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Compensating for the meltdown: The critical effective size of a population with deleterious and compensatory mutations

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In the short term, the persistence of species depends on the continued existence of suitable habitat and protection from extraordinary causes of mortality, essentially ecological and socioeconomic problems. On a longer time-scale, however, genetic problems could become paramount. Populations that have only deleterious mutations eventually decline in fitness to extinction, because of the fixation by genetic drift of a small fraction of these mutations. This proceeds fastest in small populations, because genetic drift is a more powerful factor in these circumstances. If, as is biologically reasonable, some mutations are beneficial to the population, there will exist a critical effective size above which the population can persist indefinitely, because fixation of beneficial alleles can balance the effects of deleterious mutations. This critical effective size is likely to be in the hundreds, meaning a census population size in the thousands. If some mutations act to compensate for the detrimental effects of others, then the rate of beneficial mutations will increase as fitness declines; this causes the critical effective size to be even lower. In this paper, we review the theoretical impact of beneficial and compensatory mutations on the probability of extinction, as well as the substantial theoretical and empirical literature on compensation. There are many possible mechanisms for compensatory mutations. There are insufficient data to make quantitative predictions, but it is clear that there is more hope for preserving the genetic integrity of threatened species than previously thought.

Introduction

Adaptation to a new environment proceeds by natural selection using the variation created by mutation; without mutation the pace of evolution would slow and adaptation would halt. New beneficial mutations are therefore essential to the persistence of a species. But this need for new mutations comes with a large cost: new mutations are most likely to be deleterious to an organism. The immediate effect of most new mutations is to reduce the average fitness of both the individual and its population. In species that effectively consist of a small number of

individuals, genetic drift can permit the fixation of deleterious mutations, thereby lowering the mean fitness of the species over a longer term. If this fixation of deleterious alleles continues unabated, this "mutational meltdown" can result in the eventual extinction of the species. This is thought to be one of the most serious genetic threats to the persistence of endangered populations (Charlesworth *et al*. 1993, Lynch *et al*. 1995a, 1995b, Lande 1994, 1995, 1998).

It is expected that most new mutations affecting fitness in a reasonably well-adapted population would be deleterious — if we were to randomly change a piece of machinery we would expect that it would function worse rather than better. Very occasionally, the function would improve, but this would be far outweighed by the dysfunction caused by other mindless tinkering. So it is with biological evolution: when mutations occur at random throughout the genome, on average they reduce the fitness of the individuals that carry them. This rather obvious prediction is borne out by experiments measuring the effects of new mutations; on average mutations alone will reduce the fitness of the individuals and populations that they occur in (Lynch *et al*. 1999).

But this is not to say that *all* mutations are deleterious. We know that some mutations are beneficial from the simple fact that evolution does proceed. This can be seen in a more controlled fashion by, for example, the experiments of Lenski and his collaborators, which have shown that even when genetically-uniform populations of *Escherichia coli* are begun with no genetic variation, they quickly increase in fitness by selection on *de novo* mutations (Lenski *et al*. 1991). This pattern is seen repeatedly, not only in the various replicates of these *E. coli*. experiments, but also in a long series of microbial evolution studies (Burch & Chao 1999, Holder & Bull 2001, Zeyl *et al*. 2001).

The distribution of new mutations into beneficial and deleterious categories becomes particularly important in species with relatively few individuals. Two features distinguish evolution in small populations from that in large populations. First, the allele frequency change from one generation to the next has a large random component; in other words there is random genetic drift due to the small population size. The smaller the effective size of the population, the more important genetic drift becomes relative to deterministic forces like selection. Second, there are fewer individuals in the population to have mutations, so the overall rate at which new mutations come into the population is lower; as a result there are fewer beneficial mutations available for natural selection to choose among. The effect of these two factors is that in small populations more deleterious alleles fix in populations (because they can drift to high frequencies without being as effectively opposed by selection) and fewer beneficial alleles fix (because there are fewer available to selection). At some point, this reduction in the efficacy of selection becomes critical: the population becomes small enough that the effects of fixing deleterious mutations reduce fitness faster than the effects of fixing beneficial mutations can increase fitness. At this *critical effective size*, evolution cannot increase the fitness of the species, and below this critical size the species will decline in fitness until either its situation changes or it goes extinct.

In this paper, we will review the theoretical and empirical basis of the critical effective size. In particular, we will focus on the strong possibility that the rate of beneficial mutations increases when fitness is lowered by the previous fixation of deleterious mutations. This increase is not due to discredited notions such as directed mutation, but instead comes from the simple idea that when something is broken it is easier to improve than when it is fully functional. We will review the evidence for *compensatory* mutations, that is, mutations that are more beneficial in the context of deleterious mutations than when combined with a more fit genetic background. There are many theoretical and empirical reasons to expect that compensatory mutations should be relatively common, and we explore the consequences of compensation on the probability of extinction due to mutational meltdown.

Definitions

Before proceeding further, it is worth stopping for a moment to discuss the specific defini-

tions of several terms used commonly in this article. Most critically, we will often have reason to refer to *beneficial* alleles; these are those alleles that increase the fitness of their carriers relative to the alternative alleles currently segregating at the same locus. There are several types of mutation that are subsets of beneficial mutations. First, some mutations are unconditionally beneficial to the taxa; that is, these mutations increase the fitness of their carriers in any genetic background available to the population. In a well-adapted population, these mutations are perhaps rare. *Reverse* mutations are mutations that revert the DNA sequence of a deleterious allele to the more fit ancestral state; these are also referred to as *back mutations* and by definition can only occur subsequent to a deleterious mutation event. Another subset of beneficial mutations is *compensatory* mutations; these mutations are only beneficial in the context of a previously fixed deleterious allele. The literature is ambiguous about whether back mutations should be considered to be compensatory, but in any case there are potentially many more ways to compensate for a deleterious mutation than a reverse mutation. Compensatory mutations at other loci imply epistasis for fitness, because these alleles are only strongly beneficial in the genetic context of the previous deleterious mutation. When a deleterious mutation has fixed, the fitness of the genotype carrying a compensatory allele will be higher than one not carrying this allele, but the fitness of the compensated genotype need not be as great as the original, pre-fixation state. If the fitness of the compensated genotype is higher than that of the ancestral state, we call this phenomenon *supercompensation* (Phillips *et al*. 2000).

The variance *effective population size,* N_e, is a quantitative device to describe the amount of genetic drift that is expected to occur in a population. It is the size of an ideal, randomly mating population that would have the same amount of genetic drift as the real population being described. In general, the effective population size is usually much smaller than the actual number of individuals in the population; a review of estimates of N_e has found that on average the effective size is about 10% of the census size of a population (Frankham 1995). This is just an estimate of an average, however; in reality there is a great deal of variation in the relationship between N_e and the census size.

The persistence of finite populations in the face of deleterious alleles

Fixation of deleterious alleles by drift

The possibility that the mean fitness of a population could decline as the result of deleterious alleles increasing in frequency via genetic drift was first broached by Sewall Wright in 1931, echoed by Crow in 1948 and developed further by Kimura *et al*. in 1963. At the beginning of the 1990s, Mike Lynch, his collaborators, and others developed the idea that the fixation of deleterious mutations through genetic drift could be a substantial cause for worry about the possibility of extinction of even moderately large populations (Lynch & Gabriel 1990, Lynch *et al*. 1993, 1995a, 1995b, Charlesworth *et al*. 1993, Lande 1994, 1995, 1998, Schultz & Lynch 1997). They showed that in both asexual and sexual species, populations even in the thousands had a life expectancy of a few thousand generations or less. Thus, in order to maintain biodiversity over the medium term, it is crucial to maintain a reasonably large population size. These results and those that followed had a great impact on conservation biology, forming a large part of the biological basis of the definitions of endangered and threatened species.

The conclusions of these papers are based largely on mathematical models that consider the evolutionary consequences of deleterious mutations alone. They reason that deleterious mutations are so much more common than beneficial mutations that as a first approximation it is reasonable to ignore the effects of beneficial mutations altogether. By this way of thinking, in the face of genetic drift fitness can only decline — once a deleterious mutation is fixed there is no way for the population to ever recover that loss in fitness.

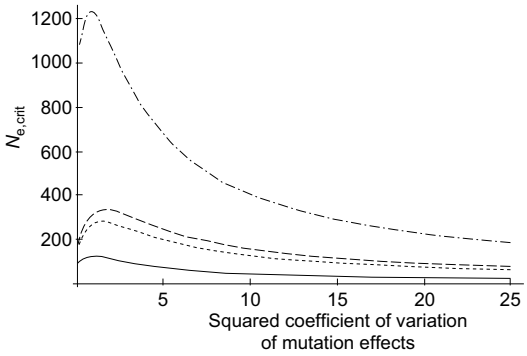

Fig. 1. The critical effective population size depends strongly on the coefficient of variation of the distribution of mutational effects. Four cases are plotted: — solid line (**a**): deleterious mutations are 1000 times more likely than beneficial and the mean effect of either is 0.02; — dotted line (**b**): same as **a** except deleterious mutations are 10 000 more likely; — dashed line (**c**) same as **a** except the mean effect of beneficial mutant is 0.005; — dot-dashed line (**d**) same as **a** except both beneficial and deleterious mutations average effect is 0.002. The critical N_e at which fixation of beneficial alleles counters the loss of fitness due to deleterious mutations is highest for values of the coefficient of variation near unity, i.e. when mutational effects are exponentially distributed. Reducing the mean effect of either beneficial or deleterious alleles increases the importance of genetic drift and therefore increases $N_{e,crit}$.

Mutational meltdown with beneficial alleles

Yet we know that beneficial mutations are possible, even if they are not common. The first analysis of the effects of beneficial mutation on the change in fitness of small populations was by Schultz and Lynch (1997) who with simulations showed that population sizes in the hundreds or thousands were required to prevent loss of fitness due to drift. Lande (1998) allowed for reverse mutations and showed that this class of mutations would not be sufficient to halt the meltdown but could slow somewhat the rate of decline towards extinction.

These models were extended by Whitlock (2000) who showed that there is a critical effective population size, $N_{e,crit}$, below which a population will decline towards extinction as a result of the fixation of deleterious alleles. However, above this critical effective size, a species would be able to persist indefinitely, because the loss of fitness from the fixation of deleterious alleles could be counterbalanced by a gain in fitness due to beneficial alleles. The critical effective size is the point at which the increase in fitness due to the fixation of beneficial mutations, ΔW_B, is exactly balanced by the decrease in fitness from the fixation of deleterious mutations, ΔW_D. These are the products of the numbers of new mutations per genome of a particular type, the probability that mutations of that sort fix, and the fitness effects of the mutations. With an exponential distribution of fitness effects of new mutations, these two quantities turn out to be approximately

$$\Delta W_B \cong 8 U_B N_e \lambda_B^2 \tag{1}$$

and

$$\Delta W_B \cong \frac{U_D}{8 N_e^2 \lambda_D} \tag{2}$$

where U_D and U_B are the numbers of new deleterious and beneficial mutations, respectively, per genome per generation, and λ_D and λ_B are the mean effects of the deleterious and beneficial mutations. (Note that $\lambda_D < 0$.) When we solve for the point at which fitness neither increases nor decreases, we find that the critical effective size occurs at

$$N_{e,crit} \cong \sqrt[3]{\frac{U_D}{64 \lambda_B^2 |\lambda_D| U_B}} \tag{3}$$

More general calculations in Whitlock (2000) allow for the effects of mutations to be gamma-distributed. The gamma distribution is a more general family of distributions (that includes the exponential), and Whitlock (2000) showed that the critical effective size is always highest when the distributions are approximately exponential. Equation 3 is therefore a conservative value in this respect.

This critical effective size is difficult to calculate quantitatively for wild populations, because a few of the parameters of the model are largely unknown from natural populations, such as the mutation rate and distribution of new beneficial alleles. Yet with plausible and conservative guesses of these parameters, the critical effective size is likely in the low hundreds (Whitlock 2000). Examples of these calculations are shown in Fig. 1.

These calculations depend critically on the distribution of mutational effects. In particular, what matters is the relative numbers of beneficial and deleterious mutations and the mean effects of these mutations, as well as the shape of the distribution. In general, we have almost no data on the values of these quantities. It is useful, therefore, to consider some theoretical predictions about what these values might be.

Moreover, the calculations in these papers implicitly assume that the values of these parameters are unchanging. However, as mentioned by Whitlock (2000), it is entirely possible that as the population declines in fitness by accumulating deleterious mutations, the relative rate and mean size of beneficial mutations may increase. This increase could be due to the possibility of compensatory mutations, mutations that are only beneficial after the fixation of other deleterious alleles. Much of the rest of this paper will explore the theoretical and empirical evidence for compensation.

Predicting the conditions when compensation is expected

Stabilizing selection

One of the most obvious conditions for which we might expect compensatory mutations is when there is stabilizing selection on a polygenic trait. With stabilizing selection, traits have an intermediate optimum. If the trait value has been able in the past to evolve to near this optimum, then large changes that either increase or decrease the value of the trait would reduce fitness. If there are a lot of loci that can potentially affect this trait, and if there are many mutations that can either increase or decrease the value of the trait, then any change away from the optimum can be repaired by changes at many other loci. If the deleterious effect of a fixed mutation were to increase the value of the trait away from the optimum, then any mutation that reduced the value of this trait would be beneficial. This idea of stabilizing selection is a common one in the evolutionary genetics literature, although there is little direct evidence for stabilizing selection.

Because stabilizing selection is based on such a simple idea, the effects of compensatory mutations can be easily modeled in this context (Hartl & Taubes 1998, Poon & Otto 2000). If we assume that there is an equal chance of a new mutation increasing the value of a character as there is of decreasing it, then some mathematical conclusions can be made. Poon and Otto investigated the expected reduction in fitness associated with finite population size for a multidimensional model with stabilizing selection. They find that for a species which has d dimensions along which it is experiencing stabilizing selection, the mean load L (i.e. the reduction in fitness of the population relative to a population in which every individual is at the optimum for each character) is given by, approximately:

$$L \cong \frac{d}{d+2N_e}. \quad (4)$$

This analysis make several assumptions. First, it assumes that the fitness of a phenotype is a linear function of its distance from the optimum. Second, it assumes that the distribution of mutational effects for each character is symmetrical around zero, and that the size of these effects are exponentially distributed. Finally, it assumes that the distribution of mutational effects is the same for each dimension and that the strength of selection is equal for all dimensions. The first assumption matters, for as Poon and Otto (2000) show in their discussion of the paper by Hartl and Taubes (1998), when the shape of the fitness function is a Gaussian curve around the optimum the load is approximately $d/(d + 4N_e)$. We have little data about the specific shape of selection around optima, but the difference in the two equations is less than a factor of 2.

Potentially more troubling is the last assumption, that all dimensions are equal. We have performed computer simulations to investigate the effects of varying the strength of selection and mutational effects among dimensions. Instead of a hypersphere model as used by Poon and Otto, we used a hyperellipse, where the strength of selection along any axis was drawn from an exponential distribution; thus some dimensions have much stronger section than others (*see* Fig. 2 for a description of the model assumptions, which are based on Fisher's (1930) model

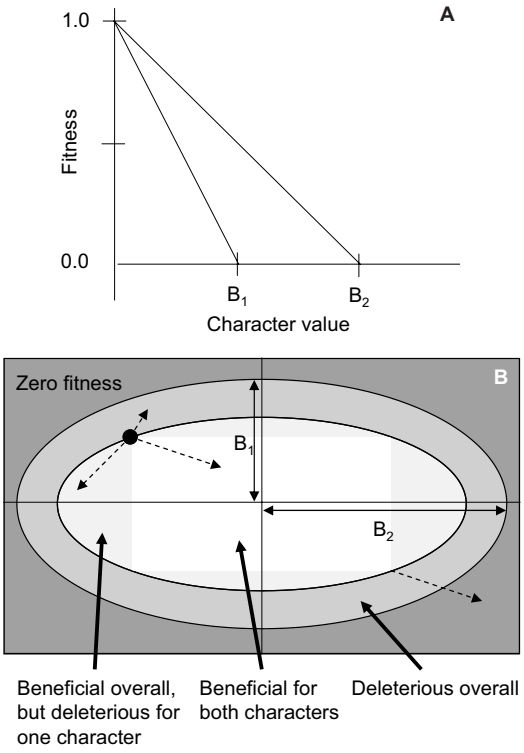

Fig. 2. A two-dimensional representation of the ellipsoid modification of Fisher's geometrical model. — **A**: Fitness as a function of the distance to the optimum for each of the characters separately. We assume a linear decline in fitness away from the optimum, with fitness of zero beyond a certain distance. — **B**: The two-dimensional fitness surface. The two axes represent the two different characters. A population starts adaptation at, for example, the black dot in the upper left quadrant, and the optimum phenotype is at the origin. Any mutation that takes the phenotype to within the ellipse results in a higher fitness, but only within the white rectangle is the phenotype improved along both dimensions. In the lightest grey region, adaptation is actually reduced on one character, even though the mutation is beneficial overall. Outside the interior ellipse, the fitness effect of a mutation is negative.

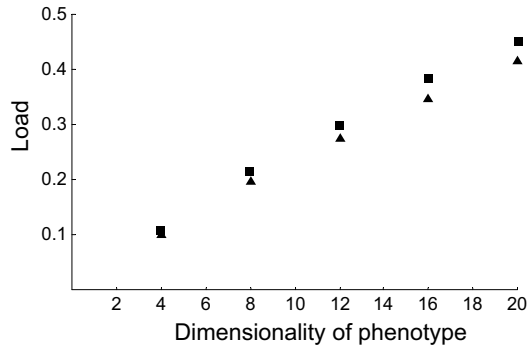

Fig. 3. The drift load on phenotypes of different dimensionalities when each dimension affects fitness equally (triangles) and when the fitness effect of a dimension is exponentially distributed (squares). A phenotype at the optimum had a fitness of one and a phenotype at a distance B_i from the optimum for dimension i had a fitness of zero; the slope of the resulting fitness function on each dimension was therefore $-1/B_i$. When each dimension affects fitness equally (triangles) $B_i = 1.0$ for all i, and when the effect of a dimension on fitness was exponentially distributed (squares), the expected effect was $B_i = 1.0$. When B was exponentially distributed, most dimensions had a large effect on fitness because B_i was often small resulting in a fitness function with a slope of large magnitude, while a few dimensions had relatively little effect on fitness. When B_i was inverse-exponentially distributed such that most dimensions had small effects on fitness and only a few had large effects it was found that drift load was not different from the case when fitness was uniform across dimensions. Fitness is determined multiplicatively across loci. The census and effective population sizes for all simulations were 10 individuals. Each population began evolution at the optimum phenotype. One-hundred thousand fixed mutations occurred prior to the measurement of drift load with the average of the last 100 iterations taken for measurement. To obtain estimates of the drift load, 100 replicates of the evolutionary process were simulated, except for the cases when the dimensionality was 16 or more, for which we simulated 50 replicates.

of the geometry of adaptation). Figure 3 shows that the effect of this change in the model is negligible. Still each dimension contributes load in indirect proportion to N_e, and the quantitative value is not far different from the model in which all dimensions are equal in selection intensity. Thus one of the more unrealistic aspects of the model, that all dimensions are equal in importance, turns out to be qualitatively unimportant to the results.

Why might this be? The load due to each dimension results from a balance between drift and selection. As a trait mean gets very close to the optimum, there is little effect of selection. This area of ineffective selection is defined by the area over which the selection differences are nearly neutral; in other words, the range of phenotypes around the optimum in which the selective differences are so small that selection cannot overwhelm drift. This zone occurs when $s < \sim 1/2N_e$. As a result, further fine-tuning would be impossible at that population size. Outside of

this range, however, selection would be effective in moving the phenotype back towards the optimum. Therefore each dimension will contribute a load roughly equal to that at the nearly neutral boundary ($\propto 1/N_e$), so the total load is proportional to d/N_e. Thus the strength of selection on an axis drops out — stronger selection causes greater load for a given deviation from the optimum, but with strong selection the population mean will not be far from the optimum. The predictive ability of these models is hampered by the fact that we do not know how to define dimensions, much less how many there might be for a given taxa.

The results of Poon and Otto (2000) and Whitlock (2000) are at first glance quite similar: both predict that beneficial mutations, in particular compensatory mutations, are capable of stopping the decline in mean fitness due to the fixation of deleterious alleles. But there is an important difference in their predictions. The critical effective size formulation from Whitlock (2000) argues that below a certain population size the species would decline to extinction, even with the occurrence of beneficial mutations. Poon and Otto (2000), on the other hand, predict that even at small population size the fitness of the population will be reasonably high, so long as the effective population size does not decline below the number of dimensions of the organism. Since we have little idea about the true dimensionality, *sensu* Fisher, Poon and Otto, of any species, it is hard to evaluate the probability of N_e dropping below d. But it seems unlikely that the critical size would be very large. What explains this difference in the two models? Besides the uncertainty in the definition of the parameters of the hypersphere model, a fundamental distinction is that a model with only stabilizing selection and bidirectional mutation can *always* have compensatory mutations. In contrast, the Whitlock model allows for the fact that there can be mutations that cannot so easily be compensated. Not all biological processes are under stabilizing selection. For example, it is likely that the peak metabolic efficiency is under directional selection. It is an open question, though, to know to what extent selection is stabilizing or purifying. How many mutations are unconditionally bad, and how many are only deleterious within the genetic context? In other words, is epistasis for fitness nearly universal, or the exception to the rule?

Metabolic pathways

One special case of stabilizing selection that has been examined in more detail is the case of an optimized flux through a metabolic pathway. Thinking about the properties of metabolic pathways from an evolutionary point of view was taken far forward by the metabolic control theory of Kacser and Burns (1973, 1981). With metabolic control theory, predictions are made about the properties of metabolic pathways. By this theory, some enzymes have high "control coefficients" while others have low control coefficients, meaning that a change in the properties of some enzymes would have a large effect on the rate of metabolism for the pathway while for others a similar change in properties would have a small effect on the flux of the pathway. Kacser and Burns (1981) have shown that most enzymes in a linear pathway would have a relatively low control coefficient, meaning that a change in their performance would have little effect on fitness. These changes can be compensated by changes in other enzymes in the pathway. Hartl and Taubes (1996) showed that under these circumstances, the ability of a genome to compensate for mildly deleterious fixed alleles would be great.

Large compensation for small deleterious effects

So far we have mainly discussed the kinds of mutations that might be expected to repair the damage caused by specific previous deleterious mutations. The intriguing possibility exists, however, that in some cases we might be able to predict that a few new mutations may be able to compensate for a large number of previous deleterious mutations. This possibility is exciting, because it could mean that, even though deleterious mutations could fix because the strength of selection against them was too weak to prevent genetic drift from increasing their frequency,

selection may be strong enough to overwhelm drift in the fixation of alleles that compensate for the damage already done.

One possible example of this comes from patterns of codon usage. The genetic code is redundant; that is, most amino acids are coded for by more than one codon. One might *a priori* expect that the different codons for each amino acid would be used in roughly equal frequencies, but in most organisms this is not the case.Especially in species with relatively large population sizes, there is *codon bias*, the preferential use of one codon over others for each amino acid. This bias is thought to exist because it is more efficient to translate proteins from messenger RNA if there are fewer transfer RNA's competing for access to the anticodons on the mRNA, and this becomes possible if most codons for the same amino acid are themselves the same. Per codon, though, the strength of selection discriminating between synonymous codons is tiny, perhaps on the order of 10^{-9}. Thus mutations to synonymous, but unpreferred, codons would have extremely weak selection against them and would therefore be very likely to fix. In fact, it is known that species with smaller population sizes have lower codon bias (Akashi 1995, Kliman *et al*. 2000).

But does this mean that in species with small population sizes (like humans), the lack of codon bias causes a large fitness loss? Not necessarily, because even though the strength of selection on each particular codon is weak, the strength of selection on the tRNA expression is much higher, and this selection can be effective to cause tRNA pools to match the current pattern of codon bias in the genome, thus minimizing the effects of the less biased codon usage. In fact, tRNA abundance and codon bias are well correlated (Moriyama & Powell 1997).

This example, while conjectural, shows that we might well be able to predict in certain cases that compensatory mutations are possible, and that moreover in some cases a single mutation (such as that which increased the concentration of a rare tRNA) could compensate for a variety of deleterious mutations (such as all of the previous mutations to the unpreferred codon corresponding to that tRNA). Other examples are possible, such as the molecular chaperones discussed in the next section.

The evidence for compensatory and beneficial mutations

Empirical evidence for compensatory evolution comes from many sources, ranging from direct experimental tests of the rate of adaptation with and without deleterious mutations, to molecular studies of suppressors, to DNA sequence comparisons, etc. In this section we will review some of these empirical studies, finding that compensatory mutations are surprisingly common.

Experimental evolution

The last two decades have seen a remarkable bloom in experimental studies of evolution, with some of the most exciting using viruses and bacteria. One of the most direct conformations of the role compensatory mutation may play in the recovery of some populations from fixed deleterious mutations is the study of the $\phi 6$ virus by Burch and Chao (1999) (*see* Whitlock & Otto 1999). In this study, a deleterious mutation was fixed in a strain, and then this strain was used to found several populations with effective sizes ranging from 60 to 60 000. This deleterious mutation reduced fitness by about 90%. In spite of the quite small size at the lower end of the range, fitness increased over generations in all of the populations (*see* Fig, 4). This recovery was near total in the larger populations (which in some cases may be due to back mutations), but fitness increased substantially even at the smallest sizes. Two aspects of this recovery deserve special mention. First, at the smaller population sizes, fitness recovery was step-wise, indicating that the increase in fitness was not due simply to back mutations, but to compensatory mutations at other sites. Second, control populations maintained at the same population sizes but without the deleterious fixed mutation did *not* increase in fitness over the same time span. This indicates that the increases in fitness of the experimental lines are not due to non-specific adaptation, but that these increases are compensatory to the original deleterious mutation. This remarkable result shows that the rate of beneficial mutations increases as fitness is lowered by previous fixation of deleterious alleles. This excellent result deserves replication.

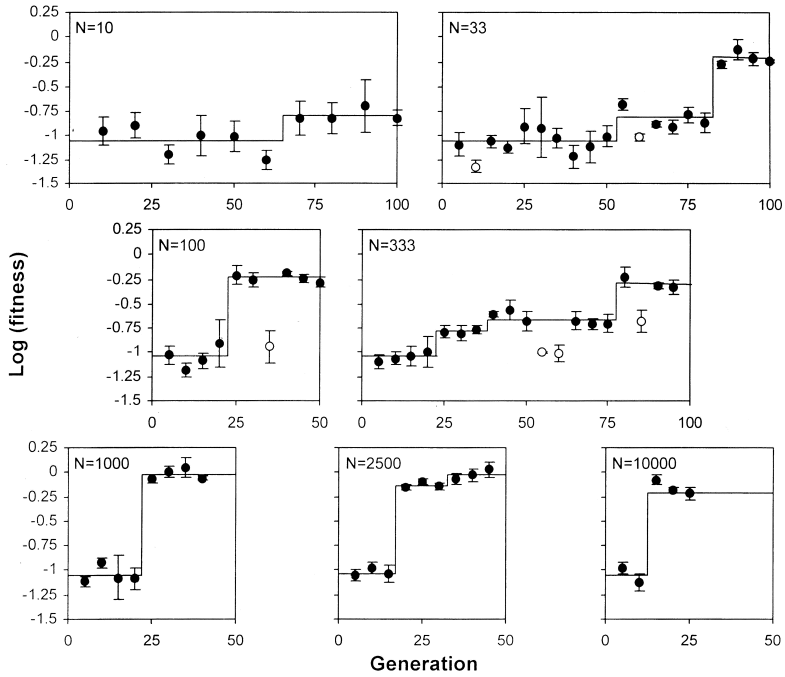

Fig. 4. Recovery of fitness in ϕ6 bacteriophage populations initially fixed with a major deleterious mutation (from Burch & Chao 1999). The effective population sizes of these populations are approximately six times greater than the bottleneck sizes given in the legends of each figure. Larger population sizes recovered quickly and nearly completely in fitness, while at smaller N_e the populations recovered by a series of steps. In all cases, fitness increased over subsequent evolution, even at the smallest population sizes, showing the power of reverse and compensatory mutations to allow recovery even at relatively small population sizes (*see* Whitlock and Otto 1999).

Compensatory mutation has also been experimentally observed in bacteriophage T7 (Rokyta *et al*. 2002). Following the deletion of the viral ligase gene, fitness dropped enormously, but most of this fitness was recovered by compensatory changes to other genes. In contrast, an experiment with ϕX174 — in which the target gene was not deleted but mutated — showed no compensatory changes, but a high rate of back mutation (Crill *et al*. 2000). Compensatory adaptation has also been observed in *Escherichia coli*. An experiment that compared the rate of adaptation of a well-adapted genotype to mutant genotypes derived from the same strain found much faster increases in fitness in the mutant strains that originally had low fitness (Moore *et al*. 2000). Rapid recovery from accumulation of deleterious mutations has also been seen in the eukaryote *Caenorhabditis elegans* (Estes & Lynch 2003). Unfortunately, it is not known whether the subsequent adaptation in these latter experiments is due to back mutation or compensation at other sites. All of these other experiments show the potential for back and compensatory mutations, but they were conducted at large population sizes so the role of compensation in recovery from small population size is undetermined.

Suppressors

While the experimental evolution studies just described intentionally looked for the evolution of compensation, a much more common occurrence is that compensatory mutations evolve in lab strains, even when they are unobserved or undescribed. A constant problem in the maintenance of lab stocks of mutant strains is that compensatory mutations (called suppressors in this context) appear, partially or completely masking the phenotypic effects of the alleles being maintained in the strain.

Suppressor mutations are so common that they have become a molecular tool themselves.

Information about the ways suppression can occur and the genes involved can be a powerful tool for understanding the molecular biology of a gene or process. Several ways have been identified by which suppression might occur, and these have been nicely reviewed by Prelich (1999). The following discussion draws heavily on his summary.

Prelich identified six mechanisms of suppression, all of which have several known examples (*see* Prelich 1999). They are:

1. *Intragenic suppression*: Changes in the same gene as the original mutation that ameliorate the effects of the first mutation. These changes can be at same codon or elsewhere in the coding region.
2. *Informational suppression*: Mutations in the translational machinery that suppress nonsense or frameshift mutations. These can involve changes in the tRNA, ribosomal subunits, etc. This class of suppressor seems unlikely to be without fitness costs, and it therefore may be unlikely in natural evolution.
3. *Changes in the amount of mutant protein*: One obvious way to correct for the deficiency caused by a mutant protein of slightly lowered activity is to increase the amount of that protein in the cell. This can be done by gene duplication, increased expression, translation or stability of the protein, or by mutations in the translational machinery.
4. *Changes in the activity of the mutant protein*: Over and above changes in the protein itself, increased activity can come from changes in other proteins that the protein directly interacts with, such as regulatory subunits. Also in this category are changes in the post-translational modification of the protein.
5. *Changes in the activity of mutant pathways*: As mentioned in the previous section, changes to the sequence or expression of other proteins in the same pathway can also compensate for deleterious changes to a protein.
6. *Changes to other pathways*: Perhaps more surprisingly, sometimes changes to other pathways can compensate for deleterious mutations. For example, changes to the lactose permease gene in *E. coli* can compensate for mutations in maltose permease, even thought the lactose transport pathway is not normally involved in maltose transport (Shuman & Beckwith 1979).

It is important to note that the study of suppressors is dominated by their effects on phenotype, not fitness. It is unclear to what extent these suppressors change fitness (indeed even the fitness effects of the original mutations are normally left unmeasured). The fitness consequences of mutants and their suppressors represents a ripe area for evolutionary biologists to use the fruits of molecular biologists' labor.

Molecular chaperones

Some examples of compensatory mutations seem to be very specific to the previous deleterious mutation, but fascinatingly some classes of compensatory mutations act more generally. One particularly good example of these mechanisms is a class of proteins called molecular chaperones. The proteins interact physically with other proteins to stabilize their configuration, thereby preserving more of their function in the presence of genetic or environmental disturbance. Chaperones include the heat shock proteins (Hsp) that have been well-studied in many taxa. Drosophila and Arabidopsis with defective Hsp express greater phenotypic effects (and presumably lower fitness) than individuals with fully functional Hsp (Queitsch *et al*. 2002, Rutherford & Lindquist 1998).

The compensatory power of molecular chaperones was dramatically demonstrated by a study of the chaperone GroEL in *E. coli* (Fares *et al*. 2002). Strains of *E. coli* with higher levels of deleterious mutations had much lower fitness than the wild type from which they were derived. However, when GroEL expression was increased, much of the fitness deficit disappeared (*see* Fig. 5). Moreover, higher GroEL expression in the wild-type flies was deleterious, showing that the effects of GroEL were epistatic and compensatory in the strictest sense.

Compensating for pleiotropic effects

Another source of evidence on the potential for compensation comes from the study of deleterious pleiotropic effects of fixed major alleles. When an allele is fixed as a result of strong selection on one aspect of physiology or morphology, very commonly this allele will have deleterious effects outside the context of this specific selection. For example, exposure to pesticides, antibiotics, or other toxins often selects for alleles of large effect that reduce the harmful aspects of the toxin (Orr & Coyne 1992), but these resistance alleles often have fitness costs in the absence of the toxin. These "resistance costs" themselves are subject to evolution, and commonly these costs are ameliorated by compensatory adaptation, even if their resistance effects remain intact.

One striking example of compensatory evolution with resistance alleles comes from the study of antibiotic resistance in *E. coli* (Schrag & Perrot 1996, Schrag *et al.* 1997, Levin *et al.* 2000). In these studies, three streptomycin resistant strains were evolved for 180 generations in the absence of streptomycin, during which time the fitness consequence of the resistance allele was reduced from a 14%–19% cost to a 3%–25% advantage, depending on the strain. Not only were the fitness costs reduced, but the resistance allele actually became advantageous even in the absence of streptomycin. Moreover, the variation among lines in the fitness advantage demonstrates that the mode of compensation varied somewhat among strains; in other words, this is evidence that there is more than one way for this compensation to occur. In short, an allele that was deleterious in a given environment (with no streptomycin) became advantageous in that same environment by compensatory adaptation at other sites. Such compensatory evolution (although not so complete) has been observed in other species and with other antibiotics (Reynolds 2000, Cowen *et al.* 2001).

Sequence comparisons

One particularly rich potential source for evidence for compensatory mutations comes from looking at correlated changes in nucleic acid sequence data. The classical example of this is the RNA–RNA bonds made in stem-loop formation. For example, in the transfer RNA cloverleaf pattern, RNA bonds by Watson-Crick pairing to form double-stranded sections from a single-stranded molecule (*see* Fig. 6). This pairing is extremely important to the secondary structure, and therefore the function, of the molecule. Parsch *et al.* (2000) have shown that changes in one part of the molecule co-occur with other, compensatory changes. This covariance could be the result of double mutations or of compensation subsequent to the fixation of a deleterious mutation, but in any case they demonstrate the potential for compensation. Parsch *et al.* (2000) have also demonstrated this sort of compensation in 5S ribosomal RNA, ribonuclease P RNA, eukaryote small subunit RNA, and mRNA from the 3´ untranslated region of *bicoid* in *Drosophila*. They have experimentally confirmed with Adh mRNA that single deleterious changes can be compensated for by subsequent changes (Parsch *et al.* 1997).

This sort of coevolution is not limited to RNA, but has also been inferred in DNA and protein evolution as well (Hancock *et al.* 1999, Peixoto *et al.* 1998, Fukami-Kobayashi *et al.* 2002). For example, the *even-skipped* stripe 2 element (S2E) is an enhancer of the expression of

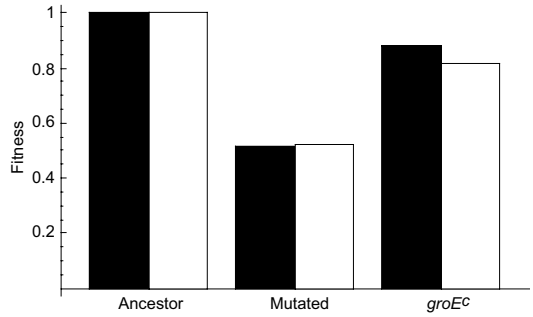

Fig. 5. Fitness in non-mutator (black bars) and mutator (white bars) strains of *Escherichia coli*. Mutated lines (which had undergone > 3000 generations of mutation accumulation) had significantly reduced fitness relative to their ancestor. Overexpression of the molecular chaperone GroEL in the *GroE^c* lines compensated for most, but not all, of this fitness loss. (Redrawn from Fares *et al.* 2002).

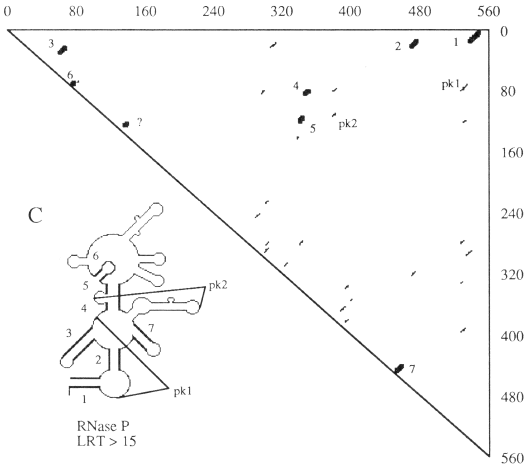

Fig. 6. RNA folding and the potential for compensatory mutation. The triangular figure in the upper right maps each position in the tRNA sequence to every other position. Dark points represent pairs of sequences identified by Parsch *et al.* (2000) as being involved in the formation of stem helices; the numbers next to these points correspond to the stems in the secondary structure of tRNA in the lower left. Individual mutations within these helices should be deleterious, but they could be compensated by mutations at the matching position. (Reprinted from Parsch *et al.* 2000).

the second transverse stripe in *Drosophila melanogaster* embryos. *Drosophila melanogaster* and *D. pseudoobscura* differ in the sequence for the enhancer, but if the sequence from one is transformed into the other, expression patterns are normal (Ludwig *et al.* 1998). However, these two species' sequences differ in multiple ways. If a chimeric construct is created with the 5´ end from one species and the 3´ end from the other, expression is no longer normal (Ludwig *et al.* 2000). Ludwig *et al.* suggest this as an example of stabilizing selection, where an initial deleterious change has been compensated for by subsequent mutations, which by themselves would have been deleterious as well.

Measuring substitution rates

Finally, sequence comparisons among related taxa can give us estimates of the numbers of beneficial mutations that have fixed over evolutionary time. Building on the logic of the McDonald-Kreitman test (1991), Smith and Eyre-Walker (2002) were able to calculate the genomic rate of fixation of beneficial mutations by comparing patterns of variation in synonymous and nonsynonymous changes in coding sequences. By comparing sequences for 35 genes in *Drosophila yakuba* and *D. simulans*, they were able to estimate that about 45% of amino acid substitutions between the two species were the result of positive selection. This translates into a new fixation of a beneficial allele about once every 450 generations. There is no way to estimate from these data the strength of selection for these changes, but nonetheless it shows that beneficial mutations are surprisingly common.

Discussion

In recent years, we have come to see that mutations affecting fitness are ubiquitous, that the average effect of these mutations before selection is to decrease the mean fitness of the population, and that many species of conservation concern are small enough that some of these mutations will fix by genetic drift. Considering deleterious mutations alone, this process would continue until the population was not able to replace itself, and extinction would result. For populations of size below approximately 1000, this decline to extinction, the mutational meltdown, would happen on a short enough time scale that we would have genetic cause for concern for the future of many species (Lynch *et al.* 1995, Lande 1995, 1998). This boundary of ~1000 is not a critical threshold, but the time to extinction increases rapidly above this point.

Yet these analyses have always begged the question — Why haven't all species, indeed all evolutionary lineages, gone extinct by now, after billions of years of biotic evolution? The answer is clearly that not all mutations are deleterious, but some fraction are beneficial. A low level of beneficial mutations can balance the effects of drift on deleterious alleles.

This is possible above a certain population size, which we have called the critical effective size. Above this $N_{e,crit}$, deleterious alleles fix at a sufficiently low rate that beneficial alleles can at least balance their effects. Below this critical size, however, the rate of fixation of deleterious

alleles increases, and fitness will decline over time. What determines this critical effective size is the relative rates of beneficial and deleterious mutations, U_D/U_B, and the mean effects of these new mutations. When beneficial alleles are very rare or have low mean effects, the critical effective size becomes higher.

As we have seen, however, the story is not so simple as this. As deleterious mutations accumulate in a population, the mean fitness declines, but with lower mean fitness there is a greater potential for beneficial mutations. The increase in the possibility of beneficial mutations comes as a result of the potential for compensatory mutation. As fitness declines, then, the ratio U_D/U_B decreases, and the critical N_e, therefore, becomes lower. Thus we need to find the value of $N_{e,crit}$ after fitness has already declined to equilibrium after previous population size declines. We should find the values of U_D/U_B, λ_D, and λ_B when the population is on the verge of sustained fitness decline, and these values would give $N_{e,crit}$. We know compensation is possible, looking at the examples given in the second half of this paper. But we still lack quantitative estimates of the beneficial mutation rates and effects with compensation. Unfortunately, we do not have good estimates of these values even in healthy populations. If we can succeed at getting estimates of beneficial mutations in any population that we know can persist, then these values would serve to give a *conservative* value of $N_{e,crit}$.

Practically, then, what should we recommend? The conclusions derived from these analyses are not that different from those of Lynch *et al.* — maintain a minimum population size in the thousands. (This comes from the idea of a minimum N_e in the hundreds, and then accounts for the fact that N_e is often an order of magnitude lower than the census size.) The difference is in the expectation of what happens above this critical size. With beneficial mutations allowed, populations will persist indefinitely if their effective size is above $N_{e,crit}$. Without beneficial mutations, a population of any size is expected to go extinct, albeit on a large time scale.

The time scale of fitness changes is important. It turns out that a population below $N_{e,crit}$ will decline in fitness much faster than a population the same degree over $N_{e,crit}$ will increase in fitness (Whitlock 2000). Thus it is key that populations not be allowed to drop even temporarily below $N_{e,crit}$, because the recovery time from this drop can be very long indeed.

The ability of genetic systems to compensate for loss of fitness in so many ways is remarkable. Moreover, the variety of mechanisms that allow this compensation is very great. Yet there must be limits to this compensation. For example, some types of mutation must be easier to compensate than others. A point mutation can always be repaired by a back mutation, but a deletion of a large part or whole of a gene would be much more difficult to fix by reverse mutation. Moreover, a point mutation can potentially be compensated by intragenic means (which are quite common form of compensation, *see* Prelich 1999), but a deletion of a gene obviously precludes this class of compensation.

Most of the evidence we have presented about compensation does not show complete compensation, but leaves the organism slightly lower in fitness than the original genotype. There are exceptions (e.g. Levin *et al.* 2000), and it is possible that not enough time has been allowed for full compensation in most of the studies. Yet the fact remains that we might expect, at least in the short term, even compensated genotypes to have lower fitness than their ancestors.

This review has left more questions than answers, yet these provoke a research program. We desperately need estimates of the rate of beneficial new mutations and of the distributions of their effects. Failing other ways of doing this, we might even be able to use the concepts of critical effective size to get rough ideas of these rates. If we could experimentally determine $N_{e,\,crit}$, we could at lest have some information about the relative rate and sizes of beneficial and deleterious mutations. Coupled with mutation accumulation experiments, which give estimates of U_D and λ_D, this would give us some leverage on finding similar values for beneficial mutations. Another line of research that would be very important would be the replication of the results of the experiments of Burch and Chao (1999), but with replication of population sizes and initial deleterious mutation and especially over a broader range of taxa. Furthermore, we need to have better theoretical predictions of the propen-

sity for compensation in order make a quantitative statement about the actual critical effective size of a species. To do this will require a lot more empirical knowledge of the mechanisms of compensation.

Finally we want to emphasize that these genetic risks facing threatened populations are not the most important threats in most cases. In the short term, populations much below the critical N_e are at great risk of extinction from purely ecological reasons, either demographic stochastic effects or more importantly ecological catastrophes. More important than either of these of course is avoiding the kind of catastrophic reduction of population size that humans are capable of; that is, solving the complex sociological and economic problems that allow a species to have enough habitat and protection from disturbance to persist. In order to make these difficult short-term fixes worthwhile, though, we must ensure that we leave enough individuals in a species to allow its future persistence in the face of mutational problems.

The likelihood of extinction in small populations by genetic meltdown is still unknown, but the possibility is a scary one. Moreover, many species are naturally small in number, and we need to understand evolution in small populations to understand evolution in perhaps most species on earth. We therefore need to understand more about the interplay between beneficial and deleterious mutations, and the epistatic role of compensatory mutations promises to be very important.

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