even when the genetic effects and variance for the phenotype are additive or involve dominance, generates epistasis for fitness (3, 4, 137). This is because the effect on fitness of an allele at a segregating locus depends on whether the allele finds itself in an individual with a slightly high or slightly low genetic value for the trait in question. If the mean of the population is at the optimal phenotype (i.e. there is no directional component of selection), then approximately equal numbers of the individuals in the population will be slightly above or slightly below that optimum. The value of the allele on fitness, then, depends on the state of all other alleles in the individual, and is therefore strongly epistatic (see Figure 2). Note that, as above, the prevalence of epistatic effects does not mean that the variance for fitness should be epistatic; the

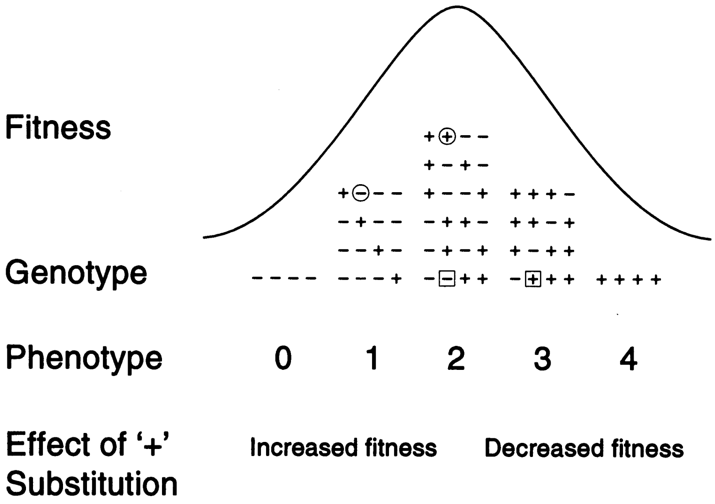

Figure 2 Stabilizing selection on an additive trait generates epistasis for fitness. The pluses and minuses represent the relative effects of alleles that affect the trait. The value of an allele for fitness depends upon the genetic background on which it finds itself: a + allele is favored in a background which would give a phenotype too small, but selected against if it is with too many other plus alleles. For example, the substitution from – to + is favored in the genetic background circled, but disfavored in the genotypes with boxes around them. Further, there are many different equally fit genotypes (e.g. —+—+ and Σ– —++) that correspond to multiple peaks in genotypic space.

degree of epistatic variance for fitness depends critically on the distribution of allelic effects (121).

There are many pitfalls in demonstrating the existence of stabilizing selection (124), including many other processes that can lead to the appearance of optimizing selection but that do not share the property of epistatic effects for fitness. For example, a trait under direct and directional selection toward one extreme, and under indirect selection toward the other extreme due to correlated response to selection on another character, can appear to be under stabilizing selection. This generates epistasis for fitness only temporarily, however, until selection can act on the component of variation for fitness that is not antagonistic. Even though in this example the hypothetical equilibrium does not necessitate epistasis for fitness, reaching this equilibrium may take a very long time, and there can be great epistasis for fitness in the meantime. The real problem is distinguishing these latter types of processes from true optimizing or stabilizing selection. Nevertheless, Travis's review shows several solid studies that demonstrate optimizing selection (124).

Selection in Subdivided Populations

As a consequence of Wright's shifting balance model, the prediction that subdivision of populations may increase the response to selection has been tested several times. This result would hold if there were strong epistatic interaction factors segregating in the population, but also if recessive alleles were sufficiently important to response to selection. Many of these studies, unfortunately, involve very high rates of mixing between subpopulations (59, 83, 103), which should eliminate the expected selection response even with strong epistasis (87). There are a few studies without too strong migration that do demonstrate stronger (36, 128) or divergent (24, 62, 75) responses to selection.

Linkage Disequilibrium in Selected Populations

As a consequence of selection on complex landscapes, selection tends to build up associations of alleles that function well together, leaving poor allele combinations in lower frequencies. This linkage disequilibrium is, of course, counterbalanced by various processes that lead to recombination, which breaks up these associations. As a result, many loci shown to interact epistatically are tightly linked, or in "co-adapted gene complexes" (see 51 for review). Linkage disequilibrium is often thought to be rare in natural populations (51), but new, more powerful techniques have demonstrated that there is more disequilibrium than formally thought (140).

The presence in natural or experimental populations of linkage disequilibrium has often been taken as good evidence of epistatic interactions for fitness, but this evidence must be taken with a few caveats. There are several studies that claim to have found disequilibria corresponding to epistatic selection (1, 21, 51, 64, 76, 119). Lewontin & White have shown that there are interactions among different grasshopper chromosomes in fitness, such that there are multiple fitness peaks that are apparently stable over time (76). Similarly, two experimental populations of cultivated barley demonstrate similar levels of disequilibrium, with the same chromosome pairs being common (21).

Unfortunately, while these results are consistent with multiple-peak epistasis, there are alternative explanations. The interactions of inversion types to produce overdominance have often been listed as evidence of coadaptation (e.g. 99), but in fact associative overdominance, due to the accumulation of different recessive deleterious alleles on different inversions, can create the same pattern without epistasis. Similarly, it is important to distinguish the effects of genetic drift or hitchhiking, which can also generate disequilibrium (93), particularly if the study is done at a geographic scale such that there is nonrandom mating.

Particularly good examples of disequilibrium in nature are given by *Cepaea nemoralis* and *C. hortensis* (57). In these snails, many of the multiple loci that control various shell patterns and shell color are in strong disequilibrium, packed into a "supergene" with low recombination among loci. These genes have been studied extensively in the field and have been shown to interact strongly to determine fitness in an environment-specific way.

MULTIPLE PEAKS FROM UNDERDOMINANCE

Underdominance at a single locus is probably the simplest type of multiple peak system. The term underdominance implies that heterozygotes are less fit than either homozygous type. With underdominance, the adaptive landscape is U-shaped, with peaks at fixation for either allele. Largely because it is a single locus system, peak shifts with underdominance have received the most complete theoretical investigation of any multiple peak system (see 6 and references cited there). One of the main results of these theoretical studies is that the underdominant valley can be traversed only if population (or neighborhood) sizes are small (6, 70), although the rate of fixation in these circumstances could be appreciable in subdivided populations, even with strong selection (1, 6, 7).

Underdominance has been studied primarily to explain the existence of the fixation of alternative chromosomal inversions in different populations and species (131). Chromosomal inversions can reduce the fitness of heterozygotes through the segregation of aneuploid gametes. Major chromosomal rearrangements of this type are widespread among animals (131, 132) and are thought to be fixed in populations at the rate of roughly 10^{-6} to 10^{-7} per generation in vertebrates (69). The existence of large numbers of cases of chromosomal inversions suggests that multiple peaks via underdominance may be a common occurrence, and this point has been used as the major evidence for the importance of genetic drift in natural populations, especially during speciation (40, 76, 132). Recent work on second- and third-chromosome pericentric inversions in *Drosophila* suggests that these conclusions may be premature, however (28, 29). Coyne and co-workers have demonstrated that some types of chromosomal inversions thought to lead to underdominance do not necessarily cause a reduction in heterozygote fitness (29), and these types have been found segregating in natural populations (28). Although most pericentric inversions are likely to be underdominant, some subsets may not be, with the obvious implication that the non-underdominant types are those fixed between populations (29). The underdominance of chromosomal rearrangements must therefore be demonstrated rather than assumed. Such a demonstration has not been performed for any polymorphic pericentric inversion (28).

Given the uncertain status of chromosomal inversions, the only demonstrable case of underdominance is shell coiling in snails (56, 78). Many, often closely related, gastropod species differ in the direction of coiling, some coiling dextrally and some sinistrally (42). Variation in coiling direction exists within some populations of the snail *Partula* (56, 78) and is known to be caused by variation at a single locus (90). Since snails with different coiling directions find mating difficult (78) or impossible (42), this is a solid example of underdominance for fitness. It has been suggested that coiling differences may lead to rapid speciation through peak-shifts (42, 95). The importance of a peak-shift is somewhat mitigated, however, by the fact that maternal inheritance of this character reduces (but does not eliminate) the depth of the valley (95).

MULTIPLE FITNESS PEAKS ON PHENOTYPIC LANDSCAPES

As discussed in the introduction, fitness functions can also be (and often are) drawn to show the relationship between phenotypes and fitness. If these phenotypes are genetically determined by genes that interact additively, then the genetic landscape will be complex as well. This section describes some of the evidence that the fitness functions of phenotypes can be complex and possibly multiple-peaked.

Disruptive Selection in Natural Populations

While it is very difficult to demonstrate multiple peaks in the fitness functions of natural populations for reasons outlined in the next section, several examples exist in which researchers have shown just that. For example, in the African *Pyrenestes* finches, there are two morphs of bill width, one that corresponds to a shape appropriate to cracking and handling one type of seed, and another that corresponds to another seed type. The phenotypic values between these forms are much less fit in certain life stages than are the forms at the adaptive peaks (113). Functional morphological and ecological analyses have demonstrated that the Galápagos finches have different species that correspond similarly to discrete niches determined by seed size and hardness (109). The analysis of the Galápagos finches was sufficiently successful to predict the presence of extinct members of the clade. Disruptive selection, caused by the presence of discrete niches in resource use, can cause multiple adaptive peaks to exist in nature.

Characters sometimes demonstrate adaptive peaks when selected in concert with other characters. One particularly good example of this is *Thamnophis* garter snakes, which have multiple color pattern morphs and multiple behavioral strategies for escape from predators. Snakes that are striped are easy to see when still, but give little indication of ground speed when racing away from a predator. On the other hand, spotted snakes are more likely to be cryptic

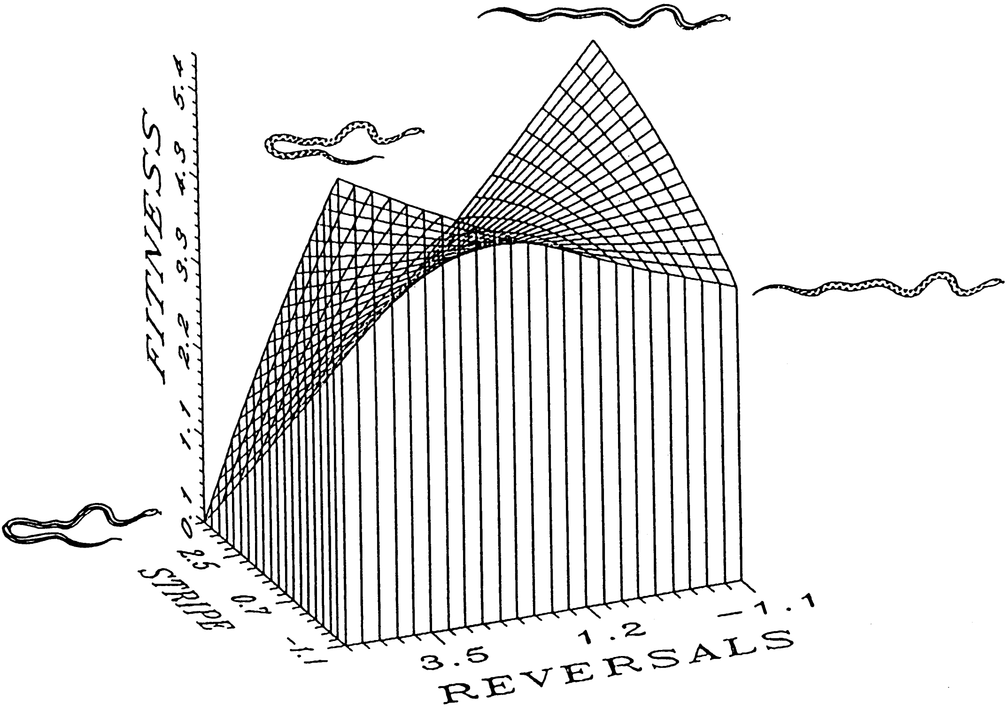

Figure 3 Brodie (9) measured the survivorship through the first year of young garter snakes (*Thamnophis ordinoides*), a highly polymorphic species. Snakes with longitudinal stripes survive better if they run straight away from predators, but snakes with blotched patterns are better able to avoid predators by reversing the direction of flight and attempting to hide cryptically. This is the presumed reason for the saddle in the fitness function of striping and the tendency to reverse direction. [From (9)] © 1992 Society for the Study of Evolution. Reprinted with the permission of the author and publisher.

when stationary but are more easily seen when moving. Brodie has shown that the stripedness of snakes is negatively correlated with the extent to which snakes reverse direction when fleeing a predator (9). Furthermore, the snakes that have the appropriate behavior to match their color pattern type are much more likely to survive through their first year (9) (see Figure 3). Similar results have been demonstrated with cryptic coloration patterns and resting site choice in *Biston betularia* (61).

Selfing rates provide another example of this type of disruptive selection. The evolution of selfing and close inbreeding in plants is facilitated if the plant is not very subject to inbreeding depression. Outcrossing is likely to be favored if the level of inbreeding depression is high. Inbreeding depression itself is a genetic property of a species, which can respond to selection; close inbreeding tends to decrease the marginal costs of inbreeding, because deleterious recessive alleles have been selected out of the population. As a result of these processes, the stable genetic states for mating systems are thought to be outcrossing (with a relatively high degree of inbreeding depression) or close inbreeding (with a relatively small associated cost to fitness) (73). This pattern has been arguably demonstrated in a collection of natural populations (108). Thus mating systems and degree of inbreeding depression are two genetically controlled characters which determine an adaptive landscape with a saddle and two peaks.

Multiple Phenotypic Solutions to the Same Evolutionary Problem

Another kind of phenotypic analysis providing evidence for the ruggedness of adaptive peaks comes from studies of genetic redundancy for fitness; there are many different ways in which different populations can solve the same environmental challenge. For example, some *Drosophila melanogaster* populations have avoided alcohol toxicity by avoiding foods that contain ethanol, while others have evolved stronger physiological mechanisms to metabolize the alcohols (23, 25, 26). Similarly, different populations of the same species of butterflies have evolved different phenotypes to mimic different forms of other toxic butterflies: hybrids between these forms are distinctly less fit (84, 85). This sort of polymorphism is extremely common in butterfly mimicry systems (125). Many other examples of this sort of genetic redundancy are known (23, 26, 134). As will be discussed further in the next section, multiple solutions to the same problem do not necessarily imply multiple fitness peaks. Only if there is a fitness cost to combining multiple solutions (as in the mimicry examples) will there be more than one peak on the adaptive surface.

LIMITATIONS TO THE STUDY OF EPISTASIS AND MULTIPLE FITNESS PEAKS

We have seen that there are many examples of epistatic interaction both at the phenotypic and genetic scale in natural populations. We also, however, have seen that there are many examples where evidence of epistasis is lacking. This section explores the overt consequences of epistatic interactions and multiple peaks, and asks, what are the strengths and weaknesses of various possible tests of the prevalence of epistatic interactions and multiple peaks?

The Problem with Epistatic Genetic Variance as a Measure of Epistasis

Because epistatic variance is rarely found in breeding experiments, it is often assumed that epistatic interactions are of little importance in creating phenotypes from genotypes. As we have seen earlier (see Figure 1), even pervasive epistatic interactions on peaked fitness landscapes are expected to demonstrate little epistatic genetic variance at equilibrium. Epistatic variance is a poor measure of the ruggedness of an adaptive landscape. There are also several reasons why the epistatic variance that does exist is very difficult to demonstrate statistically.

First, the analysis of variance techniques used for these experiments are biased against finding epistatic effects (127). The least squares analysis implicitly removes as much variation as possible to be attributed to main effects, leaving little available for attribution to interaction terms. Because correlations between variance components tend to be large (18), the hierarchical fitting of effects minimizes estimates of epistatic components. Further, since many genotypes containing epistatic alleles will be rare, estimates of the main (additive) effects of alleles are confounded with the true underlying interactions in a manner analogous to estimates arising from an unbalanced ANOVA (127). While this is appropriate for understanding the short-term response to selection (37), it is misleading if we want to understand the real genetic architecture of traits.

Second, confidence limits on genetic variance components are generally large, and this is particularly true for the epistatic variance (18, 48, 81). The magnitude of the confidence limits derives in part from the small coefficients associated with the epistatic effects in the covariances among relatives. For example, one half of the additive genetic variance contributes to the variance among full sibs, whereas one fourth of the additive-by-additive epistatic variance does so, and only one sixteenth of the dominance-by-dominance epistatic variance (37). Unfortunately, the error for each component is relatively constant, so there is one half to one eighth the power to detect the epistatic components compared to the additive variance. Experiments seeking reliable estimates of epistatic variance must therefore be quite large (80). As a result these experiments are rarely performed.

Third, in experiments conducted in artificial environments, interactions between the genotype and the environment in producing phenotypes can obscure the true nature of genetic variation in natural environments (111). Many evolutionary genetic experiments that aim to estimate nonadditive genetic variance are conducted in artificial environments and may be extremely misleading. Epistatic variance can vary strongly from one environment to another (2, 50, 55).

Finally, linkage disequilibria can hide epistasis. Tightly linked, interacting genes will appear to be additive in effect, because the alleles will, in the course of an experiment, rarely be separated. Because many examples of epistatic interactions also involve tight linkage (so-called coadapted gene complexes), the bias associated with linkage can be extreme.

The Difficulties of Estimating Epistatic Gene Effects

The problems associated with measuring epistatic genetic effects are also significant. One source of bias is that those who study genetic effects directly rarely also study fitness per se, and even more rarely are the fitness effects of gene combinations examined. A fundamental difficulty in understanding the potential for epistatic interactions is that we should expect variants that interact to affect fitness to be rare (see "simple model" section above), and therefore that they only infrequently come to the attention of biologists studying these systems.

Fortunately, there are ways of studying fitness landscapes that escape these difficulties, although they have up to now been applied only infrequently. The difficulties of estimating epistatic interactions stem from the constraints within populations to be at stable equilibria. When gene flow is restricted between two different breeding pools, the genetic systems are no longer constrained to function well together. Therefore, crosses between distant populations or closely related species should give a less biased picture of the ruggedness of genetic landscapes. In other words, the nature of genetic variation within species or populations is constrained to be at a fitness peak, and therefore biased, but the effects of genes combined across species are less constrained in this way. The genotype that occurs as a result of the combination of two distinct gene pools that have evolved completely in allopatry lands to a small extent in a random part of the adaptive landscape and gives us a less biased view of what unselected genotypes might be like. (We say "to a small extent" because even this analysis is predominantly biased: Most genes are shared by species related closely enough to allow viable offspring. The very existence of reproductive isolation between species that have had no direct selection to evolve reproductive isolation gives very strong evidence for the extremely rugged nature of adaptive landscapes.)

Difficulties in Demonstrating Disruptive Selection

The difficulties in determining the extent of disruptive selection or the density of valleys on a phenotypic adaptive landscape are very similar to the reasons why discovering epistasis on the genetic level is difficult. Sexual species are usually constrained to be near single adaptive peaks (71), and this constrains the range of phenotypes available for study. We cannot measure the fitness effects of phenotypes that do not exist; therefore we are usually limited to measuring the effects of phenotypes clustered around single peaks. The few examples we have discussed above are unusual, not because they demonstrate disruptive selection, but rather because there is some unusual situation that allows variation to be maintained in the population. In the case of the *Pyrenestes* finches, for example, the genetic variance of the trait is essentially due to a single locus (114). It is much easier to maintain variance with single macromutations than with a polygenic basis for trait variation. Other examples also are maintained by likely frequency dependent selection or recent intergradation of populations (9). The landscape for other examples is inferred across species (108). Studies based on interspecific comparisons, interpopulational transplants, phenotypic modification, and other experimental techniques should be more promising avenues for truly understanding the nature of adaptive landscapes than is possible with the strictly observational approach. Further, adaptive landscapes on the phenotypic scale to some extent reflect the functional relationship between fitness and phenotype, such that expectations based on functional morphology may point the way to the existence of multiple peaks (e.g. 109).

The Impossibility of Finding True Valleys

As has been briefly discussed in the section on speciation genetics, it is difficult to know whether epistasis for fitness translates into multiple fitness peaks. Alternatively, the multidimensional fitness function may be simply a rugged landscape with ridges connecting all high spots and no true valleys, merely depressions around which selection must detour. We face this problem not only because it is difficult to reconstruct all pertinent genotypes from the alleles which segregate within a population (and therefore the "local" landscape is difficult to measure), but also because we do not have access to all possible alleles. The number of possible genotypes in a *Drosophila* genome, for example, is close to 10 raised to the 200 millionth power, which is the sort of number that makes us glad for scientific notation but despair of ever testing even a tiny subset of the possibilities. Alleles at one locus can change the fitness function of other two-locus pairs (12, 32, 96) and eliminate the apparent adaptive valley between certain combinations. Furthermore, it is often quite difficult to reconstruct all corners of the genotype-to-fitness matrix; we most often simply do not know what trans-species homozygotes are like, for example. It is therefore impossible to ever rigorously claim that any particular landscape is necessarily multiply peaked; we can only know that there is epistasis for fitness.

Underdominance systems have the same difficulties. Like all of the cases of multiple peak systems discussed above, underdominance at a single locus is a very unstable state. We might therefore expect to see variation for underdominant alleles far more often between populations than within populations, an expectation that does match the usual pattern of chromosomal inversions (131). Indeed, there are several examples of hybrid zones in which one chromosomal inversion is on one side of the tension zone and another on the other side (132).

These problems, while substantial, do not negate the importance of studying landscapes. If alleles that allow deterministic change do not yet exist, then populations will experience constraints to adaptation until those alleles appear in the population, which could be a very long time (97). If the slopes of the fitness function are very slight in the neighborhood of the current allele frequencies, then evolution may not follow the deterministic path; mechanisms of shifts across local valleys may yet be important. Finally, adaptive landscapes may in fact be peaked; if so the only way we can test the hypothesis is by attempts at falsification. The evolutionary consequences of genetic interactions for fitness are too great not to investigate these issues as fully as we can.

CONCLUSIONS

Many examples of epistatic interactions, both for phenotypic traits and fitness, have been reported in the genetics literature. Some of these examples report multiple adaptive peaks, many others demonstrate rugged adaptive landscapes, and many show that the evolution of traits need not follow an additive model. There are many other traits, however, that do not show this kind of interaction: The additive model is adequate for many of the characters that have been studied. This review has focused on showing that this need not be the case, but rather, genetic interactions can be an important part of evolution.

There are many reasons why traditional approaches to the study of epistatic interactions and multiple adaptive peaks are biased strongly against finding evidence of these phenomena. In spite of these difficulties, there are many examples in the literature of epistasis and of multiple peaks on a phenotypic scale, which is amazing in the face of the statistical (and sociological) difficulties of studying the phenomenon. This argues that epistasis plays a much larger role in determining phenotypes and fitness than is generally thought. Furthermore, we have been looking in the wrong place; interspecific comparisons are critical to determining the ruggedness of adaptive landscapes. When these comparisons are made, epistasis (or underdominance) is most often found. The importance of epistasis in evolutionary genetics is not in the generation of epistatic variance per se, but in the particular interactions of specific loci, and the way in which these interactions structure the set of possible evolutionary outcomes. Our interpretation of the relative importance of multiple fitness peaks in the evolutionary process will depend on how prevalent these interactions turn out to be and how carefully we endeavor to find them.

A biologically significant contrast has struck us from our reading of the literature, although there is little hope of reliably testing this with the data we have now. The level of epistasis for fitness tends to increase with phylogenetic distance. Crosses performed within populations show little epistasis for fitness, but crosses from distantly related populations or between closely related species almost universally show such interactions for fitness. To a certain extent this may seem tautological: Speciation can only occur if there are gene combinations that do not function well together but do perform well with other combinations. The data strongly demonstrate this pattern. We need to lift the constraints imposed by selection and recombination, which limit the frequencies of alleles that do interact with other alleles in affecting fitness, in order to see what adaptive landscapes truly are like. Each species cross represents a random sample of the landscape. The simple fact that there are species implies that adaptive landscapes are rugged indeed.

A fundamental problem of studying adaptive landscapes, as pointed out above, is that we can never truly know whether there are ridges connecting "peaks." We cannot measure all possible genotypes, and we do not have access to all genotypes which may have existed in the history of the divergence of natural populations. We must, then, be satisfied to know that landscapes are rugged, without knowing that they are peaked, or else we must study fine-scale differentiation in much more detail, for example in laboratory experiments. Furthermore, fitness landscapes are intrinsically linked to the environments in which the species evolves; it is clear that the landscape can change as a function of environmental changes. We must learn more about the permanence of landscapes over time. Until such work is performed, we can never truly know whether peak shift models are necessary, and we run the risk, in the absence of such proof, of ignoring the genetic interactions that may be an extremely important part of the evolutionary process.

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