



Introduction

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One contribution to a Special Feature

'Experimental evolution' organized by Paul Sniegowski, Thomas Bataillon and Paul Joyce.

Evolutionary biology

As it happens: current directions in experimental evolution

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Recent decades have seen a significant rise in studies in which evolution is observed and analysed directly—as it happens—under replicated, controlled conditions. Such 'experimental evolution' approaches offer a degree of resolution of evolutionary processes and their underlying genetics that is difficult or even impossible to achieve in more traditional comparative and retrospective analyses. In principle, experimental populations can be monitored for phenotypic and genetic changes with any desired level of replication and measurement precision, facilitating progress on fundamental and previously unresolved questions in evolutionary biology. Here, we summarize 10 invited papers in which experimental evolution is making significant progress on a variety of fundamental questions. We conclude by briefly considering future directions in this very active field of research, emphasizing the importance of quantitative tests of theories and the emerging role of genome-wide re-sequencing.

1. Introduction

The early arguments that convinced the scientific world of the reality of evolution were largely based on indirect evidence: biogeography, the fossil record and comparative anatomy [1]. Indeed, in the *Origin of Species* Darwin argued that descent with modification is likely to be extremely gradual in natural populations, implying that we should not expect to observe evolution directly; instead, he invoked the obvious success of artificial selection in creating domesticated breeds and varieties to argue for the efficacy of natural selection [2]. In the twentieth century, however, it became apparent that evolution actually could be observed and analysed as it happens in appropriate study systems. This realization gave rise to the field of experimental evolution, which has grown rapidly in scope and influence in recent decades [3]. Studies in experimental evolution serve an important pedagogical purpose by providing convincing demonstrations of the reality of evolution and the power of natural selection [4,5]. The more significant contribution of experimental evolution, however, lies in its capacity to test quantitative assumptions and predictions that do not yield easily to comparative and retrospective analyses of natural populations. In this Special Feature, we bring together 10 invited papers that collectively illustrate how the field of experimental evolution is making progress on a variety of fundamental issues in evolutionary biology: namely, the fitness effects and rates of occurrence of new mutations; the nature, prevalence and evolutionary influence of genetic interactions; the nature of constraints on the evolution of sociality; and the roles of environmental variability and heterogeneity in evolution.

2. Mutation rates and fitness effects of new mutations

Mutations provide the genetic fuel for sustained evolution. Reliable estimates of the rates at which mutations that affect fitness arise, and of the distribution of mutational effects on fitness, have been elusive, although the availability of

extensive sequence data has enabled significant recent progress [6,7]. Even more elusive are how mutation rates vary due to the environment or the genetic background and how different mutations interact to determine fitness. Empirically, we know far more about deleterious mutations—mutations that ‘de-adapt’ organisms—than we know about the rare beneficial mutations which are the stuff of adaptive evolution. Newly arising mutations of all kinds are difficult to study in natural populations, which typically carry substantial amounts of standing genetic variation that complicates mutation detection and characterization and which, moreover, often live in complex and variable environments. Experimental approaches in which both the genetic background and the environment can be controlled can overcome these difficulties.

A fundamental question about mutation is the extent to which stress, defined as exposure to environmental conditions outside a population’s typical range, affects genomic mutation rate. Matsuba *et al.* [8] use a classical mutation accumulation approach, in which the effects of natural selection are minimized in a set of populations originating from a single inbred genotype, to test this question in the model metazoan *Caenorhabditis elegans* and its congener *Caenorhabditis briggsae*. They show that, at an elevated temperature that is stressful for *C. elegans* but not for *C. briggsae*, mutation rate is increased in *C. elegans* but not *C. briggsae*. Their results thus provide clear evidence for a role of stress in increasing the mutation rate, experimentally distinguishing the effect of stress from the previously known general effect of temperature on mutation rate.

Evolutionary biologists have long been interested in why haploid and diploid organisms coexist and alternate within species and in the broad implications of these two genetic states for the adaptation of populations [9]. Theoretical studies on these questions have typically assumed that beneficial mutations have identical effects in haploids and diploids—for example, that a beneficial mutation in a haploid has the same fitness effect when homozygous in a diploid. Gerstein [10] uses a set of known beneficial mutations in the yeast *Saccharomyces cerevisiae* to test this assumption, with quite surprising results: beneficial mutations placed in haploids provide higher fitness benefits than the same mutations made homozygous in isogenic diploids. In Gerstein’s experimental system, at least, all else is not equal in haploids and diploids.

The studies by Matsuba *et al.* and Gerstein illustrate the power of experimental evolution approaches to answer old questions clearly and call old assumptions into question.

3. Mutations and environments: how pervasive are interactions between factors affecting fitness?

To build sensible evolutionary models, we need to know the biologically realistic space of possibilities specifying the heritable variation available to natural selection. Ultimately, this requires understanding how mutations interact with each other (epistasis) and with the environment (genotype \times environment interactions) to determine fitness—and even how epistatic and environmental effects interact. In the era of classical genetics, progress in this general area was very difficult because isolating even individual mutations with measurable effects on fitness was possible in only a few

lucky cases [11]. Two studies in this Special Feature illustrate the power of modern experimental evolution approaches, in conjunction with molecular genetics, to make progress on this problem. Wang *et al.* [12] show that the fitness interaction between two known beneficial mutations isolated from an experimental population of *Escherichia coli* is altered substantially when these mutations are placed in the background of a diverged natural isolate of *E. coli*; moreover, they show that the interaction between these two mutations becomes more negative within the experimental population as adaptation proceeds. Lalić & Elena [13] use experimental populations to show that epistatic interactions affecting fitness in tobacco etch virus are significantly altered by host plant shifts. The results of these two studies suggest that experimental evolution has much to offer in helping sort out the space of possibilities under which selection can operate in populations.

4. Social interactions; cooperation and multicellularity

The evolution of social interactions has traditionally been studied in organisms with cognitive capacities, yet many of the central questions in this area are amenable to study using experimental populations of microbes. Indeed, there is increasing awareness that natural microbial populations themselves exhibit a variety of evolved phenomena (e.g. quorum sensing, bacteriocins and biofilm formation) that have social implications [14]. Social interactions, by their very nature, are likely to exhibit frequency and density dependence. Inglis *et al.* [15] use experimental evolution to investigate the frequency dependence of fitness effects accompanying bacteriocin production and neutralization in *Pseudomonas aeruginosa*. They show that the ability of clone mates to absorb and neutralize (‘soak’) their own bacteriocin—a capacity that would seem to provide sure benefits to relatives—markedly reduces the fitness of a bacteriocin-producing strain, compared with a non-producer, at high and low producer frequencies. Their study provides yet another illustration of the phenomenological power of experimental evolution to reveal significant complexity and contingency in even apparently simple biological systems.

Queller & Strassmann [16] describe novel microbial evolution experiments that can address fundamental conflicts in the evolution of multicellularity. Clonal reproduction entails high relatedness, which should favour cooperation possibly leading to multicellularity, but mutations arising in clonal lineages can raise evolutionary conflicts of interest. Queller & Strassmann show how relatively simple experimental systems using microbes in the laboratory have a strong potential to identify life cycles under which such conflicts will be sufficiently suppressed that multicellularity can evolve; interestingly, they point out that experimental evolution uniquely offers the possibility of exploring the implications of life cycles that have never evolved in nature.

5. Genetics of adaptation

Adaptation can be seen as the process by which a succession of beneficial alleles arise and spread to fixation in populations—a description that is deceptively simple on many levels. In order to provide the raw material for natural

selection, new beneficial mutations must first avoid stochastic loss arising from their initially small population size. Although several mathematical models have addressed the probability of fixation of new beneficial mutations, the rarity of new beneficial mutations and the difficulty of detecting them has so far frustrated most attempts to test such theories directly. Gifford *et al.* [17] make significant progress on this problem using an elegant experimental system in the fungus *Aspergillus nidulans* in which new beneficial mutations can be detected visibly as growth sectors on a solid medium. They use this system to test a specific mathematical model for the probability of fixation of new beneficial mutations in *Aspergillus*.

As a population adapts progressively in a given static environment, its state of adaptation can be seen as conditioning the availability and magnitude of effect of further beneficial mutations. Fisher [18] put forward a seminal theoretical treatment of this process that has received considerable attention from both theoreticians and experimentalists over the past few decades [19]. Inspired by very recent studies of the temporal dynamics of adaptation in experimental populations, Gordo & Campos [20] propose an extension of models based on Fisher's original geometrical model. By allowing for small, random environmental variations in an environment previously assumed to be static, their theory provides possible explanations for several interesting features of adaptive evolution observed in a set of experimental *E. coli* populations that have been propagated in the same abiotic environment for many thousands of generations.

6. Ecological variability and evolution

The environment in which many natural populations live is unlikely to remain static or only mildly variable for long periods of evolutionary time: instead, the climate may change quickly, a new competitor or host may evolve or arrive through migration, and so forth. Whether populations will go extinct in the face of such major changes or adapt, and the extent to which environmental variability governs evolutionary diversification, have been subjects of great theoretical interest to evolutionary ecologists for many years [21]. However, decisive tests of how such factors as population size, genetic system or intensity of competition determine the fate of populations in variable environments have been extraordinarily difficult to conduct as manipulative experiments in natural populations. Bell [22] investigates the factors affecting 'evolutionary rescue' from an abrupt and unfavourable change in environment using sexual and

asexual experimental populations of the green alga *Chlamydomonas reinhardtii* grown in the dark with acetate as a replacement carbon source. Most such populations succumb to extinction; interestingly, Bell observes that sexual populations are more likely to adapt to dark propagation than asexuals. Bono *et al.* [23] investigate the consequences of severe intraspecific competition for limiting resources in experimental populations of bacteriophage $\phi 6$, and show that such competitive pressure can indeed result in the rapid evolution of novel resource-use phenotypes—a prediction that was made generally for intraspecific competition by Darwin [2] but has been difficult to test in natural populations.

7. Conclusion

The series of invited papers discussed here exemplifies recent achievements in the field of experimental evolution. Multiple contributions (the papers of Gerstein [10], Lalić & Elena [13], Matsuba *et al.* [8] and Wang *et al.* [12]) illustrate the way in which experimental evolution facilitates isolation and decisive testing of the effects of individual factors on evolution in replicate populations. In general, experimental evolution is moving towards ever-tighter integration of empirical data and theory, and this trend is well represented by the work of Gifford *et al.* [17] and the theoretical contribution of Gordo & Campos [20] inspired by data from long-term evolution experiments. The potential for experimental microbial evolution to address fundamental questions in social evolution is illustrated by the work of Inglis *et al.* [15] and Queller & Strassmann [16]. Progress in testing current and longstanding ideas in evolutionary ecology is represented by the papers of Bell [22] and Bono *et al.* [23]. Clearly, quantitative tests of evolutionary theory will be an important area in which experimental evolution continues to contribute in the coming years. An emerging area that is not represented in these papers but is already beginning to transform the field is the combination of such quantitative tests with results from whole-genome resequencing data from experimental populations. Such an approach has the power, in principle, to fill in the missing link between phenotypic and genetic evolution: a subject, perhaps, for a future Special Feature in *Biology Letters*?

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