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Adaptive evolution and the footprints of history

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The genetic structure of a population at any given time is a reflection of the combined effects of many factors such as past selection history, ongoing selection, ancestry and chance, in the form of random divergence among lineages derived from a common ancestor. In this paper, I describe results from two experiments on the bacterium *Escherichia coli* and the fruit-fly *Drosophila melanogaster*, respectively, in which the contribution of these various factors to adaptive evolution was quantified in rigorous laboratory experiments. In both species, current ongoing selection obliterated the effects of past selection and ancestry on fitness-related traits. In the asexually reproducing *E. coli*, the effects of history on traits not related to fitness were still important after 1000 generations, whereas in the sexually reproducing *D. melanogaster*, traits uncorrelated with fitness showed most variation (~95%) to be among individuals within populations. When an effect of history was seen, it was largely due to past selection experienced by a population rather than ancestry *per se*. The time scale of adaptive evolution was much faster in *D. melanogaster*, and the obliteration of the effects of history much more complete, suggesting that genetic recombination may play a major role in removing historical constraints and facilitating adaptive evolution in sexually reproducing species.

BIOLOGICAL systems are characterized by the two interlinked attributes of variation and evolution[1–5]. Although much of the observed variation in populations is undoubtedly environmentally generated, a substantial part of the variation among individuals is due to genetic differences. It is this genetic variation that not only constitutes the raw material for agents of evolutionary change to act upon, but also reflects the outcome of any evolutionary change[4]. In fact, it would not be inaccurate to say that evolution largely consists of the reshaping of patterns of genetic variation within and among populations.

Evolution, it has been said, is what makes biology a different sort of subject from physics, and a key element in evolution is that of historicity[6]. The genetic structure of a population at any given time is a reflection of the combined effects of many factors. The selection pressure faced by a population in the recent past constitutes a directional force that is responsible for the adaptive effects of evolutionary change: natural selection tends to favour the increase in frequency of those genetic variants that make an individual better adapted to the present environment. At the same time, natural selection is constrained by the range of genetic variation available in

the population for it to act upon. Since natural selection is essentially little more than the differential reproductive success of different genetic variants in a given environment, it is limited to favouring the increased abundance of the most well-adapted genotype among those extant in the population. The genetic variation available in a population at any time for selection to act upon is itself affected by two major historical factors: the initial genetic composition of the ancestral population, and the past history of selection pressures experienced by the population.

In addition to the directional forces of currently ongoing selection, and the inertial effect of historical constraints upon the range of genetic variation available, any population is also subject to the stochastic influence of random genetic drift, which can cause the genetic composition of the population to change in unpredictable ways. Moreover, selection can itself be constrained by the genetic architecture of fitness traits. Very often different components of fitness exhibit negative genetic correlations among them, thereby preventing natural selection from simultaneously maximizing a number of attributes that would, when considered singly, enhance the fitness of the individual (these are constraints due to pleiotropy in formal population genetic terminology). Add to this genetic constraints on selection due to dominance and epistasis, and it should be clear that the evolutionary trajectory of a population represents the outcome of the resolution of three types of forces: (i) the deterministic force of current natural selection, (ii) the stochastic force of genetic drift, and (iii) the inertial effect due to history, which encompasses effects of ancestry and past selection history[6–10].

History in macroevolution and microevolution

Discussion of the role of history in constraining evolution has typically been restricted to studies of macroevolution (evolution at the species level and above)[11–13]. Developmental or *bauplan* constraints and other forms of phylogenetic inertia have typically been invoked by workers whose primary interest is in speciation, or in the morphological correlates of evolution of taxonomic groups above the level of species[6,7,12,13]. Early microevolutionary studies, in which the focus was on quantifying adaptive evolutionary change within and among populations of a species, tended to neglect the role of history and chance in adaptation and, instead, focused narrowly on the effects of selection (reviewed by Mayr[12]). There is, however, no reason to believe *a priori* that historicity is not important in microevolution. Indeed, the importance of contingent and random factors to adaptive evolution is implicit in Sewall Wright's shifting balance theory[14], first laid out in 1931. The neglect of historicity in microevolutionary studies, thus, has largely been a matter of convenience rather than ignorance. This neglect, nevertheless, has been unfortunate because only in studies at the microevolutionary level do we attain the degree of resolution required to quantitatively assess the role of historical factors in adaptive evolution; macroevolutionary studies, by the very nature of their subject matter, are relatively qualitative and speculative.

In more recent times, the importance of historical factors, such as ancestry, and chance to adaptation at the microevolutionary level has been explicitly considered in the design and interpretation of laboratory experiments on the evolutionary genetics of life-histories[15–17], inter-specific competitors[10,18], and ecological specialization[19]. Similarly, the importance of historicity and chance in determining patterns of within-species diversity in nature has also been receiving increasing attention from ecologists (reviewed by Thompson[20]). It is only in the last two years, however, that there have been any attempts to directly and quantitatively assess the relative contributions of natural selection, history and chance to adaptive evolution. The first such study was carried out by Richard Lenski and his co-workers, who attempted to quantify the role played by adaptation, history and chance in determining genetic variation in populations of the bacterium *Escherichia coli* adapting to novel environments[21]. The only other study of this type, and the first on a eukaryotic, sexually reproducing, and outbreeding species, was conducted by Laurence Mueller and myself[22]. We used populations of fruit-flies (*Drosophila melanogaster*) to try and measure the relative contributions of ongoing selection, past selection history, ancestry and chance to adaptive evolution in populations adapting to various densities.

Selection, chance and history in adaptation in bacteria

In two separate experiments, Lenski and his co-workers studied adaptation to novel nutritional and temperature regimes in populations of *E. coli* over 1000 generations[21]. In one experiment, 12 populations of *E. coli* were derived from a common ancestral population and propagated for 2000 generations in glucose-limited medium[23]. After 200 generations, these populations had similar fitness (assayed through competition versus the common ancestral population) on glucose as the carbohydrate resource, but differed substantially in their fitness in a medium where maltose was the sole carbohydrate. From each of these 12 populations, 3 replicate populations were derived and these 36 experimental populations were then reared on maltose-limited medium for a further 1000 generations. After 1000 generations, the fitness of these 36 populations on maltose-limited medium was again assayed. Cell size on maltose-limited medium was also assayed before and after the

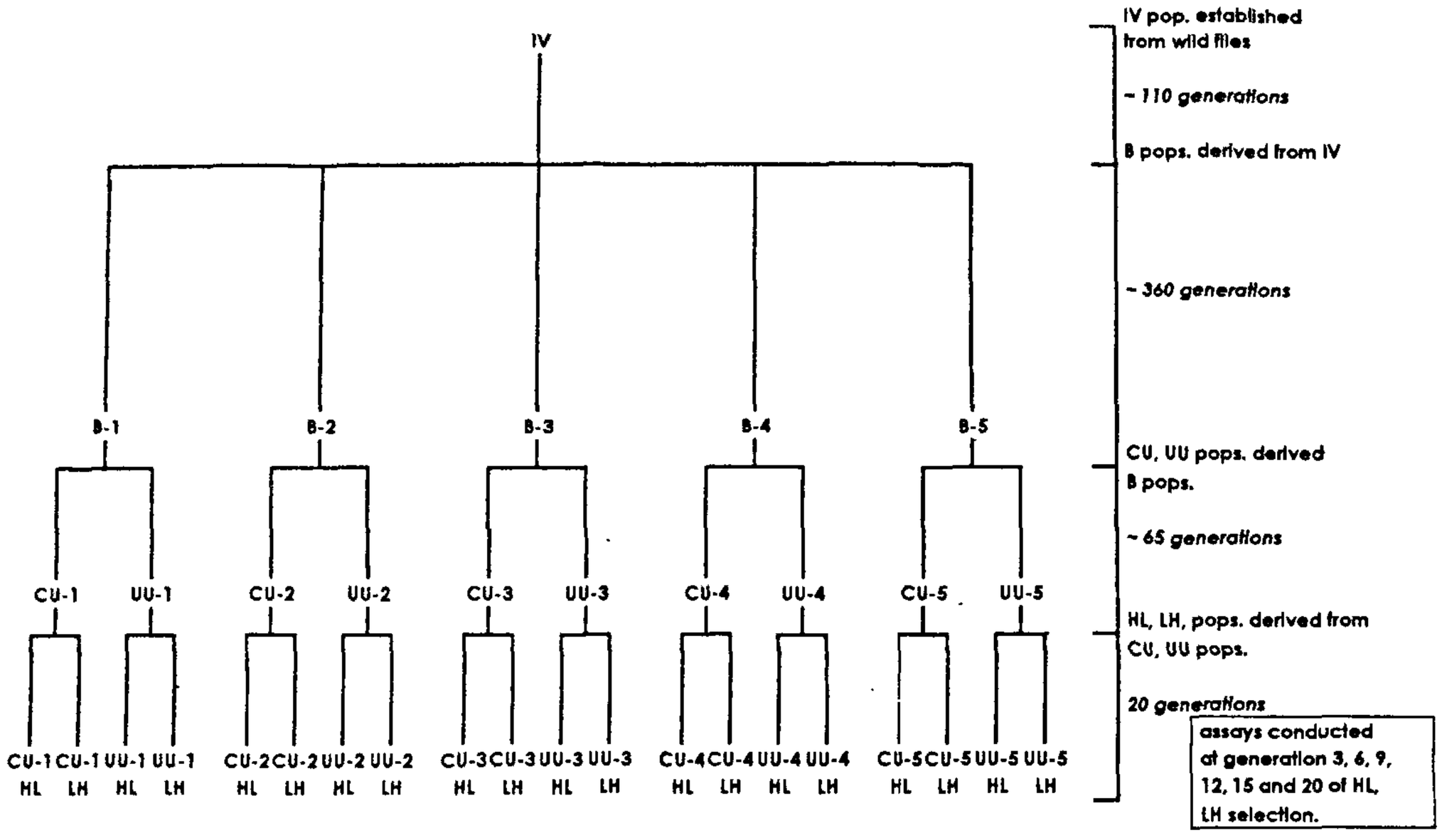

Figure 1. The genealogy of, and past selection pressure experienced by, the 20 populations of *D. melanogaster* used in the study by Joshi, Castillo and Mueller[22]. The IV population, and the five B populations were maintained at low larval density of 60–80 larvae per 8-dram vial.

1000 generations on maltose as carbohydrate source, and was seen to be uncorrelated with fitness. The entire experiment (over 3000 generations) was conducted at 37°C. Using a nested analysis of variance (ANOVA), it was possible to assess, for both fitness and cell size (a trait uncorrelated with fitness), the degree to which the final phenotypes of the 36 populations depended upon (i) history, i.e. differences that arose during the 2000 generations on glucose, (ii) chance, i.e. divergence among the 3 replicates in each of the 12 sets of populations during the 1000 generations on maltose, and (iii) adaptation, i.e. systematic changes in mean phenotype irrespective of initial genotype.

A similar experiment on adaptation to a novel temperature regime was also initiated with one of the 12 populations propagated for 2000 generations on glucose-limited medium at 37°C. From this population, 4 sets of 6 populations each were derived and subjected to different temperature regimes (32°C, 37°C, 42°C, and daily alteration between 32°C and 42°C) for 2000 generations. After these 2000 generations, each of these 24 populations exhibited adaptation to the specific temperature regime they experienced. Derivatives of each of these 24 experimental populations were then maintained at 20°C for 1000 generations. Once again, fitness and cell size at 20°C were assayed before and after the 1000 generations of adaptation to this novel environment.

The results obtained from these two experiments were qualitatively similar. In both cases, at the beginning of the 1000 generations in a novel environment, the contribution of adaptation to fitness and cell size was, by definition, zero. After 1000 generations, the contribution of adaptation to fitness had markedly increased. In the case of adaptation to maltose, the contribution of history and chance to fitness was almost negligible after 1000 generations of evolution in a maltose-limited medium. The contributions of adaptation, history and chance to cell size in maltose-limited medium were of comparable magnitude (in fact, the mean contribution of adaptation, though at par with those of history and chance, did not differ significantly from zero). In the case of adaptation to novel temperature, too, the contribution of adaptation to fitness at 20°C was significantly greater than the combined contribution of history and chance, despite a significant increase over 1000 generations in the latter. In this experiment, the contribution of history was, in part, due to the varying past selection experienced by different populations, with those that had experienced lower temperatures during the preceding 2000 generations being relatively more fit at 20°C. The contribution of history and chance to cell size at 20°C remained essentially unchanged over the 1000 generations of adaptation. Although the contribution of adaptation to cell size increased significantly over 1000

generations at 20°C, it was significantly less than the combined effect of history and chance at the end of the experiment.

The results obtained by Lenski and his co-workers, thus, clearly showed that, at least in clonally propagating populations of *E. coli*, '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.'[21] Although this path-breaking experiment was carried out with exemplary rigour, it nevertheless had several shortcomings, partly due to the nature of the experimental organism itself:

(1) Only two observations were made (before and after 1000 generations of adaptation), making it difficult to study the time course of changes in the relative contributions of adaptation, history and chance.
(2) Bacteria are asexual and, hence, depend largely upon mutations to generate the genetic variation that selection can act upon. In sexually reproducing, outbred populations, where recombination plays a major role in generating abundant genetic variation each generation, the rate of evolutionary changes similar to those seen in *E. coli* may be much greater per generation.
(3) It was not possible to clearly differentiate between the respective roles of ancestry and past selection history in determining the contribution ascribed to history.
(4) Bacterial populations being very large, the role of genetic drift in determining the contribution of chance to adaptation in bacteria may be much less than it would be in many eukaryotes, whose populations are typically, orders of magnitude smaller.

Selection, chance and history in adaptation in fruit-flies

Our experiment on *Drosophila* populations[22] avoided the above-mentioned shortcomings, with a slightly more complex design in which relative contributions of different factors to variation in a set of adapting populations were studied at regular intervals, in order to get a better idea of the time course of changes in these contributions. We used a set of 20 laboratory populations of *D. melanogaster*, whose ancestry and past-selection history, in the specific context of density, was known for over 550 generations (Figure 1). The 20 experimental populations were set up by deriving two populations from each of a set of 10 populations that had been subjected to either low (~ 70 larvae per 8-dram vial: five UU populations) or high (> 1000 larvae per 6-dram vial: five CU populations) larval density for the preceding 65 generations. Each pair of CU and UU populations (e.g. CU-1, UU-1) had, in turn, been derived from one of the five B-populations of Rose[24], which had been in the laboratory for ~ 360 generations following their derivation from a common ancestor, the IV population[24]. The maintenance regimes and evolutionary differentiation of the UU and CU populations that served as the starting point of our experiment have previously been described in detail[17,25,26], so I will restrict my discussion here to only those details as are pertinent to the experiment being described.

In the course of 65 generations of evolution under crowded larval conditions, the CU populations had evolved a higher larval feeding rate, as compared to their low density controls, the UU populations[17]. Larval feeding rate in *Drosophila* is very strongly correlated with competitive ability and, therefore, fitness, under crowded larval conditions[27]. Pupation height, the height above the food surface that the larvae pupate, had not significantly changed in the CU populations[17], suggesting that it was not significantly related to fitness at high larval density in these populations, although this trait had earlier been seen to evolve in crowded populations of *Drosophila*[28–30].

In our experiment, two populations were derived from each CU and UU population, and subjected to either crowded (LH populations) or uncrowded (HL populations) larval conditions for 20 generations. The experiment thus involved populations designated CU-HL, CU-LH, UU-HL and UU-LH, representing various combinations larval density experienced during past and ongoing selection (e.g. CU-HL populations had undergone larval crowding in the past, but during the course of the experiment were subjected to reverse selection by being kept at low density). Both larval feeding rate (20 larvae per population) and pupation height (5 vials of ~50 lar-

Table 1. Interpretation of different variance components in terms of the contribution of ancestry, chance, past selection and ongoing selection to adaptation to larval density level in 20 populations of *D. melanogaster*

Effect in variance components model	Interpretation
Block (numerical subscript)	Initial genetic composition, or ancestry, as a result of divergence among the five B-populations over ~ 360 generations.
Selection (CU *versus* UU)	Past selection for 65 generations
Treatment (HL *versus* LH)	Current selection during the 20 generations of the experiment.
Block × Selection	Random divergence among lineages during 65 generations of CU, UU selection
Block × Selection × Treatment	Random divergence among lineages during 20 generations of HL, LH selection

Adapted from ref. 22.

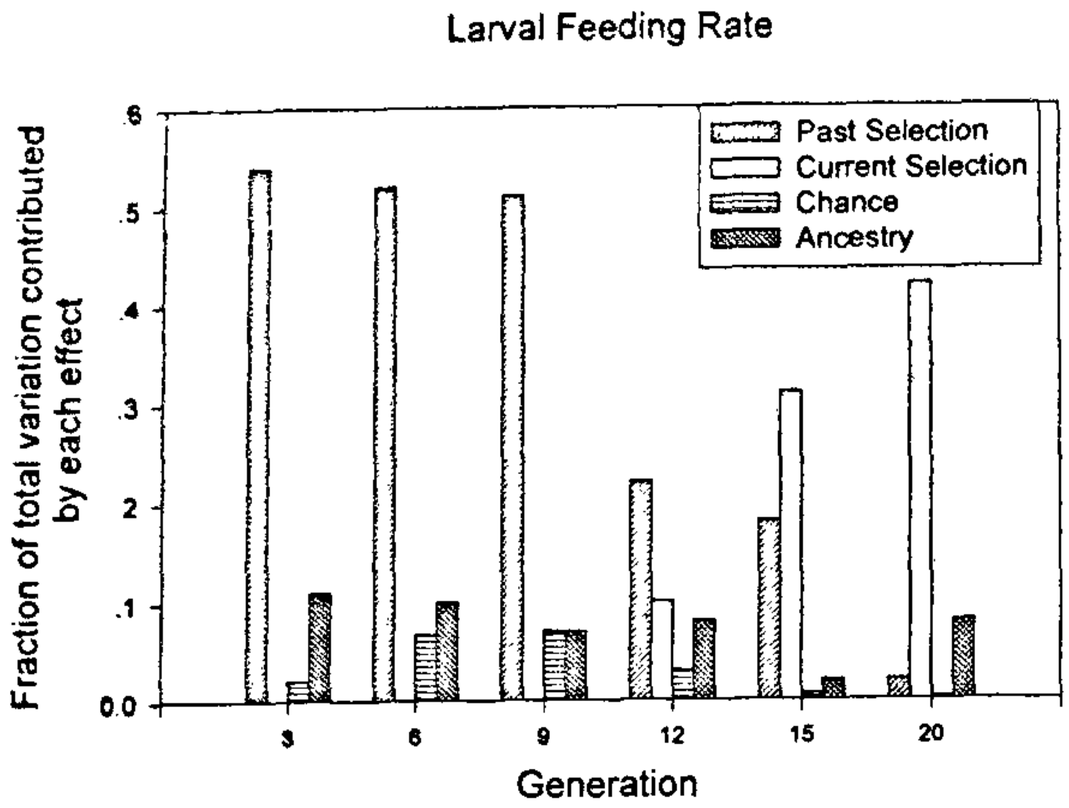

Figure 2. The relative contribution of past and current (ongoing) selection, chance (random divergence among lineages) and ancestry (initial genetic composition) to variation in larval feeding rate in 20 populations of *D. melanogaster* undergoing adaptation to different larval densities (data from ref. 22). Many interaction effects, and the error terms, are not depicted in the interests of clarity.

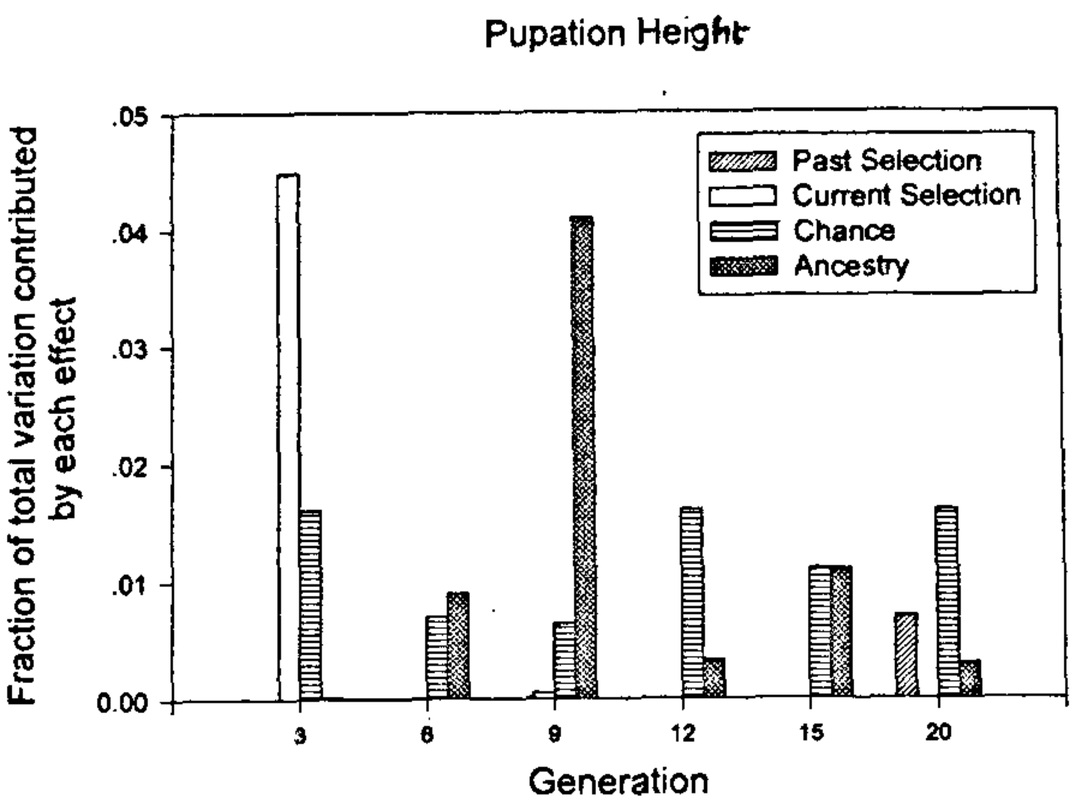

Figure 3. The relative contribution of past and current (ongoing) selection, chance (random divergence among lineages) and ancestry (initial genetic composition) to variation in pupation height in 20 populations of *D. melanogaster* undergoing adaptation to different larval densities (data from ref. 22). Many interaction effects, and the error terms, are not depicted in the interest of clarity.

vae per population) were assayed at generations 0, 3, 6, 9, 12, 15 and 20 of the experiment. The data for each generation were subjected to a variance components analysis from which an estimate could be made of the contribution of various factors to phenotypic variation in these populations at various stages of the experiment (Table 1). Due to the pattern of relatedness among the CU and UU populations (CU_i and UU_i are more closely related to each other than either of them is to other populations with which they share the same selection regime, $i = 1..5$), sets of CU-HL, CU-LH, UU-HL and UU-LH populations, matched by subscripted indices, were treated as blocks in the analysis.

The results for larval feeding rate from this experiment clearly show that by about 20 generations, the primary contribution to variation in feeding rate in the set of 20 populations was due to current or ongoing selection (Figure 2). During the first 10 generations, past selection history (CU or UU) played a major role in determining the feeding rate of a population. The effects of chance divergence over 65 generations, as well as over the course of the experiment, were negligible; the effect of ancestry remained more or less constant over the 20 generations of the experiment (Figure 2). Thus, adaptation to present conditions largely determined the larval feeding rate of the populations after 20 generations, regardless of their prior history of high or low larval density. In the case of pupation height, the major contribution to variation at all generations was actually that of the error term, representing variation among individuals within populations (data not shown). At no generation in which observations were made did the combined contribution of past and current selection, ancestry and chance exceed 5% of the total variation in the set of 20 populations (Figure 3). Moreover, no particular pattern in the relative contribution of these various factors to variation in pupation height could be discerned (Figure 3). The role of history, in the form of ancestry and past selection, and random divergence among lineages, would, therefore, appear to be of far less importance to adaptive evolution in *Drosophila* populations, as compared to the asexually reproducing *E. coli*.

Conclusion: how deep are the footprints of history and what causes them ?

There is some similarity in the results from *E. coli*[21] and *D. melanogaster*[22], most notably the fact that for fitness (or a trait strongly correlated with fitness), the effect of current ongoing selection tends to obliterate the effects of historical and random effects such as ancestry, past selection and random divergence among lineages. This is, of course, not terribly surprising, considering the well known role of natural selection in adaptive evolution. What is more interesting is to look at some of the differences in the results from *E. coli* and *D. melanogaster*, in order to get some appreciation of how the pattern and tempo of evolution may vary in asexually and sexually reproducing species.

In the case of *E. coli*, history played a major role in determining variation for cell size, a trait uncorrelated with fitness, even after 1000 generations. The footprint of history was, thus, deep enough to withstand 1000

generations of random divergence among lineages, in the absence of any strong selection. This may be partly due to the fact that the effective population size of bacterial cultures is extremely large, thus minimizing the role of random genetic drift. Moreover, the asexual bacteria rely on new mutations, and possibly transposition events, to generate new genetic variation, thus slowing down considerably the rate at which genetic variation arises (contrast this to a sexually reproducing species where, even in the absence of mutation, recombination will generate numerous new genotypes each generation).

In the sexually reproducing *D. melanogaster* populations, the footprints of history turned out to be rather shallow, even for pupation height, a trait uncorrelated with fitness under the conditions of the experiment. Indeed for this non-adaptive trait, the major contribution turned out to be that of variation among individuals within populations: eloquent testimony to the power of recombination to obliterate the imprint of past events from genomes. Even in the case of larval feeding rate, which is highly correlated with fitness, the time scale over which the footprints of history were wiped out was far more rapid than in *E. coli*: in 20 generations the contribution of history to variation in the system declined from about 65% to about 12%. Moreover, the effect of history, while it lasted, was primarily a consequence of past selection experienced by the populations rather than ancestry, as in the case of temperature adaptation in *E. coli*. Thus, the footprints of history, at least in sexually reproducing species, would, metaphorically speaking, appear to be no more than transient impressions on genomic dust. With a change in selection pressure, the genetic structure of a population is rapidly shuffled and reorganized, much as what happens to a particular sequence of sand dunes upon a sudden change in the direction of the wind.

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