sediment would solidify as intact rocks—there is no compelling reason to abandon alternative models for the interior of Eros, including that of a compacted fine-grained silt that experiences faults and fissures like any clod of dirt. A dirt-clod model for Eros and similar asteroids is defensible: It can explain why the spectral characteristics of the most abundant meteorites on Earth differ from those of the most abundant asteroids in near-Earth space, because ejected dirt clumps could not survive a meteorite's brutal fate during ejection from their parent asteroid and entry through Earth's atmosphere. Common meteorites would have to come from stronger parent asteroids.

These are exciting times for asteroid science, but one wonders how long they can go on without an unambiguous foundation. Understanding asteroids is central to understanding how planets emerged from the primordial swarm of planetesimals. Asteroids also show us how fundamental geophysical processes—such as geomorphology, impact cratering, and granular mechanics—behave in a microgravity environment. And certainly, the challenge of diverting a hazardous asteroid will require some knowledge of asteroid geology (*12*), unless we are to go shooting in the dark when that time comes.

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EVOLUTION

Epistasis in RNA Viruses

Yannis Michalakis and Denis Roze

If you cross two black guinea pigs you may get several albino pups. This could happen, for example, if the boar and sow are both heterozygous at the *C* locus for the c^a allele, which prevents the production of the pigment melanin. Guinea pig fur color is determined by many genes, but the *C* locus strongly affects the expression of these genes in homozygous c^a individuals. The *C* locus is said to be epistatic because it affects the expression of genes at other loci. Developmental biology is replete with examples of epistatic gene interactions. On the other hand, evolutionary biology is desperately searching for them. Two recent studies—by Sanjuán *et al*. in a recent issue of *Proceedings of the National Academy of Sciences U.S.A.* (*1*) and by Bonhoeffer *et al*. on page 1547 of this issue (*2*)—report analyses of epistatic gene interactions in RNA viruses and discuss their relevance to viral recombination.

Epistatic interactions are important in evolutionary biology almost whenever multilocus genetics matter. The recent explosion of interest in epistatic interactions is epitomized by publication of a book devoted to the topic (*3*). Regarding his doctoral work on guinea pig fur color and its determination, Wright has admitted that this study was one of four factors that led him to propose his "shifting balance theory" of adaptive landscapes (*4*). Epistatic interactions are also prominent in studies of speciation and reproductive isolation (*5*). And, inevitably, they are central to the evolution of genetic recombination (see the figure) (*6*).

There are very few examples of epistatic gene interactions in evolutionary biology, principally because of the difficulties inherent in performing such studies. One either needs to know all the genes determining a trait, which is typically rare in evolutionary biology, or one needs to conduct relatively laborious breeding experiments. Moreover, the relation between the trait's value and its fitness benefit is crucial and must be at least partly known. It is not surprising, therefore, that the few examples of epistasis in evolution come from microorganisms. The two new RNA virus studies provide measures of epistatic gene interactions allowing fresh insights into their effects on evolution (*1*, *2*).

In their new work, Sanjuán and colleagues (*1*) examined vesicular stomatitis virus (VSV), which does not exhibit recombination. They analyzed a set of single-nucleotide mutations generated by directed mutagenesis. The individual fitness effects of these mutations, deleterious or beneficial, were previously established (*7*). The authors now report the fitness effects of pairs of mutations that are either deleterious or beneficial when single. Remarkably, singly beneficial mutations when paired exhibit negative epistasis, whereas singly deleterious mutations when paired exhibit positive epistasis. In both cases, the mutation pairs act antagonistically: Their combined effect is less than that expected from their individual effects.

In a related study, Bonhoeffer and colleagues present evidence for positive epistasis in another RNA virus, human immunodeficiency virus 1 (HIV-1) (*2*). These authors examined the evolution of recombination in HIV-1, which unlike VSV is known to recombine frequently. If recombination in HIV-1 had been selected for because of gene interactions, then epistasis between pairs of genes should be slightly negative (see the figure). Surprisingly, Bonhoeffer and colleagues report that gene interactions in HIV-1 exhibit positive epistasis.

Their data come from an impressive analysis of the amino acid sequences of the protease and the major part of the reverse transcriptase of HIV-1 derived from 9466 patient samples. These retroviral samples were obtained from HIV-1–infected patients undergoing drug therapy. The authors then analyzed the fitness benefits conferred by these two proteins. Briefly, genes encoding the two viral proteins were introduced into NL4-3, a molecular HIV clone that can undergo only one replication cycle. Fitness of the viral progeny was measured in vitro by comparing the number of progeny produced by these constructs to that produced by the NL4-3 clone. The presence of epistasis was tested in two ways: (i) by analyzing the relation between the logarithm of fitness and number of mutations differentiating each sample from the reference clone; and (ii) by examining the fitness of pairs of di-allelic polymorphic sites for which all four possible combinations were observed. The effects of mutations at other loci were averaged. Both methods revealed positive epistasis in HIV-1.

In the results from the second method used by Bonhoeffer and co-workers, epistasis spanned negative and positive values,

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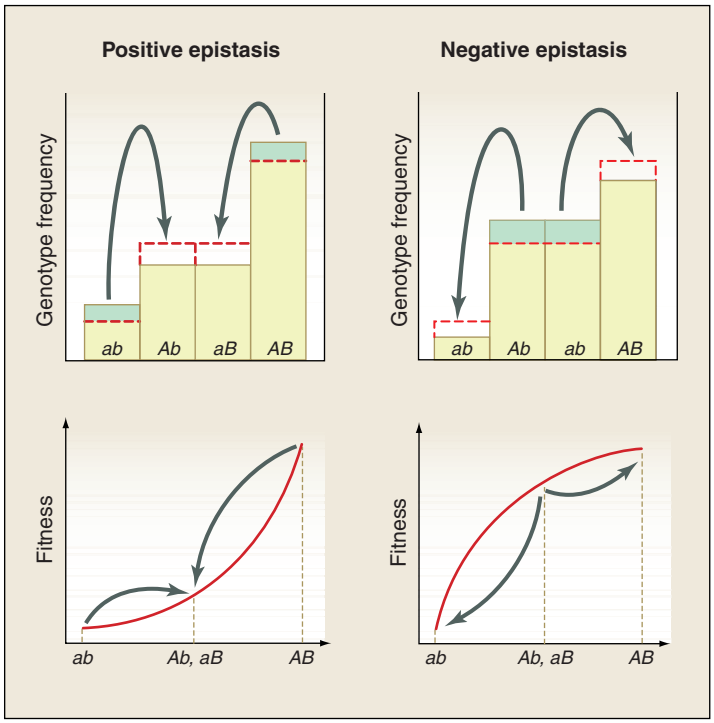

Evolution of recombination between two selected loci. Genotypes *ab*, *Ab*, *aB*, and *AB* have relative fitnesses 1, $1 + s$, $1 + s$, and $(1 + s)^2 + e$, where e is a measure of epistasis. Positive epistasis generates an excess of extreme genotypes [(**top left**) dashed lines correspond to linkage equilibrium]. Recombination tends to reduce this excess by re-creating intermediate genotypes (arrows) that are less fit on average (**bottom left**). This effect selects against recombination. Furthermore, recombination decreases the variance in fitness, which reduces the efficiency of selection and also selects against recombination. Negative epistasis (**right**) generates an excess of intermediate genotypes, which is reduced by recombination (arrows). Because extreme genotypes are less fit on average, this selects against recombination. However, recombination now increases the variance in fitness, thus increasing the efficiency of selection and resulting in selection for recombination. Theoretical models show that the benefits of recombination in terms of increased variance in fitness are stronger than the cost in terms of average fitness when epistasis is negative and sufficiently weak (*15*). Two other terms often used in the literature are "synergistic" and "antagonistic." Synergistic ("work together") means that combinations of mutations have stronger effects than those expected from the effects of the individual mutations. Thus, synergism among mutations results in positive epistasis for beneficial mutations and negative epistasis for deleterious mutations ("makes things better when things are good, and worse when they're bad"). Antagonistic ("struggle against") means that combinations of mutations have weaker effects than those expected from the effects of the individual mutations. Thus, antagonism among mutations results in negative epistasis for beneficial mutations and positive epistasis for deleterious mutations ("makes things worse when they are good, and better when they are bad").

the mean being positive. When only mutations that give rise to strong effects were considered, an even larger mean value for positive epistasis was obtained. Interestingly, these mutations are known to have arisen in response to drug therapy. Unfortunately, Bonhoeffer *et al.*'s second method—which a priori could be compared to the analysis of Sanjuán *et al*. (*1*)—did not examine whether epistasis was positive or negative when the mutations involved were singly deleterious or singly beneficial (after averaging over the genetic background) (*8*). Given that most of the HIV-1 isolates had a lower fitness than that exhibited by the NL4-3 HIV clone, we conclude that epistasis between deleterious mutations is positive (antagonistic) in HIV-1.

Why should that be the case? Epistasis between loci, like dominance between different alleles at a given locus, could be viewed as either the result of evolution or the unavoidable consequence of the way organisms function. For example, the metabolic control theory of deleterious mutations affecting enzyme activities predicts that epistasis will be either synergistic or antagonistic depending on whether the mutations in question affect the same enzyme (always synergistic) or not. If the mutations affect different enzymes, the nature of epistasis will depend on whether selection acts to maximize metabolic flux (antagonistic) or the concentration of an intermediate in the pathway (partly synergistic), or to optimize either the flux or the quantity of metabolic product (mostly synergistic) (*9*).

We do not know of any models explicitly addressing the question of the evolution of epistasis. Models of the evolution of genetic robustness, however, provide some basis for discussion (*10*). Organisms with high mutation rates, such as RNA viruses, are expected to evolve flat fitness landscapes, meaning that they comprise many genotypes with equal or almost equal fitness (*11*, *12*). This kind of fitness landscape should lead to negative epistasis for deleterious mutations (synergistic) (*13*), and also negative epistasis for beneficial mutations (antagonistic).

Environmental variation as a factor affecting whether epistasis is negative or positive has only been touched on. In a simulation study of the effects of deleterious mutations on bacteriophage T7 growth, severe deleterious mutations were always found to interact antagonistically, whereas the interactions between mildly deleterious mutations depended on the quality of the environment. In rich environments, the mutations were also antagonistic, but in poor environments they were synergistic (*14*). It is difficult to generalize and extrapolate the results of this study to other organisms, which may have different metabolic activities and live in different environments. This study, however, underlines that the effect of the environment on epistasis deserves more attention, both theoretically and experimentally. Future studies should complement our understanding of how and whether a particular kind of epistasis is dependent on the environment.

The Sanjuán *et al*. and Bonhoeffer *et al*. studies show that the pattern of epistasis in RNA viruses is not compatible with current genetic theories of sexual reproduction and recombination, which assume that mutations affecting fitness exhibit negative epistasis. Given that VSV exhibits no recombination, perhaps this is not a big problem. In contrast, HIV-1 recombines frequently and Bonhoeffer and colleagues propose that it may do so to repair single-strand breaks in the RNA genome. This and the fact that VSV and HIV-1 are subject to exceptionally high mutation rates caution us against extrapolating results obtained with these simple organisms. Nevertheless, their contribution to our understanding of evolution remains invaluable.

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