

REVIEW SUMMARY

EVOLUTION

Contingency and determinism in evolution: Replaying life's tape

Zachary D. Blount*, Richard E. Lenski, Jonathan B. Losos

BACKGROUND: Evolution is a strongly historical process, and evolutionary biology is a field that combines history and science. How the historical nature of evolution affects the predictability of evolutionary outcomes has long been a major question in the field. The power of natural selection to find the limited set of high-fitness solutions to the challenges imposed by environments could, in principle, make those outcomes deterministic. However, the outcomes also may depend on idiosyncratic events that an evolving lineage experiences—such as the order of appearance of random mutations or rare environmental perturbations—making evolutionary outcomes unrepeatable. This sensitivity of outcomes to the details of history is called “historical contingency,” which Stephen Jay Gould argued was an essential feature of evolution. Gould illustrated this view by proposing the thought experiment of replaying life's tape to see if the living world that we know would re-evolve. But, Gould wrote, “The bad news is that we can't possibly perform the experiment.”

Gould's pessimistic assessment notwithstanding, experimental evolutionary biologists have now performed many replay experiments, albeit on a small scale, while comparative biologists are analyzing evolutionary outcomes in nature as though they were natural replay

experiments. These studies provide new examples and insights into the interplay of historical contingency and natural selection that sits at the heart of evolution.

ADVANCES: Biologists have devised a variety of approaches to study the effects of history on the repeatability of evolutionary outcomes. On the experimental side, several designs have been employed, mostly using microbes, including “parallel replay experiments,” in which initially identical populations are followed as they evolve in identical environments, and “historical difference experiments,” in which previously diverged populations evolve under identical conditions (see the figure). Our review of many such experiments indicates that responses across replicate populations are often repeatable to some degree, although divergence increases as analyses move from overall fitness to underlying phenotypes and genetic changes. It is common for replicates with similar fitness under the conditions in which they evolved to vary more in their performance in other environments. Idiosyncratic outcomes also occur. For example, aerobic growth on citrate has evolved only once among 12 populations in an experiment with *Escherichia coli*, even after more than 65,000 generations. In that case, additional replays showed that the trait's evo-

lution was dependent on the prior occurrence of particular mutations.

Meanwhile, comparative biologists have cataloged many notable examples of convergent evolution among species living in similar environments, illustrating the power of

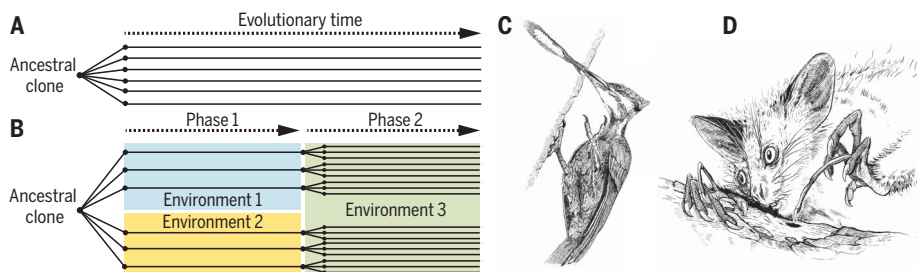
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natural selection to produce similar phenotypic outcomes despite different evolutionary histories. Nonetheless, convergence is not inevitable—in many cases, lineages adapt phe-

notypically in different ways to the same environmental conditions. For example, the aye-aye (a lemur) and woodpeckers have evolved different morphological adaptations to similar ecological niches (see the figure). An emerging theme from comparative studies, tentatively supported by replay experiments, is that repeatability is common when the founding populations are closely related, perhaps resulting from shared genetics and developmental pathways, whereas different outcomes become more likely as historical divergences become greater.

OUTLOOK: Gould would be pleased that his thought experiment of replaying life's tape has been transformed into an empirical research program that explores the roles of historical contingency and natural selection at multiple levels. However, his view of historical influences as the central feature of evolution remains debatable. Laboratory replay experiments show that repeatable outcomes are common, at least when defined broadly (e.g., at the level of genes, not mutations). Moreover, convergence in nature is more common than many biologists would have wagered not long ago. On the other hand, as evolving lineages accumulate more differences, both experimental and comparative approaches suggest that the power of selection to drive convergence is reduced, and the contingent effects of history are amplified. Recognizing the joint contributions of contingency and natural selection raises interesting questions for further study, such as how the extent of prior genetic divergence affects the propensity for later convergence. Theory and experiments indicate that the “adaptive landscape”—that is, how specific phenotypes, and ultimately fitness, map onto the high dimensionality of genotypic space—plays a key role in these outcomes. Thus, a better understanding of these mappings will be important for a deeper appreciation of how fate and chance intertwine in the evolutionary pageant. ■



Replaying the tape of life. The tape of life is replayed on a small scale in evolution experiments of different designs. (A) In a parallel replay experiment, initially identical replicate populations evolve under the same conditions to see whether evolution is parallel or divergent. (B) A historical difference experiment explores the influence of earlier history in phase 1 on later evolution during phase 2. In nature, diverged lineages exposed to similar environmental conditions are similar to a historical difference experiment, in that the potential for convergence on the same adaptive response may depend on their earlier evolutionary histories. In the case of (C) the woodpecker and (D) the aye-aye, they have adapted to the same ecological niche (locating grubs, excavating through dead wood, and extracting them), but they evolved different anatomical traits to do so, reflecting the legacy of their evolutionary histories (e.g., primates lack beaks, birds lack fingers).

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REVIEW

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Contingency and determinism in evolution: Replaying life's tape

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Historical processes display some degree of “contingency,” meaning their outcomes are sensitive to seemingly inconsequential events that can fundamentally change the future. Contingency is what makes historical outcomes unpredictable. Unlike many other natural phenomena, evolution is a historical process. Evolutionary change is often driven by the deterministic force of natural selection, but natural selection works upon variation that arises unpredictably through time by random mutation, and even beneficial mutations can be lost by chance through genetic drift. Moreover, evolution has taken place within a planetary environment with a particular history of its own. This tension between determinism and contingency makes evolutionary biology a kind of hybrid between science and history. While philosophers of science examine the nuances of contingency, biologists have performed many empirical studies of evolutionary repeatability and contingency. Here, we review the experimental and comparative evidence from these studies. Replicate populations in evolutionary “replay” experiments often show parallel changes, especially in overall performance, although idiosyncratic outcomes show that the particulars of a lineage's history can affect which of several evolutionary paths is taken. Comparative biologists have found many notable examples of convergent adaptation to similar conditions, but quantification of how frequently such convergence occurs is difficult. On balance, the evidence indicates that evolution tends to be surprisingly repeatable among closely related lineages, but disparate outcomes become more likely as the footprint of history grows deeper. Ongoing research on the structure of adaptive landscapes is providing additional insight into the interplay of fate and chance in the evolutionary process.

The world in which we live—with all its splendor, tragedy, and strangeness—is the product of a vast, tangled web of events that form what we call history. Had history taken another route, the world of today would be different. Indeed, the historical record is filled with accidents and coincidences that shaped the course of events, critical twists of fate in which wrong turns and stalled cars helped start wars, dropped cigars changed military outcomes, and mutations contributed to toppling empires (1–3). These instances illustrate a property of history called “contingency,” which makes outcomes sensitive to the details of the interacting events that led up to them. Contingency is why even though some trends may be predictable over the long-term and the past may be explicable, the future is unknowable.

Unlike many natural phenomena, evolution is a historical process, and evolutionary biology is a field in which science and history necessarily come together. Just as historians debate the extent to which certain historical events were inevitable, so too similar debates

have raged in evolutionary biology. One person was especially influential in forcing biologists to grapple with the role of history in evolution: Stephen Jay Gould. In many of his writings, and most forcefully in his 1989 book *Wonderful Life* (4), Gould argued that historical contingency is central to evolution. He asserted that the living world is the product of a particular history, and had that history gone differently, the world of today would be utterly unlike the one we know.

In *Wonderful Life*, Gould illustrated his view with the now-famous gedankenexperiment of replaying life's tape and seeing whether the outcome would be at all like the original. Gould's conclusion was “Replay the tape a million times... and I doubt that anything like *Homo sapiens* would ever evolve again.” But, Gould lamented, “The bad news is that we can't possibly perform the experiment.” In recent years, however, evolutionary biologists have shown that Gould's experiment can, indeed, be conducted, at least on smaller scales. Indeed, a thriving subfield of experimental evolution has performed many replay experiments in both the lab and the field. Moreover, many paleontologists and comparative biologists contend that evolution in nature has conducted natural experiments that can be interpreted as replay experiments. These empirical studies are providing new insights into the interplay of contingency and determinism at the heart of evolution.

“Replaying life's tape” and the meaning of “contingency”

Any attempt to review the body of empirical research on contingency's role in evolution must first grapple with two sources of confusion that Gould himself introduced. The first comes from inconsistencies in how Gould described the replay metaphor. As pointed out by the philosopher John Beatty (5), in *Wonderful Life*, Gould first describes his gedankenexperiment as a strict replaying of the tape of life from identical earlier conditions (6), but later on Gould asks how slight variations at the outset would have altered the outcome (7). One can quibble about which idea Gould really favored, but a number of quotes from *Wonderful Life* suggest he was thinking more about the latter scenario (8). In any case, different researchers have designed tests of the replay hypothesis based on Gould's alternative versions, which both complicates and enriches the synthesis of their findings.

Gould also introduced confusion about the concept of contingency itself. Despite its centrality to his thinking, Gould never formally defined “contingency.” He gave various informal descriptions, but these tended to be unfulfilling and circular. Moreover, he often conflated the two common meanings of the word “contingency”: “dependence on something else” and “an accidental or chance event.” Other writers have attempted to define contingency based on their interpretations of Gould's works, and different researchers have, again, designed work based on different notions of contingency (9–13). The definitions largely boil down to two alternatives that correspond to the different versions of the replay metaphor (5): unpredictability in outcomes from identical starting conditions, and causal dependence on the history leading to an outcome.

Philosophers of science have worked to clarify and formalize the concept of contingency. Beatty (14, 15) points out that contingency ultimately means that an outcome depends on a history that did not necessarily have to happen. Desjardins (16–18) has further identified this property as intrinsic to path-dependent systems in which there are multiple possible paths from an initial state, multiple possible outcomes, and “probabilistic causal dependence” that links the two. These characteristics make path-dependent systems sensitive to differences over their entire history, including initial conditions, as well as later events that may cause paths to diverge even when starting from identical conditions (16, 17). Thus, Gould's two alternative notions of contingency are just facets of the same thing. These characteristics also mean that a system's historical sensitivity will vary. In extreme cases, certain events along a historical path might completely preclude a given outcome, or render another outcome inevitable.

Desjardins' identification of contingency as a property of path-dependent systems is important because evolution inevitably has characteristics of path dependency. In particular, the stochastic processes of mutation and genetic drift virtually guarantee that different histories

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will occur even when populations start from the same state and evolve under identical conditions (Box 1) (19). Such differences, in turn, constitute the sort of unpredictable antecedent events that might preclude populations from evolving the same solutions when confronting the same selective circumstances or, at least, change the relative likelihoods of different outcomes (5). These effects arise from how mutations and the order in which they occur affect later evolution. Indeed, the particular mutations that occur, their effects, and their fates can alter the rates of occurrence, phenotypic and developmental effects, and fates of later mutations, thereby shifting the probabilities of alternative evolutionary paths (20). These differences may be further amplified or dampened by environmental perturbations that may themselves be stochastic. In short, past genetic changes that originate stochastically through mutations can become the contingencies that shape subsequent evolution. Therefore, just like human history, evolution permits different historical paths, the instantiation of which is governed by probabilistic causal dependence. The central question that remains is whether, and under what conditions, those different paths lead to meaningfully different outcomes. Evolution involves the strongly deterministic force of natural selection, which has no clear analog in human history. Is evolution still meaningfully contingent, despite this deterministic element?

One reason that evolution might be meaningfully contingent, even with the deterministic force of natural selection, is the extraordinarily complex relationship of genotype to fitness. This relation is often described using the metaphor of an “adaptive landscape” (21). The metaphor is often drawn as a vista or topographical map, in which genotypes are arranged according to their mutational distance, while the elevation represents each genotype’s fitness in a given environment. As a population evolves, new genotypes arise and their relative abundances shift, and the population thereby moves through the landscape. Absent any changes in conditions, natural selection tends to push the population uphill to higher average fitness, whereas the stochastic processes of mutation and drift tend to increase dispersion. If a landscape is smooth, with a single peak, then selection will eventually drive a population to that peak. If the landscape is rugged, with multiple peaks, then not all possible paths will lead to the highest peak, and evolutionary outcomes will be more sensitive to the population’s initial state. Moreover, environmental changes may alter the shape of the adaptive landscape, potentially moving peaks or even turning hills into valleys and vice versa. Of course, this analogy of the adaptive landscape to a physical landscape is flawed, in part because the extreme high-dimensionality of potentially relevant genotypic states makes it impossible to identify and represent the possible paths that an evolving population might take. Moreover, the adaptive landscape metaphor as usually put forth implicitly ignores the role of developmental

processes in translating genotypes into phenotypes. Nonetheless, while imperfect, the adaptive landscape metaphor remains widely used and is helpful when discussing the role of history in evolution.

Approaches to “replaying the tape” in evolutionary biology

Gould’s writings have inspired many studies of evolutionary contingency using a variety of approaches. Some comparative and paleontological analyses have used “macroevolutionary” data to examine contingency and convergence in key innovations and other phenotypic features (22–26). Others have reconstructed ancestral genes to examine contingency in the historical transitions in protein function (27–29). However, the main approach has been to perform

Gould’s replay experiment, albeit on a smaller scale. In some studies, this approach has been used to evolve replicate populations of digital organisms—programs that replicate, mutate, compete, and evolve—in which all parameters can be controlled and histories reconstructed perfectly (30–32). More often, however, replay studies have employed three other approaches: (i) experiments in the laboratory with fast-evolving organisms; (ii) experiments in nature; and (iii) comparative studies of lineages that have experienced similar environments.

A note on the issue of development

The field of evolutionary developmental biology, or “evo-devo,” has shown that development is a key aspect of the evolution of multicellular life, affecting the relationship between genotype,

Box 1. Contingency, determinism, and related words in an evolutionary context.

The vocabulary of evolution includes many words used both in ordinary language and to convey specific scientific ideas. Some of them also have different technical definitions in different scholarly contexts. Here we clarify what we mean by some of these words. To do so, we will build up from the basic processes that govern evolution to the conceptual issues that are the focus of this review.

At its core, evolution occurs by four fundamental processes: mutation, recombination, natural selection, and genetic drift. The first two produce genetic variation, whereas the last two govern the fate of variants. (Gene flow, interspecific hybridization, and horizontal gene transfer are special forms of recombination. The first describes the movement of genes across a spatial landscape; the second and third involve genes moving between species and microbial lineages, respectively.) Three of the processes—all except natural selection—are stochastic, in the sense that the specific variants produced or lost in a given generation are (or appear to be) a matter of chance. Chance is a tricky concept, however. There may well be some underlying cause for a chance event, such as a UVB (ultraviolet B) photon hitting DNA to produce a particular mutation or an asteroid striking Earth at a particular moment, but whether any specific event happens is unknowable or, at the least, impossible to incorporate into a mathematically efficient and useful theory of evolution. By contrast, natural selection is a deterministic process that reflects systematic differences in the propensity of alternative genotypes to survive and reproduce, depending on their fit to the environment. Thus, the “determinism” in our paper’s title makes reference to the systematic effects of natural selection that promote repeatable outcomes in evolution. Of course, natural selection can act only on variation that exists within the realm of physical and biological constraints, which might thus be viewed as also contributing to that determinism.

Determinism implies inevitability in some philosophical contexts, but it does not in an evolutionary context because of the interplay between natural selection and the various stochastic processes. For example, a deleterious mutation might reach fixation in a small population by genetic drift, and a beneficial mutation may go extinct by drift, even in a large population, because the number of individuals initially carrying the mutation is small. Thus, our paper attempts to review studies that provide evidence about the repeatability of evolution, rather than to resolve conflicting philosophical positions.

To be sure, evolutionary theory involves higher-level processes, such as speciation and extinction, but they emerge from these four fundamental processes playing out in time and space. This situation is comparable to that in physics, in which a few fundamental forces—gravity, electromagnetism, and the weak and strong nuclear forces (the second and third of these are now unified as the electroweak force)—together gave rise to chemical elements and galaxies.

The words “parallel” and “convergent” are widely used to describe repeatable evolutionary outcomes. If two lineages are ancestrally similar or identical, and if they evolve similar adaptations, then that is often called parallel evolution (although several other definitions of parallel evolution are sometimes used as well). By contrast, if they diverged substantially in the past, but subsequently evolve similar structures or functions, then that is called convergence. However, the distinction is often unclear, especially for organisms in nature and even sometimes in long-running experiments. For this reason, we follow Arendt and Reznick (134) in referring to all cases of independently derived similarity as convergent evolution.

phenotype, and fitness (33–35). Indeed, the evolution of developmental systems can introduce the various constraints and biases that preclude or predispose subsequent evolutionary outcomes, making development an important factor in evolutionary contingency (36, 37). In this review, we couch our discussion in terms of genetic changes and gloss over the details of how development affects the contingency of evolution. However, this approach is not intended to discount the role of development. Rather, development is generally encoded by genes (including developmental responses to environmental perturbations), so although our presentation emphasizes genetic changes, we recognize that genes produce phenotypes in multicellular organisms via the developmental process. Moreover, our review places substantial emphasis on experiments with unicellular microbes, for which development is less relevant. Although we discuss studies with multicellular plants and animals with complex developmental programs, we aim to present a view that integrates them with the microbial work, and thus have focused on genetics. For these reasons, we do not dwell on the manner in which the evolution of developmental systems can produce the historical contingencies that are the subject of this essay. Such a topic provides excellent material for dissecting the role of evolutionary

contingency, but is beyond the scope of this review.

Laboratory evolution experiments

In these experiments, replicate populations of a given species (or sometimes a community of two or more species) are propagated under controlled conditions, and their evolution monitored (38). History can play out repeatedly in these experiments, with initial and ongoing conditions that are either kept as identical as feasible or subtly changed, depending on the experiment, providing a valuable tool with which researchers can probe and even quantify the effects of contingency. Candidate events upon which particular outcomes are putatively contingent can then be identified, and their effects tested in further experiments. Although these experiments take place in laboratories, their results illuminate the potential role of contingency in the natural world.

The experiments have been performed with a variety of organisms. Microbes have been particularly useful because they are easy to handle and manipulate, they have fast generation times and large populations, and their (typically) asexual reproduction allows researchers to found replicate populations from the same clonal genotype. Moreover, some microbes can be frozen

and later revived, allowing the preservation of living “frozen fossil records” of evolving populations (39). These fossil records provide direct access to population histories, making them particularly useful in contingency studies (40).

Alternative experimental designs

Three basic designs have been used to examine contingency and repeatability in laboratory evolution experiments (40) (Fig. 1). The simplest and most common is the “parallel replay experiment” in which initially identical replicate populations evolve under identical conditions, thus effectively playing the same tape several times simultaneously (Fig. 1A). In parallel replay experiments with frozen fossil records, the contingency of a particular outcome can later be tested with “analytic replay experiments,” which are often called simply “replay” or “re-evolution” experiments (Fig. 1B). These experiments highlight the probabilistic nature of evolution and contingency. In an analytic replay experiment, archived samples are used to restart a population from multiple time points in its history. The resurrected populations are then allowed to evolve, and the patterns of recurrence of the outcome of interest examined (41, 42). Researchers use this design to probe for critical historical points at which the probability of a particular eventual outcome shifted to become more or less likely to occur than beforehand. These points can then be examined to identify the critical mutations or other events upon which the outcome’s occurrence or nonoccurrence was contingent. Analytic replay experiments come closest to representing Gould’s thought experiment, as they involve rerunning evolution from a previous point in history and seeing whether (and when and how often) the outcome is the same as the original.

Finally, “historical difference experiments” use a two-phase design to examine the effect of divergent evolutionary histories on subsequent evolution (40). In the simplest design, initially identical populations evolve in a single condition, just as in a parallel replay experiment. During this phase, each replicate acquires a unique history. In the second phase, the replicates are moved to a new environment where they evolve for another period (43) (Fig. 1C). Typically, the purpose of the second phase is to see whether the replicates adapt in the same way despite the differences accumulated during the first phase. There are several variations on this design; in all cases, the object of the first phase is for replicate populations to accumulate different histories, whereas the effect of those different histories on subsequent evolution is assessed in the second phase. In one variation, the populations evolve under multiple conditions in the first phase, before being shifted to a single condition in the second phase (Fig. 1D). In another variation, populations are founded from natural isolates and then evolved in a common laboratory environment; in this case, their prior evolution in the wild constitutes the first phase (Fig. 1E).

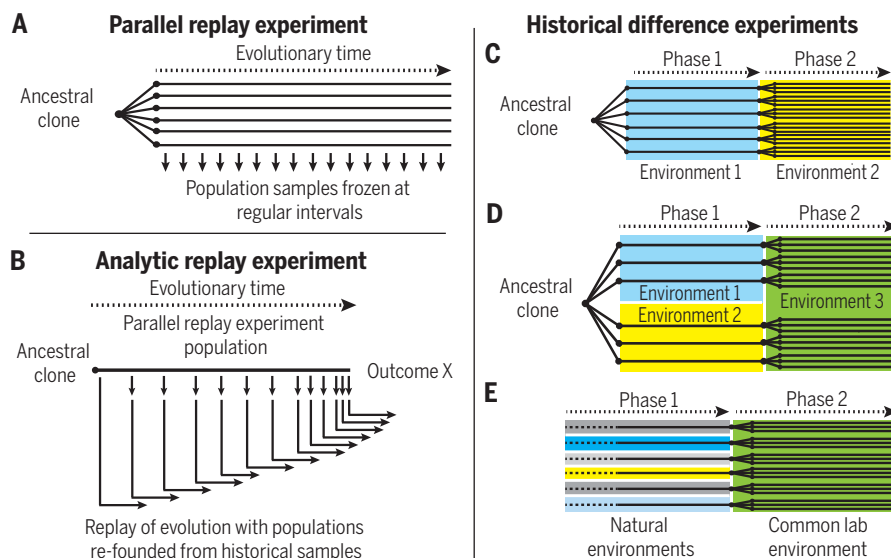


Fig. 1. Designs of microbial evolution experiments to explore historical contingency in parallel replay experiments. (A) Initially identical replicate populations are evolved under the same conditions to see whether evolution is parallel or divergent. Analytic replay experiments (B) are used to assess the contingency of a given outcome observed in a parallel replay experiment by replaying the population’s evolution from various points in its history to see whether the likelihood of that outcome changes over time. Historical difference experiments explore the influence of differences caused by earlier history in phase 1 on later evolution during phase 2. In the simplest historical difference experiment design (C), initially identical populations evolve under one condition for a period of time. They are then shifted to a second condition, in which they evolve for another period, typically to see whether they evolve convergently despite differences accumulated in the first period. In one variant historical difference experiment design (D), the first phase of evolution is carried out under multiple conditions before the populations are shifted to a single, common condition. In another (E), wild isolates are used to found populations that evolve under a common, laboratory environmental condition. In this case, prior evolution in the wild constitutes phase 1.

Survey of findings

In recent years, the number of laboratory evolution experiments relevant to historical contingency has increased greatly. Both the parallel replay and historical difference experimental designs have often been used to address various questions other than contingency. Indeed, the parallel replay experiment is effectively the default design for replicated evolution experiments. Consequently, many studies can be evaluated for what they say about evolutionary contingency, even when they were not explicitly designed for that purpose. A formal meta-analysis of the full body of experiments would be difficult because of their heterogeneity, and it is beyond the scope of this review. Instead, we surveyed 51 studies chosen for their variety of designs and organisms. These studies include 35 that used a parallel replay experiment design, 5 that involved some type of analytic replay experiment, and 14 with variations of the historical difference experiment design (these sum to more than 51 because some studies used multiple designs). Altogether, they involved 17 different species, including bacteria, viruses, and unicellular and multicellular eukaryotes (table S1). For each study, we noted the experimental design, organisms used, specific questions asked, and the sources of any historical differences either among the founding populations or that arose during the experiments. We then evaluated whether and how history affected the measured outcomes. Collectively, the studies present a complex, and sometimes contradictory, picture that suggests a more nuanced role for contingency in evolution than Gould envisioned.

The Long-Term Evolution Experiment with *Escherichia coli* (LTEE) is the most extensively studied example of a parallel replay experiment. The LTEE has followed 12 populations for over 65,000 generations since they were founded from a single clone in 1988 (44) (Fig. 2). The populations have been serially propagated in a glucose-limited medium that is considerably different from their natural environment, providing substantial opportunities for adaptation. Evolution in the LTEE occurs by de novo mutations, drift, and natural selection, making it a good model for investigating the contributions of these core processes to contingency. The populations have evolved in parallel (i.e., repeatedly) in several ways (45, 46). All have evolved much higher fitness, faster growth, and larger cells than the ancestor. Also, beneficial mutations have accumulated in many of the same genes across some or all of the populations, although the mutations are rarely the same at the nucleotide level. The populations have also diverged in various ways (45, 46). Each has accumulated a unique suite of mutations. Half evolved much higher mutation rates, causing the number of mutations accrued in each population to vary greatly. Most populations have evolved very similar fitness levels under the conditions of the experiment, but even so there are persistent differences in fitness between them, suggesting that they are ascending different peaks on the adaptive land-

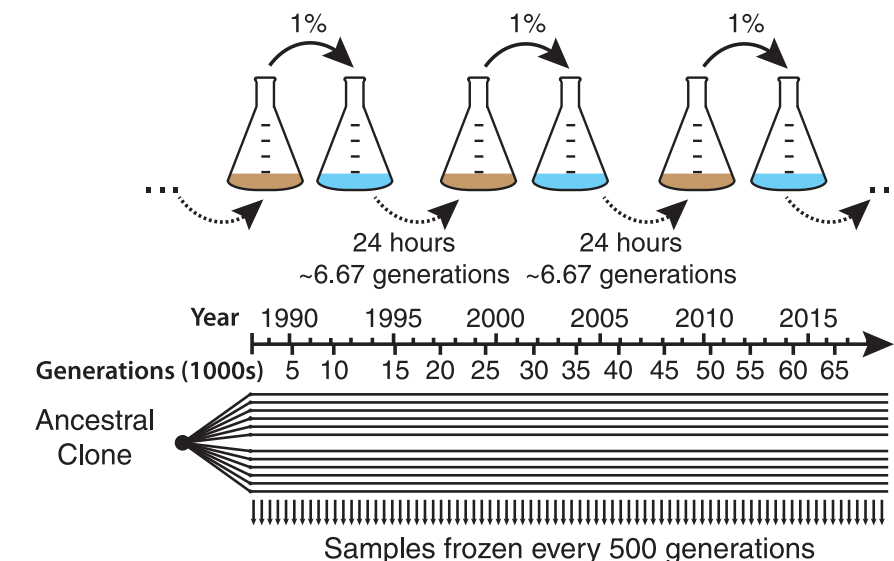


Fig. 2. The Long-Term Evolution Experiment with *E. coli* (LTEE). The LTEE is a paradigmatic parallel replay experiment that has studied 12 initially identical populations of *E. coli* for more than 65,000 generations of laboratory evolution under conditions of serial batch culture with daily 100-fold dilution into fresh medium. Samples of each population are frozen every 500 generations to provide a fossil record of viable bacteria.

scape. Moreover, the evolved populations vary considerably in their fitness under other conditions, including on different resources (47). Finally, many of the populations have evolved simple ecosystems in which two or more lineages stably coexist (48–50), although it remains to be seen whether coexistence typically involves the same ecological and genetic mechanisms. Overall, the LTEE populations seem to be following subtly different evolutionary paths, albeit in the same general direction, with one major exception that we will address later.

Broadly speaking, other parallel replay experiments, although much shorter in duration, show a similar pattern of generally consistent evolutionary responses across replicate populations under a variety of conditions. In some instances, these responses have been markedly parallel (51–57). However, heterogeneity in evolutionary responses across replicates is not uncommon (58–60). Such divergence is often more evident as analyses move from fitness per se to underlying phenotypic and genotypic responses (61). For instance, phenotypic parallelism often involves more variable genotypic changes, although instances of phenotypic variability with genotypic parallelism, at least at the level of genes mutated, have also been reported (54, 62–64). Similarly, as in the LTEE, it is not unusual for replicates with similar fitness under the conditions in which they evolved to have genetic differences that cause significant variation in fitness and phenotype under other conditions (65). This cross-condition variability makes it difficult to compare levels of divergence among experiments. This difficulty is exacerbated by logistical differences in obtaining genetic and phenotypic information. Modern genome sequencing and bioinformatics make the detection and comparison of evolved genetic

changes easy and cost-effective. By contrast, measuring phenotypes is difficult, costly, and time consuming, so most studies have examined relatively few phenotypic changes under a restricted set of conditions.

Divergence among replicates, when it occurs, is not always subtle (66–71). Collins and Bell (66), for example, observed two starkly different adaptive responses among five replicate algal populations that evolved under an elevated CO₂ level. Another notable example comes from the LTEE. After more than 31,000 generations, one population evolved the capacity to grow aerobically on citrate (Cit⁺), which was included in the culture medium as a chelating agent. Although many bacteria are Cit⁺, *E. coli* has been historically defined as a species in part by its inability to grow aerobically on citrate (Cit⁻). Occasional environmental isolates of *E. coli* have been found to be Cit⁺, but as the result of the acquisition of foreign plasmids, not chromosomal mutations. The Cit⁺ mutant that arose in the LTEE was only the second case ever reported (72), despite decades of study of this organism in hundreds of laboratories. A recent study found additional spontaneous Cit⁺ mutants, but their isolation required prolonged, intense, and focused selection (73). When this new ability arose in the LTEE, it changed the population's ecological circumstances and evolutionary direction in several important ways—allowing the cell numbers to increase several-fold, causing metabolic by-products to accumulate, changing the bacteria's stoichiometric evolution, and perhaps even setting the Cit⁺ lineage on a path toward incipient speciation (41, 74–76).

The ability to grow on citrate is highly beneficial in the LTEE environment, yet the Cit⁺ trait has evolved in only 1 of 12 populations,

even after more than 65,000 generations. There are two plausible explanations for this seeming paradox. The trait might have been caused by a single extremely rare mutation that could have occurred at any time in any of the populations. Alternatively, the ability to grow on citrate might have required multiple mutations. If so, selection for the Cit⁺ trait per se would not have facilitated spread of the earlier mutations that, nonetheless, were required for the evolution of the Cit⁺ trait under the experimental conditions. According to that second hypothesis, the evolution of the Cit⁺ trait was therefore contingent on a particular history during which one or more required mutations happened to accumulate, “potentiating” the trait’s appearance. To test these ideas, Blount *et al.* (41) devised the analytic replay experiment design, recognizing that a contingent outcome should be more likely after the potentiating event (or events). In several experiments, they restarted the population with clones isolated at 16 time points in its frozen fossil record, replayed evolution thousands of times, and examined the outcomes. The Cit⁺ trait re-evolved only in populations founded by clones from 20,000 generations onward, implying that some potentiating mutation had arisen by then.

Subsequent work has revealed the complex evolutionary history that led to the Cit⁺ trait. Leon *et al.* (77) found that the trait was slightly beneficial in the ancestral genetic background. However, early evolution in the population was dominated by high-fitness, glucose-adapted mutations against which any rare Cit⁺ mutants could not effectively compete. This adaptation led to a genetic background in which the Cit⁺ trait had become detrimental. Further mutations, some of which seem to have been involved in adaptation to growth on acetate (a by-product of glucose metabolism), accumulated between 10,000 and 29,000 generations. The Cit⁺ trait was slightly beneficial again on this new background (78). At this point, high-fitness mutations were no longer sweeping through the population, and the weakly beneficial Cit⁺ cells were able to persist long enough to accumulate refining mutations that made the trait highly beneficial (74, 75, 79).

The analytic replay experiment design has since been used to test the contingency of other outcomes seen in parallel replay experiments. Using four closely related clones isolated very early from another LTEE population, Woods *et al.* (80) performed an analytic replay experiment to investigate why one lineage had eventually prevailed over another, even though the clones representing the eventual winner had demonstrably lower fitness than clones from the lineage that later went extinct. Replays showed that the eventual winners prevailed because they were more evolvable; that is, they were more likely to generate beneficial mutations of large effect. Genome sequencing and genetic manipulations showed that this difference reflected a strong epistatic interaction between mutations at two specific loci. Meyer *et al.* (81) performed a multispecies analytic replay exper-

iment, which showed that the evolution of a phage λ variant able to infect *E. coli* via an alternative receptor was contingent on mutations in the coevolving host population. This work highlights how evolutionary contingency can play a key role in community dynamics that are more typically addressed in purely ecological terms.

The analytic replay design is relatively new, and few have been performed to date. However, they show that particular outcomes can hinge on small historical differences between populations that can then lead to substantial divergence even under identical conditions. They also indicate that genetic and ecological interactions can play critical roles in generating the events that drive such divergence. Altogether, analytic replay experiments provide compelling examples of how evolutionary outcomes can hinge on the particulars of history.

Parallel replay experiments show that differences can arise among initially identical populations evolving under identical conditions, and analytic replay experiments show that those differences can alter evolutionary potentials in important ways, even in the absence of environmental change. By contrast, historical difference experiments examine how different histories can affect subsequent evolution when the environment is changed. Forerunners to this design included experiments in which bacteria were challenged to grow in different environments to see whether the sequence of challenges affected the propensity to acquire an altered metabolic or resistance phenotype (82, 83). In the first historical difference experiment to explicitly quantify the effect of history, Travisano *et al.* (43) isolated clones from each LTEE population after 2000 generations of adaptation to the glucose-limited medium. They then founded three replicate populations from each clone, which evolved for 1000 generations in the same medium except with glucose replaced by maltose. Owing to their different histories, the clones varied greatly in their initial fitness in the maltose environment. However, they rapidly converged in their fitness on maltose during evolution in that new environment (Fig. 3). Several later historical difference experiments have also shown that adaptation to new conditions can drive convergence at the level of fitness, despite initial differences, although the mark of history often lingers at the genetic level (84–89).

Some historical difference experiments, however, have shown stronger historical effects that preclude complete convergence, although those effects can vary with the environment used for the second phase of adaptation (7). Burch and Chao (89) found that two closely related phage $\phi 6$ genotypes had distinctly different capacities for further adaptation after prior evolution left them in different regions of the adaptive landscape, and Flores-Moya *et al.* (90) found that history strongly affected the evolution of two dinoflagellate strains. Moore and Woods (91) also found that *E. coli* strains isolated from different hosts varied significantly in the rate at

which they adapted to a glucose-limited medium; this variation did not simply reflect differences in their initial adaptation to the laboratory environment, but instead indicated more idiosyncratic effects of prior history. Similarly, a study of 230 *Saccharomyces cerevisiae* strains (produced by crossing two highly diverged parental strains) showed a strong tendency for later, convergent adaptation to erase prior history, although the degree of erasure was subtly affected by specific genotypes (92). Taken together, historical difference experiments indicate that the capacity of selection to overcome historical differences has limits. Specifically, the

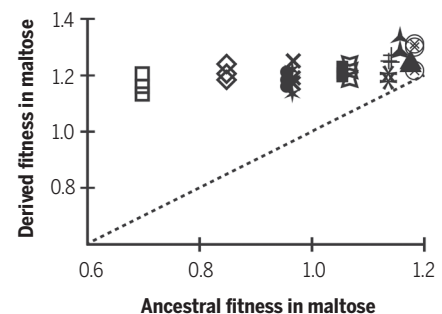


Fig. 3. Rapid convergence in a historical difference experiment. Single clones of *E. coli* were isolated from each of the 12 LTEE populations after 2000 generations of evolution in glucose-limited medium. Three replicate populations of each were founded and then evolved for 1000 generations in a maltose medium. Despite substantial initial variation due to their independent histories of adaptation to glucose, the replicate populations rapidly converged in their fitness on maltose. [Redrawn from Travisano *et al.* (43)]

historical difference experiments suggest that adaptation’s ability to drive convergence declines as populations have spent more time diverging from one another, and when that divergence occurred in more distinct environments.

Synopsis of laboratory studies

These replay experiments present a rich and complex picture of the repeatability and contingency of evolutionary outcomes. The direction of evolutionary change typically seems to be broadly consistent in a given condition, regardless of history, and phenotypic and genetic parallelisms are often striking (45). Even so, there remains scope for history to drive substantially divergent outcomes. These divergences are often subtle, such as differences in genotype that nonetheless lead to parallel evolution in phenotypes, including especially fitness itself. But subtlety of immediate effects does not necessarily negate the importance of long-term effects, as differences can build on one another. The evolution of the Cit⁺ trait in the LTEE is a case in point, illustrating how seemingly minor changes can shift the potential for further

evolution in ways that lead to marked divergence (41). Moreover, subtle divergences that matter little in the environment where they emerge can have major effects when conditions change, as a consequence of mutations that have not been tested under the new conditions (45, 88). On the other hand, historical difference experiments show that selection in the new environment can sometimes overcome those previously evolved differences. The deeper the imprint of history, however, the less likely it becomes that evolution can reverse the prior divergence.

One interpretation of the results of the laboratory replay experiments is that the potential for contingency to matter is determined, in part, by the structure of the adaptive landscape encountered by the replicate evolving populations. As might be expected, a rugged landscape that presents multiple adaptive peaks makes distinct outcomes possible, and starting conditions, as well as the form and strength of interactions between mutations (epistasis), will affect the probabilities of those outcomes. Alternatively, a smooth landscape will tend to yield more repeatability if the time scale examined allows replicates to find the peak (67). However, these inferences are potentially circular, because our knowledge of adaptive landscapes typically comes from such experimental outcomes. This issue highlights the need for further investigation into landscape parameters. One factor that may affect ruggedness is environmental complexity; an environment with spatial structure or multiple resources, for instance, may often provide more opportunities for divergent adaptive responses (61, 68). Exogenous events and how organisms modify their environments complicate things further by changing the structure of the landscape in ways that can affect opportunities for subsequent divergence (69, 70). However, a genotype may have multiple distinct paths to higher fitness even in a homogeneous, single-resource environment (71).

Altogether, laboratory experiments on contingency support a nuanced view. Evolution is more likely to be historically insensitive and repeatable if the adaptive landscape offers few alternative paths or many that lead to similar outcomes. If, however, the landscape is rugged, with multiple avenues available that lead to dissimilar adaptations, then outcomes are likely to be more variable and more sensitive to historical contingencies. Evolutionary repeatability varies because the degree to which outcomes are contingent varies.

Experimental evolution in nature

Although most replay experiments have been conducted in the laboratory, an ambitious new direction involves replicated evolution experiments in natural settings. The realization that natural selection can produce rapid evolutionary change (93–96) opened the door to evolution experiments in nature. To date, results are available from only a few such experiments, but many more are now under way (97). Some of

these studies take advantage of long-running ecological experiments, including the Park Grass Experiment, which was started in 1856 (98, 99).

These studies have focused on hypotheses about adaptation in the wild. However, they often also constitute *de facto* replay experiments, as replicate populations can be compared to examine variation in evolutionary responses. Several differences should be kept in mind when comparing these studies to laboratory experiments. In particular, the experiments in nature often involve vertebrate animals, rather than the microorganisms and invertebrates typically used in laboratory experiments; therefore, populations are smaller, generations are longer, and founding populations are genetically heterogeneous. These factors make it more likely that evolutionary responses in field experiments rely on standing genetic variation present at the outset, rather than on *de novo* variation generated during the experiment. They therefore increase the opportunity for parallel responses based on shared variation, on the one hand, and the scope for differences in initial conditions between replicate populations to produce contingent evolutionary responses, on the other hand. Furthermore, in some experiments, such as those on color and life histories in guppies, different populations were used to establish the experimental populations, making these studies more akin to historical difference experiments than to parallel replay experiments (97).

It is perhaps too early to generalize from the field evolution experiments reported to date. Nonetheless, the results so far—including guppies evolving slower life histories in the absence of predators (100) and lizards evolving shorter limbs when forced to use narrow substrates (101)—tend to indicate a high degree of repeatability in evolutionary responses (97).

Comparative studies: Evolutionary replays across space and time

The ideal experiment for characterizing repeatability and contingency in evolution would be to expose initially identical populations to the same conditions in nature and allow them to evolve not for a few years or tens of years, but for thousands and even millions of years. Even if funding were available for such studies, we would have to wait a long time to get the results. But fortuitously, nature has already conducted such experiments for us, albeit not quite as precisely as those performed in the laboratory.

Convergent evolution is broadly defined (Box 1) as the independent evolution of similar features in multiple species or clades (102). Convergent evolution can occur for many reasons. For instance, shared developmental programs may predispose species to evolve in the same way for reasons unrelated to natural selection (103, 104). However, convergence occurring in distinct lineages living in similar environments has long been considered strong evidence of the operation of natural selection (102, 105, 106). For example, both the C_4 and CAM (crassulacean acid metab-

olism) photosynthetic pathways have evolved independently many times in plants, almost always in lineages that now occur in arid or semiarid regions; this evolutionary correlation suggests that the lower rates of water loss and other physiological features of these pathways are advantageous under these conditions (107, 108). Similarly, strikingly convergent carnivorous pitcher plants have evolved in several unrelated genera as an adaptation to waterlogged soils with low nutrient availability and high light (109, 110). Until fairly recently, such cases of convergence were considered relatively rare exceptions. In recent years, however, myriad examples of adaptive convergence have been reported (23, 111, 112). Particularly impressive are cases in which convergence involves not just two (or more) lineages adapting to the same niche, but entire multispecies assemblages evolving similarly, such as evolutionary radiations of Caribbean lizards and Pacific Ocean snails on multiple islands, and frog and bird faunas on different continents (113).

The extent of convergence has led some to argue that the repeated evolution of the same feature under similar circumstances means that evolution is predictable and that contingencies of history hold little sway in directing evolution. More specifically, they argue that the ubiquity of convergence indicates that optimal solutions exist to problems posed by the environment and that lineages have repeatedly, almost deterministically, found these solutions (23, 111, 112).

This argument assumes that the same selective conditions occur repeatedly, that there are a limited number of high-fitness phenotypic solutions (“adaptive peaks”) to these challenges, and that populations inevitably evolve these phenotypes. According to Conway Morris (23), “the evolutionary routes are many, but the destinations are limited.” McGhee (114) put it this way: “Convergent evolution is the result of the fact that there are limited numbers of ways to solve a functional problem within the constraints imposed by the laws of physics and geometry.”

One prerequisite for adaptive convergence is that species respond to similar selective pressures by adopting the same ecological role [i.e., the same niche in the original Grinnellian sense (115)]. This need not be the case, however, because communities of species do not necessarily partition resources in similar ways. Moreover, even when species converge upon the same ecological role, they may evolve distinct non-convergent phenotypic adaptations. For example, considering the aye-aye (a lemur) and the woodpecker to be convergent misses the point that they evolved very different phenotypic means to accomplish the same task of locating and extracting grubs from inside wood. They occupy the same niche but adapted in divergent, rather than convergent, ways.

Assuming that multiple lineages independently adopt the same ecological niche, how might contingency lead them to adapt in different ways to the same environmental challenge? We see three main possibilities. First, populations might evolve

different solutions to the same challenge. For example, some plants may adapt to the presence of a herbivore by evolving physical defenses such as thorns, others by acquiring chemical defenses, and yet others by becoming cryptic. Second, populations may evolve the same function, but by means of different phenotypic changes. For example, the hammering beak and long bristly tongue of the woodpecker accomplish the same ends as the chiseling teeth and long, flexible finger of the aye-aye [more generally, the “many-to-one” phenomenon in biomechanics (116)]. Third, some populations may get stuck on a lower adaptive peak (local optimum) and be unable to evolve the best possible phenotype (global optimum). In all three cases, historical contingencies may predispose a lineage to adapt one way or another (birds lack teeth and hands, and primates lack beaks, explaining the different routes taken by the aye-aye and woodpecker). Their different histories thus may explain why two lineages fail to converge despite experiencing the same selective conditions for millions of years.

In evaluating the extent to which convergence is evidence of evolutionary determinism, several points must be considered. Most generally, we need to ask what constitutes convergence. Birds, bats, and insects all fly, but their wings are constructed differently and their aerodynamics also differ. Are these convergent adaptations, or divergent adaptations accomplishing the same task? At some level, drawing a line becomes arbitrary. Another difficulty is that convergence is identified after the fact. The saber-toothed condition evolved at least three times in the Carnivora, as well as once each in creodonts and South American marsupials, presumably as an adaptation to a particular predatory strategy (117). But how many other taxa, faced with the same selective conditions, failed to evolve this adaptation? Knowing the denominator is key to determining how repeatable a convergent trend is (45), but rarely does one know how many other lineages experienced similar circumstances, yet failed to evolve the trait in question. Moreover, although recent compilations of convergence (23, 111, 112) are impressive, one could just as easily compile lists of adaptive types lacking a convergent doppelgänger: the two-leaved *Welwitschia mirabilis*, the platypus, chameleons, kiwis, elephants, octopuses, and hominins—all adaptive types that have evolved just once—to name a few (Fig. 4).

Finally, the occurrence of convergent evolution is not necessarily inconsistent with the evolutionary importance of contingency. Genetic changes can become the contingencies that shape subsequent evolution. To the extent that shared genetic and developmental systems predispose species to evolve in similar ways (103, 104, 106, 118), then adaptive convergence may often be shaped by the particular history that sculpted the genetics and development of their shared ancestors (119). In such cases, evolution may be deterministic within a clade but contingent at deeper phylogenetic levels when comparing species across clades (104, 112, 119). Moreover, the shared regulatory mechanisms

planets, it will look much like what we see here (23). But we need not look to the stars to test that hypothesis: All we need to do is go to New Zealand, an island lacking any native terrestrial mammals. In their absence, New Zealand's flora and fauna evolved to bear little resemblance to any other ecosystem in the world. In addition to kiwis, there are both carnivorous and flightless parrots, adzebills, moas, giant eagles, and flightless wrens, as well as a semi-terrestrial bat [“the bat family's attempt to make a mouse” (123)], giant snails and orthopterans, and divaricating shrubs with leaves that grow in the interior of the bush. And going back in time, one would be hard-pressed to find many similarities between the Mesozoic world of the dinosaurs and today's faunas.

In short, lineages adapting to similar environmental conditions in nature can be thought of as evolutionary replays, even if these “natural experiments” are not as precise as carefully designed and controlled laboratory experiments. Because the lineages will have different genetic constitutions and will have experienced different histories, these cases are analogous to the historical difference experiments in laboratory studies. Unfortunately, however, the evidence boils down to one list of cases in which convergence occurred and another where it did not, rendering quantitative conclusions unsatisfactory. Nonetheless, the many impressive cases of convergence show that repeated outcomes can arise from similar environmental challenges. Conversely, the many cases in which convergence did not occur suggest that contingent effects can play a strong role in shaping divergent adaptive responses.

Against that murky conclusion, one trend stands out (despite some exceptions): Conspecific populations and closely related species seem to evolve in similar ways more often than distantly related taxa (124). Such a trend is expected in part because closely related species tend to interact with the environment in similar ways. Moreover, they share more of their history, and thus share more of the past changes in their genetic and developmental systems that can shape later evolution. Closely related lineages are thus predisposed to evolve in the same way. Indeed, some cases of parallel evolution have occurred by selection on shared variation that was present in a common ancestral population (125, 126). By contrast, convergence between distantly related lineages is less likely to result from selection on shared variation. A related finding is that when convergence

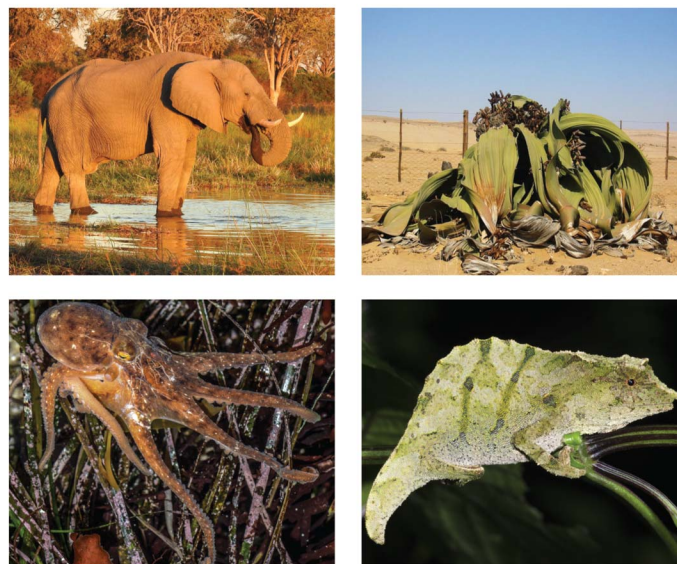


Fig. 4. Evolutionary one-offs. Evolutionary one-offs are species or clades that evolved unique adaptations to their ecological circumstances that have not been convergently evolved by other lineages. Clockwise from top left: African elephant, *Welwitschia*, Moyer's pygmy chameleon, red octopus. (Note that similarity among, for example, species of elephants or chameleons is not convergent; rather, their shared features are the result of inheritance from a common ancestral species that evolved their trademark features a single time.) [Photo credits: African elephant: Jonathan Losos. *Welwitschia*: Thomas Schoch, CC BY-SA 3.0 license; original at [https://en.wikipedia.org/wiki/Welwitschia#/media/File:Welwitschia_mirabilis\(2\).jpg](https://en.wikipedia.org/wiki/Welwitschia#/media/File:Welwitschia_mirabilis(2).jpg). Moyer's pygmy chameleon: Martin Neilsen, CC BY-SA 4.0 license; original at https://commons.wikimedia.org/wiki/Category:Rhampholeon_moyeri#/media/File:Stump-tailed_chameleons_Rhampholeon_moyeri.jpg. Red octopus: Jerry Kirkhart, CC BY-SA 2.0 license; original at https://commons.wikimedia.org/wiki/Category:Octopus_rubescens#/media/File:Red_Octopus_rescued.jpg]

and sometimes cryptic genetic similarities that underlie deep homologies indicate that contingent historical events can shape convergence even among distant relatives (36). The evolutionary reactivation of previously silenced, but still functional, developmental programs is another example of how distant relatives can exhibit evolutionarily derived phenotypic similarity as a result of contingent genetic events (120–122).

Some convergence proponents go so far as to say that if life has evolved on Earth-like exo-

occurs, the extent to which the response involves the same gene is greater when the taxa are closely related (127). This pattern accords with the tentative conclusion from laboratory studies that parallel replay experiments (with replicate populations founded by the same ancestor) tend to produce parallel outcomes more often than historical difference experiments (with populations founded by different ancestral strains or species).

Conclusions and future prospects

Gould's gedankenexperiment that "we can't possibly perform" has been transformed into a real experimental program, one in which increasingly sophisticated and audacious studies are exploring the roles of contingency and determinism at ever deeper levels. Although Gould's ideas on contingency have stimulated a great deal of productive work, his view that contingent effects were pervasive throughout evolution remains debatable. The laboratory replays performed to date typically show that repeatable outcomes are common, at least when the founding populations are similar, when repeatability is defined broadly (e.g., at the level of affected genes and pathways, as opposed to precise mutational changes), and over the time scales accessible to experiments. Moreover, evolutionary convergence across lineages that share similar natural environments has proven more common than most biologists would have wagered even two decades ago—its prevalence attests to the power of natural selection to repeatedly sculpt the same adaptive solutions. That it does so more often among closely related taxa, which share similar genetics and developmental programs, illustrates the yin and yang of contingency and determinism.

Where to now? Clearly, evolution can be both contingent and deterministic, and often in complicated and fascinating ways. Recognizing this mixed nature will allow future research to investigate how contingency and determinism interact. Many questions remain to be addressed; for example, what circumstances promote contingent and deterministic outcomes, how does the extent of prior genetic divergence affect the propensity for future parallelism versus contingency, what types of divergence—say, a few mutations of large effect versus the accumulation of minor variants over long periods—lead to which outcomes, and what circumstances allow convergence even in distantly related taxa? Theory and experiments show that the structure of the adaptive landscape plays a critical role in determining the potential for contingent outcomes. Therefore, a deeper understanding of adaptive landscapes will be important for understanding evolutionary contingency (89, 128–133). In short, there's no shortage of work to do, and interesting outcomes to be discovered and quantified. Gould would be pleased that the field he inspired has such bright prospects, as the tape of life plays on.

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- "I call this experiment 'replaying life's tape.' You press the rewind button and, making sure you thoroughly erase everything that actually happened, go back to any time and place in the past...Then let the tape run again and see if the repetition looks at all like the original" [p. 48 in (4)].
- "[A]ny replay, altered by an apparently insignificant jot or tittle at the outset, would have yielded an...outcome of entirely different form..." [p. 248 in (4)].
- "Alter any early event, ever so slightly and without apparent importance at the time, and evolution cascades into a radically different channel" [p. 51 in (4)]. "A historical explanation...[rests]...on an unpredictable sequence of antecedent states, where any major change in any step of the sequence would have altered the final result. This final result is therefore dependent, or contingent, upon everything that came before—the unerasable and determining signature of history" [p. 283 in (4)]. "Historical explanations take the form of narrative: E, the phenomenon to be explained, arose because D came before, preceded by C, B and A. If any of these earlier stages had not occurred, or had transpired in a different way, then E would not exist" [p. 283 in (4)].
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SUPPLEMENTARY MATERIALS

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Supplementary Text
References (135–150)

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Contingency and determinism in evolution: Replaying life's tape

Zachary D. Blount, Richard E. Lenski and Jonathan B. Losos

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Replaying the tape of life

The evolutionary biologist Stephen Jay Gould once dreamed about replaying the tape of life in order to identify whether evolution is more subject to deterministic or contingent forces. Greater influence of determinism would mean that outcomes are more repeatable and less subject to variations of history. Contingency, on the other hand, suggests that outcomes are contingent on specific events, making them less repeatable. Blount *et al.* review the numerous studies that have been done since Gould put forward this question, both experimental and observational, and find that many patterns of adaptation are convergent. Nevertheless, there is still much variation with regard to the mechanisms and forms that converge.

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Supplement 1: Digest of Selected Laboratory Replay Experiments

Citation: J. B. Anderson, J. Funt, D. A. Thompson, S. Prabhu, A. Socha, C. Sirjusingh, J. R. Dettman, L. Parreiras, D. S. Guttman, A. Regev, L. M. Kohn, Determinants of divergent adaptation and Dobzhansky–Muller interaction in experimental yeast populations. *Curr. Biol.* **20**, 1383–1388 (2010).

Organism: *Saccharomyces cerevisiae*

Type of Experiment: Parallel Replay Experiment with Multiple Conditions

Experimental Question: What are the genetic determinants of incipient reproductive isolation in strains adapting to different conditions?

Experiment Description: Six populations were evolved in a high salinity medium, while six others were evolved in a low glucose medium under conditions of serial batch culture for 500 generations. (Same populations as Dettman et al. 2007, from which this experiment was derived.) Evolved isolates were derived from three populations, two evolved under high salt conditions, and one under low glucose conditions, and sequenced.

Findings: The same specific genetic changes underlying adaptation evolved repeatedly in independent lines, and these same adaptations reliably produced incipient reproductive isolation.

Sources of Historical Differences: Mutation and genetic drift

Did History Matter? No.

Citation: H. J. E. Beaumont, J. Gallie, C. Kost, G. C. Ferguson, P. B. Rainey, Experimental evolution of bet hedging, *Nature* **462**, 90-93 (2009).

Organism: *Pseudomonas fluorescens*

Type of Experiment: Parallel Replay Experiment

Experimental Question: Can a fluctuating environment lead to the evolution of bet-hedging, that is the capacity for stochastic switching between phenotypic states?

Experiment Description: Twelve initially identical populations were founded and evolved through 16 rounds of alternating selection. In each round, the populations were evolved via serial passage in a shaken or unshaken environment, with plating each day. When a novel colony type was noted, that colony was chosen and placed into the opposite environment type for the next round.

Findings: Variant genotypes that underwent rapid colony-morphology switching between translucent and opaque colonies that either produced or did not produce capsules was observed in two of the 12 replicates. Genome sequencing found that a mutation in the *carB* gene was necessary and sufficient to produce the switching phenotype. However, it did not occur until after multiple rounds of selection. Tests with isogenic constructs showed that, while the mutation could occur and produce the phenotype in the ancestor, it was detrimental in the ancestral background. By contrast, the mutation was beneficial in the evolved background. Evolution of the bet-hedging phenotype was therefore historically contingent, and potentiated by earlier mutations.

Sources of Historical Differences: Mutation and genetic drift.

Did History Matter? Yes. Evolution of bet-hedging phenotype was contingent upon particular histories that altered its fitness effects. The trait only evolved twice out of 12 replicates.

Citation: S. Bedhomme, G. Lafforgue, S.F. Elena, Genotypic but not phenotypic historical contingency revealed by viral experimental evolution. *BMC Evol. Biol.* **13**, 46 (2013).

Organism: Tobacco etch potyvirus (TEV)

Type of Experiment: Historical Difference Experiment

Experimental Question: Does prior different evolutionary history impact the phenotypic or genotypic evolution of TEV populations upon evolution on a single, common host?

Experiment Description: Sixty viral lines had been previously evolved under conditions of 15 serial passages on one of four single hosts, or through alternation of three combinations of two hosts, with 10-fold replication. The evolved clones were then evolved via 15 serial passages on a single host, *N. tabacum*.

Findings: The experimental populations all evolved similar phenotypes during the second round of evolution, with the effects of prior adaptation largely disappearing. However, the effects of past adaptation left clear marks at the genetic level, with possible effects on what new adaptive mutations were fixed across populations during the second phase of evolution. This result was taken to suggest that compensatory mutations were common, and functioned to overcome prior evolution.

Sources of Historical Differences: Mutation and genetic drift, as well as prior evolutionary history accrued during adaptation to different hosts or alternating hosts.

Did History Matter? Not at the phenotypic level, but clearly yes at the genotypic level.

Citation: A. F. Bennett, R.E. Lenski, An experimental test of evolutionary trade-offs during temperature adaptation. *Proc. Natl. Acad. Sci. U.S.A.* **104**, 8649-8654 (2007).

Organism: *Escherichia coli*

Type of Experiment: Historical Difference Experiment with multiple phase one conditions

Experimental Question: Does adaptation to a given environmental condition carry tradeoffs under other conditions?

Experiment Description: An ancestral clone was isolated from a population that had evolved for 2,000 generations at 37°C under conditions of serial batch culture. It was then used to found 24 populations that were evolved for another 2,000 generations of serial batch culture under conditions of 32°C, 37°C, 42°C, or alteration between 32°C and 42°C with six-fold replication. A clone was then isolated from each of the evolved populations, and used to found a new population that then evolved for another 2,000 generations at 20°C. The fitness of the evolved clones was assayed at 20°C and 40°C.

Findings: All populations showed increased fitness at 20°C. However, only 15 of the 24 showed evidence of a trade-off, as indicated by a concurrent significant loss of fitness at 40°C. One population showed significantly increased fitness at 40°C. No effect of prior history was noticed in either the degree of adaptation to 20°C, or the degree of tradeoff at 40°C.

Sources of Historical Differences: Prior history of evolution under different adaptive conditions, together with mutation and genetic drift.

Did History Matter? In terms of adaptation to new conditions, no, but there was great variation in correlated fitness effects in other conditions.

Citation: A. J. Betancourt, Genomewide patterns of substitution in adaptively evolving populations of the RNA bacteriophage MS2, *Genetics* **181**, 1535-1544 (2009).

Organism: Phage MS2

Type of Experiment: Parallel Replay Experiment

Experimental Question: What is the genome-wide spectrum of substitution during adaptation to a novel environment?

Experiment Description: Three lines of MS2 evolved under conditions favoring rapid growth and lysis at cold temperatures for 50 generations, and their genomes were then sequenced.

Findings: All three lines showed significant increases in fitness over the experiment. Many substitutions occurred in parallel across populations.

Sources of Historical Differences: Mutation and genetic drift.

Did History Matter? No.

Citation: Z. D. Blount, C. Z. Borland, R. E. Lenski, Historical contingency in the evolution of a key innovation in an experimental population of *Escherichia coli*. *Proc. Natl. Acad. Sci. U.S.A.* **105**, 7899-7906 (2008).

Organism: *Escherichia coli*

Type of Experiment: Analytic Replay Experiment

Experimental Question: Was the evolution of aerobic growth on citrate in one of twelve long-term populations of *E. coli* historically contingent?

Experiment Description: Cit⁻ clones were isolated from many time points in the history of the population, and their capacity to yield Cit⁺ mutants assayed via evolutionary replays under two different conditions.

Findings: Clones isolated from later time points in the population's history were found to have higher rates of mutation to Cit⁺, implying that mutations had accumulated over its history that potentiated the trait's evolution.

Sources of Historical Differences: Mutation and drift originally, also extent of prior history and hence selection experienced by clones from different points along the same time line.

Did History Matter? Yes, strongly.

Citation: J. J. Bull, M. R. Badgett, H. A. Wichman, J. P. Huelsenbeck, D. M. Hillis, A. Gulati, C. Ho, I. J. Molineux, Exceptional convergent evolution in a virus. *Genetics* **147**, 1497-1507 (1997).

Organism: Phage φX174

Type of Experiment: Parallel Replay Experiment with multiple conditions

Experimental Question: What is the extent of convergent evolution?

Experiment Description: Five lineages of this phage, four of which started from the same genotype, were propagated for 11 days under conditions in which temperature was elevated to 43.5°C. Two populations were propagated on *E. coli* C, and three on *Salmonella typhimurium*. A subset of two lineages evolved for longer periods of time under the same conditions as the initial adaptation. Two others, derived from a *S. typhimurium* lineage, evolved for another 11 days on *E. coli*.

Findings: All populations showed substantial fitness improvement. Sequencing of exemplars from each line identified 119 mutations at 68 loci, half of which were identical across multiple lineages, both in terms of those propagated on the same host, and in general. Switching of hosts led to additional convergent fixation of mutations, including reversions of previously adaptive mutations.

Sources of Historical Differences: Mutation and drift

Did History Matter? No.

Citation: C. L. Burch, L. Chao, Evolvability of an RNA virus is determined by its mutational neighbourhood. *Nature* **406**, 625-628 (2000).

Organism: Phage $\phi 6$

Type of Experiment: Historical Difference Experiment

Experimental Question: Do different mutations that compensate for the same detrimental mutation alter evolvability?

Experiment Description: A single phage $\phi 6$ clone with a deleterious mutation was used to found two populations that were then propagated through bottlenecks of 100 or 33 phage to permit fitness recovery by acquisition of compensatory mutations. The two populations then evolved for 100 generations and their fitness was assayed.

Findings: Population A showed a significantly higher fitness after phase 2 evolution, while B showed no increase in fitness after 100 generations, suggesting that A was more evolvable. Examination of mutants of each during evolution was used to examine the distribution of fitness effects of available mutations. While the distribution for A showed many beneficial mutations, that of B included only detrimental mutations. The authors conclude that the difference in the evolvability of A and B was due to each having evolved up different fitness peaks of different height, which altered the range of accessible mutations for further evolution.

Sources of Historical Differences: Prior history, mutation, and genetic drift.

Did History Matter? Yes, history strongly altered propensity for further adaptive evolution.

Citation: S. Collins, G. Bell, Phenotypic consequences of 1,000 generations of selection at elevated CO₂ in a green alga. *Nature* **34**, 566-569 (2004).

Organism: *Chlamydomonas reinhardtii*

Type of Experiment: Parallel Replay Experiment with multiple conditions

Experimental Question: What are the consequences of selection for growth at increased CO₂ levels?

Experiment Description: Two ancestors were used to found 10 replicate populations each. Half of each type evolved under ambient CO₂ concentrations for 1000 generations, and half under conditions of increasing CO₂ for 600 generations and then for 400 generations under a maximum of 1050 ppm CO₂. Phenotypic changes were then examined.

Findings: The lines evolved at high CO₂ failed to significantly improve in growth at high CO₂, while losing fitness at the ambient level, as well as having a lower limiting density at high CO₂. Two distinct groups evolved with different physiological characteristics. One group showed no changes in chlorophyll a content, photosynthesis or respiration rates, and when growing at high CO₂ seemed the same as control cells growing at ambient CO₂. The second group had variable responses, but all had increased rates of photosynthesis at high CO₂, but apparently could not channel the carbon fixed into appreciably faster growth. Two of the three evolved lines with increased rates of photosynthesis had elevated respiration rates at high CO₂.

Sources of Historical Differences: Mutation and genetic drift.

Did History Matter? Yes for the high CO₂ condition, in which the replicate populations evolved in two distinct ways.

Citation: T. F. Cooper, R. E. Lenski, Experimental evolution with *E. coli* in diverse resource environments. I. fluctuating environments promote divergence of replicate populations. *BMC Evol. Biol.* **10**, 11 (2010).

Organism: *Escherichia coli*

Type of Experiment: Parallel Reply Experiment with multiple conditions

Experimental Question: How does environmental variability impact evolutionary dynamics?

Experiment Description: In total, 42 initially identical populations of *E. coli* B evolved for 2,000 generations in seven different environmental regimes. (Glucose only, maltose only, glucose and maltose in alternation, glucose and maltose together, lactose only, glucose and lactose in alternation, and glucose and lactose together.) After evolution, fitness was measured for each population under different conditions to examine the effects of the selective environment on adaptation and degree of fitness divergence among replicate populations.

Findings: The populations evolved increased fitness under the conditions in which they evolved, but the magnitude of the fitness gains varied across the conditions, as did their correlated fitness responses to other regimes. Among-population genetic variation for fitness was highest and most sustained over time in those groups that evolved under fluctuating regimes. The greater variation among the replicate populations in the fluctuating environments suggested they were climbing different fitness peaks.

Sources of Historical Differences: Mutation and genetic drift.

Did History Matter? Yes in the fluctuating conditions, as indicated by the replicate populations apparently climbing distinct fitness peaks.

Citation: D. E. Deatherage, J.L. Kepner, A.F. Bennett, R.E. Lenski, J.E. Barrick, Specificity of genome evolution in experimental populations of *Escherichia coli* evolved at different temperatures. *Proc. Natl. Acad. Sci. U.S.A.* **114**, E1904–E1912 (2017).

Organism: *Escherichia coli*

Type of Experiment: Parallel Replay Experiment with multiple conditions

Experimental Question: To what extent are environmental differences and commonalities reflected in genomic evolution?

Experiment Description: Thirty populations evolved for 2,000 generations at five different temperatures (20°C, 32°C, 37°C, 42°C, and daily alternation between 32°C and 42°C), with six-fold replication. An evolved clone was then isolated from each end-point population, sequenced, and compared. Populations that had evolved for 20,000 generations at 37°C were also examined and compared to the others.

Findings: Clones that evolved under the same conditions showed ~17% overlap in mutated genes, while those evolved under different conditions showed only ~4% overlap. However, populations that had evolved for more than 18,000 more generations at one condition (37°C) also showed the accumulation of mutations in the same genes that were particular to populations that had evolved under other thermal conditions over the 2000-generation experiment. The authors suggest that prolonged evolution might eventually eliminate early divergences as populations adapt to the shared features (e.g., the same growth medium) across thermal conditions.

Sources of Historical Differences: Mutation and genetic drift.

Did History Matter? The genomic signatures of adaptation to particular conditions identified suggest no. Authors conclude that the degree of predictability in adaptation to particular conditions is striking.

Citation: J. R. Dettmann, C. Sirjusingh, L. M. Kohn, J. B. Anderson, Incipient speciation by divergent adaptation and antagonistic epistasis in yeast. *Nature* **447**, 585-588 (2007).

Organism: *Escherichia coli*

Type of Experiment: Parallel Replay Experiment with multiple conditions

Experimental Question: What is the relationship between adaptation to divergent environments and the evolution of reproductive isolation?

Experiment Description: Six populations evolved in a high salinity medium, while six others evolved in a low glucose medium under conditions of serial batch culture for 500 generations.

Findings: All populations evolved increased fitness in their environment, though the degree of adaptation varied. The divergent adaptation to the different environments drove the accumulation of mutations producing incompatibility between strains adapted to the different conditions.

Sources of Historical Differences: Mutation and genetic drift.

Did History Matter? Not at the level of the derived incompatibilities.

Citation: R. Dhar, R. Sägesser, C. Weikert, J. Yuan, A. Wagner, Adaptation of *Saccharomyces cerevisiae* to saline stress through laboratory evolution. *J. Evol. Biol.* **24**, 1135–1153 (2011).

Organism: *Saccharomyces cerevisiae*

Type of Experiment: Parallel Replay Experiment

Experimental Question: How do genomes and gene expression change during adaptive evolution affecting complex traits? Does evolutionary adaptation mirror physiological response? What is the genetic basis of evolutionary adaptation of yeast to high-saline stress?

Experiment Description: Three initially identical populations of *S. cerevisiae* strain BY4741 were founded from a single haploid clone and evolved for 300 generations under conditions of 0.5 M NaCl. Physiological, transcriptomic, and genetic changes were then investigated.

Findings: All experimental lines showed significant fitness gains, higher growth rates, increased ploidy, and increased cell size over time in the high salt environment, but significant variation was observed between populations. Significant variation in evolved DNA context was also seen between populations, and at least one adaptive SNP was particular to one population. Significant parallelism was observed in gene expression between populations.

Sources of Historical Differences: Mutation and genetic drift.

Did History Matter? Significant differences between the populations suggest that it did.

Citation: M. Dragostits, V. Mozhayskiy, S. Quinones-Soto, J. Park, I. Tagkopoulos, Evolutionary potential, cross-stress behavior and the genetic basis of acquired stress resistance in *Escherichia coli*. *Mol. Sys. Biol.* **9**, 643 (2013).

Organism: *Escherichia coli*

Type of Experiment: Parallel Replay Experiment with multiple conditions

Experimental Question: How does cross-stress adaptation evolve, and by what genetic changes?

Experiment Description: Initially identical populations of *E. coli* evolved for 500 generations in five environments in which they were exposed to osmotic, acid, oxidative, n-butanol, or no stresses (control) with four-fold replication. Degree of tolerance to selected and unselected stressors was then examined. Attempts were made to identify genetic determinants of observed changes.

Findings: Populations evolved in a given condition displayed similar response to selection and correlated fitness changes in other stressor environments. Similar genetic changes were observed among those evolved under same conditions as well.

Sources of Historical Differences: Mutation and genetic drift.

Did History Matter? No.

Citation: W. W. Driscoll, M. Travisano, Synergistic cooperation promotes multicellular performance and unicellular free-rider persistence. *Nat. Commun.* **8**, 15707 (2017).

Organism: *Kluyveromyces lactis*

Type of Experiment: Parallel Replay Experiment

Experimental Question: Can multicellularity evolve in the unicellular yeast, *K. lactis*? If so, how? And what facilitates the maintenance of unicellular forms in co-culture with newly evolved multicellular yeast?

Experiment Description: Ten initially identical *K. lactis* populations were subjected to settling selection for ~400 generations, and assessed for evolution of multicellularity. Instances of multicellularity evolution were then studied, along with attendant interactions with cells that retained unicellular modes of life.

Findings: All 10 populations evolved multicellularity via very similar means. All populations also saw the persistence of unicellular types throughout the experiment.

Sources of Historical Differences: Mutation and genetic drift.

Did History Matter? No.

Citation: A. Flores-Moya, M. Rouco, M. J. García-Sánchez, C. García-Balboa, R. González, E. Costas, V. López-Rodas, Effects of adaptation, chance, and history on the evolution of the toxic dinoflagellate *Alexandrium minutum* under selection of increased temperature and acidification. *Ecol. Evol.* **2**, 1251–1259 (2012).

Organism: *Alexandrium minutum*

Type of Experiment: Historical Difference Experiment

Experimental Question: How do adaptation, chance, and history impact *Alexandrium minutum* evolution under conditions of increased heat and lower pH?

Experiment Description: Two strains, AL1V and AL2V, of *A. minutum* were used to found ten independent populations each. Half were evolved under conditions of 20°C and pH 8, and half were evolved at pH 7.5 and 25°C for 720 days, or 180 to 250 generations. Phenotypic change in growth rate and toxin cell quota were then examined and compared.

Findings: History strongly altered the evolutionary trajectories of the populations in terms of both growth rate and toxin cell quota.

Sources of Historical Differences: History of the two strains prior to experiment, together with mutation and genetic drift.

Did History Matter? Yes, strongly.

Citation: S. S. Fong, A. R. Joyce, B. Ø. Palsson, Parallel adaptive evolution cultures of *Escherichia coli* lead to convergent growth phenotypes with different gene expression states, *Gen. Res.* **15**, 1365-1372 (2005).

Organism: *Escherichia coli*

Type of Experiment: Parallel Replay Experiment with multiple conditions

Experimental Question: How reproducible are growth phenotypes and global gene expression states during adaptive evolution?

Experiment Description: Fourteen initially identical populations of *E. coli* were founded. Seven of the populations evolved for over 1000 generations (60 days) in lactate minimal medium (M9), and the remaining seven evolved for over 600 generations (44 days) in a glycerol minimal medium (M9). Growth rates, substrate uptake rates, oxygen uptake rates, and growth rates on alternate carbon substrates, as well as transcription levels were measured at different time points during adaptation.

Findings: Growth phenotypes were found to be convergent and reproducible, though these were underlain by different gene expression states, which were in turn due to a number of different compensatory changes in expression as well as other adaptively beneficial expression changes that were common across all replicates.

Sources of Historical Differences: Mutation and genetic drift.

Did History Matter? Not at the growth-phenotypic level, but clearly yes at the transcriptomic and genetic levels.

Citation: M. L. Friesen, G. Saxer, M. Travisano, M. Boebeli, Experimental evidence for sympatric ecological diversification due to frequency dependent competition in *Escherichia coli*. *Evolution* **58**, 245-260 (2004).

Organism: *Escherichia coli*

Type of Experiment: Parallel Replay Experiment

Experimental Question: What are the ecological and evolutionary dynamics underlying sympatric diversification of *E. coli* in a mixed resource environment?

Experiment Description: Twelve initially identical populations of *E. coli* B evolved under a serial batch culture regime for 1000 generations in a medium containing a mixture of glucose and acetate.

Findings: All populations evolved a diversity of colony morphology, with varying modes of colony size presumed to indicate differences in composition of ecological types. Four populations showed unimodality, three showed a range of colony sizes suggestive of multiple types, and five populations showed clear bimodality in colony size.

Sources of Historical Differences: Mutation and genetic drift.

Did History Matter? Yes, differences in history of adaptation appears to underlie the difference between those populations that evolved bimodal ecologies and those that did not.

Citation: E. T. Granato, R. Kümmerli, The path to re-evolve cooperation is constrained in *Pseudomonas aeruginosa*. *BMC Evol. Biol.* **14**, 214 (2017).

Organism: *Pseudomonas aeruginosa*

Type of Experiment: Parallel Replay Experiment

Experimental Question: Can cheating mutants revert back to cooperation when the conditions that favor cheating change?

Experiment Description: Two mutants of *Pseudomonas aeruginosa* PAO1, each deficient in pyoverdine production owing to different mutations, were used to found three initially independent replicate populations in six conditions (unstructured shaken medium with low, medium, or high iron, and structured agar medium with low, medium, or high iron), and evolved for 200 generations. Twenty clones were then isolated from each population and screened for re-evolution of pyoverdine production. The genetic basis of recovery was investigated for those clones in which recovery was found.

Findings: Re-evolution of increased pyoverdine production was very rare, being observed in only ~0.7% of evolved clones, and complete re-evolution of cooperative pyoverdine production levels was never seen. Evolution of variants that had totally lost pyoverdine production was more common, albeit still rare, being observed among ~4% of evolved clones. The authors conclude that the evolutionary path to recovery of complete cooperation was highly constrained.

Sources of Historical Differences: Mutation and genetic drift.

Did History Matter? Yes, as indicated by the variability in evolved pyoverdine production levels.

Citation: D. R. Hekstra, S. Leibler, Contingency and statistical laws in replicate microbial closed ecosystems. *Cell* **149**, 1164-1173 (2012).

Organisms: *Chlamydomonas reinhardtii*, *Escherichia coli*, *Tetrahymena thermophila*

Type of Experiment: Parallel Replay Experiment

Experimental Question: How unique are each play of ecology and evolution, and what extent do they display a common statistical structure?

Experiment Description: Initially identical, closed ecosystems composed of three species of microbes were established, and allowed to run for multiple years under controlled, unchanging conditions, with light being the only interaction with the world outside the microcosm. Eleven ecosystems in the first run, and 5 sets of 10 were constructed in the second. The local population densities of the three co-existing species were tracked in each microcosm over time to examine covariation and ecological dynamics.

Findings: The three species co-existed in a majority, though not all, of replicates over at least 1000 days. The density of the species in each microcosm covaried according to stable structures described as “ecomodes” around which population densities fluctuate. The fluctuations followed power laws that were consistent with a geometric random walk, which is intrinsically contingent. However, the authors found that the effects of the past were not amplified exponentially.

Sources of Historical Differences: Mutation and genetic drift in each species population, together with random ecological fluctuations.

Did History Matter? Yes, but in a constrained manner.

Citation: K. L. Hillesland, R. E. Lenski, G. J. Velicer, Experimental evolution of a predator's ability to find prey, *Proc. Royal Soc. B* **276**, 459-467 (2009).

Organism: *Myxococcus xanthus*

Type of Experiment: Parallel Replay Experiment with multiple conditions

Experimental Question: How does prey density affect the evolution of a predator's behavior during search and handling?

Experiment Description: Sixteen initially identical populations of *M. xanthus* were evolved for over a year (~300 generations) on agar plates inoculated with patches of *E. coli* prey. The populations were transferred to new plates every fourteen days. Eight evolved with prey patches separated by 1 cm, and eight with 2 cm separation. Changes in fitness, searching, and handling were examined.

Findings: 15 of 16 populations evolved faster swarming speeds and prey-handling speeds. Only one showed significantly higher prey-handling speed. All showed reduced fruiting body formation. Significant heterogeneity in between-population response to selection among high-density-evolved populations

Sources of Historical Differences: Mutation and genetic drift.

Did History Matter? Heterogeneity suggests yes, but overall evolutionary directions were largely concordant.

Citation: E. R. Jerison, S. Kryzahimskiy, J. K. Mitchell, J. S. Bloom, L. Kruglyak, M. M. Desai, Genetic variation in adaptability and pleiotropy in budding yeast. *eLife* **2017**, 6e27167 (2017).

Organism: *Saccharomyces cerevisiae*

Type of Experiment: Historical Difference Experiment

Experimental Question: How does genetic variation in adaptability affect evolution?

Experiment Description: 230 offspring of a cross between the divergent yeast strains RM and BY were isolated, and the adaptability of the genotypes was measured in two different environments, one in rich medium at the optimal temperature of 30°C (OT), and one in synthetic medium at a high temperature of 37°C (HT). Each hybrid was used to found eight new replicates for a total of 1840 populations. Half evolved for 500 generations in the OT environment, and half in the HT environment, after which the fitness of each population was determined in each environment. Adaptability was defined as the average rate of adaptation in a specific environmental condition. The authors also examined heritability, predictability, and genetic basis of adaptability.

Findings: There was significant variation in evolved fitness. Strains with lower initial fitness showed a pronounced tendency for great improvement than those with higher initial fitness, consistent with convergence. Initial fitness therefore strongly altered adaptability and subsequent evolution by affecting the spectrum of available beneficial mutations. Moreover, there were subtle, albeit significant effects associated with several quantitative trait loci that caused specific genotypes to improve more or less than expected given initial fitness level. A number of genes were mutated in parallel across multiple populations.

Sources of Historical Differences: Genotypic heterogeneity following cross of previously diverged strains.

Did History Matter? Yes.

Citation: Jochumsen, R. L. Marvig, S. Damkiær, R. L. Jensen, W. Paulander, S. Molin, L. Jelsbak, A. Folkesson, The evolution of antimicrobial peptide resistance in *Pseudomonas aeruginosa* is shaped by strong epistatic interactions, *Nat. Commun.* **7**, 13002 (2016).

Organism: *Pseudomonas aeruginosa*

Type of Experiment: Parallel Replay Experiment, Analytic Replay Experiment

Experimental Question: What are the molecular evolutionary pathways by which high-level colistin resistance evolves?

Experiment Description: Sixteen replicate populations were founded from *Ps. aeruginosa* PAO1, and evolved in parallel via serial culture in Luria Bertani medium with increasing concentrations of colistin for 62 cycles. Five other replicate cultures were propagated in a colistin-free environment as a control. At the end of the experiment, seven of the colistin condition replicates were dead, while all of the controls remained colistin-sensitive. One evolved clone was isolated from each of the surviving nine experimental and five control populations,

and subjected to whole genome sequencing. Seven loci were identified as having evolved in parallel in at least three of the colistin-evolved populations. Adaptive mutations that provided resistance to colistin were placed into an ancestral background in all combinations, and the MIC of the constructs tested to examine the available evolutionary trajectories. Constructs carrying observed mutations in *phoQ* and *pmrB* were then tested for potentiation of high colistin resistance in a microtiter resistance assay.

Findings: Nine of sixteen populations evolved high colistin resistance. Genome sequencing identified a number of loci mutated in parallel across the populations. Isogenic constructs were generated and used in replay experiments, showing that they cause increased likelihood of evolving colistin resistance. The authors conclude that evolution of resistance is complex and multistep, requiring mutations in at least five loci that interact epistatically to generate the phenotype. The strong epistatic interactions also limit viable pathways to the phenotype. Mutations in transcriptional regulators potentiate resistance evolution, as shown by replay experiments with isogenic constructs.

Sources of Historical Differences: Mutation, genetic drift, engineered mutations.

Did History Matter? Yes, the evolution of high-level resistance was contingent upon the occurrence of potentiating mutations in transcriptional regulator genes.

Citation: R. Korona, C. H. Nakatsu, L. F. Forney, R. E. Lenski, Evidence for multiple adaptive peaks from populations of bacteria evolving in a structured habitat. *Proc. Natl. Acad. Sci. U.S.A.* **91**, 9037-9041 (1994).

Organism: *Comamonas* sp. strain TFD41

Type of Experiment: Parallel Replay Experiment with multiple conditions

Experimental Question: Can natural selection drive divergence in sympatry when alternative adaptive solutions are available?

Experiment Description: Eighteen initially identical populations were founded from the newly isolated *Comamonas* sp. strain TFD41. Twelve evolved for 1000 generations under conditions of serial transfer in a shaken broth medium with 2,4-D as the carbon source, while the remaining six evolved via serial transfer on the surface of the same medium solidified with agar. Fitness was examined every 200 generations and colony morphologies were examined at 200 and 1000 generations.

Findings: The shaken populations showed very similar fitness responses, whereas those on the solid medium showed significant divergence among them that increased over time, suggestive of differing trajectories up distinct fitness peaks.

Sources of Historical Differences: Mutation and genetic drift.

Did History Matter? Yes, for the populations in the structured surface environment. Early mutational steps seem to have put replicate populations on different paths up distinct peaks.

Citation: K. E. Kram, C. Geiger, W. M. Ismail, H. Lee, H. Tang, P. L. Foster, S. E. Finkel, Adaptation of *Escherichia coli* to long-term serial passage in complex medium: evidence of parallel evolution. *mSystems* **2**, e00192-16 (2017).

Organism: *Escherichia coli*

Type of Experiment: Parallel Replay Experiment

Experimental Question: Is there more variation in the types of mutations that benefit cells when evolved in a more complex medium than most evolution experiments typically use?

Experiment Description: Three parallel cultures were founded from *E. coli* K-12 and evolved in LB medium in tubes, with serial transfer every 4 days via 1:1000 dilution into fresh medium for 300 generations. Growth phenotypes and genotypes were examined every 30 generations.

Findings: Rapid improvements in fitness were observed for all three replicates within the first 30 generations. A number of genes were mutated across populations. The authors conclude that the number of genetic routes may be quite limited, but that each population took a different path to more or less the same adaptive peak.

Sources of Historical Differences: Mutation and genetic drift.

Did History Matter? Only transiently.

Citation: S. Kryazhimskiy, D.P. Rice, M.M. Desai, Population subdivision and adaptation in asexual populations of *Saccharomyces cerevisiae*. *Evolution* **66**, 1931-1941 (2012).

Organism: *Saccharomyces cerevisiae*

Type of Experiment: Historical Difference Experiment

Experimental Question: Does epistasis impact adaptive predictability and make evolutionary trajectories contingent on chance effects of initial mutations?

Experiment Description: 432 lines were founded from a single clone, and evolved in rich medium for 240 generations. Half were maintained at large and half at small population size during evolution. A single evolved clone was chosen from 64 of the population, with clones chosen with the aim of assembling a collection that spanned a range of different fitness values relative to the original ancestor. Ten replicate populations were then founded from each clone, to give 640 lines that were then evolved for another 500 generations at large population size. Fitness was assayed at 250 and 500 generations.

Findings: All populations showed increased fitness over time, the increase occurred at different rates across the populations. Those that started off at lower fitness increased at a faster rate, regardless of the initial mutation they possessed. Though the effects of specific genotypes on adaptability were rare or weak, they were found to be significant. Most mutations identified were unique at the nucleotide level, though there was significant gene-level evolution. However, the authors ultimately conclude that accurate prediction of evolutionary rate is possible based solely on starting fitness. This pattern is attributable to global diminishing returns epistasis regardless of genotype. They conclude that the pattern of convergent evolution is striking at the level of fitness, though evolution is still highly stochastic at the genotype level due to the number of mutational paths to similar fitness levels.

Sources of Historical Differences: History in initial adaptive phase, mutation, and genetic drift.

Did History Matter? Not at the level of fitness, but yes at the genotypic level.

Citation: J. Lachapelle, N. Colegrave, The effects of sex on the repeatability of evolution in different environments. *Evolution* **71**, 1075-1087 (2017).

Organism: *Chlamydomonas reinhardtii*

Type of Experiment: Parallel replay experiment with multiple conditions

Experimental Question: How does sex impact evolutionary repeatability?

Experiment Description: Sexual and asexual populations of varying ancestry were evolved under four different environmental conditions with six-fold replication for 300 generations. Fitness was then determined and compared.

Findings: Sex impacted evolutionary repeatability in an inconsistent manner by changing to what degree selection, chance, and ancestral constraints affected evolutionary outcomes.

Sources of Historical Differences: Mutation, genetic drift, recombination, different ancestral composition.

Did History Matter? Tentatively yes, but it is hard to say owing to confounding aspects and the complexity of the experiment.

Citation: M. Le Gac, T. F. Cooper, S. Cruveiller, C. Médigue, D. Schneider, Evolutionary history and genetic parallelism affect correlated responses to evolution. *Mol. Ecol.* **22**, 3292–3303 (2013).

Organism: *Escherichia coli*

Type of Experiment: Parallel Replay Experiment with multiple conditions

Experimental Questions: What is the relation between genomic and phenotypic evolution? What is the relation between parallel genetic change and correlated phenotypic response?

Experiment Description: Initially identical replicate populations of *E. coli* evolved for 1000 generations in four different environments with four-fold replication. Evolved clones were isolated, and their phenotypes assayed in all four environments to examine direct and correlated fitness responses. Genome sequencing of the evolved clones was used to examine genetic parallelism, and to investigate the effect of genetic parallelism and evolutionary history on the correlated responses. The authors examined whether phenotypes in alternative environments (correlated responses to evolution) were more similar for clones sharing mutations in the same genes (genetic parallelism) irrespective of the environment in which they evolved (evolutionary history) or for clones that evolved in the same environment irrespective of genetic parallelism.

Findings: Clones evolved in different environments showed significant phenotypic parallelism even when genetic parallelism was lacking. Clones evolved in different environments showed a higher level of parallelism in correlated response when they shared mutated genes. This finding implied that experience of similar adaptive histories results in a propensity for similar adaptive evolution under changed conditions.

Sources of Historical Differences: Mutation and genetic drift.

Did History Matter? Yes, there were historical differences that arose at the level of genotype.

Citation: R. E. Lenski, Convergence and divergence in a long-term experiment with bacteria. *Am. Nat.*, **190**, S57-S68 (2017).

Organism: *Escherichia coli*

Type of Experiment: Parallel Replay Experiment

Experimental Question: How repeatable is evolution?

Experiment Description: Twelve populations of *E. coli* were founded from a single clone and evolved under identical conditions for more than 67,000 generations. Analyses of the experiment have examined many questions and phenomena using a variety of methods and techniques over the course of dozens of papers, reviewed in this paper.

Findings: The populations have shown a complex mix of convergence and divergence. The overall direction of evolution has been remarkably parallel, with similar changes in fitness, cell size, and growth rate. Significant parallelism in genotypic evolution has also occurred. However, significant divergence has been observed as well, with half the populations becoming mutators, each population accruing unique suites of mutations, variation in cell shape, several evolving stable polymorphisms indicative of frequency-dependent interactions, and one evolving a historically contingent novel trait.

Sources of Historical Differences: Mutation and genetic drift.

Did History Matter? Varies with level of analysis. In most respects, relatively little for high-level features (fitness and gene targets of selection), but yes at finer levels (specific phenotypes and mutations).

Citation: H. A. Lindsey, J. Gallie, S. Taylor, B. Kerr, Evolutionary rescue from extinction is contingent on a lower rate of environmental change. *Nature* **494**, 463-467 (2013).

Organism: *Escherichia coli*

Type of Experiment: Parallel Replay Experiment with multiple conditions

Experimental Question: What are the genetic bases of evolutionary rescue?

Experiment Description: Over a thousand initially identical populations of *E. coli* evolved by serial transfer every two days for two months in microtiter plates under three conditions of exposure to rifampicin. In the Sudden condition, populations were immediately introduced to the maximum concentration of rifampicin (190 µg/mL); in the Moderate condition, rifampicin was slowly increased to the maximum at the experiment's mid-point; and in the Gradual condition, the level was increased at a rate such that the maximum was reached only at the end. Thirty Moderate evolved populations and 30 Gradual evolved populations were selected at random, and a single clone isolated from each. A single clone was also isolated from each of the 13 populations evolved under the Sudden condition that did not go extinct during the experiment. The *rpoB* locus was then sequenced for these 73 clones, and the accumulated mutations noted. Constructs were then engineered with every combination of *rpoB* mutations seen in the Moderate and Gradual clones, and their fitness assessed across a range of rifampicin concentrations.

Findings: Even within the same conditions, replicates show a wide variation in growth rate at the maximum rifampicin concentration. The authors also demonstrate that a substantial number of beneficial alleles are inaccessible under the Sudden condition, limiting the opportunity for evolutionary rescue. The authors conclude that evolutionary rescue was contingent upon the history of environmental change encountered.

Sources of Historical Differences: Mutation, genetic drift, rate of environmental change.

Did History Matter? Yes. Even within a condition, replicate populations showed a range of evolved growth rates at maximum rifampicin concentration. More importantly, the authors show that environmental history can matter to whether or not evolutionary rescue takes place.

Citation: R. C. MacLean, G. Bell, Experimental adaptive radiation in *Pseudomonas*. *Am. Nat.* **160**, 569-581 (2002).

Organism: *Pseudomonas fluorescens*

Type of Experiment: Parallel Replay Experiment with multiple environments.

Experimental Questions: How do selection and constraints determine the limits of adaptive radiation? What are the consequences of this influence?

Experiment Description: Four initially identical replicate populations of *Pseudomonas fluorescens* strain SBW25 were founded, and used to found another 95 replicates that each evolved for 1,000 generations on a different substrate in 96-well BIOLOG plates. The fitness of the evolved populations were then assayed on all ninety-five substrates.

Findings: The populations that evolved on the 51 substrates on which the ancestor could grow each showed improved fitness on those substrates. The ability to grow on 31 substrates on which the ancestor could not also evolved over the course of the experiment. All populations evolved wider niche breadth. Loss of ability to grow on substrates varied significantly across populations. On average, each population lost the ability to grow on ~2.5 substrates, but 69 of 380 populations showed no loss of ability to grow on a substrate on which the ancestor could grow. The bulk of the cost of adaptation was explained by neutral mutation accumulation rather than antagonistic pleiotropy.

Sources of Historical Differences: Mutation and genetic drift.

Did History Matter? The variability in correlated fitness responses, as well as the loss of some niche breadth suggest that it did.

Citation: A. H. Melnyk, R. Kassen, Adaptive landscapes in evolving populations of *Pseudomonas fluorescens*. *Evolution* **65**, 3048-3059 (2011).

Organism: *Pseudomonas fluorescens*

Type of Experiment: Historical Difference Experiment

Experimental Questions: How rugged are real adaptive landscapes? How does this impact evolutionary repeatability?

Experiment Description: Eight replicates were founded from each of six genotypes and evolved for 500 generations under conditions of serial batch culture. Half of the replicates evolved in medium supplemented with glucose, while the other half evolved in medium supplemented with xylose. Changes in fitness and the ability to metabolize ninety-five substrates were assessed every 100 generations.

Founding genotypes:

P. aeruginosa strain SBW25*lacZ*

Wrinkly-spreader morphotype isolated from a static microcosm grown in King's B medium.

Clone isolated from a population founded from strain SBW25*lacZ* and evolved for 400 generations in glucose medium.

Clone isolated from a population founded from strain SBW25*lacZ* and evolved for 400 generations in mannose medium.

Clone isolated from a population founded from strain SBW25*lacZ* and evolved for 400 generations in xylose medium.

Clone isolated from a population founded from strain SBW25*lacZ* and evolved for 400 generations in glucose, mannose, xylose medium.

Findings: All populations evolved higher fitness under both conditions. The xylose-evolved populations varied more in evolved fitness and changes in metabolic capacity than the glucose-evolved populations. The authors concluded that the xylose environment provided a more rugged adaptive landscape, which led to different populations climbing distinct adaptive peaks due to differences in early adaptive steps. They also found that history was a greater influence on the evolution of the populations grown on xylose, while adaptation had a greater effect on those grown in glucose. Together, the results suggested that the environment affects the degree to which contingency can affect evolution.

Sources of Historical Differences: Past adaptive history, mutation, and genetic drift.

Did History Matter? Yes, though more in one condition than the other.

Citation: J. R. Meyer, D. T. Dobias, J. S. Weitz, J. E. Barrick, R. T. Quick, R. E. Lenski, Repeatability and contingency in the evolution of a key innovation in phage lambda. *Science* **335**, 428–432 (2012).

Organism: Phage λ and *Escherichia coli*

Type of Experiment: Parallel Replay Experiment, Analytic Replay Experiment

Experimental Question: What processes contributed to the evolution of a virus's ability to use a novel receptor?

Experiment Description: Six initially identical populations of λ evolved for 28 days of serial transfer with *E. coli* B as a host, and were tested for capacity to infect hosts through a new target receptor, OmpF, instead of the ancestral target, LamB. The new ability evolved in one population by day 15. Genome sequencing was used to identify genetic changes associated with the novel trait's evolution. The experiment was then repeated with 96 more populations that were sampled daily and tested for novel target use. Twenty-four populations re-evolved the new trait, and sequencing was performed to identify mutations in relevant host and phage genes. Evolution was then replayed from certain time points of phage and hosts to examine the roles of chance and contingency in the novel trait's evolution.

Findings: Evolution of the ability to use the OmpF receptor involved four canonical phage mutations that displayed all-or-nothing epistasis. Some of these changes were adaptive on the ancestral receptor, and potentiated infection through the new receptor. Evolution of the novel trait was contingent upon the host evolving partial resistance via the LamB receptor and not evolving another form of resistance that would preclude further evolution of the phage toward using the new receptor.

Sources of Historical Differences: Mutation and genetic drift for both the phage and host bacterial populations. Previous evolutionary history in the Analytic Replay Experiment.

Did History Matter? Yes, critically.

Citation: F. B.-G. Moore, R. Woods, Tempo and constraint of adaptive evolution in *Escherichia coli* (Enterobacteriaceae, Enterobacteriales). *Biol. J. Linn. Soc.* **88**, 403-411 (2006).

Organism: *Escherichia coli*

Type of Experiment: Historical Difference Experiment (Parallel Replay Experiment using natural isolates)

Experimental Questions: How do different genotypes adapt to a given novel environment? What does this say about the relationship between macroevolutionary pattern and microevolutionary forces?

Experiment Description: Five strains of *E. coli* that had each been isolated from a different animal host were each used to found four replicate populations that then evolved for 2000 generations of serial batch culture in DM250 minimal glucose medium.

Findings: Each population showed improvements in fitness ranging from 30% to 70% over the course of the experiment. Though, replicates founded from the same ancestor varied significantly, founding genotype strongly affected evolution rate.

Sources of Historical Differences: Past adaptive history in nature, mutation, genetic drift, and adaptive change within the experiment.

Did History Matter? Yes.

Citation: L. Notley-McRobb, T. Ferenci, Adaptive *mgl*-regulatory mutations and genetic diversity evolving in glucose-limited *Escherichia coli* populations. *Env. Microbiol.* **1**, 33-43 (1999).

Organism: *Escherichia coli*

Type of Experiment: Parallel Replay Experiment

Experimental Question: What is the genetic basis of adaptation to a glucose resource environment? (Note: The authors specifically focused on mutations affecting the high-affinity Mgl glucose accumulation system.)

Experiment Description: Six initially identical populations evolved for over 280 generations in a glucose minimal medium in chemostats, and the mutations affecting the Mgl glucose uptake system they accumulated were examined.

Findings: All populations accumulated mutations affecting the *mgl* system. Mutations in the *mgl* operator gene, *mglO*, were found in all six populations.

Sources of Historical Differences: Mutation and genetic drift.

Did History Matter? No and yes. The parallelism at the level of genes mutated was remarkable, but there was significant diversity in the specific mutant alleles that arose.

Citation: J. Plucain, T. Hindré, M. Le Gac, O. Tenaillon, S. Cruveiller, C. Médigue, N. Leiby, W. R. Harcombe, C. J. Marx, R. E. Lenski, D. Schneider, Epistasis and allele specificity in the emergence of a stable polymorphism in *E. coli*. *Science* **343**, 1366–1369 (2014).

Organism: *Escherichia coli*

Type of Experiment: Parallel Replay Experiment, focused on one particular LTEE population

Experimental Question: How did two *E. coli* lineages evolve to exploit ecological opportunities and diverge from their common ancestor at the beginning of a long-term coexistence?

Experiment Description: This work examines a stable polymorphism that evolved in one of the twelve LTEE populations. Genome sequencing, molecular analysis, and allele transfer techniques were used to reconstruct the history by which the two coexisting lineages diverged.

Findings: Three mutations in regulatory genes were sufficient to establish the frequency-dependent interaction that allowed long-term coexistence of the two lineages. The authors found that other populations in the LTEE also accrued mutations in these genes, but without evolving such a stable coexistence. They infer that subtle differences between mutations in the same genes can influence whether or not coexistence is established. This population had evolved a suitable combination of mutations.

Sources of Historical Differences: Mutation and genetic drift.

Did History Matter? Yes. Had different mutations accumulated, or had they accumulated differently, coexistence would not have occurred.

Citation: J. Plucain, A. Suau, S. Cruveiller, C. Médigue, D. Schneider, M. Le Gac, Contrasting effects of historical contingency on phenotypic and genomic trajectories during a two-step evolution experiment with bacteria. *BMC Evol. Biol.* **16**, 86 (2016).

Organism: *Escherichia coli*

Type of Experiment: Historical Difference Experiment

Experimental Question: What is the effect of past evolutionary history on future phenotypic and genotypic adaptation?

Experiment Description: Sixteen replicate populations were founded from a single clone. These were then divided into four groups of four replicates each, which evolved for 1,000 generations in one of four conditions: Davis Mingioli (DM) medium supplemented with acetate in shaken flasks, DM supplemented with glycerol in static Petri dishes, DM supplemented with D-gluconate in shaken test tubes, and DM supplemented with glucose in shaken 96-well plates. Mixed samples of each population were frozen. One random clone was also isolated from each evolved population. The evolved clones and population samples were then each used to found a new population to give a total of 32 new populations that evolved for 1,000 generations in DM supplemented with glycerol in shaken flasks. The fitness and maximum growth rate of each population was assayed at the end of both phase one and phase two. The genomes of clones isolated at the end of each phase were also sequenced. (Note: At the end of this second phase of evolution, two of the cultures started from phase one clones were discarded due to contamination. The corresponding population founded from mixed phase one samples were also discarded.)

Findings: The growth rate and fitness of the evolved populations after phase two were highly contingent upon the conditions under which they evolved in phase one. However, the genes that were mutated during phase two were not affected by evolution during phase one, suggesting that phenotypic evolution, but not genotypic evolution was impacted by prior history.

Sources of Historical Differences: Prior history during phase one, mutation, genetic drift.

Did History Matter? Yes at the phenotypic level, but not at the level of genes mutated.

Citation: W.C. Ratcliff, M.D. Herron, K. Howell, J.T. Pentz, F. Rosenzweig, M. Travisano, Experimental evolution of an alternating uni- and multicellular life cycle in *Chlamydomonas reinhardtii*. *Nature Communications* **4**, 2742 (2013).

Organism: *Chlamydomonas reinhardtii*

Type of Experiment: Parallel Replay Experiment

Experimental Question: Can an organism that has never had a multicellular ancestor evolve multicellularity?

Experiment Description: An outbred population of *C. reinhardtii* with high standing genetic diversity was used to found twenty replicate populations in 24-well, unshaken plates. The replicates evolved under a serial propagation regime in which they were transferred to fresh medium every three days for 73 transfers, or ~315 generations. Ten populations were subjected to selection for cell cluster formation during each transfer. In this selection, a milliliter sample of each population was centrifuged at 100 g for five seconds, and the bottom 100 μ L was then transferred to fresh medium. The other 10 populations were not subjected to selection at transfer as a control group.

Findings: Multicellularity evolved in only one of the ten experimental populations.

Sources of Historical Differences: Mutation and genetic drift.

Did History Matter? Yes. Only one population discovered a path to evolving multicellularity.

Citation: G. Saxer, M. Doebeli, M. Travisano, The repeatability of adaptive radiation during long-term experimental evolution of *E. coli* in a multiple nutrient environment. *PLOS ONE* **5**, e14184 (2010).

Organism: *Escherichia coli*

Type of Experiment: Parallel Replay Experiment

Experimental Question: What are the relative contributions of chance and necessity to an experimental adaptive radiation?

Experiment Description: Twelve initially identical populations of *E. coli* evolved for 1000 generations in a medium containing both glucose and acetate. The divergences in fitness, mean colony size, and colony size diversity within and between evolved populations were then examined. Tests for the evolution of negative frequency dependence between lineages within populations were also performed.

Findings: Colony size diversity rapidly evolved in parallel across all populations. The evolved populations showed fitness improvement of ~32% relative to their ancestor. Within populations, fitness variance among individual clones showed no significant increase. The strength of

negative frequency dependent selection varied among population, and strong frequency dependent interaction noted in only three populations. Partial investigation of the genetic basis of acetate usage showed substantial variation at genetic level despite parallel phenotypic evolution.

Sources of Historical Differences: Mutation and genetic drift.

Did History Matter? No with regard to several phenotypic traits, yes with others, and yes at genetic level.

Citation: P. Simões, I. Fragata, S. G. Seabra, G. S. Faria, M. A. Santos, M. R. Rose, M. Santos, M. Margarida, Predictable phenotypic, but not karyotypic, evolution of populations with contrasting initial history. *Sci. Rep.* **7**, 913 (2017).

Organism: *Drosophila subobscura*

Type of Experiment: Historical Difference Experiment (Parallel Replay Experiment using natural isolates)

Experimental Questions: How predictable is laboratory evolution of *D. subobscura* populations founded from isolates adapted to different conditions? Is the impact of prior differences at the initial stages of experimental evolution in a novel environment repeatable across years of sampling? Can evolutionary rates and outcomes be predicted when populations differ initially? How does the ability to predict evolutionary patterns depend on the biological level studied?

Experiment Description: Wild populations were sampled in the Netherlands and Portugal in 2010 and 2013. Isolates from these samples were used to found populations that evolved for 30 generations in the lab. Changes in life-history, morphological, and physiological traits, as well as in karyotype, were then examined.

Findings: Repeatable historical effects were observed at the phenotypic and karyotypic levels in the initial states of populations. The populations showed predictable, convergent evolution in phenotype, though body size did not converge. However, evolution at the level of karyotype was unpredictable.

Sources of Historical Differences: Past adaptive history in nature, sampling, and then mutation, drift, and adaptive evolution during the experiment.

Did History Matter? Yes. The authors conclude: "...the predictability of evolution is contingent on the trait and level of organization, highlighting the importance of studying multiple biological levels with respect to evolutionary patterns."

Citation: A. Spor, D.J. Kvitek, T. Nidelet, J. Martin, J. Legrand, C. Dillmann, A. Bourgais, D. de Vienne, G. Sherlock, D. Sicard, Phenotypic and genotypic convergences are influenced by historical contingency and environment in yeast. *Evolution* **68**, 772–790 (2014).

Organism: *Saccharomyces cerevisiae*

Type of Experiment: Historical Difference Experiment with multiple conditions during phase two.

Experimental Question: How do selection and historical contingency affect phenotypic and genotypic evolution?

Experiment Description: Six strains of yeast evolved with three-fold replication under four different regimes (72 total populations) for 162 to 325 generations. Twelve populations were discarded due to contamination during the experiment, leaving 60 that were analyzed. An evolved clone was isolated from each evolved population, and nine life history and four metabolic traits were assayed under two conditions.

Findings: The populations evolved in each condition converged in phenotype, though the extent of convergence was constrained by prior history. Examination of changes in a gene that was mutated across multiple populations showed that history altered the types of mutations that arose, their locations in the gene, and their pleiotropic effects.

Sources of Historical Differences: Prior history, mutation, and genetic drift.

Did History Matter? Yes. The direction of evolution was largely convergent in each condition, but historical contingency constrained the degree of phenotypic and genotypic convergence.

Citation: H. Teotónio, I. M. Chelo, M. Bradić, M. R. Rose, A. D. Long, Experimental evolution reveals natural selection on standing genetic variation. *Nat. Genet.* **41**, 251–257 (2009).

Organism: *Drosophila melanogaster*

Type of Experiment: Historical Difference Experiment

Experimental Question: How does adaptation to different selection regimes affect the ability of populations to re-adapt to the ancestral condition at the level of fitness and allele frequency?

Experiment Description: Twenty-nine replicate populations evolved under varying selective regimes (late-life reproductive success, very early-life reproductive success, starvation) and then evolved for another 50 generations under ancestral conditions.

Findings: Populations converged back to the ancestral level of adaptation, but allele frequencies did not return to ancestral state.

Sources of Historical Differences: Prior history of evolution under different adaptive conditions, mutation, and drift.

Did History Matter? No at the fitness level, but yes on the genetic level.

Citation: M. Travisano, J. A. Mongold, A. F. Bennett, R. E. Lenski, Experimental tests of the roles of adaptation, chance, and history in evolution. *Science* **267**, 87-90 (1995).

Organism: *Escherichia coli*

Type of Experiment: Historical Difference Experiment

Experimental Questions: What are the contributions of adaptation, chance, and history to the evolution of fitness and cell size?

Experiment Description: During phase one, 12 replicate populations of *E. coli* were founded from a single clone, and evolved for 2,000 generations of serial transfer at 37°C in a glucose minimal medium. For the first phase-two experiment, an evolved clone was isolated from each evolved phase-one population, and used to found three new replicate populations each, for 36 populations total, which then evolved for another 1,000 generations under identical conditions, but with maltose replacing glucose in the medium. Fitness and cell size were assayed in maltose before and after the second phase of evolution. In the second experiment, a clone was isolated

from one population that had evolved at 37°C, and it was used to found 24 phase-one populations, which then evolved with six-fold replication under four different thermal regimes: 32°C, 37°C, 42°C, and alternation between 32°C and 42°C for 2,000 generations, after which fitness was assayed and compared to the founding clones. A single clone from each population was then isolated and used to found 24 new phase-two populations that then evolved for 1000 generations at 20°C. Fitness and cell size were then compared at the beginning and end of this final phase of evolution.

Findings: The replicates consistently converged in fitness during the second phase in the new conditions, whereas prior history had a major impact on the final cell size that was achieved.

Sources of Historical Differences: History during phase one, mutation, and genetic drift.

Did History Matter? The effect of history on the fitness reached in the second phase was small in both experiments, as adaptation to the new environment swamped initial differences caused by earlier history. However, history had a stronger effect on cell size, which was not subject to direct selection.

Citation: D. S. Treves, S. Manning, J. Adams, Repeated evolution of an acetate-crossfeeding polymorphism in long-term populations of *Escherichia coli*. *Mol. Biol. Evol.* **15**, 789-797 (1998).

Organism: *Escherichia coli*

Type of Experiment: Parallel Replay Experiment

Experimental Questions: Is divergence in an evolution experiment replicable? What is the genetic basis of the polymorphism underlying the divergence?

Experiment Description: Twelve initially identical populations of *E. coli* evolved in a glucose minimal medium for 1,750 generations under conditions of continuous chemostat culture, and the genetic basis of observed instances of polymorphism was investigated.

Findings: Six populations evolved polymorphisms associated with acetate cross feeding. The acetate-cross feeding phenotype was always due to mutations in the regulatory region of the acetyl-CoA synthetase gene that caused overexpression of the gene. All of these mutations were either an IS insertion or a T→A SNP at position -93 relative to the gene's start site.

Sources of Historical Differences: Mutation and genetic drift.

Did History Matter? Yes. There were clearly multiple available paths along which the populations could evolve. Two of these paths led to evolution of an acetate cross-feeding-based polymorphism. Each path involved a separate mutation affecting the acetyl CoA synthetase.

Citation: C. B. Turner, Z. B. Blount, R. E. Lenski, Replaying evolution to test the cause of extinction of one ecotype in an experimentally evolved population. *PLOS ONE*, **10**: e0142050 (2015).

Organism: *Escherichia coli*

Type of Experiment: Analytic Replay Experiment

Experimental Question: Was the extinction of the Cit⁻ lineage in an LTEE population after the evolution of the Cit⁺ lineage a deterministic outcome of ongoing evolution in the population, or a chance event contingent upon some random perturbation?

Experiment Description: Twenty replicate populations were founded from a sample frozen within 500 generations of the extinction, and another 20 from a sample frozen several thousand generations earlier. These populations then evolved under the same LTEE conditions for 500 generations, and the patterns of extinction were examined.

Findings: No extinction events occurred in any population. Moreover, experiments showed that the Cit⁻ type could re-invade after the point of extinction, including even several thousand generations later. This led the authors to conclude that the extinction was not deterministic, but a chance, contingent event caused by a random perturbation of unknown cause.

Sources of Historical Differences: Mutation, genetic drift, differences in history experienced along the same time line.

Did History Matter? Yes, the failure to observe any repetitions of the extinction in the replays imply that it was contingent upon the particular history experienced by the original population.

Citation: J. Tyerman, N. Havard, G. Saxer, M. Travisano, M. Doebeli, Unparallel diversification in bacterial microcosms. *Proc. R. Soc. B.* **276**, 1392 (2005).

Organism: *Escherichia coli*

Type of Experiment: Parallel Replay Experiment with multiple conditions

Experimental Question: Does similar diversification imply parallel evolution in response to similar forms of disruptive selection?

Experiment Description: Thirty-six initially identical populations of *E. coli* evolved for 1,000 generations of serial batch culture in three different media. Twelve populations were evolved in Davis Mingioli (DM) medium supplemented with glucose alone, twelve others were evolved in DM supplemented with acetate, and twelve more were evolved in DM supplemented with both glucose and acetate. All glucose and mixed resource-evolved populations evolved similar polymorphic communities composed of an L form that specialized on glucose and an S form that specialized on acetate. In the principal experiment, the authors conducted competition experiments between cognate L and S pairs from the same replicate populations, and non-cognate L and S pairs from different populations to test to see if the independently-evolved pairs were ecologically equivalent.

Findings: L and S pairs from the same replicate displayed frequency dependent selection that caused convergence on stable intermediate frequencies. This was also observed between non-cognate L and S pairs from replicates evolved under the same conditions. By contrast, competitions between pairs from different populations evolved under different conditions, did not converge on stable intermediate frequencies, but either experienced ongoing oscillations in frequency, or one competitor was nearly driven extinct. The authors concluded that differences in evolutionary history can lead to similar diversifications via different adaptations that result in non-equivalence of similar ecotypes.

Sources of Historical Differences: Mutation and genetic drift.

Did History Matter? Yes, though it is difficult to parse from differences in selective conditions.

Citation: D. van Ditmarsch, K.E. Boyle, H. Skhtah, J.E. Oyler, C.D. Nadell, É. Déziel, L.E. Dietrich, J.B. Xavier, Convergent evolution of hyperswarming leads to impaired biofilm formation in pathogenic bacteria, *Cell Rep.* **4**, 697–708 (2013).

Organism: *Pseudomonas aeruginosa*

Type of Experiment: Parallel Replay Experiment with multiple conditions

Experimental Question: How does evolution during selection for swarming motility impact biofilm formation?

Experiment Description: Three initially identical populations evolved for nine days, or ~100 generations, under conditions of daily, 1/1500-fold dilution on swarming plates. The populations were then tested for hyperswarmer phenotypes, and studied at the phenotypic, genotypic, and fitness levels.

Findings: All three populations evolved hyperswarming phenotypes after five or more days of evolution. The hyperswarming clones were defective in biofilm formation, and all had mutations in the flagellar synthesis regular gene, *fleN*.

Sources of Historical Differences: Mutation and genetic drift

Did History Matter? No. The parallelism was striking.

Citation: G. J. Velicer, L. Kroos, R. E. Lenski, Loss of social behaviors by *Myxococcus xanthus* during evolution in an unstructured habitat. *Proc. Natl. Acad. Sci. U.S.A.* **95**, 12376-12380 (1998).

Organism: *Myxococcus xanthus*

Type of Experiment: Parallel Replay Experiment

Experimental Question: Are social traits lost during evolution under asocial conditions?

Experiment Description: Twelve initially identical populations of *M. xanthus* evolved for 1,000 generations in an unstructured liquid habitat under serial batch culture conditions. Social traits were then examined.

Findings: Improved fitness under asocial conditions occurred in all 12 populations, and in all cases this improvement was associated with the partial or complete loss of one or more social behaviors. There was, however, variation in what traits were lost, suggesting the populations followed different genetic and phenotypic pathways.

Sources of Historical Differences: Mutation and genetic drift.

Did History Matter? Yes. Although the general trends were parallel, there was substantial variation in the actual evolutionary paths taken.

Citation: G.J. Velicer, R. E. Lenski. Evolutionary trade-offs under conditions of resource abundance and scarcity: experiments with bacteria. *Ecology* **80**, 1168-1179 (1999).

Organism: *Burkholderia* strains TFD2 and TFD13

Type of Experiment: Parallel Replay Experiment (Phase One), and Historical Difference Experiment (Phase Two)

Experimental Question: Do tradeoffs associated with growing well under resource abundance result in a reduced ability to compete during scarcity?

Experiment Description: Phase one: Two *Burkholderia* strains that differ in their growth rate were each used to found four populations. Half evolved for 75 days of serial batch culture in a medium supplemented with succinate, while the other half were used to found chemostat cultures in the same medium that were maintained for ~75 days. A clone was then isolated from each population. The fitnesses of each clone relative to its own ancestor under both conditions were determined. Phase two: The eight clones from phase one were used to found four populations each in a medium supplemented with 2,4-dichlorophenoxyacetic acid as the carbon source. Half of the populations evolved for 75 days of serial batch culture, while the other half evolved for the same time in chemostats. Clones were isolated from each population, and their competitive fitness relative to their proximate ancestor for phase two determined under both batch and chemostat culture conditions.

Findings: There was clear heterogeneity of evolutionary responses as indicated by fitness in the two conditions. Most lines did not show significant tradeoffs, but two showed strong tradeoffs. Five lines improved in both regimes.

Sources of Historical Differences: History prior to experiment, mutation and genetic drift within the experiment, and different histories during phase one for the phase-two populations.

Did History Matter? Yes.

Citation: H. A. Wichman, M. R. Badgett, L. A. Scott, C. M. Boulianne, J. J. Bull, Different trajectories of parallel evolution during viral adaptation. *Science* **285**, 422–424 (1999).

Organism: Phage ϕ X174

Type of Experiment: Parallel Replay Experiment with multiple conditions.

Experimental Question: What are the dynamics of the molecular basis of adaptation?

Experiment Description: Two lines of ϕ X174 were grown on a novel host, *Salmonella typhimurium*, in chemostats at 43.5°C for ~1,000 generations. Cultures were sampled every 24 hours during the 10-day experiment. Genetic and phage growth rate changes were examined during and after evolution.

Findings: Most of the amino acid changes identified appeared to be adaptive. Half occurred in parallel between the two lines, but in different order. The authors conclude that the differences they observed indicate that the two populations were not following the same trajectory.

Sources of Historical Differences: Mutation and genetic drift.

Did History Matter? Yes.

Citation: R. J. Woods, J. E. Barrick, T. F. Cooper, U. Shrestha, M. R. Kauth, R. E. Lenski, Second-order selection for evolvability in a large *Escherichia coli* population. *Science* **331**, 1433–1436 (2011).

Organism: *Escherichia coli*

Type of Experiment: Analytic Replay Experiment

Experimental Question: Does competition between asexual lineages lead to second-order selection for evolvability?

Experiment Description: Four genetically distinct clones were isolated from a frozen sample of one of the LTEE populations. The fitness of these clones was determined, and their long-term fates in the population examined. Two of the clones were shown to have lower fitness than the other two, despite having beneficial mutations that later fixed in the population. Evolution was replayed many times from each of these clones and their capacity to yield adaptive variants was assayed.

Findings: The two eventual winner clones were found to display greater evolvability than the two eventual losers, owing to the particular beneficial alleles they carried and how those alleles interacted with subsequent mutations.

Sources of Historical Differences: Prior history.

Did History Matter? Yes, in terms of both fitness gains and identity of subsequent beneficial mutations.