Experimental Tests of the Roles of Adaptation, Chance, and History in Evolution

Michael Travisano, Judith A. Mongold, Albert F. Bennett, and Richard E. Lenski*
The diversity of organisms is the product of three fundamental evolutionary influences: adaptation, chance, and history. Their relative contributions to evolutionary change have been the subject of intense debate. Adaptation has sometimes been regarded as the sole influence on evolution, and some biologists have invoked natural selection to explain almost any phenotypic difference. Unsubstantiated claims that adaptation is the cause of all biological diversity have prompted critics to offer two alternative causes, chance and history, that might account for any particular phenotypic difference. Chance effects include mutation and drift, which govern the stochastic appearance of new traits. Chance is usually invoked in a context of molecular genetic traits that are selectively neutral; however, chance is also important for phenotypic evolution, because beneficial mutations arise at random and may be lost soon after they appear, or fixed in a population. This chance divergence might reflect mutation or drift or the effects of selection. A third possible outcome is that the trait had not evolved. Alternatively, one might find that, although there was no significant change in the grand mean of the derived populations, there was significant variation among the derived populations (Fig. 1B). One would attribute this systematic change to chance, because the derived populations had identical ancestors and were subject to identical environments. This chance divergence might reflect mutation or drift on their interactions with other evolutionary processes; attributing this variation to chance makes no specific claims in that regard. A third possible outcome is that the grand mean of the derived populations changed significantly from the value for the ancestor but without significant variation among the replicate populations (Fig. 1C). One would attribute this systematic change in mean value of a trait to adaptation. By invoking adaptation, we do not necessarily mean that the trait was the actual target of selection; it might instead be correlated with some other trait that was selected. Nor do we mean that stochastic processes were not involved; for example, adaptation may depend on random mutations, but similar mutations may be common enough to permit parallel evolution in the replicate populations. A fourth possibility is that both chance and adaptation contribute significantly to the trait's evolution (Fig. 1D). To visualize the effects of history, imagine that a similar experiment is done using several different ancestral genotypes. One might observe that any initial variation in the value of some trait among ancestral genotypes was eliminated from the derived populations because of the effects of adaptation or chance or both (Fig. 1E). That is, the statistical contribution of initial genetic composition to the value of the derived trait was lost, so that one cannot reconstruct a derived genotype's ancestry using that trait. Alternatively, one might observe

![Image](https://via.placeholder.com/150)

**Fig. 1. Schematic representation of effects due to adaptation, chance, and history on evolutionary change and diversification. (A) No initial variation in any evolutionary parameter and hence no effects. (B) An effect due to chance only. (C) An effect due to adaptation only. (D) Effects due to both chance and adaptation. (E) An effect due to history is eliminated by subsequent effects due to chance and adaptation. (F) An infinitesimal effect due to history is maintained, with subsequent effects due to chance and adaptation superimposed. See text for further explanation.**
Therefore, we expect that fitness, as a trait, experimental evolution; relative fitness is neontological research, where other are widely used in paleontological (as well as genetic) research, where other differences have no discernible effect on the initial value of some trait may constrain subsequent evolution so that the effect of ancestry becomes evident only lat- er. The effects of chance, history, and varia- tion are not mutually exclusive; all three may simultaneously influence a par- ticular lineage. As we will now show, one can rigorously quantify the contributions of these different influences. Bacteria have several properties that make them well suited for evolution experi- ments (3). Their rapid growth allows evolving populations to be tracked for hun- dreds of generations. They can be frozen indefinitely and then revived, which allows ancestral and derived genotypes to be com- pared directly, including measurement of their relative fitness in competition. Be- cause bacteria reproduce asexually, one can initiate replicated populations that are identical, consisting of a single ancestral; evolutionary change in these populations thus depends entirely on mutations that occur during the course of the experiment. Hence, experiments may encompass the orig- ination, as well as the fate, of genetic variation and phenotypic novelties.

We analyzed the contributions of adap- tation, chance, and history in two experi- ments with Escherichia coli. The first exam- ined evolution in a novel carbon source, mal- tose. Error bars represent 95% confidence intervals. The mean fitnesses, obtained before and after 1000 generations in maltose, are shown in Fig. 1E, in which the effect of history is shown by elevation of points above the isocline). We can formalize the contributions of adaptation, chance, and history to fitness by estimating the change in grand mean, which reflects adaptation, and by doing a nested analysis of variance (ANOVA) to estimate variance compo- nents corresponding to chance and history (11). Figure 2B shows the relative contri- butions of adaptation, chance, and history to fitness before and after evolution in maltose. By design, the initial effects of adap- tion were diminished (shown by compression of the regression line above the isocline). We can formalize the contributions of adaptation, chance, and history to fitness by estimating the change in grand mean, which reflects adaptation, and by doing a nested analysis of variance (ANOVA) to estimate variance compo- nents corresponding to chance and history (11). Figure 2B shows the relative contri- butions of adaptation, chance, and history to fitness before and after evolution in mal- tose.

Ancestral cell size (9)

Derived versus ancestral val- ues for mean fitness in the 36 experimental populations. Symbols A to L indicate the 12 progenitor genotypes. (B) Relative contributions of adaptation, chance, and history to mean fit- ness before (a) and after (b) 1000 generations in maltose. Error bars represent 95% confidence intervals.

Fig. 2. Evolution of fitness during 1000 genera- tions in maltose: (A) Derived versus ancestral values for mean fitness in the 36 experimental populations. Symbols A to L indicate 12 different progenitor genotypes. (B) Relative contributions of adaptation, chance, and history to mean fitness before (a) and after (b) 1000 generations in maltose. Error bars represent 95% confidence intervals.

Fig. 3. Evolution of cell size during 1000 genera- tions in maltose: (A) Derived versus ancestral values for average cell volume in the 36 experimental populations. Symbols A to L indicate 12 different progenitor genotypes from Fig. 2; fl, femtoliters. (B) Relative contributions of adaptation, chance, and history to average cell volume before (a) and after (b) 1000 generations in maltose. Error bars represent 95% confidence intervals.
During 1000 generations in identical environments (identical genotypes) was highly significant (12). After 1000 generations in maltose, the grand mean fitness of the 36 populations had significantly increased at both the beginning (20) and end (21) of the 1000 generations in maltose. In fact, a large fraction of the initial contribution of history to cell size was maintained, and the final historical contribution was at least comparable to that of adaptation (Fig. 3B).

In the second experiment, a single genotype from 1 of the 12 populations propagated for 1000 generations in glucose at 37°C became the common ancestor (and competitor). This genotype was cloned to found 24 populations, 6 of which were propagated in the same glucose medium under each of four thermal regimes (22): constant 32°C, 37°C, and 42°C and daily alternation between 32°C and 48°C. After 2000 generations, fitnesses at the temperatures at which each group had evolved were systematically correlated with changes in fitness, which indicates that size was effectively an independent trait (27). Cell size showed to significant overall change (18), as shown by the confidence limits for the contributions of adaptation and chance-plus-history throughout the experiment. Chance divergence was not significant (15).

During evolution in maltose, changes in cell size (Fig. 3A) were not significant, but correlated with changes in fitness, which indicates that size was an effective independent trait (17). Cell size showed to significant overall change (18), as shown by the confidence limits for the contributions of adaptation and chance-plus-history throughout the experiment. Chance divergence was not significant (15).

In the second experiment, a single genotype from each of these 24 populations was then used to found a new population, which was propagated in the same medium at 20°C for an additional 1000 generations. Thus, this experiment examined evolution at a novel temperature of 20°C. Mean fitnesses before and after 1000 generations at 20°C are shown in Fig. 4A. As in the previous experiment, changes in fitness were highly significant at both the beginning (20) and end (21) of the 1000 generations (24). The effect of chance plus history on fitness was significant (27). Even after 1000 generations, chance divergence was not significant (15). The effect of chance plus history also was not from statistically significant (25) to highly significant (26). We asked whether populations whose ancestors had adapted to lower temperatures (32°C and alternating between 32°C and 42°C) eventually became more fit at 20°C than did populations whose ancestors had adapted to higher temperatures (37°C and 42°C). In fact, this effect of ancestral selection history on fitness was significant (27). Even so, the effect of adaptation on fitness was significantly greater than that of chance plus history.

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We have shown that the contributions of adaptation, chance, and even history to phenotypic evolution can be disentangled and rigorously quantified by appropriately designed experiments. Bacterial populations (29) and even even convergent evolution in fitness. In contrast, the effects of chance and history were more important for changes in cell size, a trait only weakly correlated with fitness. These results are consistent with the view that the footprint of history may be obliterated for traits that are subject to strong selection, whereas the effect of history is preserved in traits that are less important. However, experiments can span only short stretches of time. Over much longer periods, the footprint of history might eventually become too deep to be obscured even by intense selection (22).
11. The variance component due to measurement error indicates variation among populations. We report the square roots of the variance components, and they are asymmetric (34).

12. Cell sizes were measured with fivefold replication for both the 12 ancestral genotypes and the 36 derived populations. 11 df was used to obtain confidence limits for the point estimate of the final variance component. Hence, we cannot confidently claim that the effect of chance-plus-history was amplified during 1000 generations in identical environments, nor can we exclude this possibility.

13. Although the confidence intervals for chance-plus-history and for adaptation overlap, neither includes the point estimate of the final variance component. Hence, we cannot confidently claim that the effect of chance-plus-history was amplified during 1000 generations in identical environments, nor can we exclude this possibility.

14. The effect due to adaptation is based on the change in grand mean and thus reflects the muddled component of selection on cell size. Stabilizing selection on cell size might be suggested by a reduction in the combined effects of chance and history, but this was not observed.

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REFERENCES AND NOTES

17. \( r^2 = 0.042, n = 12, P = 0.525 \).

18. \( F = 2.10, 11 \text{ df}, P = 0.144 (22) \).

19. \( F = 0.02, 11 \text{ df}, P = 0.986 (22) \).

20. \( F = 0.07, 11 \text{ df}, P = 0.801 (22) \).

21. \( F = 3.59, 11 \text{ and } 24 \text{ df}, P = 0.024 (22) \).

22. A. F. Bennett, R. E. Lenski, J. E. Mittler, Evolution 46, 16 (1992). Again, half the populations were Ara- and the other half were Ara+. The marker was used to explore the possible effects of adaptation.

23. R. E. L. dedicates this paper to the memory of his mother, Jean Lenski. We thank A. Cullum, L. Forney, R. Hudson, S. Kalisz, M. Rose, D. Straney, J. Tedje, and S. Tonsor for discussions; A. Inouye and B. Korona for assistance in the lab; and two reviewers for comments. Supported by NSF grants DEB-9249916 (to R.E.L.) and IBN-9208662 (to A.F.B. and R.E.L.) and by the NSF Center for Microbial Ecology (DBI-9118999). 1 July 1994; accepted 3 October 1994

24. \( F = 1.20, 24 \text{ and } 34 \text{ df}, P = 0.240 \).

25. \( F = 2.40, 12 \text{ df}, P = 0.028 \).

26. The progenitor strains were isogenic, except for an arabi- and the other half were Ara+. The marker enabled us to monitor possible cross-contamination and to score competitors in assays of relative fitness.

27. \( t = 6.10, 23 \text{ df}, P < 0.001 (35) \).

28. Although the confidence intervals for chance-plus-history and for adaptation overlap, neither includes the point estimate of the other. Hence, the difference is significant.

29. Cell sizes were measured with fourfold replication for both the 24 ancestral genotypes and the 24 derived populations. 11 df was used to obtain confidence limits for the point estimate of the final variance component. Hence, we cannot confidently claim that the effect of chance-plus-history was amplified during 1000 generations in identical environments, nor can we exclude this possibility.

30. \( t = 2.09, 13 \text{ and } 138 \text{ df}, P = 0.035 \).

31. \( F = 6.10, 12 \text{ df}, P < 0.001 (35) \).

32. \( F = 3.93, 11 \text{ and } 24 \text{ df}, P = 0.024 (22) \).

33. \( t = 2.43, 23 \text{ df}, P = 0.023 (35) \).


35. The effect due to adaptation is based on the change in grand mean and thus reflects the muddled component of selection on cell size. Stabilizing selection on cell size might be suggested by a reduction in the combined effects of chance and history, but this was not observed.

36. The effect due to adaptation is based on the change in grand mean and thus reflects the muddled component of selection on cell size. Stabilizing selection on cell size might be suggested by a reduction in the combined effects of chance and history, but this was not observed.

7. The progenitor strains were isogenic, except for an arabi- and the other half were Ara+. The marker enabled us to monitor possible cross-contamination and to score competitors in assays of relative fitness.