Genes, proteins, and genomes are pervasively shaped by biases in mutation that exert their influence by biasing rates of evolutionary change. Indeed, methods of phylogeny inference routinely assume that evolutionary rates will reflect transition–transversion bias and other common mutational biases. This influence is typically attributed to neutral evolution, presuming that mutation is a weak force easily overcome by selection, so that any noticeable effect of mutation must signal a lack of selection. However, in PNAS Galen et al. (1) propose a case of mutation-biased adaptation, in which the course of evolution reflects both a bias in mutation and a fitness benefit. How important are mutational biases in shaping adaptation? Are internal sources of orientation or direction in evolution—once dismissed as improbable (2)—more important than previously imagined?

Galen et al. (1) report detailed molecular and biogeographic evidence for an adaptation to altitude in Andean house wrens (Troglodytes aedon) involving hemoglobin, a protein with a central role in oxygen transport. In this species, site 55 of the β-chain of hemoglobin has either Val (valine), which is the ancestral amino acid, or Ile (isoleucine). Using genetic engineering and biochemical measurements, Galen et al. show that this change increases oxygen affinity by 34%. Furthermore, their surveys of wren populations living at different altitudes show that the β55Ile allele is highly unusual (compared with other variants) in showing a positive correlation with altitude. In other words, this change appears to be adaptive: birds at higher altitudes are more likely to have the feature (a hemoglobin with higher oxygen affinity) that we expect a priori to be beneficial because of the reduced oxygen levels in high-altitude environments.

The idea of altitude adaptation taking place by changes in hemoglobin is not new. High altitudes pose a special challenge for birds: not only does the lower partial pressure of oxygen make it harder to transport oxygen to muscles, an aerial lifestyle requires more energy because of lower air density and generally stronger winds (3). For example, the bar-headed goose, which migrates over the Himalayas, exhibits a variety of behavioral, morphological, and physiological adaptions to high-altitude flight, including an increase in hemoglobin affinity because of a single Proto-Ala change at site 119 in the α-chain (4).

The twist in Galen et al.’s (1) report is the possible role of a CpG mutational hot spot. The change from Val to Ile at β55 is a G→A transition mutation. In context, this G is part of a CpG dinucleotide, and such sites (in birds and mammals) typically have higher mutation rates, especially for C→T or G→A transitions (5). Galen et al. (1) don’t measure the mutation rate directly, but use a phylogenetic model to infer a fivefold higher rate of evolution at this site in related birds, consistent with an elevated mutation rate.

How does this elevated mutation rate fit into the story? The evidence for adaptation is independent of whether the mutation happened at a high or low rate. What Galen et al. (1) are suggesting is that we see this particular adaptive change (rather than something else) because its rate of mutational origination is higher. Surely there are other ways to adapt, and other ways to adapt by increasing affinity, given that previous studies (cited by Galen et al.) found other hemoglobin mutations that increase affinity in high-altitude species; indeed, the authors themselves discovered a different affinity-increasing mutant at a low frequency in house wrens (see figure 4 in ref. 1). We see the Val-to-Ile change, not because it is the fittest alternative, but because it is so strongly favored by the “arrival of the fittest.” This effect arises because the chance of seeing a particular change is the joint probability of its origination by mutation and its subsequent spread as a result of any beneficial effects; in some cases, one can even write down a simple equation for the dual influence of mutational biases and selective preferences (6, 7).

What other evidence suggests a role for mutational hot spots or mutational biases in adaptation? Some general classes of mutations that occur at high rates have been associated with adaptive or putatively adaptive changes. For example, mutations that change the number of repeats in simple sequence repeats (e.g., triplet repeats) have been implicated in a few studies of adaptive or putatively adaptive features, including social behavior in voles and morphological diversification in dogs (8). Loss-of-function mutations occur at a high rate because of the many different ways of breaking a gene (deletion, frameshift, premature stop, and so forth), and also have been implicated in reports of adaptation in the wild (9, 10).

Studies of laboratory adaptation provide more direct evidence of mutation-driven dynamics. Couce et al. (11) used an experimental design focused directly on demonstrating mutation-biased adaptation. These authors began with two different mutator strains of Escherichia coli with widely differing mutation spectra, and repeatedly selected for increased resistance to the antibiotic cefotaxime. Whereas both types of mutator
evolved over 1,000-fold improved resistance, the spectrum of changes observed (in two key loci of adaptation) strongly reflected the strain-specific mutation spectrum. In a study focused on parallel evolution, Rokytá et al. (12) showed that the genotype that recurs most often in replicate adaptation of phage ΦX174 was not the genotype with the highest fitness, but the fourth-most-fit alternative, which happened to be favored by a considerable bias in mutation, specifically a transition-transversion bias.

Sometimes one has to dig a bit to find the effect of mutation, because it typically is not a focus of attention. For example, MacLean et al. (13) repeatedly selected rifampicin resistance in *Pseudomonas aeruginosa*, noting that some of the most common changes had high rates of mutation. In fact, the data reported by MacLean et al. show a striking correlation between the probability of recovering a genotype at high frequencies in adapted cultures, and the measured rate of mutation to that genotype (Fig. 1). Similarly, Meyer et al. (14) reported extensive parallel changes when they carried out laboratory adaptation of phage λ to two hosts, but they didn’t draw attention to the fact that, of 22 substitutions observed in parallel in replicate cultures, there were 16 types of transition changes observed 181 times, and 6 types of transversion changes observed 42 times. This ratio of transitions to transversions is far above the null expectation of a 1:2 ratio, and suggests that transitions are overrepresented among adaptive changes because they occur more frequently by mutation.

Relative to prior work, what do we learn from Galen et al. (1)? As noted, the studies that establish mutation-biased adaptation most directly involve laboratory evolution of bacteria and phages. One doesn’t know how well these dynamics would apply outside the laboratory. The natural cases mentioned above tend to involve special classes of mutations (loss-of-function mutations, simple sequence repeat mutations), rather than the single-nucleotide substitutions that change proteins or regulatory sites, and which typically take center stage in studies of molecular evolution.

In contrast, Galen et al. (1) present us with adaptation of a charismatic animal in a natural setting, involving a large-effect, activity-increasing mutation at a well-known type of mutational hot spot, a CpG site. Indeed, it is possible that such CpG sites have played a role in other examples of high-altitude adaptation. A previous study from some of the same authors on altitude adaptation in Andean hummingbirds (15) reported Gly→Ser changes at globin site β13 (and also at site β83) that increase oxygen affinity and that occur in parallel in high-altitude species, suggesting that these changes are adaptive. BLASTing their sequence KP222510 against other hummingbird genes and inspecting the relevant codons suggests that β13 Gly→Ser is another CpG → CaA hot spot mutation. Interestingly, the β13 Gly→Ser change is also found in two species of Andean waterfowl, the yellow-billed pintail and the speckled teal, according to a report from a different group (16).

Perhaps we could find more evidence by digging more deeply. The issue simply has not been on the radar in evolutionary biology. However, a variety of sources indicate that the dynamics of evolution reflect the dynamics of mutation in predictable ways. Some of this evidence has been staring us in the face for years (17).

Although Galen et al.’s (1) results are tantalizing, further work is required to establish the conclusions more firmly and to explore their broader significance. For example, the mutation rate at the implicated CpG site has not been measured directly. Doing so is probably impractical, but the next best thing is to measure the level of germ-line methylation of this site, because the elevated mutation rate at CpG sites is entirely a matter of methylation (5, 18).

Similarly, although the correlation of j55ile frequency with altitude is evidence that the affinity change is adaptive, this isn’t the same as measuring fitness. Again, it may be impractical to measure fitness of wrens with different hemoglobins at various altitudes, but one could measure whole-animal physiological performance. Such studies might address the additional question of whether heterozygotes are selectively favored at intermediate altitudes.

In addition to establishing the claim for adaptation more firmly, there are other open questions. Are all j55ile alleles the product of a single mutation, or did multiple mutations contribute? Was the mutation segregating in the population before the invasion of higher altitudes? Did it play a role in facilitating an upward expansion of habitat range? Or did the range expansion occur first, with the mutation occurring subsequently and then spreading upwards? Galen et al. (1) show that the j55 Val→Ile replacement has occurred in several other passerine lineages. Does this produce a similar increase in oxygen affinity, and if not, what lineage-specific differences are implicated in potentiating or blocking an affinity increase?

Finally, although carefully demonstrated cases linking adaptation to mutationally favored changes are invaluable, case studies can only take us so far. To establish mutational bias as a general cause of direction in adaptation will require a set of quasi-replicate cases large enough to support statistical hypothesis-testing. Only then will it be possible to make statements about the degree to which the course of adaptation, in general, is determined by mutational biases.

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