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Genetic Divergence and Fitness Convergence Under Uniform Selection in Experimental Populations of Bacteria

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ABSTRACT

Replicate populations of bacteria were propagated for 1000 generations in the laboratory. The growth substrate was periodically renewed, so that during most generations (cell doublings) it was not limiting. The final clones demonstrated about a 40% fitness increase when competed against their common ancestor. This increase was uniform both among and within populations despite extensive differentiation in correlated traits: cell size, resistance to starvation and dry mass of culture. It is suggested that genetic diversity developed because selection promoted any changes directing cell activity toward a higher maximum growth rate. Evolution of this trait halted at a similar level when some basic constraints on bacterial metabolism were met. The selective values of emerging mutations must have depended on the genetic background. They would be beneficial early in evolution but ineffective near the limit of adaptation. This hypothesis was tested for one mutation that affected both fitness and colony morphology. In some clones it was the first adaptive mutation and provided a third of the total fitness increase, but it was not assimilated by the clones that reached the adaptive ceiling in some other way. Near the limit of adaptation, epistasis levels off the fitnesses of genetically variable clones.

THERE is no guarantee that evolution replicated in identical environments will lead to similar adaptations. One reason is that spontaneous mutations are likely to be different in separate populations. But even similar sets of mutations may result in unequal fitnesses. This can happen when the sequences of mutations are different (LEWONTIN 1966; MANI and CLARKE 1990) or the number of mutants is small (JOHNSON *et al.* 1995).

The problem of adaptation and differentiation in evolution can be studied in the laboratory. Especially promising are long-term selection experiments with microbial populations (DYKHUIZEN 1990; LENSKI 1992). Large populations of microorganisms can be derived from a single cell and maintained for many generations in a strictly controlled environment. Genetic diversity arises in them only from *de novo* mutations and may or may not be maintained by natural selection. Recent selection experiments with *Escherichia coli* yielded some interesting data. Laboratory populations of these bacteria propagated for many generations usually converged around more or less similar values of competitive ability (fitness). This occurred when the experimental populations were started either from laboratory clones (DYKHUIZEN and HARTL 1981; LENSKI *et al.* 1991; LENSKI and TRAVISANO 1994) or from different wild isolates (MIKKOLA and KURLAND 1992).

Laboratory studies of evolution provide a unique opportunity for direct manipulation of the environment. KORONA *et al.* (1994) attempted to facilitate evolutionary differentiation between replicate bacterial populations. They used a freshly isolated strain of soil bacteria (Comamonas), a novel nutritional substance (halogenated chloroaromatic compound 2,4-D) and two different experimental environments. In the "mass-action" environment, cultures were kept in flasks with constantly agitated liquid medium. This type of environment is common in selection studies; it was used in the previously mentioned experiments with *E. coli.* In the "structured" environment the bacteria grew on agar surfaces, forming cell layers. This environment was not homogeneous because the physical and chemical conditions might differ in different spots of the cell layer. Only in the structured environment did populations reach adaptive peaks with clearly different heights (KORONA *et al.* 1994). Thus in some environments initially identical replicate populations of bacteria may undergo substantially different adaptive evolutions. This makes the observed evolutionary parallelism in mass-action cultures especially intriguing.

The uniformity of the final adaptive values in bacterial populations may also be of interest to students of higher organisms. In their views, the outcome of selection over a long period is not very predictable: the initial populations are likely to be genetically different, sampling of genes during breeding is random, and the mutations are chaotic (FALCONER 1989). Indeed, the experimental evolution of Drosophila is influenced by initial genetic differences between populations (COHAN

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et al. 1989) and genetic drift (COHAN and HOFFMANN 1986), which could lead to even further divergence in both selected and correlated traits under uniform selection (COHAN and HOFFMANN 1989). In the case of Comamonas in the mass-action habitat, natural selection probably tested the limit of a particular adaptation. This would be impossible when adaptations are complex and conflicting, as in the structured habitat, or the populations relatively small, as for Drosophila.

The present study extends the previous work (KORONA *et al.* 1994) and involves different experiments addressing the following problems. Was fitness really uniform after 1000 generations of selection, both among and within replicate populations? What adaptations led to the increase in fitness? Was the genetic basis of adaptation similar or different among clones? Since it turned out that the final clones were of uniform fitness but genetically diverse, how did this polymorphism originate and why did it persist?

MATERIALS AND METHODS

Bacterial strain: The original clone was isolated from soil in Michigan and identified as *Comamonas acidovorans* strain TFD41 (N. TONSO and W. HOLBEN, personal communication). It harbors a plasmid encoding a catabolic pathway similar to that described for plasmid pJP4 (KORONA *et al.* 1994). The latter is a well-known plasmid enabling bacteria to degrade 2,4-dichlorophenoxyacetic acid (2,4-D) (DON and PEMBERTON 1985; CHAUNDRY and CHAPALAMADUGU 1991). From this progenitor strain, two marked variants were derived by selecting for resistant mutants on plates containing either streptomycin or nalidixic acid.

Selection experiment: Six experimental populations were started from one Str^r progenitor bacterium and another six from one Nal^r progenitor bacterium. The populations were cultured in 50-ml Erlenmeyer flasks containing 10-ml liquid medium and kept in a shaking incubator (120 rpm) at 25°. The medium consisted of MMO minimal salts (STANIER *et al.* 1966) supplemented with 0.5 mg/ml 2,4-D as the sole source of organic carbon. The culture depleted this resource after reaching a density of 2.6×10^8 cells, or more precisely, colony-forming units (cfu) per 1 ml. (In the stationary phase most cfu consisted of single cells.) Every 48 hr, when the bacteria were in the stationary phase, samples of 0.039 ml ($\sim 10^7$ cfu) were transferred to 10 ml fresh medium. The ratio of the volume of inoculum to the volume of fresh medium was 1/256, or $1/2^8$. Thus there were eight generations of binary fission within one transfer. Every 24 or 26 days ($\sim$100 generations), samples of cultures were mixed with glycerol and stored at −80°. All 12 populations were maintained in this way for 1000 generations, although the six Nal^r populations reverted to Nal^s between generations 200 and 400. The loss of marker excluded them from the fitness assay because they were no longer distinguishable. However, they were useful as a control for cross-contamination between experimental cultures because transfers of differently marked populations were always alternated. No such cross-contamination was found; the absence of external contamination was confirmed by DNA-fingerprinting (KORONA *et al.* 1994).

Colony morphology and relative fitness: Starting from generation 200, the experimental populations consisted of two types of bacteria, which formed colonies either of the wild type (W), that is, of the progenitor, or of a new translucent type (T). The morphology types were easily distinguishable, heritable, and phenotypically stable over the whole experiment. The stability and heritability of both morphs were tested in several blind trials and confirmed by the absence of revertants on the numerous Petri dishes used in the fitness assays.

Relative fitness was estimated in pairwise competition experiments between the derived Str^r strains and the unmarked ancestral strain. Samples of both competitors were thawed and acclimated to the experimental conditions by twice transferring them serially as in the selection experiment. In the third transfer, the competitors were mixed together. At the beginning and end of this transfer, samples of the mixed culture were appropriately diluted to get 100–500 colonies per plate (numbers were easy to count and reliable). This was done separately for nonselective broth plates and broth plates containing streptomycin (15 mg/liter). The density of the ancestor was found by subtracting the counts on the selective medium from those on the plain medium. The data on the initial and final densities were used to calculate the number of doublings achieved by the derived strain (D_d) and the ancestral one (D_a). The relative fitness of a derived clone is defined as the ratio of these two values, $w_d = D_d/D_a$ (DYKHUIZEN 1990; LENSKI *et al.* 1991).

The derived and the ancestral strains were mixed in a ratio of about 1:1000 at the beginning of competition. Thus the course of resource depletion and medium conditioning was determined by the ancestor, so that the fitness of every derived strain was tested in practically identical conditions. It should also be mentioned that the average number of cells in a colony-forming unit varied in time. In the stationary phase, most of the cells were separate, but in the growth phase, they were often aggregated. Since the proportion of single cells increased with the age of the culture, fitness assays were always done at 48-hr intervals.

Other traits: The following traits were assayed for clones from generation 1000.

Maximum growth rate: Assays were done under the standard experimental conditions, except that the initial population density was about twice that of the regular transfers. To ensure that the cultures entered growth phase, the measurements were started after the populations at least doubled. The light absorbance of the cultures was checked with a spectrophotometer (wavelength 600 nm) about every hour. The measurements were terminated when the population density reached about half its maximum value. The maximum growth rate was estimated by regressing $\log_e$-transformed absorbance against time over a period of $\sim$14 hr.

Cell size: Average cell size and the number of cells in a fixed volume were estimated using a Coulter electronic particle counter (model ZM with channelizer 256). Only particles of sufficiently large size (channel 20 and higher) were included, to avoid counting small objects that were probably parts of dead cells. Intact dead cells could not be excluded. Therefore, when the samples were prepared for the Coulter counter, dilutions of the cultures were spread on broth plates to estimate the number of cfu.

Long-term survival rate: Cultures were starved for 10 days, starting with 48-hr cultures. Samples of the populations were diluted and spread on agar plates to estimate the density of living cells. The same assays were repeated after 1, 3, 5, 7, 9 and 10 days. The liquid cultures were kept in loosely capped flasks. To estimate average daily water loss, six flasks were weighed every day. Volumes of distilled water equal to the average loss were added daily to every flask, so that total volumes were maintained between 9.7 and 10.3 ml. The $\log_e$-transformed data on population density were regressed against time to determine the rate of density change per day.

Dry mass: A 1.5-ml sample of stationary phase culture was centrifuged and 1.4 ml of the supernatant removed. The pel-

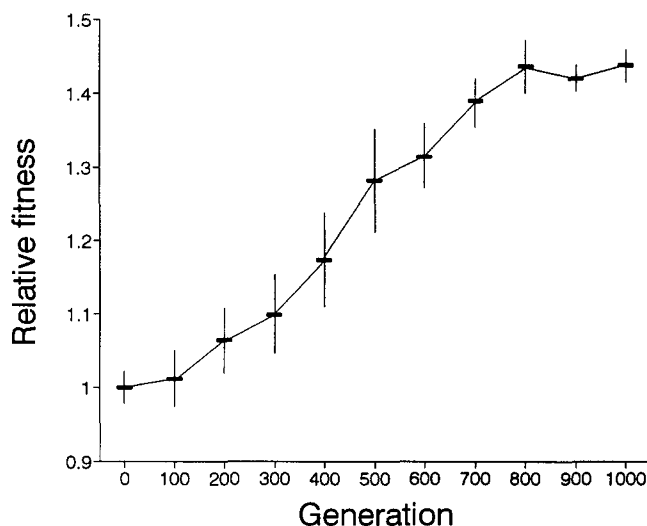


FIGURE 1.—Relative fitness of Comamonas during 1000 generations of selection. Every 100 generations, the relative fitness of a whole evolving population (not single clones) was estimated in competition with the progenitor clone. The graph presents the mean and 95% confidence limits for six independent populations. (data from KORONA *et al.* 1994).

let was then diluted in 1.4 ml distilled water, the mixture was centrifuged again, and 1.4 ml supernatant was removed. This procedure for washing out the salts was repeated three times. The resulting samples of condensed cultures were loaded into small tin vials and dried at 100°. The samples were then weighed to an accuracy of 0.001 mg. The procedure of rinsing the salts was effective because the average dry mass of the samples was about one-hundredth the mass of salts in the same volume of medium. On the other hand, the same dry mass was equal to ~12–20% of the growth substrate in pure medium. Therefore the loss of cells due to ineffective centrifugation or cell lysis had to be relatively small.

Experimental design: Estimates of four traits (relative fitness, dry mass, cell size and long-term survival rate) were obtained for the ancestral clone and four clones from each final population. These four clones were chosen at random from each of six cultures at generation 1000 and stored at −80°. The different traits were always assayed in separate rounds of thawing and conditioning of cultures. The assays of a given trait were done simultaneously for eight independent replicates (separate cultures started from different colonies) of the ancestral clone and two independent replicas for each of 24 derived clones. This orthogonal design was completed successfully with two exceptions. One survival rate estimate had to be based on four instead of seven time points. One dry mass estimate was lost. It was repeated, strictly following the same procedure. The result was close to that of the other replicate and the data were included in the analysis to generate balanced nested ANOVAs (six populations, four clones in each population and two replicas of each clone for each trait). This design was not followed in the case of maximum growth rate. These measurements were not done for four separate clones but for the whole final population (generation 1000), because it was not possible to handle a large number of cultures with the available spectrophotometer and incubator.

RESULTS

Relative fitness: Figure 1 presents the fitness increase trajectory over the entire selection experiment. The mean fitness of all six independent populations initially rose rapidly, and the variation between them also increased. The pattern was reversed in the second half of evolution: the fitness increase gradually slowed down and the variation declined. The data were collected in the "population assays," in which the whole derived population was competed against the progenitor strain. It is possible, however, that the clones constituting the populations would have different fitnesses when competed individually against the ancestor. In the present study, replicated fitness assays were done for single clones from generation 1000. The results of nested ANOVA (Table 1 and Figure 2A) confirm the absence of statistically significant variation in relative fitness among populations at generation 1000 and do not show any variation among clones within populations. Although there could

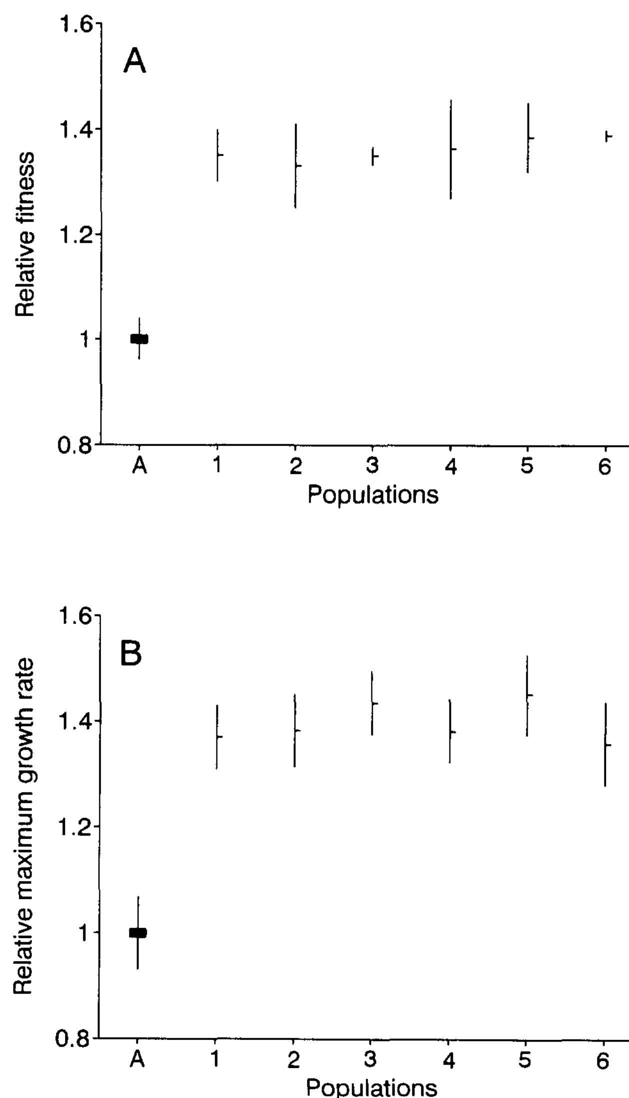


FIGURE 2.—(A) Fitness of the initial Str^r clone (A) and the clones from six experimental (one to six) populations after 1000 generations. The mean and 95% confidence limits were estimated using eight replicas of clone A and averages of two estimates for four clones in each derived population. (B) Maximum growth rate of the initial Str^r clone and the six derived populations at generation 1000. The mean and 95% confidence limits were based on 16 time points.

TABLE 1

Nested ANOVA for relative fitness at generation 1000

Source of variation	d.f.[a]	MS × 10[6][a]	F[a]	Variance components[b]
Among populations	5 (4)	3706 (4353)	1.33[c] (1.32[c])	4.1 (4.0)
Within populations	18 (15)	2794 (3319)	1.08[c] (1.13[c])	3.8 (6.0)
Error	24 (20)	2578 (2924)		92.1 (90.0)

[a] Numbers in parentheses are from analyses that included only five populations (1, 2, 4, 5, and 6).
[b] Values in parentheses are percentages.
[c] Not statistically significant.

be rare clones of different fitnesses that were missed in these assays, the overall result indicates that practically all clones in all populations evolved toward a similar level of competitive ability. (The estimates for clones are slightly lower than those for populations at generation 1000. Such a difference is not surprising. Clonal cultures were started a few months later, from single colonies, not from samples of cultures frozen with glycerol. This resulted in a different starting point for conditioning, which in turn might influence performance in the competition experiments.)

Maximum growth rate: The population means of relative fitness were very similar to those of relative maximum growth rate. Figure 2, A and B, shows that the differences between them were on the order of a few percent and their confidence limits mostly overlapped. Thus the increase in the maximum growth rate was the major, if not the only, factor responsible for the fitness improvement. The reason is that the ratio of maximum growth rates is equal to the ratio of the number of generations achieved by each competitor. Fitness was not enhanced by decreasing mortality because the proportion of dead cells in 48-hr cultures was similar for the ancestral and derived clones. This proportion was found by comparing two estimates of population density. One was obtained by counting colonies on the broth plates (the number of live cells or groups of cells), the other by using a Coulter counter (the number of all appropriately large particles). The ratios of these two numbers were very similar: 0.900 ± 0.023 (mean ± SE) for the ancestral strain and 0.907 ± 0.017 for the derived ones. Another analogy between fitness and maximum growth rate is the absence of detectable variation among the final populations (Figure 2). Thus, different populations attained selective peaks of comparable heights and in the same way, that is, by enhancing their maximum growth rate. Does this mean that they were genetically uniform?

Phenotypic differentiation: Enhancement of the maximum growth rate might occur by a variety of means: improved 2,4-D transport and/or degradation, adaptation to physical conditions, deletion of unnecessary DNA or elements of the cell wall, *etc.* Searching for all of them would be practically impossible. Therefore the study was limited to a few major bacterial traits: cell size, insusceptibility to starvation (long-term survival), and efficiency of resource utilization (dry mass in stationary phase). The goal of this analysis was to test whether correlated traits (PRICE and LANGEN 1992), and thus the genetic basis of adaptations, were homogenous or heterogenous among the final clones.

The increase in relative fitness was associated with extensive changes in cell size, yield and survival (Figure 3, A–C). However, these modifications were not unidirectional because the measurements for the derived clones could fall both above and below the ancestor clone. For example, although dry mass and cell size generally decreased, there were single clones and populations that remained unchanged through generation 1000. Nested analysis of variance revealed statistically significant differences both within and among replicate populations, except for the differences in survival rates among populations (Table 2, numbers without parentheses). Population 3 deserves special attention because only in this population did some (three out of four) of the randomly selected clones turn out to have the wild-type colony morphology (W). It is possible that such a conspicuous morphological difference was associated with other phenotypic traits and made their variation significant. Therefore nested analysis of variance was repeated for the remaining five populations containing only translucent colonies (T). Most of the variance among clones and populations remained statistically significant (Table 2, numbers in parentheses). This implies that the populations contained different clones within type T.

Genetic background and the selective value of mutants: As stated above, at about generation 200 in all populations, some of the cells spread on agar plates produced translucent colonies T instead of the ancestral type W (Figure 4A). This mutation was unique because it not only enhanced fitness but also was easily distinguishable. It was therefore possible to estimate its selective value at different points of evolution, that is, on different genetic backgrounds.

Two populations (2 and 3) were chosen for further tests because they retained relatively many cells of type W at the end of the experiment. In the standard assays of fitness, both types were found to be equally fit at generation 1000, although in generation 200 the novel type had a 10–15% advantage (Figure 4B). In other words, the new type spread quickly in the original popu-

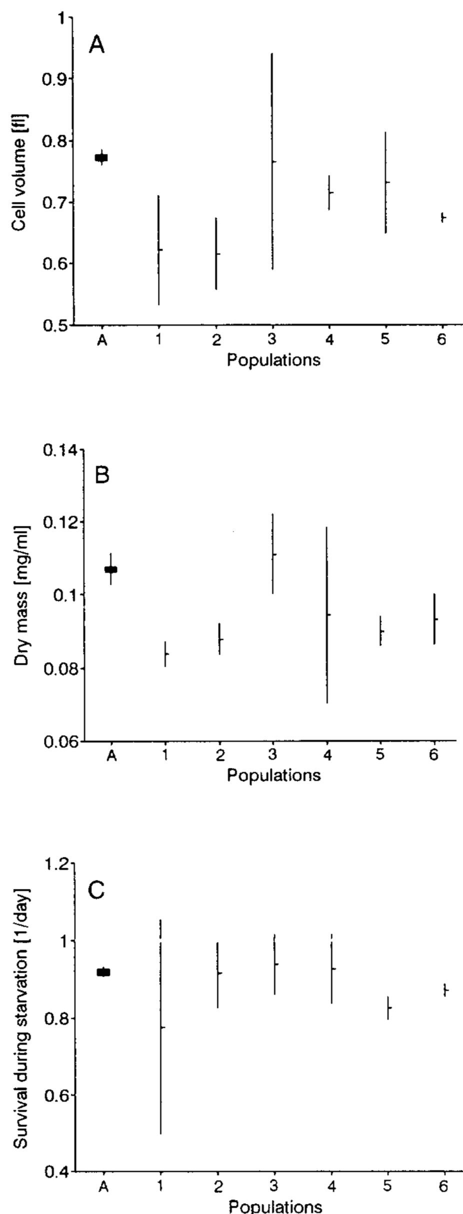


FIGURE 3.—Estimates of cell volume (A), dry mass (B) and survival (C) during 10-day starvation for the initial clone and the six derived populations at generation 1000. The mean and 95% confidence limits were estimated using eight replicas of clone A and the averages of two estimates for four clones in each derived population.

lations because it made about nine doublings in one transfer while the ancestor had only eight. It is easy to convert these estimates into difference in the Malthusian parameter Δr, where $\Delta r = \ln(2^9) - \ln(2^8) = 0.693$ per transfer. The rate of increase in the frequency of an advantageous mutant is governed basically by Δr as long as the new form is in the minority. After the initial 25 transfers (200 generations), the frequency of type T would increase according to the following formula: $N_{200}/N_0 = e^{\Delta n} = e^{0.693*25} = 3.34 \times 10^7$. Thus, if in the first inoculum (10^7 cells) there was only one cell of type T, it would constitute ~13% of the population after 200 generations.

The above experimental data and calculations lead to two conclusions. First, the mutation that manifested itself by a change in colony morphology was among the first derived clones to reach a high frequency. This follows from the fact that they were found when "unfit" clones were still present (Figure 4B). Some of the clones that retained the original colony morphology must have acquired genetically different but similarly beneficial mutations at the same time or soon after. Otherwise they would have been swept away. Second, the selective value of the mutation marked by a new colony morphology depended on the genetic background. It provided 10–15% of the fitness increase and therefore was fixed after ~200 generations. However, these estimates apply only to the ancestral clone. To see this, note that type W clones persisted in some populations in the last 300 generations (Figure 4A). Their relative fitness was ~1.4, like the fitness of type T (Figure 1). A mutation from type W to T in generation 700 would have resulted in a fitness of 1.50–1.55 in generation 900 if its effect were as great as at the beginning of evolution. However, such clones were not detected by fitness assays even at generation 1000.

DISCUSSION

The above data are seemingly contradictory. The final clones were genetically variable, but their maximum growth rates and fitnesses were uniform. The mutation manifesting itself in a change in colony morphology was not incorporated into some clones, although it had a profound effect in others. The very fact of extensive polymorphism in asexual populations propagated in a homogeneous environment is intriguing. A model of selection and gene interaction incorporating the above findings is suggested below.

The trajectories of relative fitness steadily decelerated and from generation 700 remained basically unchanged. One suggestion is that the beneficial mutations were gradually exhausted. Depletion of mutations would be more or less ordered: the mutations with the highest adaptive effects would be incorporated among the first ones and those with the smallest effects would be at the end (LENSKI *et al.* 1991; DYKHUIZEN 1992). The adaptive mutations would not be the same in every derived clone because of broad phenotypic diversity. This interpretation leads to two difficulties. First, why could some mutations be incorporated in one clone but not another? An answer was suggested by WRIGHT (1932, 1988) who insisted that epistasis could modify the selective value of new mutations. Applied to this experiment, this concept would mean that in some clones of Comamonas, the initial mutation directed evolution toward solutions that precluded (made inefficient) a mutation marked by a change in colony morphology. Such interactions could involve different mutations in different clones. But if the populations and clones were directed toward different adaptive peaks,

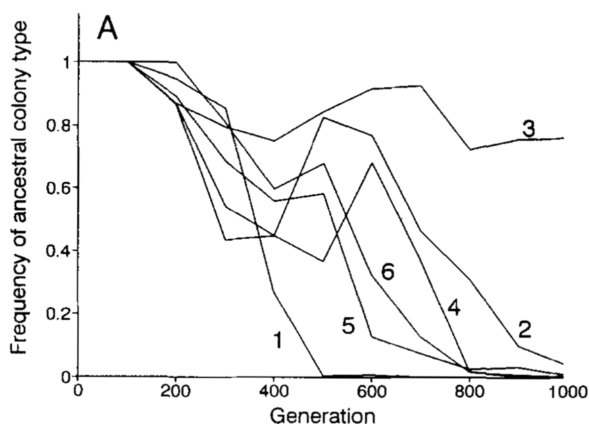
TABLE 2

Nested ANOVA for dry mass, long-term survival and cell volume at generation 1000

Source of variation	d.f.[a]	MS × 10[6] [a]	F[ac]	Variance components[b]
Cell volume				
Among populations	5 (4)	29614 (2236)	4.71* (7.31*)	45.3 (59.2)
Within populations	18 (15)	6623 (3067)	21.1** (11.7**)	49.7 (34.3)
Error	24 (20)	315 (263)		5.0 (6.4)
Survival				
Among populations	5 (4)	33112 (31865)	2.50 NS (2.13 NS)	26.5 (21.5)
Within populations	18 (15)	13261 (14953)	26.9** (28.5**)	68.2 (73.2)
Error	24 (20)	493 (523)		5.3 (5.3)
Dry mass				
Among populations	5 (4)	722 (141)	6.87** (1.34 NS)	57.2 (7.2)
Within populations	18 (15)	105 (111)	9.97** (10.3**)	35.0 (76.4)
Error	24 (20)	10 (11)		7.8 (16.4)

[a] Numbers in parentheses are from analyses that included only five populations (1, 2, 4, 5, and 6).
[b] Values in parentheses are percentages.
[c] Statistical significance: $*P < 0.01$; $**P < 0.001$; NS, not significant.

why were so many peaks of equal height? (Four clones from each of six populations were tested.) This is the second, more serious dilemma. In a parallel selection experiment carried out in a structured habitat, the final clones reached clearly different selective values (Korona *et al.* 1994). In strong contrast, in a mass-action environment only correlated traits underwent differentiation. The last observation has also been made in *E. coli* (Vasi *et al.* 1994; Travisano *et al.* 1995).



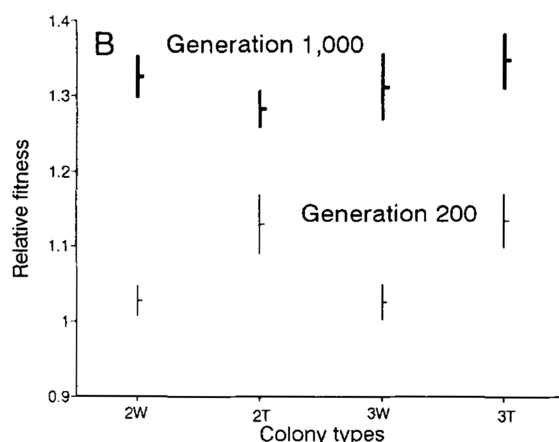


Figure 4.—Evolution of colony morphology polymorphism. (A) Changes in the frequency of the wild type of colony (W) for the six experimental populations. (B) Relative fitness of the wild-type (W) and translucent (T) types of colony in two selected populations (2 and 3).

The problem of flattening trajectories can be approached by considering not the depletion of adaptive mutations but the existence of constraints. The environment provides a clue. It was represented by serial transfers of bacterial cultures between agitated fresh media. In such a habitat, practically all generations encounter unlimited resources. Adaptation is strictly unidirectional, that is, only toward a higher growth rate. An isolate could become better adapted by reducing the burden of functions hindering its rate of growth. It might also change more specifically by intensifying transport of the substrate, adapting to the temperature, *etc.* Thus the adaptive mutations would direct metabolic activity toward fast growth, but the rate of metabolism itself would have to meet its constraint(s). A possible candidate for such a constraint, common to all clones, is the ultimate efficiency of protein synthesis (Marr 1991; Mikkola and Kurland 1992). Mutations intensifying growth need not be ordered nor be the same in different clones. The most beneficial and relatively frequent mutations should be the first to ascend. But the selective effect of a mutation would depend not only on its "absolute" value but also on the distance to the limit of adaptation. The closer to the limit the less could be done. According to this scenario, the mutation associated with the change in colony morphology, and presumably other adaptive mutations, need not have interacted with any specific former adaptation(s). They were without detectable effect after generation 700 because the organisms were already adapted. The overall performance would be more important than the genetic details. This also can be classified as an epistatic effect, such where the differences in fitness among ge-

netically variable clones are reduced near the limit of adaptation.

Both models—one positing depletion of favorable mutations, the other suggesting their ineffectiveness near the limit of adaptation—assume epistatic interactions among multiple genes. It is impossible to completely verify any of them, since the genes in question remain unknown. The second explanation is probably simpler and more convincing: it is easier to accept that equality of adaptive peaks results from a common constraint than that it is purely accidental. However, more work is necessary to corroborate this empirically. For example, the effects produced by an adaptive mutation inserted into clones of different fitness could be studied.

The standard model of evolution in a population of asexuals living in a homogeneous environment is known as "periodic selection." It predicts generally low diversity (ATWOOD *et al.* 1951; LEVIN 1981; DYKHUIZEN 1992); the fittest clone must eventually be fixed, resulting in a decline of genetic variation. This variation will then be rebuilt by new mutations within the selectively superior clone. The most successful mutant of the second order will again outcompete other clones, and so on. Diversity is allowed if frequency-dependent selection operates (LEVIN 1972, 1988; HELLING *et al.* 1986; ROSENZWEIG *et al.* 1994). Such a mechanism need not be invoked here. Different genotypes could coexist because their fitnesses were equal, although they were measured for each clone separately so that no interactions were possible.

The classical model of periodic selection is probably correct when adaptive mutations are rare. It may less accurately describe evolution in large populations where beneficial mutations are relatively abundant, genetically diverse and similarly effective. In such a situation, a few superior clones may ascend and codominate the population. The next waves of beneficial mutations may also occur in more than one clone. Genetic variation will not be immediately purged by a single clone as long as the differences in fitness are small. The above suggested mechanism of leveling epistasis will facilitate this process because it reduces the differences in relative fitness. Thus not only numerous and different beneficial mutations but also an "epistatic buffer" are required for the buildup and persistence of genetic variation.

The proposed model suits the present data perhaps better than do the classical concepts: FISHER's (1930) idea of selection as a basically uniform process or WRIGHT's (1932, 1988) suggestion of an uneven adaptive landscape. The idea of periodic selection is also inapplicable here in its strict form. One must remember, however, that the available data on long-term selection experiments usually account for joint effects of mutation and selection. The actual frequencies of adaptive mutants—as well as their genetic bases, selective values and interactions with other genes—are unknown even for well-controlled laboratory systems. Without information on the whole spectrum of mutations, every new model of selection will at most be a closer approximation, not a solution.

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