Adaptation and incipient sympatric speciation of *Bacillus simplex* under microclimatic contrast at "Evolution Canyons" I and II, Israel

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The microevolutionary dynamics of prokaryotes in natural habitats, such as soil, is poorly understood in contrast to our increasing knowledge on their immense diversity. We performed microevolutionary analyses on 945 soil isolates of *Bacillus simplex* from "Evolution Canyons" I (Carmel, Israel) and II (Galilee, Israel). These canyons represent similar ecological replicates, separated by 40 km, with highly contrasting interslope abiotic and biotic conditions in each (within a distance of only 100–400 m). Strains representing genetic groups were identical in their 16S sequences, suggesting high genetic similarity and monophyletic origin. Parallel and nested phylogenetic structures correlated with ecological contrasts rather than geographical distance. Additionally, slope-specific populations differed substantially in their diversity. The levels of DNA repair (determined by UV sensitivity) and spontaneous mutation rate (resistance to rifampicin) relate to ecological stress and phylogeny. Altogether, the results suggest adaptive radiation at a microscale. We discuss the observed adaptive population structures in the context of incipient sympatric speciation in soil bacteria. We conclude that, despite different biology, prokaryotes, like sexually reproducing eukaryotes, may consist of true species and parallel ecological speciation in eukaryotes.

adaptive radiation | *Bacillus* soil isolates | environmental stress | microbial population genetics | natural selection

Bacterial microevolution in nature proceeds in the complex context of changing natural environments at local, regional, and global scales and is being driven by the intertwined evolutionary forces of natural selection, mutation, varying degrees of parasexuality (lateral gene transfer), migration, and genetic drift. It is still largely unknown how bacterial biodiversity evolves in natural systems, such as soil. Is soil bacterial microdivergence primarily adaptive and selected by the environment or primarily stochastic and only selected on the macroscale level of communities? Does soil bacterial evolution differ substantially from other prokaryotes, e.g., hyperthermophiles (1, 2)? How does adaptive progress relate to speciation? Moreover, do adaptive and speciational patterns of prokaryote and eukaryote evolution differ?

Insights into bacterial microevolution have come mostly from theoretical and computational studies (3–6) and from multigenerational laboratory model systems (7, 8). Few population genetic studies on bacteria from natural environments, such as soil or sediment, have been performed. These studies frequently lack the linking of changing or contrasting environments to geographical distance, and to the evolutionary dynamics of both adaptation and speciation. Some studies focus on world-wide populations, with less emphasis on local dynamics (9, 10); others focus entirely on very localized populations (11, 12), thereby neglecting migration and geographical distance. Some studies refer to local and global populations but focus on populations from highly similar (13) or very different types of habitats (14, 15). This procedure impedes linkage of varying geographical distance to varying ecology. Interestingly, other studies have observed indications of natural selection (16–18); however, the results were not linked either to ecology (17) or to the effect of geographic distance (18).

The environmental model of "Evolution Canyon" (EC) (19–21) is highly suitable for studying the intertwined evolutionary processes of adaptation and speciation under microscale environmental stress. ECI (Lower Nahal Oren, Mount Carmel, Israel) and ECII (Lower Nahal Keziv, western Upper Galilee, Israel) are separated by 40 km and represent similar ecologically replicating canyons with sharply contrasting interslopes in each, separated by only 100–400 m (19–21) (see Fig. 4, which is published as supporting information on the PNAS web site). The open-park forest of African–Asian savanna-like, south-facing slopes (SFSs) that are tropical, warm, and xeric receives 200–800% more solar radiation (22). Consequently, these slopes are warmer, drier, spatiotemporally more heterogeneous and fluctuating and, thus, more stressful than the green and lush European-like, north-facing slopes (NFSs) that are temperate, cool, and mesic (22). Among 2,500 species recorded in ECI, several mainly eukaryotic model species (fungi, plants, and animals) have been studied in detail (19, 20, 23–32). The spatially very close ecological contrast of African-like and European-like slopes (100–400 m distance) in ECI, similarly replicated at a distance of 40 km, is optimal for studying the soil bacterial microevolution of adaptation and speciation both locally and regionally.

We expected significant differences in divergence and diversity among strains from the African-like and European-like slopes. According to the niche-width variation hypothesis (33) and the environmental theory of genetic diversity (34–36), populations from spatiotemporally variable and ecologically more stressful habitats should display higher morphological and genetic polymorphism. We discuss the observed divergence patterns with respect to selection and migration. Likewise, we phenotypically investigated DNA repair and mutation rates and relate their levels to ecology and phylogeny. Finally, we discuss the observed adaptive evolutionary processes in the ECs in the context of the prokaryotic species concept and speciation processes in bacteria.

Materials and Methods

Sampling of Soil and Isolation of Bacteria. Seven sampling stations were designated in each canyon: three on the African-like SFS (stations 1–3), one at the valley bottom (station 4), and three on the European-like NSF (stations 5–7) (see Fig. 4). In ECI, six soil samples were taken per station, three from shady sites (underbush or tree canopy) and three from sunny, exposed sites

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Abbreviations: RAPD, randomly amplified polymorphic DNA; EC, Evolution Canyon; GL, genomic lineage; NFS, north-facing slope; SFS, south-facing slope.

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(microniches). Because of the absence of sunny sites on the European-like ECII slope, all ECII European-like samples were obtained from shady sites, and all ECII African-like samples were obtained from sunny sites (three soil samples were taken per station). Samples (each ≈50 g) were taken from the top, 1-cm soil layer in February 2003, at the end of the winter rains. Sampling sites per station were spaced horizontally by 5–10 m. For bacterial isolation, a 10% soil slurry in 0.9% NaCl (excluding plant or stone material) was plated on an LB agar plate and incubated for 12 h at 37°C. We screened bacterial isolates by a colony morphology-based approach, assuming that colonies of identical morphology taxonomically represent very closely related strains. Strains giving a distinct colony morphology type and present in all soil samples were purified by single-cell isolation and subsequently stored in 20% glycerol at −80°C.

Genomic Comparison of Bacterial Strains by Randomly Amplified Polymorphic DNA (RAPD)-PCR. We conducted a high-resolution genetic fingerprinting by RAPD-PCR (37) with two primers. Thorough initial control experiments for primer choice and PCR reproducibility included (*i*) separate RAPD-PCR runs on the same or different DNA template preparations of the same strains, (*ii*) variation of DNA template concentration, and (*iii*) variation of polymerase enzyme and $MgCl_2$ concentration. Only primers that yielded highly reproducible PCR band patterns despite methodically varied PCR conditions were applied. Details are given in *Supporting Materials and Methods* and Table 1, which are published as supporting information on the PNAS web site. For each strain, the presence or absence of a RAPD-PCR product was scored as a 0 or 1, respectively, with GENEIMAGIR 3.52 (Scanalytics, Rockville, MD), culminating in a string of zeros and ones, i.e., the RAPD sequence.

Gene Sequence Determination, Analyses, and Phylogenetic Reconstructions. The 16S rDNA sequence (≈1,500 nt) was determined as described in ref. 14. Details on sequence determination of parts of the genes for glyceraldehyde-3-phosphate dehydrogenase (*gapA*), phosphoglycerate kinase (*pgk*), and the gene for the DNA damage repair protein UvrA are given in *Supporting Materials and Methods* and Table 1. The DNA sequences were aligned manually, and PAUP* 4.0 (38) was used for phylogenetic reconstructions.

UV-C Survival Measurement. For growth of the preculture, cells grown on LB agar were inoculated in 2 ml of LB medium by using a TU-400 orbital shaker incubator at 30°C for 15 h. Cells were then diluted 1:4,000 in 2 ml of LB medium and allowed to grow as in the preculture. After 10 h (early stationary phase), appropriate dilutions were spotted on an LB plate for viable count determination and for irradiation for 5 sec with UV-C light at 254 nm with a 15 W Philips G15T8 lamp at a distance of 23 cm from the agar surface (2.3 mJ/cm^2, as determined with a RAMSES-ACC-UV radiometer).

Mutation Rate Measurement by Fluctuation Tests. Cells from a preculture (see above) were diluted 1:4,000 in 12 ml of LB medium, aliquoted into 25 cultures of 400 μl each, and allowed to grow as the preculture. After 12 h, each culture was spread on an LB plate with 20 μg/ml rifampicin. The plates were incubated at 37°C and scored after 18 h for mutant colonies resistant to rifampicin antibiotic. The viable cell count was averaged from four randomly chosen cultures per strain. Mutation rates were estimated by using the MSS maximum likelihood approach (39).

Statistical Analyses. Generally, calculations were performed as implemented in STATISTICA 6.0 and ARLEQUIN 2.000 (40). The index of association, I_A, a measure of linkage disequilibrium of alleles, was calculated by using the S.T.A.R.T. package (41), and intragenic recombination rates were estimated on a coalescent-based approach (42). The diversity of a station is given as the mean number of differences in the RAPD sequence. The significance of these differences was assessed by a permutation procedure by mixing and randomly reseparating the RAPD sequences according to the proportions in the original data set (5,000 permutations per comparison of interest). The percentage of permutated data sets obtaining a smaller or equal difference in diversity, as compared with the original data, is given as the *P* value.

Results

Isolation of Strains, RAPD Patterns, and Taxonomic Affiliation. From ECI, ≈90 strains were obtained per station, of which ≈45 were from sunny sites and ≈45 were from shady sites. From ECII, ≈45 strains were obtained per station. Cells expressing the chosen colony morphology were present at a frequency of 10^4 to 10^5 per gram of soil of each sample. We observed several RAPD clusters (clusters A–E) of varying sizes (Fig. 1*a*, values i), which were dominated by African-like or European-like strains (Fig. 1*a*, values iii). This distribution pattern suggests a strong impact of ecology on the observed divergences. Each cluster contained twice as many strains from ECI than ECII (Fig. 1*a*, values ii), which reflects the sampling regime. Cluster representative strains were all identical in their 16S sequence to each other and to *Bacillus simplex* LMG 21002 (Fig. 1*b*). In general, *B. simplex* strains have up to 1% 16S sequence diversity (43). Therefore, the 16S results suggest conspecificity of the EC isolates and affiliation to *B. simplex*.

The RAPD Clusters Are Corroborated by Gene Sequences and Represent Specific Ecological Evolutionary Lineages. The metapopulation consists of two genomic lineages (GL): GL1 and GL2 (Fig. 1*c*). GL1 is primarily African-like, containing two African-like lineages A and C, and one European-like lineage B. GL2 is primarily European-like, consisting of a dominant European-like lineage E and a small African-like lineage D. Lineage B shows an apparently ecologically driven nested substructure (Fig. 1*a*). First, an African-like lineage Ba is nested, with strains from all six African-like stations (stations 1–3 of both ECs), yet predominantly from ECII (Fig. 1). Second, a European-like lineage, Bae, emerges within Ba with strains from European-like stations 5 and 6 from each of the two ECs. The phylogenies deduced from the nucleotide sequences of parts of three genes (*gapA*, 654 bp; *pgk*, 426 bp; *uvrA*, 1,135 bp) of representative strains from each RAPD cluster and from both canyons (n = 21–27) (Fig. 1*b*) clearly corroborate the RAPD clustering at the A–B–C versus D–E split and with very good congruence at the levels of the individual lineages A, B, C, D, and E. A striking difference occurs in the *uvrA* phylogeny compared with RAPD, *gapA*, and *pgk*. Three of four Ba strains clearly cluster apart from the GL1 clade (Fig. 1*b*, *uvrA* tree). The valley bottom strains represent a mixture of slope strains, with a tendency to larger abundance in the African-like lineage A (Fig. 1*a*). Altogether, 837 strains were grouped to the evolutionary lineages and provided the basis for all further analyses.

Remarkably, in ECI, African-like strains (lineages A, Ba, C, and D) inhabit predominantly sunny open microniches (211 strains as opposed to 174 strains), irrespective of the slope type on which they reside. Similarly, European-like genomic strains (lineages B, Bae, and E) inhabit predominantly shady microniches (101 strains as opposed to 83 strains). Details are given in Table 2, which is published as supporting information on the PNAS web site. Thus, the African-like or European-like slope-specificity of ecological–evolutionary lineages is significantly reflected by their strain distribution in either sunny or shady microniches (n = 569 strains; P = 0.0236, sign test).

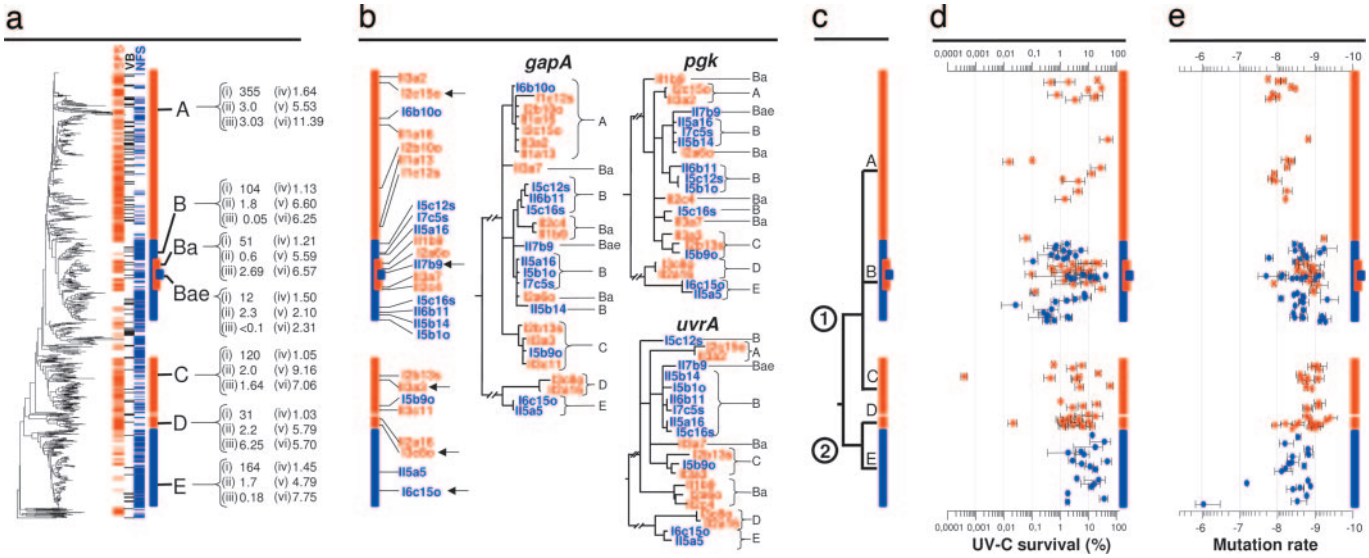

Fig. 1. Evolutionary lineages in the metapopulation of *B. simplex* and their physiological properties of UV survival and mutation rate. (*a*) Neighbor-joining tree (Jukes–Cantor distances) from RAPD data of all isolates. Thin horizontal lines to the right of the tree indicate origin from African-like slope (SFS, red), valley bottom (VB, black), and European-like slope (NFS, blue). For each lineage, six characteristic values are given and denoted with the numbers of strains (i), the ECI/ECII ratio of strains (ii), the ratio of African-like to European-like strains (iii), the ratio of isolates to haplotypes (iv), the genetic diversity given as mean number of pairwise differences of the RAPD sequence (v), and the variance of RAPD pattern diversity (vi). The depicted RAPD clusters are supported by unweighted pair group method with arithmetic mean and maximum parsimony analyses (data not shown). (*b*) Representative RAPD cluster strains chosen from both canyons and their phylogenies of portions of the *gapA*, *pgk*, and *uvrA* genes. Names of the strains reflect their origin and are explained in *Supporting Materials and Methods*. The 16S sequence was determined from strains marked with an arrow. The phylogenetic trees were reconstructed from the results of neighbor-joining, minimum evolution, maximum parsimony, and maximum likelihood analyses of the gene sequences. Only nodes that were unambiguously resolved in the same way in all four reconstruction methods are shown. Divergence values are given in Table 5, which is published as supporting information on the PNAS web site. Outgroup sequences (which are not shown) were obtained from *Bacillus subtilis*, *Bacillus cereus*, *Bacillus halodurans*, *Bacillus licheniformis*, and *Oceanobacillus iheyiensis*. (*c*) Phylogenetic tree of ecological genomic evolutionary lineages summarizing the results from the gene tree topologies and of the RAPD tree. (*d* and *e*) UV-C survival (percentage) (*d*) and spontaneous mutation rates to rifampicin resistance of representative isolates from the evolutionary lineages A-E (*e*). Experiments were done twice, and results are given as mean and deviation from the mean.

Genomic Divergence of Strains Is Significantly Correlated with Ecology But Not with Geography. To correlate genetic divergence to ecology and geography independently from the aforementioned phylogenetic approach, we applied the fixation index F_{st} on RAPD data for pairwise station comparisons (*i*) within slopes ($n = 3$ per slope) (Fig. 2, columns a–d), (*ii*) among either African-like or European-like slopes across both canyons [$n = 9$ for SFSs (Fig. 2, column e) or NFSs (Fig. 2, column f)], and (*iii*) among slopes of the same canyon [$n = 9$ for ECI (Fig. 2, column g) or ECII (Fig. 2, column h)]. The F_{st} values within slopes in both canyons indicated little divergence (Fig. 2, columns a–d). The F_{st} values across canyons but within the same slope type (either African-like or European-like of ECI and ECII) were slightly higher, although mostly not significantly (Fig. 2, columns e and f). Remarkably, for interslope stations within the same canyon, the F_{st} values increase sharply and significantly (Fig. 2, columns g and h). In summary, *B. simplex* on the African-like and European-like slopes in each canyon was genetically significantly different, whereas the African-like and European-like slopes of both canyons were very similar. Thus, the genetic divergence significantly relates to ecology and clearly less, if at all, to geographical distance.

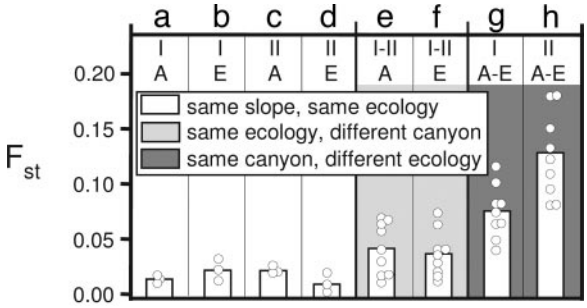

Fig. 2. Genomic divergence of *B. simplex* across and within two ECs, as estimated from pairwise station comparisons of RAPD data within slopes ($n = 3$) (columns a–d), between slopes of same ecology but different canyon ($n = 9$) (columns e and f), and of interslope stations within canyons ($n = 9$) (columns g and h). Open circles indicate a pairwise station comparison; bars indicate the mean of all pairwise station comparisons. I and II denote ECI and ECII, respectively; and A and E denote African-like and European-like slopes, respectively. The *P* values (Mann–Whitney U test) between the indicated columns are as follows: a–e, $P = 0.100$; c–e, $P = 0.482$; b–f, $P = 0.372$; d–f, $P = 0.036$; a–g, b–g, c–g, d–g, a–h, b–h, c–h, and d–h, $P < 0.0001$; e–g, $P = 0.018$; f–g, $P = 0.003$; e–h and f–h, $P < 0.0001$.

Diversity Is Higher Under European-Like Shady Conditions. In ECI and ECII, the genetic diversity of strains is significantly higher in European-like stations 5–7 than in the bottom station 4 or in the African-like stations 1–3 ($P < 0.0002$) (Fig. 3). Interestingly, this pattern is maintained in shady and sunny microniches of ECI (Fig. 3). In five of seven stations (stations 1, 2, 4, 6, and 7), the diversity is higher in shady sites, which is significant for stations 2 and 4 ($P = 0.01$ and $P = 0.006$, respectively).

Characteristics of African-Like and European-Like Evolutionary Lineages Strains in UV-Survival and Mutation Rates. We observed a tendency for higher UV survivals in African-like strains (Table 3, which is published as supporting information on the PNAS web site), although the difference in the averages was insignificant. The African-like strains of lineages A, Ba, and C (amounting to 81% of the GL1 metapopulation) were significantly more UV-resistant than the European-like strains of lineage B (*P* =

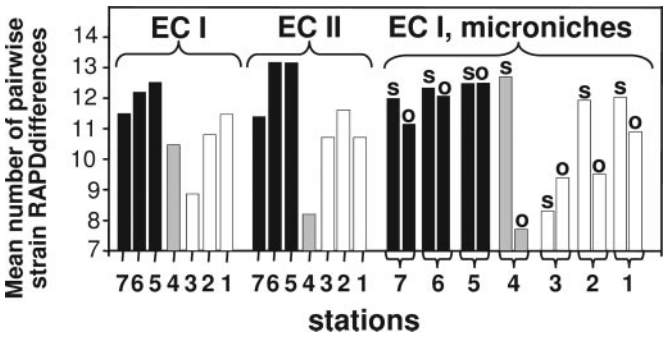

Fig. 3. Genomic diversity of *B. simplex* in stations of ECI and ECII and of shady (s) and sunny (o) microniches on ECI. Black denotes stations 5–7 of European-like slopes; gray denotes valley bottom station 4; and white denotes stations 1–3 of the African-like slopes.

0.014, Scheffé post hoc test to one-way ANOVA analysis on GL1 evolutionary lineages). In the European-like lineage E, we observed two strains with substantially higher mutation rates (10- to 100-fold) than all other strains (Fig. 1*e* and Table 3). There was no tendency for African-like or European-like strains to express higher mutation rates.

However, we found significant differences between African-like and European-like strains from ECI in distributions of log-transformed UV-survival ($P < 0.02$) and mutation rate ($P < 0.0001$) values (Wald–Wolfowitz series test). When we examined the distribution values of both traits more closely, we found them to differ strongly in their kurtosis values. For UV survival, kurtosis was high in African-like strains (4.9) but low for European-like strains (−0.02). For the mutation rates, the situation was opposite. Kurtosis was low in African-like strains (−0.8) but high in European-like strains (9.99). Distributions with high kurtosis tend to have a distinct peak near the mean, decline rather rapidly, and have heavy tails. In contrast, distributions with low kurtosis tend to have a flat top near the mean, rather than a sharp peak, and low extremes. We will discuss the biological significance below.

Recombination. Small but significant incongruencies in the *gapA*, *pgk*, and *uvrA* gene trees of GL1 strains (Fig. 1*b*) prompted us to test the sequence data for recombination. We measured the degree of linkage disequilibria of the *gapA*, *pgk*, and *uvrA* genes among 17 strains from the GL1 lineage (Fig. 1*b*). The I_A value was close to zero ($I_A = 0.258$), which suggests some influence of recombination. The I_A values for African-like ($n = 8$) and European-like ($n = 9$) strains were 0.720 and −0.055, respectively, suggesting a probably larger influence of recombination on the European-like slope. The intragenic recombination rates (*pgk* and *uvrA*) were low but higher within lineages (e.g., lineage B) than across genomic lineages (Table 4, which is published as supporting information on the PNAS web site), which could suggest increasing sexual isolation with phylogenetic distance.

Discussion

Overview of Evidence. The isolation strategy applied here yielded a set of 945 taxonomically closely related strains well suited for microevolutionary studies with respect to genetics, ecology, and spatial distance. First, the *B. simplex* populations on the African-like and European-like slopes in each canyon are genetically significantly different, whereas they are very similar on African-like and European-like slopes across canyons (Figs. 1*a* and 2). Second, parallel and nested phylogenetic evolutionary lineages were observed (Fig. 1*a–c*). Third, the slope specificity of lineages is mirrored by their strain distribution in sunny or shady microniches irrespective of slopes. Fourth, the higher genetic diversity observed in slopes and microniches was associated with the more heterogeneous European-like shady soil ecology (Fig. 3). Fifth, the population structure suggests influence of parasexual recombination, probably larger on the European-like slope. Sixth, the UV survival phenotype is apparently under more pressure for diversification on the African-like slope, yielding a substantial proportion of strains in the upper and lower extremes of the distribution of UV survival phenotypes. Finally, the mutation rate phenotype is apparently under more pressure for adaptive diversification on the European-like slope (Fig. 1*e*). These results suggest the twin evolutionary processes of adaptation and incipient sympatric speciation of soil bacteria in EC as discussed below.

Adaptive Evolution. ***Selection overrides migration and random distribution.*** The highly similar phylogenetic structure of the *B. simplex* populations in both canyons (Fig. 1 *a* and *b*) and their genetic similarity on either African-like or European-like slopes of both canyons (Fig. 2 *e* and *f*) is best explained by migrational exchange, which is expected for *Bacilli* because of spore dissemination (9, 13). Yet, despite the high equalizing factor of migration, certainly higher within (100–400 m) than between canyons (40 km), the *B. simplex* interslope divergence within a canyon is by far higher than for the same slopes across canyons (Fig. 1 *g* and *h*). The slope specificity of lineages is additionally reflected by their strain distribution in microniches. For example, the European-like B strains reside predominantly in shady microniches, irrespective of the African-like slope, valley bottom, or European-like slope. In contrast, the African-like Ba strains reside predominantly in open sunny microniches in both the African-like and European-like slopes (Table 1). We hypothesize that Ba strains diverged from the European-like lineage B by an adaptive switch to the African-like slope. The strong (4-fold) affiliation of lineage Ba strains to ECII suggests an ECII-specific adaptive pattern of this specific lineage. Overall, the results emphasize a strong slope-specific selective regime that overrides migration and stochasticity. *B. simplex* populations, similar to other prokaryotic or eukaryotic EC model organisms, adaptively reflect the sharp interslope ecological divergence of EC (20, 21, 32). Here, *B. simplex* contrasts strongly hyperthermophilic prokaryotes, which seem to diverge according to geographical separation (1, 2).

Diversity is higher under European-like shady conditions. A positive correlation of genetic diversity with abiotic and biotic stresses has been widely documented (21, 32, 44–46). *B. simplex* strains are strongly influenced by the soil specificities, which are undoubtedly caused by the interslope microclimatic differences and resulting divergent macrobiota (47). Soil encompasses a vast reservoir of numerous specialized niches. Although the soil of both slopes is terra rossa (47), small but significant interslope differences exist. The European-like soil shows significantly higher biomass, humification, and nutrient minerals (47). Also, various stages of decomposition of plant material and larger bioturbation features, e.g., abundant excrements of soil mesofauna and earthworms, make the European-like soil more heterogeneous for bacteria (47). The African-like slope soil represents higher drought and lower productivity (22, 27), contrasting with the higher moisture availability, nutrition, and productivity of the European-like slope soil (22, 27, 47). We hypothesize that the higher resource concentration and heterogeneity of the European-like slope soil select for higher diversity of European-like *B. simplex* isolates between and within slopes, corroborating the niche-width hypothesis (33) and the environmental theory of genetic diversity (34–36), i.e., higher variation with niche-breadth and stress, respectively. Our results fit previous studies on prokaryotes from laboratory experiments of *Pseudomonas fluorescens* (8) and from natural soil populations of *Burkholderia cepacia* (formerly *Pseudomonas*) (48).

In contrast to the soil population of *B. simplex*, most of the mainly macrobiotic model organisms from EC (11 of 14 species, including the rock-residing cyanobacterium *Nostoc linckia*), show higher genetic diversity on the African-like slope, probably because they are directly exposed to the more heterogeneous surface conditions on the African-like slope (21, 32). The genetic diversity of the soil *B. simplex* seems to reflect inner soil complexity rather than surface stresses.

Higher UV resistance correlates with the African-like slope. Solar radiation is a strong surface stress affecting the African-like slope and soil and is up to 8-fold higher than the European-like slope exposure (22). This higher exposure probably led to a higher UV resistance on the African-like slope. The kurtosis of the distributions for log-transformed UV survival values of African-like strains is high, which indicates an overrepresentation of the extreme upper and lower values compared with the normal distribution. We explain this kurtosis according to the niche-width variation hypothesis (33): A strong diversification pressure on the UV-survival phenotype specifically in African-like strains is exerted by the higher African-like mosaic solar radiation, which probably resulted in the evolution of a substantial proportion of high and low UV-adapted strains. In contrast, the significantly lower kurtosis of the distribution of log-transformed UV survival values for European-like strains, which is characterized by a lack of extremes, could be explained by the relatively much lower UV stress on the European-like slope, resulting in a relaxed pressure for diversification into extremes of this phenotype on the European-like slope.

The higher solar radiation probably selected for more effective nucleotide excision repair by UvrABC proteins under SOS regulation. Likewise, recombination repair or UV-light-shielding effects could also contribute to the hardier African-like phenotype observed. The observed adaptive response is again exemplified by the African-like lineage Ba, which has a strongly (Table 3), although insignificantly, increased UV survival ($P = 0.52$, Scheffé test post hoc to one-way ANOVA analysis on GL1 evolutionary lineages) compared with the European-like lineage B, from which it apparently originated. Remarkably, Ba strains differ substantially in DNA and amino acid sequence from lineage B and GL1 strains (Fig. 1*b*, *uvrA* phylogeny). This lineage-Ba-specific sequence could perhaps confer an elevated DNA repair function, which could have aided in the adaptive switch from the European-like slope to the African-like slope. However, it should be noted that European-like lineage E also shows considerable UV resistance that might be explained by other soil-inherent stresses that specifically affect lineage E but not other European-like lineages, such as B. We regard the high UV survival of the European-like lineage Bae as nontypical for the European-like slope. Apparently, Bae strains express the high UV survival properties of the African-like lineage Ba, to which they are genetically very closely affiliated.

The mutation rate is affected by the greater inner complexity of the European-like slope soil. The levels of mutation rates are, although indirectly, environmentally selected (49). Remarkably, African-like and European-like strains differed significantly in log-transformed distributions. However, in contrast to UV-survival, we observed a high kurtosis for European-like strains, which can be explained by a strong pressure for diversification in the mutation rate ability specifically exerted by the larger diversity of microhabitats of the more heterogeneous European-like soil complex. Additionally, this broader niche-width could have led to the emergence of two European-like lineage E strains with 10- to 100-fold higher mutation rates, which could be mutator strains. The importance of shifts in environmental conditions in mutator evolution has been recently highlighted (3). The apparent lack of extremes in the mutation rate distribution of African-like strains might consequently be explained by the lower inner niche-width of the African-like soil.

Speciation of the *B. simplex* Population. Incipient speciation is the branching and robust coherence of new lineages by evolutionary forces, irrespective of prokaryote and eukaryote organismal form. The formation of the obviously separated evolutionary lineages (Fig. 1 *a–c*) present strong evidence for multiple incipient speciation events in the EC *B. simplex* metapopulation. The speciation boundaries are clearly set by the ecological constraints of the African-like and European-like slope characteristics. Speciation proceeds mainly sympatrically in EC, as shown by the presence of several evolutionary genomic lineages per slope type. We regard the evolutionary lineages as true evolutionary species driven and cohered by natural selection. Temporal bacterial population dynamics might change strain abundances within lineages, as compared with the sampling series presented here, but would not change the fact of the existence of ecologically separated lineages. The overall results strongly parallel ecologically driven eukaryote incipient speciation in EC. The fruit fly *Drosophila melanogaster* and the soil fungus *Sordaria fimicola* speciate across the slopes (28, 29). Notably, under the current prokaryotic species concept (50, 51), all evolutionary lineages described here, considered by us as true evolutionary species, would have been pooled to one taxonomic species (43). However, this pragmatic and applicable concept lacks a theoretical basis and does not refer to the evolutionary processes of adaptation and speciation due to ecological divergence (52–54). Here, our data add to the endeavors of developing a theory-based prokaryotic taxonomy (55).

Natural selection is the dominant evolutionary force driving and cohering prokaryotic evolutionary lineages (53). Parasexual recombination (lateral gene transfer) in bacteria, the level of which could be probably environmentally selected (56, 57), could exert different but not mutually exclusive effects. First, it could initiate speciation by entering adaptive genetic information into the recipients' genome, allowing for an adaptive boost of the recombinant (53, 58). The aberrant *uvrA* gene sequence of lineage Ba could perhaps be such an example. Second, it could blur and retard to various extents the divergence of genomic lineages by frequent gene shuffling (52). The linkage disequilibrium analyses of the *gapA*, *pgk*, and *uvrA* genes suggest some influence of parasexual recombination in the *B. simplex* metapopulation. However, lateral gene transfer was not strong enough to avoid or blur the genetical separation of the sympatrically living African-like or European-like evolutionary lineages, respectively. Previously, it was argued that recurrent recombination between incipient ecotypes is too rare to constrain adaptive divergence (59, 60). Additionally, the intragenic recombination rate analyses could hint toward incipient sexual isolation across evolutionary lineages. In summary, natural selection overrides the gene mixing effects of parasexuality.

Conclusions and Prospects

The genomic and physiologic characteristics of the *B. simplex* metapopulation in EC are clearly nonrandom and show strong interslope and intraslope divergence shaped by natural selection overriding migrational patterns and leading to adaptive incipient sympatric speciation. The findings of this study of the microevolution of soil bacteria in EC concur well with other prokaryotic and eukaryotic model organisms of EC, including the cyanobacterium *N. linckia*, the fungus *S. fimicola*, the spiny mouse *Acomys cahirinus*, the fruit fly *D. melanogaster*, and the wild barley *Hordeum spontaneum* (19, 20, 23–32, 61). Most of the macrobiotic model organisms (11 of 14) express higher genetic diversity on the generally more stressful African-like slope (21). Higher DNA repair capabilities were observed in the more thermotolerant *D. melanogaster* flies from the African-like slope (61). Higher mutation rates were observed in *D. melanogaster* and *S. fimicola* from the African-like slope (23,

25). Incipient speciation due to nonrandom mating was observed in *S. fimicola* (28) and *D. melanogaster* (29). The *B. simplex* metapopulation, as characterized by the complex phylogenetic–ecological structure presented here, provides an excellent basis for further evolutionary analyses. For example, what is the population genome-wide physiological variation (62)? Are expression patterns similar in African-like lineages A, Ba, C, and D, and do they differ substantially from European-like lineages B, Bae, and E? Such patterns would exemplify ecological convergent evolution in closely related strains overriding phylogenetic history and would confirm active adaptive ecological incipient speciation not only in eukaryotes (21) but also in prokaryotes.

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