Speciation

Table 1. A proposed strategy for developing and validating models of bacterial evolution that might eventually be used to classify genetic diversity data and provide a firm foundation for a bacterial species concept.

1. Collect samples according to systematic ecological stratification. Focus on longitudinal studies, geographical studies, and measurement of physical and chemical gradients affecting bacterial growth. Consider biotic factors such as the presence of other competing bacteria or parasitic phage.
2. For each isolate, sequence as much as possible and affordable (16S rRNA, MLSA, auxillary genes, full genomes, etc.).
3. Use empirical classification algorithms that use genetic and ecological data to jointly map isolates.
4. To guide model formulation, use population genetic tests on observed clusters, focusing on tests for selection, population structure, and gene flow.
5. Generate evolutionary models and simulate populations.
6. Test, then reject or adapt, evolutionary models according to agreement between simulations and real populations; if necessary, return to step 1.
7. For successful models, develop model-based methods for interpreting pure genetic data (without ecological covariates) and test on new data.
8. If one or more validated models emerge, use these to classify genetic data and to develop bacterial species concepts.

References and Notes

14. See supplementary information in (16) for a statistical comparison of the ecolytic model with an effective neutral model and an implicit estimate of Ns.

REVIEW

Is Genetic Evolution Predictable?

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Ever since the integration of Mendelian genetics into evolutionary biology in the early 20th century, evolutionary geneticists have for the most part treated genes and mutations as generic entities. However, recent observations indicate that all genes are not equal in the eyes of evolution. Evolutionarily relevant mutations tend to accumulate in hotspot genes and at specific positions within genes. Genetic evolution is constrained by gene function, the structure of genetic networks, and population biology. The genetic basis of evolution may be predictable to some extent, and further understanding of this predictability requires incorporation of the specific functions and characteristics of genes into evolutionary theory.

One hundred and fifty years ago, Charles Darwin and Alfred Russell Wallace proposed that biological diversity results from natural selection acting on heritable variation in populations. Both Darwin and Wallace recognized the importance of heritable variation to evolutionary theory, but neither man knew the true cause of inheritance. Early in the 20th cen-
tury, the rediscovery of Mendel’s studies allowed for a formal mathematical treatment of alleles in populations, generating the field of population genetics. Population geneticists treated genes and alleles as generic entities, particles that were inherited and somehow caused variation in the appearance, behavior, and physiology of organisms—what we call collectively the phenotype. This level of abstraction was appropriate given that a molecular understanding of gene function lay many decades in the future. Even with this rudimentary view of gene function, however, population genetics greatly clarified how real populations evolve, and this theoretical understanding spurred the New Synthesis, combining population genetics with ecology, systematics, and biogeography to explain and explore many questions in evolution.

In the past 40 years, molecular biologists have elucidated how genes regulate biological processes, but only the most basic mechanistic observations have been integrated into evolutionary biology. For example, evolutionary theory has effectively absorbed the distinction between coding (nonsynonymous) and silent (synonymous) substitutions in protein-coding regions, but other aspects of molecular biology currently contribute little to evolutionary thought. The time has now come to integrate the specifics of molecular and developmental biology into evolutionary biology. Over the past 15 years, many examples of the genes and mutations causing evolutionary change have been identified (1). Patterns in these data suggest that a synthesis of molecular developmental biology with evolutionary theory will reveal new general principles of genetic evolution.

Nonrandom Distribution of Evolutionarily Relevant Mutations

Recent studies suggest that the mutations contributing to phenotypic variation [evolutionarily relevant mutations (2)] are not distributed randomly across all genetic regions. The most compelling evidence comes from cases of parallel genetic evolution: the independent evolution of similar phenotypic changes in different species due to changes in homologous genes or sometimes in the same amino acid position of homologous genes.

Many cases of parallel evolution have been discovered across all of the kingdoms. At least 20 separate populations of the plant Arabidopsis thaliana have evolved null coding mutations (mutations that completely eliminate protein function) in the Frigida gene that cause early flowering (3). Resistance to DDT and pyrethroids has evolved in 11 insect species by mutations in either amino acid Leu

\[10^{14}\] or Thr

\[29^{2}\] of the voltage-gated sodium channel gene para (4). Two virus populations independently subjected to experimental evolution in a novel host accumulated many of the same amino acid mutations (5). In total, about 350 evolutionarily relevant mutations have been found in plants and animals, and more than half of these represent cases of parallel genetic evolution (1).

One explanation for parallel genetic evolution is that most genes play specialized roles during development, and only some genes can evolve to generate particular phenotypic variants. For example, mutations in rhodopsin can alter light-wavelength sensitivity (6), and mutations in lysozyme may enhance enzyme activity at the particular pH of a fermenting gut (7). But the reverse would not be true. Mutations in rhodopsin are unlikely to enhance fermentation, and mutations in a digestive enzyme will not aid detection of a particular wavelength of light, even if each protein was expressed in the reciprocal organ.

Gene function explains part but not all of the observed pattern of parallel genetic evolution. In several cases, parallelism has been observed even though mutations in a large number of genes can produce similar phenotypic changes. For example, although more than 80 genes regulate flowering time (8), changes in only a subset of these genes have produced evolutionary changes in flowering time (3). Hundreds of genes regulate the pattern of fine epidermal projections, called trichomes, on Drosophila melanogaster larvae. But only one gene, called shavenbaby, has evolved to alter larval trichome patterns between Drosophila species, and this gene has accumulated multiple evolutionarily relevant mutations (9). What is special about these hotspot genes?

Developmental biology illuminates why hotspot genes such as shavenbaby exist. During development, multiple cell-signaling pathways and transcription factors act together to progressively divide the embryo into a virtual map that specifies when and where organs will form. The interactions between the genes encoding these signaling molecules and transcription factors can be represented as a genetic network. Gene interactions are modulated in large part by the cis-regulatory regions of patterning genes. (All genes are composed of two fundamentally different regions: a region encoding the gene product—a protein or an RNA—and adjacent cis-regulatory DNA that encodes the instructions governing when and where the gene product will be produced.) Transcription factors bind to cis-regulatory regions of target genes, and the summed effect of many such interactions at a target gene determines whether the gene is expressed or not. Patterning genes act within complex genetic networks, and usually each patterning gene contributes to the development of multiple cell types. For example, most patterning genes that are active during embryonic development of the epidermis contribute to the development of muscle-attachment sites, sensory organs, tracheal pits, trichomes, or other cell types.

The importance of regulatory networks in determining which genes may be evolutionary hotspots can be illustrated with the genetic network that governs larval trichome development in D. melanogaster (Fig. 1). In this network, developmental patterning genes first collaborate to divide the embryonic epidermis into domains expressing distinct transcription factors. These patterning genes then regulate the expression of the shavenbaby gene, a so-called input-output gene (10). Input-output genes integrate complex spatiotemporal information (the input) and trigger development of an entire program of cell differentiation (the output). The Shavenbaby protein activates expression of a battery of target genes that transform an epidermal cell into a trichome cell. Each target gene triggers a specific aspect of cell differentiation, and production of a differentiated trichome requires coordinated expression of all target genes. The pattern of trichomes over the body is thus determined by the distribution of Shavenbaby protein in the epidermis, which is controlled by the cis-regulatory region of the shavenbaby gene. The shavenbaby gene serves as a nexus for patterning information flowing in and for cell-fate information flowing out.

In the entire regulatory network governing development of the Drosophila embryo, only shavenbaby, with its specialized function to rally the entire module of trichome morphogenesis, can accumulate mutations that alter trichome patterns without disrupting other developmental processes. Genetic changes in upstream developmental genes will alter trichome production, but these mutations also disrupt other organs. Changes in any one of the downstream genes are not sufficient to create or eliminate a trichome; concerted changes in multiple downstream genes are required to build a trichome (11). Furthermore, all of the evolutionarily relevant mutations in shavenbaby that have been identified so far alter the cis-regulatory region and not the protein-coding region. Mutations in the protein-coding region would alter shavenbaby function in every cell that accumulates Shavenbaby protein, and this would alter every trichome produced in larvae and adults. Thus, a developmental perspective clarifies why shavenbaby is a hotspot for evolutionarily relevant mutations and why these mutations occur in the cis-regulatory region of the gene. We predict that the cis-regulatory regions of other input-output genes may be hotspots for other phenotypic characteristics.

The shavenbaby gene provides one example of a more general principle: that mutations af-

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one effect of a pleiotropic mutation provides a major improvement in fitness, the other effects may be deleterious and will reduce the likelihood that the mutation will become established in the population (15).

Epistasis. When examined in a single genetic background, a mutation may have a specific or a pleiotropic effect. But in another genetic background, the same mutation may produce a different phenotypic effect because of nonadditive interactions of alleles: so-called epistasis. For example, one allele in A. thaliana increases growth in one genetic background but reduces growth in a different genetic background (16). The second genetic background is not simply deleterious in general, because a variant allele at a second locus causes higher growth in this background. Thus, the effects of one mutation can depend on the genetic variation present at other loci.

Epistasis is extremely common in natural populations and it may sometimes reduce the rate of evolution (17). Epistasis increases the phenotypic variance associated with a particular mutation, causing a mutation to have a fluctuating fitness effect dependent on the genetic background. Thus, in an Arabidopsis population containing multiple genetic backgrounds, we expect that selection for increased size will tend to favor nonpessimistic alleles that increase growth in all backgrounds rather than epistatic alleles that increase growth in only one genetic background.
from the mean phenotype in a population, mutations causing large phenotypic changes toward the new optimum will be favored, at least initially (18). For example, recently domesticated races have probably experienced strong selection by farmers, and many recent domestication traits result from mutations that cause large phenotypic effects, including pleiotropic deleterious effects. As an example, six different null-coding mutations in the myostatin gene cause muscle hypertrophy in different breeds of cattle (19). Myostatin is a member of the transforming growth factor–β superfamily of growth factors and acts as a negative regulator of muscle development. Although null mutations of myostatin generate cattle with more and leaner meat, these cattle experience difficulties in calving and have reduced stress tolerance. Strong selection during domestication can obviously overcome the negative pleiotropic effects of null myostatin mutations.

Population history. The past and current sizes of a population also influence genetic evolution. Small population size increases the effects of random sampling of alleles, so-called genetic drift. In small populations, genetic drift will allow deleterious alleles to occasionally increase in frequency. For example, a small inbred population of Bedouins in Israel has evolved a high frequency of a recessive allele that causes deafness (20). With stronger genetic drift in small populations, natural selection will fail to promote the spread of adaptive mutations of small effect. Instead, in comparison with large populations, adaptive mutations of relatively large effect will tend to evolve by natural selection in small populations.

Small populations also have another critical effect on evolution: They limit the total number of new mutations introduced into the population each generation. Thus, small populations may end up selecting far-from-ideal mutations (those with pleiotropic consequences and epistatic effects) simply because potentially superior mutations occur at a lower rate.

The abundance of null Frigida mutations in populations of A. thaliana highlights the importance of population history in genetic evolution. Null Frigida mutations have the negative consequence of reducing

**Fig. 2.** Bristle patterns on the dorsal thorax of Drosophila species have evolved within species and between species because of different kinds of mutations. (A) A mutation generating a null allele of the poils au dos gene within a population of D. melanogaster increases the number of large bristles on the dorsal thorax (white triangles indicate normal bristles and green triangles indicate extra bristles) (13). In contrast, the increased number of bristles in D. quadrilineata results at least in part from changes in the cis-regulatory region of the scute gene (12). The extra bristles caused by the poils au dos mutation are not as precisely positioned as the extra bristles caused by the scute mutation (indicated by purple triangles). (B) The two evolving genes, poils au dos and scute, occupy different locations in the genetic network that generates the pattern of bristles. The scute gene is an input-output gene, whereas the poils au dos gene is a developmental patterning gene. The null mutation in poils au dos increases sensory organ numbers not only in the thorax but also in the wing.
plasticity for flowering time. These mutations also have pleiotropic effects [they reduce fruit production (21)] and display epistasis with respect to other genes that control flowering time (22). These observations suggest that null Frigida mutations are not ideal alleles for controlling flowering time. In fact, null Frigida mutations must only rarely, if ever, be involved in phenotypic divergence between species because homologs of the Frigida gene exist in diverse plant species. But natural selection has overcome the deleterious effects of null Frigida mutations to promote the spread of these mutations in small populations. A. thaliana has migrated from Scandinavia around the world in the footsteps of agriculture. Subpopulations have adapted to local conditions, including the relatively warm and short winters of more temperate regions. A. thaliana plants are self-fertile, so even a single plant can give rise to a new population. These small subpopulations provide fewer opportunities for beneficial mutations of specific effect to appear, and strong selection for rapid flowering has favored whatever mutations of strong effect arose in the population, such as null Frigida mutations. The abundance of null Frigida mutations probably reflects the fact that these mutations occur at a higher rate than mutations without associated deleterious consequences.

The Genetic Basis of Short-Term and Long-Term Evolution

The Frigida example is not unique. In many plants and animals, evolution over long periods (variation between species) appears to differ in several ways from evolution over shorter periods (variation between domesticated races and between individuals within a species) (1). Here are three general ways in which long-term and short-term genetic evolution differ.

First, epistasis is commonly found for the mutations that contribute to phenotypic variation within species, whereas it is rarely observed for the mutations that cause differences between species. Within D. melanogaster, variation in bristle number is caused by multiple loci of relatively small effect, and these loci have epistatic effects of the same order of magnitude as the additive effects (23, 24). In contrast, morphological differences between Drosophila species result from multiple loci of intermediate-to-small effect that only rarely show epistasis (25, 26). Studies of body size variation in chickens show a similar pattern, with alleles segregating within species showing more epistasis than alleles differentiating species (27, 28).

Second, null mutations, which arise frequently and often cause pleiotropic and epistatic effects, seem to contribute more to phenotypic variation within species than to phenotypic differences between species. About 55% of the 99 mutations known to cause domestication traits are null-coding mutations, whereas only 7% of the 75 mutations known to cause interspecific differences are null-coding mutations (Fig. 3). For example, although domesticated cattle stocks have evolved multiple null mutations in the myostatin gene, all mammal species investigated so far possess a functional myostatin gene.

Third, the frequency of cis-regulatory mutations causing morphological variation differs between taxonomic levels. Morphological changes may occur either through coding changes or through cis-regulatory changes (Fig. 2). Because mutations in cis-regulatory regions often have fewer pleiotropic effects than mutations in coding regions, morphological changes are expected to involve mainly cis-regulatory mutations (1, 2, 29). Within species, most mutations that cause morphological variation have been found in protein-coding regions (Fig. 3). In contrast, between species most mutations that cause morphological differences have been found in cis-regulatory regions. Presumably, many of the coding mutations found within species fail to spread through populations, perhaps because of pleiotropic deleterious effects.

These striking and unexpected differences between short-term and long-term genetic evolution have emerged only recently with the accumulation of a sufficient number of case studies. These patterns are consistent with theoretical expectations of how the five parameters discussed earlier (pleiotropy, epistasis, plasticity, strength of selection, and population structure) should influence genetic evolution. Evolution over long periods, reflected in the differences between species, should result from mutations relatively devoid of pleiotropic and epistatic effects. In contrast, evolution over shorter periods, reflected in the differences between domesticated races and in the variation segregating within species, may often result from mutations that disrupt plasticity or that have pleiotropic and epistatic effects. In summary, differences between species are caused by a biased subset of the mutations that have appeared within natural populations (1).

Conclusions

Although mutations are thought to occur randomly in the genome, the distribution of mutations that cause biological diversity appears to be highly nonrandom. Gene function, gene structure, and the roles of genes and gene products in genetic networks all influence whether particular mutations will contribute to phenotypic evolution. Thus, for some phenotypic changes, evolutionarily relevant mutations are expected to accumulate in a few hot-spot genes and even in particular regions within single genes. In addition, population biology and ecology influence the spectrum of evolutionarily relevant mutations. Over short periods, adaptive mutations with deleterious pleiotropic effects may be selected because mutations without deleterious effects have not yet appeared. In contrast, over long periods adaptive mutations without pleiotropic deleterious effects have more opportunity to arise and be selected.

The genetic basis of phenotypic evolution thus appears to be somewhat predictable. These emerging patterns in the distribution of mutations causing phenotypic diversity derive, however, from a limited set of data culled from the published literature. It is possible that these patterns reflect biases in the way scientists have searched for evolutionarily relevant mutations (1). For
example, many researchers focus on candidate genes, which precludes the discovery of previously unknown genes. In the future, we expect that widespread adoption of unbiased experimental approaches, for example genetic mapping, will provide data for robust tests of the predictability of genetic evolution. Genetic mapping can be performed within species and, in rare cases, between closely related species. Alternatively, gene-by-gene replacement of all genes from one species into a second species, although experimentally tedious, may allow unbiased surveys for species that cannot be crossed. This approach would allow comparisons of distantly related taxa and provide a direct test of whether distantly related taxa have accumulated different kinds of evolutionarily relevant mutations than have closely related species.

More precise quantitative predictions about the mutations responsible for phenotypic evolution will probably result from further synthesis of molecular biology and population genetics. New theoretical models will encompass multiple population-genetic parameters within a genomic and developmental framework. These models may provide insight into how the distribution of spontaneously arising mutations is translated into the distribution of mutations segregating within populations and how these two distributions impact short-term and long-term evolution.

Finally, the fact that long-term genetic evolution may represent a biased subset of mutations has applied consequences, from the development of more efficient computer algorithms that utilize evolutionary search strategies to the improvement of agricultural crops and animals. Domestication often selects for mutations that have pleiotropic deleterious effects. Long-term evolution, in contrast, selects for mutations with specific phenotypic effects, and this class of mutations might be exploited to engineer domesticated races that possess desirable characteristics without associated unfavorable properties.

References and Notes
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