Evolution: Towards a genetical theory of adaptation
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The population genetic basis for adaptation has remained obscure despite a longstanding body of theory. Microbial selection experiments are beginning to provide some answers.

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Knowledge of the genetic basis of adaptation should provide the centerpiece of a unified theory of evolution. Such a theory would greatly aid in the determination of past evolutionary changes, such as those leading to the evolution of Homo sapiens. Perhaps more importantly, forward-looking evolutionary predictions would have a much stronger foundation. Exactly how adaptation occurs at the genetic level is still uncertain. Recent studies have focused on two broad questions: are adaptive mutations of large effect, and what factors restrict rates of adaptation?

Current thinking on the genetic basis of adaptation has its roots in the great debate that occurred at the end of the 19th century on the genetics of speciation and the apparent contradiction between Darwinian natural selection and Mendelian genetics [1]. One view was that speciation resulted from the gradual fixation of a large number of changes, each of small effect. The opposing view held that speciation required mutations of large effect, and that the rules of Mendelian genetics apply equally well to genes of small effect as to those of large effect.

The reconciliation of Mendelian genetics and natural selection provided a mathematical basis for the study of evolution and adaptation, upon which hypotheses for the genetics of adaptation have been made. One hypothesis states that most adaptive mutations fixed in a population are of small effect [2]. Mutations having large phenotypic effect would have a greater likelihood of having wide-ranging deleterious pleiotropic effects, negating any adaptive benefit. A second hypothesis states that the rate of adaptation should decline over time as a result of the limited number of possible adaptive mutations [3–5].

Assessment of these hypotheses has often relied upon indirect or static measurements of adaptation. Paleontology has provided important information on the tempo of evolutionary change, but it is much less useful in the assessment of selective benefits of phenotypic change or the genetic basis for adaptation. Surveys of extant diversity, either phenotypic or genetic, have identified substantial diversity, but have been less able to quantify selective effects. An ideal approach would be to document adaptation as it occurs. Recent work has taken just this approach, using rapidly growing microbial populations to directly observe evolution in action.

In a series of experiments, Lenski and colleagues [4,6,7] propagated twelve replicate populations of Escherichia coli for over 10,000 generations. The goal was to investigate the dynamics of adaptation, using replicate populations propagated under the same environmental conditions and having the same immediate ancestor. Under these conditions, the improvements in Darwinian fitness could be directly associated with the fixation of novel adaptive mutations arising within each of the replicate populations. Using step-model regression to detect adaptive events, three step-like fitness increases were found on average within each population over the first 2,000 generations, each step of roughly a 10% fitness increase. However, the rate of adaptation declined over the course of propagation: between 1000 and 2000 generations, the average rate of improvement was approximately 0.108 per 1000 generations, but the rate fell to approximately 0.008 between 5,000 and 10,000 generations.

Subsequently, over far fewer generations but with greater precision, Burch and Chao [8] measured the fitness benefit due to adaptive mutations in populations of the RNA virus φ6. A low fitness ancestral genotype was generated by the fixation of a single deleterious mutation, reducing fitness by about 90%. Phage populations, of different sizes, were then allowed to recover and the fitness step size of the first adaptive mutation was measured. Fitness was measured every five generations for 100 generations and at least one beneficial mutation was always observed prior to 100 generations of selection. The fitness benefit conferred by the adaptive mutations were generally smaller than the fitness reduction due to the deleterious mutation, but population size was an important determinant of fitness benefit: the smaller the effective population size, the smaller the benefit. Further investigation demonstrated that the adaptation of φ6 was limited “by the accessibility of advantageous genotypes within the mutational neighbourhood (the set of mutants one or a few mutational steps away)” [9].

A difficulty for most selection studies, including those mentioned above, has been the identification of adaptive
events. If an adaptive mutation yields only a 10% fitness improvement, identifying the time frame for the appearance of the mutation is technically difficult. Initially, an adaptive mutation is at a low frequency (1/population size) and has little effect on the fitness of a population. In addition, multiple adaptive mutations may occur simultaneously, obscuring the fitness effect of any single mutation [10]. Burch and Chao [8] overcame these difficulties, but began with an ancestral genotype of very low initial fitness and focused primarily on the very first adaptive mutation.

Imhof and Schlotterer [11] have recently been able to perform a detailed examination of step size over 1,000 generations of selection, by an ingenious approach using the high rate of mutation inherent in microsatellite DNA. A microsatellite DNA locus — (GA)n — was cloned into a relatively low copy plasmid (pBR322), which was then transformed into a single E. coli genotype. The plasmid-bearing genotype was used to initiate ten replicate populations that were then subsequently propagated for 1,000 generations. Carriage of the plasmid was ensured by periodic treatment of the cultures with ampicillin, resistance to which was encoded on the plasmid. During propagation of the replicate populations, the rapid evolution and diversification of the microsatellite DNA provided a marker for different clonal lineages within each population. The populations were assayed every 90 generations by restriction analysis of the plasmid DNA, and biologically significant changes in allele frequency were determined relative to the signal intensity of the restriction bands.

The ability to track clonal lineages within populations allowed Imhof and Schlotterer [11] to make several important observations. Firstly, numerous adaptive events were identified, 66 across all ten populations over the course of the propagation. Each event was identified by a shift in microsatellite allele frequency within a population, resulting from the evolution of new microsatellite alleles and a combination of genetic drift and the selection of adaptive mutations associated with bacterial lineages having different microsatellite alleles. It was shown that selection, and not drift, was the primary cause of the frequency shifts by restarting four populations, with five-fold replication, at least 90 generations prior to the observation of a significant microsatellite allelic shift in the original four populations. In 19 of the 20 populations evolution was recapitulated and the same allelic shift was observed as in the original populations.

Secondly, the rate of adaptation was not observed to decline over the course of the selection (Table 1). The occurrence and persistence of selective sweeps did not decline over time, nor did the selective benefit of adaptive mutations. This result stands in contrast with most other selection experiments, either with *E. coli* [6] or with other species [12]. Imhof and Schlotterer [11] suggest that the discrepancy may simply have been due to the relatively ‘short-term’ length of the experiment, but declines in the rate of adaptation have been observed in other 1000-generation selection experiments with *E. coli* [13]. The difference is more likely to have alternative explanations, two of which are the particular nutrient environment that was used in the experiment, and the manner in which fitness was determined.

Imhof and Schlotterer [10] chose for their experiments a nutrient-rich environment, containing abundant peptides and vitamins, in contrast with most other bacterial selection experiments, which were performed in minimal environments containing a single carbon and energy source. Rich nutrient conditions have been shown to greatly increase the range of adaptive possibilities for bacterial populations, primarily because of increases in ecological interactions [14]. It is probable that there were a greater number of highly beneficial adaptive mutations in the nutrient-rich environment than has been observed in the nutrient-poor environments of other selection studies. The manner in which fitness assays were performed may have been important, as fitness was determined by the changes in allele frequency within the evolving populations, and not by separate competition experiments against the common ancestor, as has been typically performed. If frequency-dependence is important for fitness, as has been observed in microbial populations [15–17], then the rate of fitness improvement will vary depending upon the competing genotypes.

Finally, and most importantly, Imhof and Schlotterer [11] were able to make an empirical measure of the distribution of fitness benefits for adaptive mutations. The fitness measures ranged from 0.006–0.059, with a median fitness effect between 0.01 and 0.02. The distribution of fitness effects appears to be exponentially distributed, in agreement with the hypothesis that most adaptive mutations

<table>
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<tr>
<th>Table 1</th>
<th>Time course of the number of alleles that significantly increase in frequency for each population.</th>
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<tbody>
<tr>
<td>Generation</td>
<td>0</td>
</tr>
<tr>
<td>90</td>
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<tr>
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<td>270</td>
<td>0</td>
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<td>900</td>
<td>0</td>
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<td>990</td>
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are of relatively modest effect. As the authors note, however, the estimates obtained are likely to be somewhat downward biased, given the manner in which fitness was measured and the somewhat imprecise nature of the microsatellite marker.

Understanding the genetics of adaptation is one of the most vexing and complex problems in Biology. Adaptation is a process common to all life and plays a central role in shaping biological diversity. However, quantitative hypotheses on the genetics of adaptation, devised over half a century ago, have remained essentially untested because of the lack of appropriate data. The primary difficulty has been identifying those mutations which are important during adaptation and then quantifying their selective effect. The combination of appropriate molecular techniques and microbial selection experiments shows great promise for the derivation of a genetical theory of adaptation.

References