source of many alkali intraplate hotspot magmas—such sources are at extreme depths in the mantle[24–26]. Recent discoveries of coesite and diamond in eclogites, metamorphic sedimentary rocks[1] and even granitic gneisses[27] in continental subduction orogenic belts reveal that large, coherent volumes of continental materials could be subducted to depths of more than 120 km. The present study further extends continental subduction to depths of more than 200 km. Experiments and density calculations[8] show that the densities of the continental crust are substantially smaller than that of the surrounding pyrolitic mantle ($3.4\,g\,cm^{-3}$) at pressures lower than 6 GPa ($3.0\,g\,cm^{-3}$), slightly less-dense in the interval of 6.5–8.5 GPa ($3.2–3.3\,g\,cm^{-3}$), and much denser above 9–10 GPa until 24 GPa ($3.7–3.9\,g\,cm^{-3}$)[8]. Once the subducted continental materials reach a depth of about 200 km, their densities approach that of the surrounding mantle, and exceed the density of the surrounding mantle below 300 km. Thus, the buoyancy forces are effectively cancelled and entrainment by the sinking slab becomes the predominant influence. In the Sulu case, a subducted oceanic slab is thought to have hauled the following continental materials to depths greater than 200 km. Only one mechanism is reasonable for exhumation of such deeply subducted rocks: that they are rafted by rocks beneath them, propelled by buoyancy forces[2]. Thus, a detachment was required along the subducting slab at some depths between 200 and 300 km, so that the buoyancy forces could drive the deeply subducted continental materials back to the crustal level.

Much larger-scale extremely deeply subducted crustal materials may be present in the Sulu UHP belt. Such materials would be indicated by the presence of appropriate mineralogical or micro-textural evidence—such as TiO_2 with the α-PbO_2 structure (stable at 5–7 GPa), K-wadeite (6.5–7 GPa), or high-pressure C2/C clinoenstatite (6–7 GPa). We note that the presence of high-pressure C2/C clinoenstatite was previously predicted[28] for the Alpe Arami peridotite, and that this was subsequently confirmed[3]. □

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Correspondence and requests for materials should be addressed to K.Y. (e-mail: yekai@hotmail.com).

The population genetics of ecological specialization in evolving *Escherichia coli* populations

Vaughn S. Cooper & Richard E. Lenski

Center for Microbial Ecology, Michigan State University, East Lansing, Michigan 48824, USA

When organisms adapt genetically to one environment, they may lose fitness in other environments[1–4]. Two distinct population genetic processes can produce ecological specialization—mutation accumulation and antagonistic pleiotropy[5–8]. In mutation accumulation, mutations become fixed by genetic drift in genes that are not maintained by selection; adaptation to one environment and loss of adaptation to another are caused by different mutations. Antagonistic pleiotropy arises from trade-offs, such that the same mutations that are beneficial in one environment are detrimental in another. In general, it is difficult to distinguish between these processes[5–8]. We analysed the decay of unused catabolic functions in 12 lines of *Escherichia coli* propagated on glucose for 20,000 generations[9,10]. During that time, several lines evolved high mutation rates[11]. If mutation accumulation is important, their unused functions should decay more than the other lines, but no significant difference was observed. Moreover, most catabolic losses occurred early in the experiment when beneficial mutations were being rapidly fixed, a pattern predicted by antagonistic pleiotropy. Thus, antagonistic pleiotropy appears more important than mutation accumulation for the decay of unused catabolic functions in these populations.

We founded 12 populations from a strain of *E. coli*, and they all adapted to minimal medium supplemented with glucose[9,10]. Competitive fitness increased rapidly in the first few thousand generations. Fitness continued to improve, but the average rate of

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improvement decelerated sharply over time and was only about one-tenth as fast between generations 15,000 and 20,000 as during the first 5,000 generations (Fig. 1). While the evolving populations adapted to the glucose medium, unused catabolic functions decayed and their diet breadth became narrower and more specialized[12].

We sought to identify the population genetic process primarily responsible for the association between increased fitness in glucose and reduced diet breadth by following both characteristics over time. If most losses of catabolic function happened early in the experiment, when adaptation to glucose was most rapid, then antagonistic pleiotropy would be supported (Fig. 2, AP). Moreover, if the same functional losses occurred in most populations, then this parallelism would imply that the losses were adaptive and caused by antagonistic pleiotropy. Note that adaptation to glucose may result from mutations that either improve some aspect of glucose catabolism or eliminate unnecessary functions that are costly to fitness in glucose. In either case, the mutations improve fitness on glucose while adversely affecting performance on other substrates.

We do not expect this association between the dynamics of adaptation and decay under mutation accumulation. Rather, mutation accumulation predicts that losses of unused functions should accumulate stochastically, and different functions should decay in replicate populations. If one assumes that such losses occur by neutral mutations that knock out unused functions, then diet breadth should decay exponentially, that is log-linearly (Fig. 2, MA). 'Bottlenecks' caused by selective sweeps of beneficial alleles should not affect the expected rate of substitution of strictly neutral mutations, which depends on mutation rate but not on effective population size[13]. However, three of the 12 lines evolved defects in DNA mismatch repair, which led to genome-wide mutation rates about 50-fold higher than the rates experienced by the ancestor and other lines[11]. These three lines became mutators around 2,500, 3,000 and 8,500 generations[11], and they retained high mutation rates through generation 20,000 (P. D. Sniegowski, unpublished data); therefore, all three experienced elevated mutation rates for most of the experiment. (A fourth line became a mutator between 16,500 and 18,000 generations (P. D. Sniegowski, unpublished data). Because most of this line's history occurred at the ancestral mutation rate, it was excluded from statistical comparisons between mutator and non-mutator populations at generation 20,000.) If mutation accumulation were the primary cause of ecological specialization, then the mutator lines should have evolved much narrower diet breadth than the lines that retained the ancestral mutation rate (Fig. 2, MA*).

We used Biolog ES plates to measure the catabolic function of the ancestor and three clones isolated from each population at generations 2,000, 10,000 and 20,000. The wells in these plates contain 95 different carbon sources and an indicator dye that reflects the amount of growth on the substrate; 64 substrates were informative for this study. On the basis of the proportion of genes in *E. coli* that encode these catabolic pathways, the plates can resolve mutations in several hundred genes (B. Bochner, personal communication). The weighted average of the 64 catabolic phenotypes provides an estimate of total catabolic function, or diet breadth.

Two predictions of antagonistic pleiotropy are that replicate lines should exhibit parallel decay of catabolic functions (those that trade-off with fitness in glucose), and that most losses should occur early (when adaptation to glucose is fastest). To test these predictions, for each substrate we compared the ancestral strain with the 12 evolved populations, as a group, at generations 2,000, 10,000 and 20,000. Because this approach entails multiple comparisons[14], we were very conservative in our statistical criteria; we employed two-tailed *t*-tests, assumed unequal variances for the ancestor and evolved lines, and used 0.0005 as the critical *P*-value for hypothesis testing. A significant test indicates a parallel change in catabolic function that is common to most, if not all, of the lines (Fig. 3). Sixteen of the 64 informative substrates showed parallel decay in the first 10,000 generations. However, there was no further increase in this number over the next 10,000 generations, even though most of the informative substrates remained at risk (Fig. 3). In fact, nine substrates showed parallel decay after only 2,000 generations, at which time none of the populations had yet evolved a mutator phenotype. Average total catabolic function declined by 32% (from 1 to 0.68) in the first 10,000 generations, but declined only 15% (from 0.68 to 0.58) between 10,000 and 20,000 generations (Fig. 4). The initial decline was significant ($t = 9.712$, 11 d.f., one-tailed $P < 0.0001$), whereas the later decline was not ($t = 1.623$, 11 d.f., one-tailed $P = 0.0664$); the difference in proportional decay between the two periods was also significant (paired $t = 1.899$, 11 d.f., one-tailed $P = 0.0420$). The findings that there were many parallel reductions of catabolic function and that reductions were concentrated early in the experiment indicate that antagonistic pleiotropy contributed to ecological specialization.

This evidence does not exclude the possibility that mutation accumulation is also important, however. To that end, we tested whether the mutator populations accumulated more losses of catabolic function than did the populations that retained the ancestral mutation rate (Fig. 4). Although the observed trend was in the direction predicted by mutation accumulation, there was no significant difference in the catabolic diet breadth of the two groups after 20,000 generations ($t = 0.887$, 9 d.f., one-tailed $P = 0.1991$). Any difference between these groups in their extent of catabolic decay is small and subtle in comparison with the roughly 50-fold

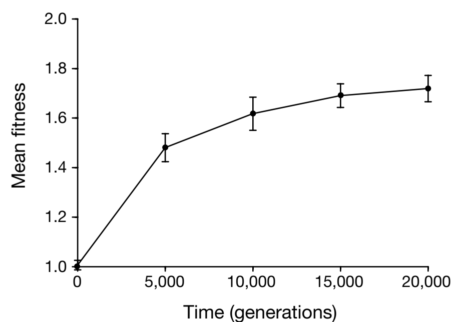

Figure 1 Trajectory for mean fitness of *E. coli* during 20,000 generations in minimal glucose medium. Each point is the mean of all 12 populations, and fitness of each population relative to the ancestor was measured with fivefold replication. Error bars are 95% confidence intervals based on the replicate populations.

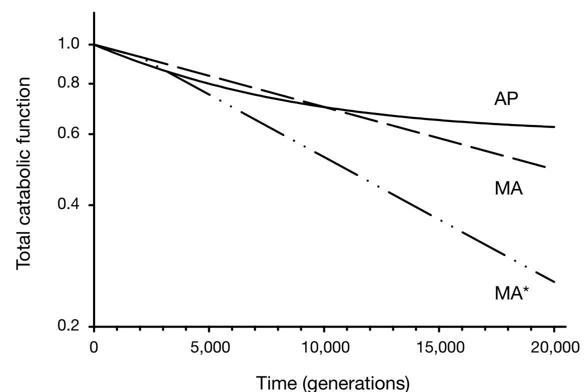

Figure 2 Hypothetical trajectories for the evolution of ecological specialization, as reflected by the decay of total catabolic function. AP, antagonistic pleiotropy, in which functional decay is inversely parallel to gains in fitness; MA, mutation accumulation (ancestral mutation rate), in which functional decay occurs at a constant rate and is independent of the pace of adaptation; MA*, mutation accumulation at an accelerated rate that occurs when a population becomes a mutator. Total catabolic function is shown on a log-transformed scale, such that predicted decay under mutation accumulation is linear.

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Carbon source	Time (generations) 2,000	10,000	20,000
bromosuccinic acid	7	11	12
D-alanine	1	3	6
D-malic acid	5	12	12
D-ribose	12	12	12
D-saccharic acid	9	11	11
D-serine	12	11	10
D-sorbitol	12	11	11
fructose-6-phosphate	11	10	9
fumaric acid	9	12	12
glucose-1-phosphate	12	11	10
glucose-6-phosphate	11	12	8
glucuronamide	0	4	8
L-asparagine	8	12	12
L-aspartic acid	9	12	12
L-glutamine	12	12	12
L-lactic acid	11	12	10
L-malic acid	7	12	12
malic acid	9	12	12
mono-methylsuccinate	2	12	12
mucic acid	12	8	9
P-hydroxyphenylacetic acid	5	12	11
succinic acid	9	12	12
uridine	12	12	10
Sum of parallel losses	9	16	16

Figure 3 Summary of parallel changes in catabolic functions, based on comparisons between the evolved populations and common ancestor at three time points. Stringent significance ($P < 0.0005$) was demanded to account for multiple tests[14] (64 substrates tested at each time point). Red, catabolic functions that consistently decayed; green, significant gains in function. The number in each cell is the number of populations (out of 12) whose average catabolic function on a substrate was less than that of the ancestor.

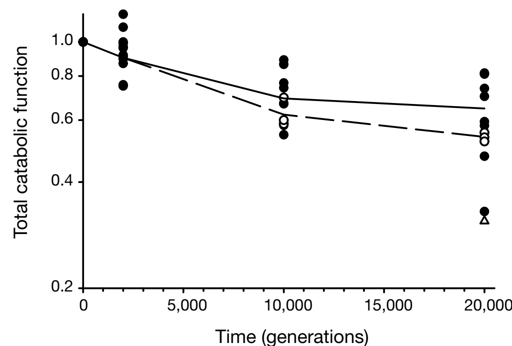

Figure 4 Evolution of total catabolic function during 20,000 generations in minimal glucose medium. Total catabolic function is a weighted average across 64 informative substrates; 1.0 is the ancestral level, while lower values indicate decay. Values are shown on a log-transformed scale, where the decay predicted by mutation accumulation is linear. Each point is the mean of three clones from each population. Solid line, mean value of populations that retained the ancestral mutation rate at that generation; dashed line, mean of three mutator populations; closed circles, populations with the ancestral mutation rate; open circles, mutator populations; open triangle, late appearing mutator (excluded from both classes).

difference in mutation rates that they experienced for most of that time.

On balance, our data indicate that antagonistic pleiotropy was the main contributor to the resource specialization during evolution of *E. coli* lines in minimal glucose medium. That is, the mutations responsible for catabolic decay did not simply accumulate under mutation pressure but were themselves adaptive in the glucose environment. Two recent studies support this inference further. We have shown elsewhere that loss of the capacity to use D-ribose, which occurred in all 12 populations during the initial 2,000 generations, resulted from deletions that confer a selective advantage in glucose medium[15]. Funchain *et al.*[16] studied *E. coli* mutator lines that evolved under a regime with severe population bottlenecks, which allowed deleterious mutations to accumulate. In contrast to our study, they found that (1) fitness declined over time; (2) losses of catabolic function identified using Biolog plates were not concentrated early in the experiment, but rather accumulated at a constant and perhaps even an accelerating rate; and (3) these defects were scattered across catabolic pathways, with no single defect being present in more than a small minority of their lines. The large population sizes and intense competition in our experiment would have prevented most deleterious mutations from being fixed as they would have been under a severe bottleneck regime.

We now consider three possible caveats concerning the hypothesized effects of mutation accumulation and antagonistic pleiotropy. First, we assumed that the amount of antagonistic pleiotropy was equivalent in all lines, which might be invalid if mutator populations experienced faster adaptive evolution than their less mutable counterparts. Consistent with our assumption, the three mutator lines did not attain significantly higher final fitness than the other eight lines ($t = 1.322$, 9 d.f., two-tailed $P = 0.2188$); nor does the mutation supply limit the rate of adaptive evolution under these circumstances[17]. Moreover, most parallel reductions in catabolic function occurred in the first 2,000 generations (Fig. 3), before any populations became mutators[11]. Second, the prediction that unused functions should decay at a constant rate under mutation accumulation holds true for neutral mutations[13]. But some of the decay might reflect deleterious mutations that hitch-hiked to fixation with beneficial mutations; during the early period of rapid adaptive evolution, there would have been more opportunities for deleterious mutations to hitch-hike, possibly generating a false indication of antagonistic pleiotropy. However, this explanation cannot account for the parallel decay of catabolic functions across replicate populations (Fig. 3). Also, we have demonstrated elsewhere that the mutations responsible for the decay of ribose catabolic function were indeed beneficial in the glucose medium[15]. Third, unused catabolic functions may not decay log-linearly under mutation accumulation if successive mutations in the same pathway interact epistatically[18], or if some functions present larger mutational targets than others. These complications should have similarly affected the losses of catabolic function in the study by Funchain *et al.*[16], which depended on mutation accumulation but not antagonistic pleiotropy. However, in that study, the rate of decay of catabolic functions tended to accelerate, in contrast to the deceleration observed here (Figs 3 and 4). Therefore, the deceleration in functional decay cannot be attributed to a more complex model of mutation accumulation, but instead indicates the important role of antagonistic pleiotropy.

In summary, a long-running evolutionary experiment with bacteria allowed us to distinguish between the effects of two processes that can contribute to ecological specialization, mutation accumulation and antagonistic pleiotropy. Our findings indicate that antagonistic pleiotropy played a greater role in specialization than did mutation accumulation. This conclusion does not contradict theory[19,20] and experiments[16,21,22] on the importance of mutation accumulation in populations that become less fit because of high mutation rates, small population sizes, or both. Rather, our work emphasizes the distinction between adaptive evolution, in which unused functions may be lost as a consequence of natural selection for other traits, and nonadaptive evolution, in which deleterious mutations accumulate precisely because selection is ineffective. □

Methods

Evolution experiment

The design of the long-term evolution experiment has been described in detail[9,10]. In brief, 12 lines were founded from two variants of *E. coli* B and propagated daily by 1:100 dilution into Davis minimal medium supplemented with glucose at 25 μg ml^{-1}. The populations were maintained in this manner for 20,000 cell generations (3,000 days); every 500 generations, samples from each population were stored at −80 °C. Six of the populations

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were founded from a strain unable to grow on arabinose (Ara⁻), and the other six were founded with a spontaneous Ara⁺ mutant; the two ancestors were otherwise isogenic, and the Ara marker itself is neutral in the glucose medium[9].

Fitness assays

The protocol for estimating the competitive fitness of evolved lines relative to their ancestor has been described[9]. In brief, samples of the evolved lines (containing whatever genetic diversity was present when they were sampled) and ancestral strains were removed from the freezer and separately acclimated to the medium and culture conditions used in the evolution experiment. Each evolved line was mixed with an equal volume of the reciprocally marked ancestor, and the two types then grew and competed under the same conditions that prevailed during the evolution experiment. Initial and final densities of the two competitors were enumerated by plating cells on a tetrazolium-arabinose indicator agar that allowed them to be distinguished by the Ara marker. (A different plating procedure was used for competitions with one evolved line that no longer produced distinct colonies on the indicator agar.) The net growth rate of each competitor was calculated from the data, and the relative fitness of an evolved line is then expressed simply as the ratio of its growth rate to that of the ancestor. Assays were run in blocks with fivefold replication for all 12 lines.

Biolog assays

Catabolic diet breadth was assayed using Biolog (Hayward, California) ES plates for the two ancestral variants and three clones randomly chosen from each evolved line at generations 2,000, 10,000 and 20,000. Assays were run in three sets, each set comprising all of the clones for four lines plus three replicates of each ancestral variant. The bacteria were grown for two days in LB broth; on the next day, each culture was diluted 1:100 into fresh LB and incubated for 6 h. (LB was used instead of minimal glucose medium to avoid catabolite repression, which depresses other functions and may yield fewer positive readings[23].) The cultures were centrifuged at 12,000 *g* for 10 min and resuspended in saline to remove residual medium; this suspension was used to inoculate each well at a constant density. At 0, 4, 12, 24 and 48 h, optical densities were measured at 590 nm using an automated plate reader, and all measurements were adjusted by subtracting out the reading from the blank well. A trapezoidal area approximation[24] was used to integrate the five measurements for each well into one value, which reflects the area beneath the curve of optical density versus time; this area value is sensitive to both the rate and final level of catabolic function. Of the 95 substrates, glucose and arabinose were excluded *a priori* because glucose was the target of adaptation and arabinose use was a marker in the evolution experiment. Another 29 substrates were excluded because repeated measurements on the ancestor were statistically unreliable (coefficient of variation > 1), leaving 64 informative substrates. To test the evolutionary change in each individual catabolic function, the values for the three clones from a line at a given generation were averaged. The 12 evolved lines as a group were compared with the two ancestral variants using a two-tailed t-test with unequal variances (given divergence among the replicate lines) and a very stringent P-value of 0.0005 (to adjust for multiple tests[14]). Also, for each informative substrate, the catabolic function of an evolved clone was standardized to the ancestral value to give equal weight to all substrates, and then log-transformed to give equal weight to proportionally equivalent gains and losses of function. The anti-log of the average of these transformed values provides a measure of total catabolic function; the ancestral total equals 1.0 (by definition), whereas values less than 1.0 indicate an overall loss of function.

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Correspondence and requests for materials should be addressed to V.S.C. (e-mail: cooperva@msu.edu).

Natural selection and sympatric divergence in the apple maggot *Rhagoletis pomonella*

Kenneth E. Filchak, Joseph B. Roethele & Jeffrey L. Feder

Department of Biological Sciences, Galvin Life Science Center, University of Notre Dame, Notre Dame, Indiana 46556-0169, USA

In *On the Origin of Species*, Darwin proposed that natural selection had a fundamental role in speciation[1]. But this view receded during the Modern Synthesis when allopatric (geographic) models of speciation were integrated with genetic studies of hybrid sterility and inviability[2,3]. The sympatric hypothesis posits that ecological specialization after a host shift can result in speciation in the absence of complete geographic isolation[4,5]. The apple maggot, *Rhagoletis pomonella*, is a model for sympatric speciation in progress[4,5]. Hawthorn (*Crataegus* spp.) is the native host for *R. pomonella* in N. America[5]. But in the mid-1800s, a new population formed on introduced, domesticated apple (*Malus pumila*)[4,5]. Recent studies[6–10] have conferred 'host race' status on apple flies as a potentially incipient species, partially isolated from haw flies owing to host-related adaptation. However, the source of selection that differentiates apple and haw flies is unresolved. Here we document a gene–environment interaction (fitness trade-off) that is related to host phenology and that genetically differentiates the races.

Because *Rhagoletis* flies mate exclusively on or near the fruit of their host plants[11], differences in host preference can result in virtually complete premating isolation among species[12]. However, mark-release experiments have shown that such 'host fidelity' only partly restricts gene flow between apple and haw races to about 6% per year[9] (*R. pomonella* is univoltine). Despite this exchange, the races are not fusing; allozyme loci mapping to three different regions of the fly's genome display consistent allele frequency differences over time[10] (see Methods). Therefore, some form of strong host-dependent selection must be acting on these allozymes (or linked genes).

Evidence suggests that the interplay between host phenology, temperature and diapause is responsible for differentiating the

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