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Evolution in action across life at "Evolution Canyons", Israel

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Abstract

The "Evolution Canyon" model reveals evolution in action across life at a microscale involving biodiversity divergence, adaptation and incipient sympatric ecological speciation across life. The model highlights diverse taxa species richness, genomics, proteomics and phenomics phenomena by exploring genetic polymorphisms at protein and DNA levels and, recently, genome-wide gene expression and regulation. Genetic diversity and divergence reveal evolutionary dynamics of natural populations exposed to sharp-interslope, ecologically divergent, tropical and temperate microclimates on a xeric, tropical, "African" south-facing slope (AS) abutting with a mesic, temperate, "European" north-facing slope (ES) separated by 200 meters on average. Four "Evolution Canyons" (EC) are currently being investigated in Israel in the Carmel, Galilee, Negev, and Golan Mountains (EC I-IV), respectively. We identified 2,500 species in ECI (Carmel) from bacteria to mammals in an area of 7,000 m^2. Local biodiversity patterns parallel global patterns. Higher terrestrial species richness was found on the AS. Aquatic species richness prevails on the ES. In 9 out of 14 (64%) model organisms across life, we identified a significantly higher genetic polymorphism on the more stressful AS. Likewise, in some model taxa, we found largely higher levels of mutation rates, gene conversion, recombination, DNA repair, genome size, small sequence repeats (SSRs), single nucleotide polymorphism (SNPs), retrotransposons, transposons, candidate gene diversity, and genome-wide gene expression and regulation on the more stressful AS. Remarkably, interslope incipient sympatric ecological speciation was found across life from bacteria to mammals. The "Evolution Canyon" model represents the Israeli ecological analogue of the Galapagos Islands. Microclimatic selection overrides gene flow and drift, and drives both interslope adaptive divergence and incipient sympatric ecological speciation at a microscale. The EC model could potentially highlight many mysteries of evolutionary biology including the genetic basis of adaptation and speciation, especially now with the rapid high-throughput techniques of whole genome analysis.

Introduction

Evolution in action at "Evolution Canyon"

Local microcosmic natural laboratories, dubbed the "Evolution Canyon" (EC) model (Figure 1A and B) mirror regional and global macrocosmic ecological theaters across life. They represent global domains squeezed into microscales and are very suitable for resolving many problems of evolution in action.[1-4] Likewise, microsites contrasting topographical, thermal, chemical, rock and soil variations [5-12] permit the tracking of evolutionary processes in action at a microscale caused by sharply divergent ecologies. Here, I will attempt a comprehensive updated review of our key studies of the "Evolution Canyon" model. There are four microsites across Israel that have been dubbed "Evolution Canyons" (EC I, II, III, and IV) in the mountains of Carmel, Galilee, Negev, and Golan, respectively[4,12] (Figure 2 and Table 1). These canyons present sharp ecological contrasts at a microscale, permitting observations and experiments in diverse prokaryote and eukaryote taxa across life at small interslope distances (50-100 m at bottom, 200 m at mid-slopes, and 400 m at canyon tops). They harbor thousands of taxa from phages and viruses through bacteria, fungi, plants and animals across phylogeny, sharing the same slope or distributed on abutting, sharply ecologically subdivided slopes. The four canyons generate theoretical and predictable models of *convergent* and *divergent* biodiversity, genome, proteome, and phenome adaptive evolutions and ecological speciational modes and evolutionary rates. The tropical-xeric "African" slopes (AS), or south-facing slopes (SFS), in canyons north of the equator, receive higher solar radiation (200-800% at EC I) than on the opposite temperate-mesic "European" slopes (ES), or north-facing slopes (NFS).[13] This solar radiation is associated with higher temperature and drought on the AS compared to the low-light, shadier, more humid and cooler ES causing dramatic abiotic and biotic interslope divergence which may have originated by water erosion several million years ago after mountain uplifts (Figure 2). These divergences are also particularly accentuated in special slope orientations opened to south-westerly winds. Essentially, *ECs represent the global meeting of xeric Africa and mesic southern Europe at a microsite*. This model facilitates tracking divergent adaptive and speciational evolutions and the identification and ranking of driving forces of evolution, e.g., mutation, genetic drift, gene flow, and natural selection. Even strongly sedentary organisms, e.g., lichens and cyanobacteria, can "migrate" between slopes. Thus, migration is basically excluded from the evolutionary scenario as a determinant divergent force.

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These canyons are extraordinary natural microscale evolutionary laboratories. If rocks, soils, and topography are similar on the opposite slopes then the interslope microclimatic differential remains the major divergent factor. The interslope divergence of biodiversity (e.g., genes, sequences, genomes, proteomes, populations, and species) can be examined *within any species distributed on the slopes* across the opposite *dry* and *humid* ecosystems. This *intraspecific interslope* divergence can be compared in many species across life in an attempt to unravel *intraslope adaptive convergence and interslope adaptive divergence leading to incipient sympatric ecological speciation*.[1-4,12-14] A critical review on soil microfungal communities at the four ECs indicates similarities among EC I, II, IV, and remarkable differences with the extreme desert EC III.[15] Great similarities were found in soil bacteria between EC I and EC II separated by 38 km.[16]

In a genomic and post-genomic era[17,18] all available prokaryote and eukaryote completely or partially sequenced and expressed genomes, including stress genes, are comparable by structural SNPs[19] and microarray whole genome expression[20-22] on both slopes, along with their proteomes and phenomes, i.e., at the interrelated molecular and organismal levels.[4,23] Note that as of December 2007, the complete sequencing of 3,300 species has been completed.[24] Whole genome analysis permits structural, functional, and regulatory comparisons in diverse model organisms. Evaluating interslope *convergence* across life and interslope *divergence* within model organisms on both slopes, adapting to their slope-unique stresses, could unfold *adaptive complexes* and *speciational* trends.

These natural laboratories also permit in depth and long-term stress studies of genome and phenome evolution in adaptation and speciation. Ecological hypotheses could be advanced to test speciation[25-28] and reproductive isolation (e.g., wild barley,[29-30] beetles,[31] and *Drosophila* as conducted at ECI[32]) by hybridization or by behavioral reproductive patterns.[33,34] Microevolution rates, patterns, and processes[35,36] could be deciphered. Using the dissection of quantitative traits loci (QTLs)[37] and the sequencing of stress and reproductive candidate genes[19,32,38,39] interslope molecular divergence can be tracked. Moreover, the dynamics of molecular polymorphisms, transposable elements, lateral transfers, alternative splicing and small RNA regulatory processes are tractable. Likewise, also the *entire genome expression comparison (transcriptomes) and their regulation through hybridization with tiling arrays*[21,22] can be elucidated under the differential interslope stress regimes, either by the tropical "African" (AS) or temperate "European" (ES) climatic contrasting stresses. It is noteworthy that the EC model permits the *exclusion of migration* and even stochastic processes from the evolutionary equation as the evolutionary determinants. Interslope migration is easy from both slopes[40] and large populations exclude stochasticity.

Microclimatic interslope differences underlying biodiversity contrasts at "Evolution Canyon" I, Mount Carmel, Israel

Microclimatic interslope differences of illuminance, temperature and humidity were measured on the "African" south-facing slope (AS) and the "European" north-facing slope (ES) at the EC I microsite over different time periods from September 1996 to February 1998.[13] Illuminance on the AS was significantly higher (200-800%) than on the ES during April-October 1997. Mean daily temperatures and daily temperature ranges were higher on the AS than on the ES, and relative humidity was 1-7% higher on the ES. The AS was more illuminated, less humid and microclimatically more fluctuating than the opposite ES which is covered by a typical Mediterranean maquis forest. Clearly, microclimatic stress at the EC is responsible for the drastic interslope abiotic divergence leading to the dramatic biodiversity divergence across life.[1-4]

Evidence

Species richness

As of mid-2009, we have identified 2,500 species in EC I and more than 1,000 species in EC II, in an area of 7,000 m^2 in each. The *tropical* AS is generally richer in species (in both canyons) of "terrestrial" taxa, and the *temperate* ES is richer in humid taxa, locally reflecting global patterns (Table 1).[1] Recently, we analyzed species richness again at EC I.[41] The analysis included 12 phylogenetic groups representing bacteria, lower plants, vascular plants and invertebrates. Four major taxa (76.5%) could be distinguished according to their site in the canyon. Water-dependent taxa (algae, soil fungi, springtails, mosses, agaricales, woody plants and oribatid mites) ranged primarily on the mesic, cooler, more humid, shadier *temperate* slope. The "heat-dependent" taxa (e.g., reptiles, butterflies, and beetles) prevailed on the *tropical* AS behaviorally adapting for heat gain, and they were significantly more speciose on the AS. Higher speciose richness on the AS included darkling, carpet, leaf and hister beetles as well as grasshoppers. The results indicate that climatic factors, mainly *water, energy*, and *water-energy* dynamics influence species richness across phylogeny and global distributional patterns.[41] Our results suggest similar adaptive and speciational responses to climatic stress across life, signifying that microclimate evolution is active at EC across life.

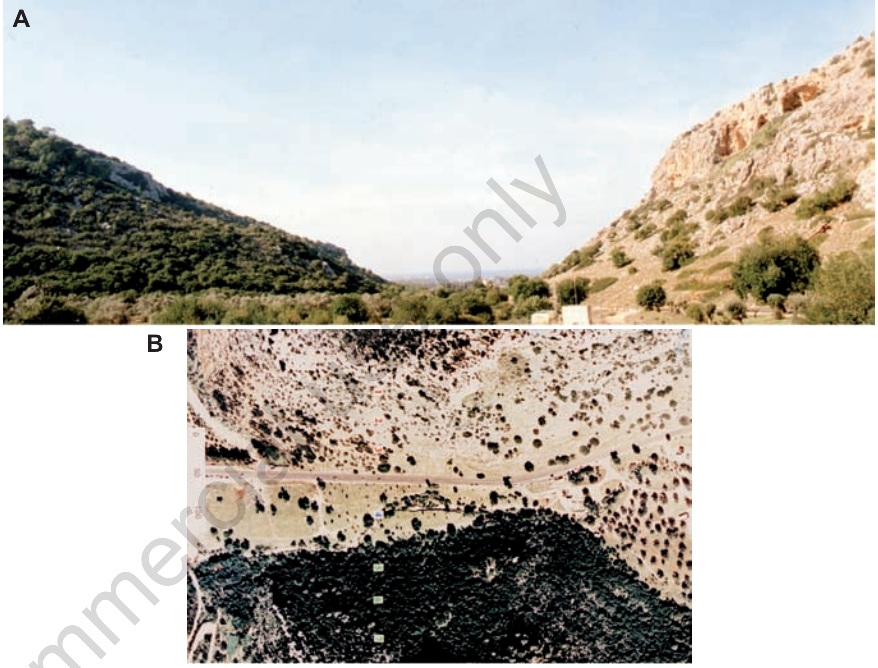

Figure 1. A. "Evolution Canyon", Lower Nahal Oren, Mount Carmel, Israel. A cross-section. B. "Evolution Canyon", Lower Nahal Oren, Mount Carmel, Israel. Air view with the seven assigned stations: three on the SFS (1-3), one at the valley bottom (4), and three on the NFS (5-7).[2] Note the plant formation on opposite slopes. The green, lush, "European", temperate, cool-mesic, north-facing slope (NFS) sharply contrasts with the open park forest of warm-xeric, tropical, "African-Asian" savanna on the south-facing slope (SFS).

Figure 2. The four "Evolution Canyons" in Israel (EC I – IV). Note the interslope divergence in vegetation, even in EC III in the Negev Desert.[4]

Adaptive complexes and incipient sympatric ecological speciation patterns in diverse model organisms at "Evolution Canyon" – an overview of major findings

A major dilemma relates to prokaryote and eukaryote evolution. Are they basically similar in adaptation and speciation patterns responding to environmental stresses?[42] The following evidence indeed suggests similar adaptive and speciational responses across life, regardless of the complexity level, i.e., prokaryotes and eukaryotes. Detailed results appear in the cited papers representing this 20-year long-term project.[1,12] The present overview is systematic from bacteria to mammals aimed at displaying similarity across life.

Table 1. The four "Evolution Canyons" in Israel (EC I-IV): location, climate, annual rainfall, and major taxa plus number of species identified up until the end of 2006. AS:"African slope", ES:"European" temperate slope.

	Identified species*	Annual rainfall (mm)	Coordinates and general climate		ECs
AS = 1044 ES = 1036 bacteria, fungi, plants, invertebrates, vertebrates	2500	600	32°43′N, 34°58′E Mediterranean	Lower Nahal Oren, Mt. Carmel	EC I
AS = 471 ES = 225 lichens, soil fungi, insects, higher plants	1000	700	33°02′N, 35°11′E Mediterranean	Lower Nahal Keziv, Upper Galilee	EC II
AS = 184 ES = 184 lichens, soil fungi, beetles	272	50	29°56′N, 34°58′E Saharo-Arabian Desert	Nahal Shacharut, Southern Negev Desert	EC III
AS = 27 ES = 60 soil fungi, lichens	84	350	32°44′N, 35°43′E Mediterranean	Nahal Mezar, Golan Heights	EC IV

*The AS/ES proportion is probably biased towards the water-dependent taxa that were more extensively studied in the last few years. The total species count includes all identified species, together with the species of the valley bottom, with the exception of higher plants at EC I where the count of frequent species was used.

Prokaryotes

Adaptation and incipient sympatric speciation of *Bacillus simplex* under microclimatically contrasting stresses at "Evolution Canyons" I and II, Israel

The microevolutionary dynamics of prokaryotes in natural habitats, such as soil, is poorly understood in contrast to our increasing knowledge on their immense diversity.[16,43] We performed microevolutionary analyses on 945 soil isolates of *Bacillus simplex* from "Evolution Canyons" I and II *representing similar ecological replicates*, separated by 38 km with highly contrasting interslope abiotic and biotic conditions in each (within an interslope distance of only 50-400 m). Strains representing genetic groups were identical in their 16S sequences, suggesting high-genetic similarity and monophyletic origin (Figure 3). Parallel and nested phylogenetic structures were correlated with ecological contrasts rather than with 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. Interslope DNA repair resistance was also shown in yeast,[44] *Drosophila*[45] and wild barley.[46] Altogether, the results in *B. simplex* suggest similar *interslope* adaptive divergence at a microscale where ecological stress causes adaptive and speciational divergence by climatic selection.

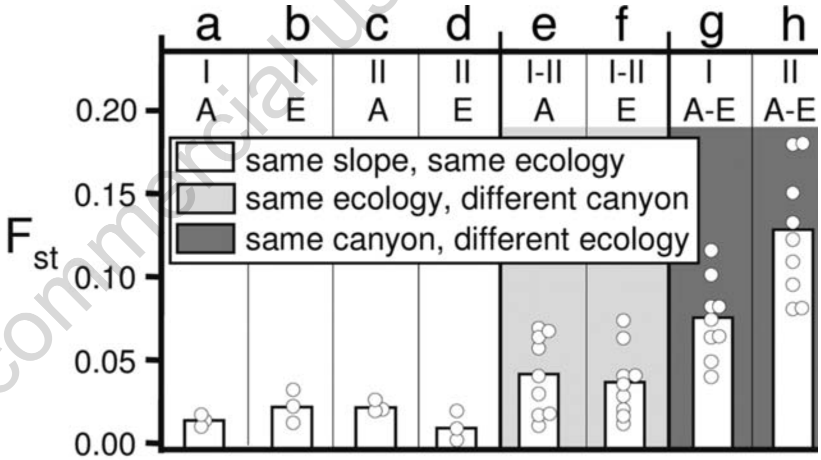

Figure 3. Genomic divergence of *B. simplex* across and within two "ECs", as estimated from pairwise station comparisons of RAPD data within slopes (n = 3) (columns a–d), between slopes of same ecology but different canyon (n = 9) (columns e and f), and of interslope stations within canyons (n = 9) (columns g and h). Open circles indicate a pairwise station comparison. Bars indicate the mean of all pairwise station comparisons. I and II denote EC I and EC II, respectively. "A" and "E" denote African- 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.[16]

We identified *slope-specific* ecotypes with differential thermotolerance matching slope microclimate.[39,47,48] "African" strains grow better than "European" strains at a physiologically stressful high temperature (43.25°C). The process of thermal adaptation of growth rates is currently ongoing at 43.25°C and 20°C, specifically among "African" strains. This reflects further adaptation to the broader temperature range of the AS; hot during the day and cold at night. Likewise, we identified the biological species status of the "African" and "European" ecotypes. We concluded that, despite different biology, prokaryotes, like sexually reproducing eukaryotes, might consist of true species ("ecotypes") and parallel ecological speciation in eukaryotes.[49,50] This may elucidate current confusing kinships in bacteria[42] and shift empirically to a theory based on clustering. A new approach, ecotype simulation, was advanced[50] for identifying ecologically distinct lineages in DNA sequence-based bacterial systematics[49,50] (*J. Sikorski, E. Perry, A. Koeppel, D. Krizanc, A. Rooney, R. Pukall, M. Roberts, N. Field, J. Francisco, S. Vergarg, N. Connor, E. Nevo, F. Cohan, "Incorporating Ecology and Evolution into the Classification of*

Species: Proposal of Bacillus negevensis sp. nov.", in preparation). Intriguingly, we found higher density on the AS at EC I in drought tolerance enhancing bacterium *Paenibacillus polymyxa* in the rhizosphere of wild barley, *Hordeum spontaneum*, apparently promoting drought resistance in the latter.[51] *H. spontaneum* on the AS is indeed more drought-resistant than on the ES.[52]

Adaptive mutations in RNA-based regulatory mechanisms in *Bacillus subtilis* at "Evolution Canyon" III in the Negev Desert

Traditional Darwinism considers that mutations occur at *random*, regardless of the environmental fitness of the resulting mutants,[53] before the second *selective* stage. Indeed, if mutations occur at random, then only selection determines mutation survivorship and evolutionary trajectories. This nonrandom or adaptive mutation hypothesis is still highly controversial.[54] Can mutations at the molecular level that will be favored by natural selection be predicted? Usually, the answer is negative. We studied a bacterial case in EC III in the extreme Negev Desert, in which the regulation system is simple and functionally predictable by dramatically altering the structure. To explore *non-randomness of mutations*, we recorded the effect of extreme environmental stress on RNA-based regulatory mechanisms of the riboswitch in *Bacillus subtilis* at EC III.[54] Empirical results indicate that in the promoter region of the thiamine pyrophosphate (TPP) riboswitch all mutations increase nucleotide GC content in the xeric AS, whereas AT predominates in the *mesic* ES.

Our observations may indicate that the hotter AS strains have, in general, more efficient biosynthesis regulation. This regulation could have evolved under the energetically more stressful conditions of the AS. If so, this may be a case of *nonrandom adaptive* mutations. Further critical confirmation is needed to support this hypothesis. Incorporating ecology and evolution into the classification of bacterial species, we proposed *Bacillus negevensis* sp. nov. from EC III (*J. Sikorski, E. Perry, A. Koeppel, D. Krizanc, A. Rooney, R. Pukall, M. Roberts, N. Field, J. Francisco, S. Vergarg, N. Connor, E. Nevo, F. Cohan, "Incorporating ecology and evolution into the classification of species: Proposal of Bacillus negevensis sp. nov.", in preparation*) as a new species.

Adaptive genome polymorphism in the cyanobacterium *Nostoc linckia* at "Evolution Canyon" I, Israel

Are the oldest known living organisms, the cyanobacteria, still actively evolving at EC I? We examined the cyanobacterium *Nostoc linckia*, a sessile microorganism growing on rock surfaces and constantly exposed to environmental fluctuations of solar radiation, temperature and desiccation. We demonstrated remarkable interslope and intraslope genetic divergence of the *coding* and *non-coding* genomes of *N. linckia* using 211 AFLP (amplified fragment length polymorphism) DNA molecular marker loci from EC I.[55] Genetic polymorphism of *N. linckia* populations on the ecologically *harsher* AS was significantly ($p<0.05$) higher than on the climatically mild ES (p=99.53% vs. 85.78%; $p<0.05$, respectively). Genetic polymorphism (P) and gene diversity (*He*) were significantly correlated with variables influencing aridity stress: solar radiation (r_p=0.956; p=0.046), temperature (r_p=0.993; p=0.0068), and day-night temperature difference (r_p=0.975; p=0.025). We suggest that the AS selects for higher genetic polymorphism in *Nostoc linckia* and other model terrestrial taxa (Figure 4) which is maintained by the combined evolutionary forces of diversifying and balancing selection, as is true regionally in Israel.[3,5,56,57] This emphasizes the similar effects of ecological stress and selection in evolution of prokaryotic and eukaryotic genomes. Is this true only for the AFLP anonymous markers?

Ecological stress and HIP1 genome evolution in the cyanobacterium *Nostoc linckia* at "Evolution Canyon" I

To assess functional interslope differential fitness we examined the highly iterated regulatory palindrome (HIP1) (5'-GCGATCGD-3') in the genome of *N. linckia* at EC I.[58] The interslope divergence was demonstrated by significantly higher diversity (*He* and *v*) and polymorphism (*P*) indices on the more heterogeneous and climatically stressful AS (Figure 4). The intraslope divergence on the AS reflects an upslope increase in genetic diversity indices with a xeric microclimatic increase across the slope. Correlations were found between *P*, *He*, and climatic variables influencing aridity stress: solar radiation, temperature and day-night temperature differences suggesting that interslope adaptive divergence was caused by natural selection to environmental stress. HIP1 presumably promotes genome diversity to cope with climatic stress in cyanobacteria.[58] Is this pattern also true for candidate genes?

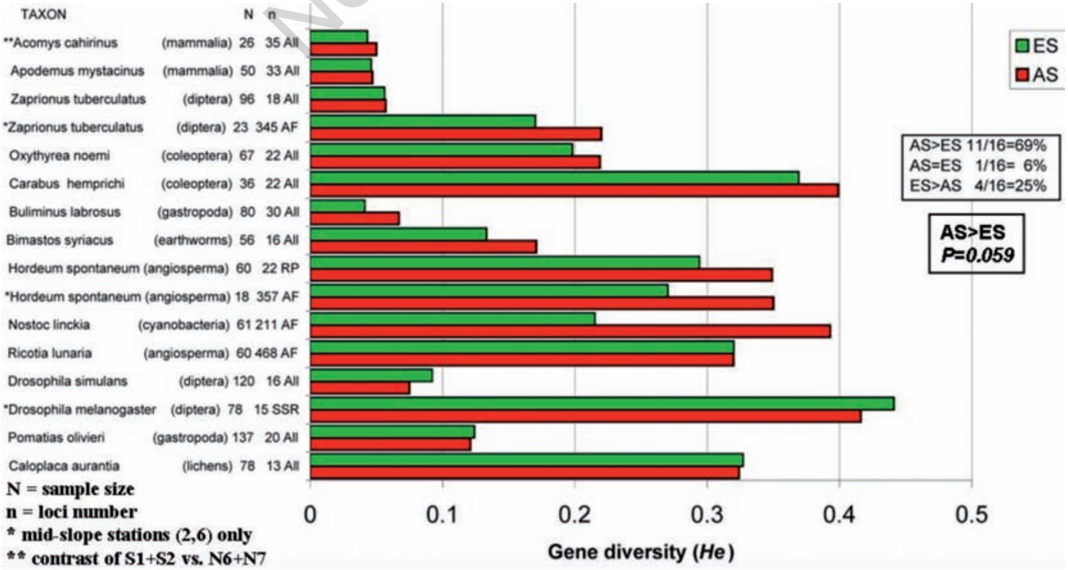

Figure 4. Genetic diversity of diverse taxa from bacteria (*Nostoc linckia*) to mammals (*Acomys cahirinus*). Lower Nahal Oren, Mount Carmel: comparison between the "African" (AS) and "European" (ES) slopes. The scientific name is written under the taxon and the taxonomic affiliation appears in parenthesis. Symbols in right hand column: all, allozymes; AF, AFLP; RP, RAPDs; SSR, small sequence repeats (microsatellites).

Long-term microclimatic stress causes rapid adaptive radiation of *kaiABC* clock gene family in a cyanobacterium, *Nostoc linckia,* from "Evolution Canyons" I and II

Can we find interslope divergence in the biological clock genes? Cyanobacteria are the first prokaryotes possessing circadian rhythms, controlled by a cluster of three genes: *kaiA*, *kaiB*, and *kaiC*. We demonstrated considerably higher genetic polymorphism and extremely rapid evolution of the *kaiABC* gene family in the cyanobacterium *Nostoc linckia*, permanently exposed to acute natural stress, parallel in *both* EC I and EC II,[59] as shown in *Bacillus simplex*.[16] The family consists of five distinct subfamilies (*kaiI-kaiV*) comprising at least 20 duplicated functional genes and pseudogenes. The obtained data suggest that the duplications reinforce function (*superfunctionalization*) of *kai* genes that have adaptive significance to extreme and fluctuating environments, and some of them are evolutionarily quite recent (~80,000 years ago). The observed patterns of within- and between-subfamily polymorphisms of the clock gene family controlling circadian rhythmicity, one of the fundamental features in most organisms, indicate that positive diversifying, balancing, and purifying selection regimes are the *principle driving forces* of the *kai* gene family's evolution in *Nostoc linckia*.

Do eukaryotes display interslope adaptive divergent complexes as prokaryotes?

Eukaryotes

Fungi and lichens

Adaptive oxidative and UV stress responses in yeast at "Evolution Canyon" I

We identified a total of 25 yeast species at EC I. The total number and frequency of occurrence of yeast species was higher on the ES than on the AS.[60] Genome-wide gene expression analysis of the baker's yeast *Saccharomyces cerevisiae* indicated that sunny AS strains were significantly more adaptively tolerant than ES strains to peroxide stress. [20] Population genetic analysis of 19 microsatellite loci revealed high allelic diversity and variation of diploids, triploids and tetraploids largely reproducing clonally.[61] UV radiation is one of the most important physical parameters influencing yeast growth in nature. We have also shown by analyzing 46 strains higher adaptive tolerance of *S. cerevisiae* on the "African" slope to UVA and UVC radiations and to methyl methansulfonate MMS to DNA damaging agents.[44] Parallel higher AS DNA repair response was also demonstrated in soil bacteria,[16] fungi,[62] *Drosophila* and wild barley.[45,46]

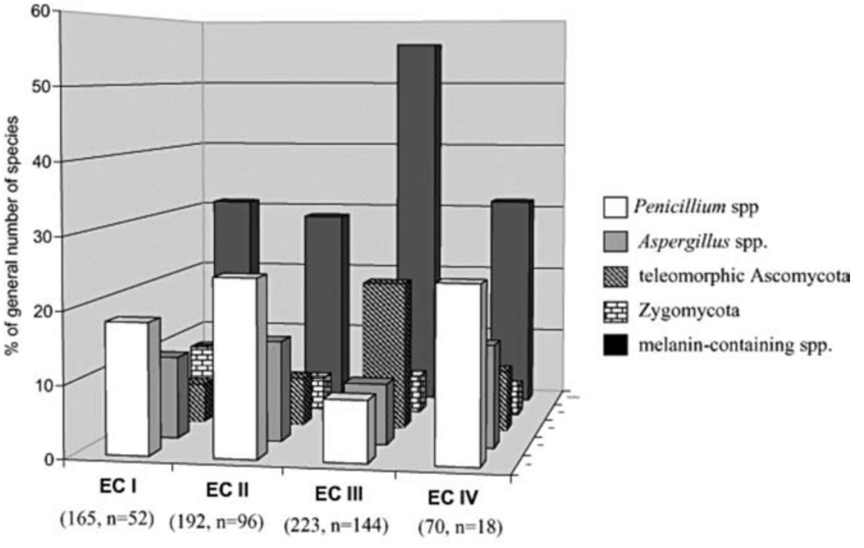

Figure 5. Proportions of main groups in mycobiotas of "Evolution Canyons" (the general number of species and number of samples examined are shown in parenthesis). Differences between "Evolution Canyons" (χ^2 test): EC III vs. EC I, $\chi^2(4) = 25.07$, $p < 0.0001$; EC III vs. EC II, $\chi^2(4) = 34.90$, $p < 0.0001$; EC III vs. EC IV, $\chi^2(4) = 17.91$, $p = 0.001$. EC I vs. EC II, $\chi^2(4) = 5.92$, $p = 0.205$; EC I vs. EC IV, $\chi^2(4) = 4.22$, $p = 0.377$; EC II vs. EC IV, $\chi^2(4) = 0.27$, $p = 0.992$.[15]

Adaptive complexes in soil fungi: local and regional comparisons at all four "Evolution Canyons" (EC I-IV)

How does soil microfungi diversity respond to climatic stresses? We compared and contrasted interslope soil microfungi across the four "Evolution Canyons" (EC I-IV), both *locally* in each EC[63-66] and *regionally* in all four ECs.[15]

We analyzed the number of colonial-forming units (CFU), species composition, species richness, diversity level, and dominant groups of species. We identified the following number of species: 166, 192, 223, and 70 species in EC I, EC II, EC III, and EC IV, respectively. Fungal communities were more diverse on the stressful xeric AS than on the mild mesic ES and the valley bottom (VB). The AS was also characterized by a higher level of population diversity. The greatest intra- and interslope differences were found by comparing sunny open niches with shady niches under trees on the AS. Dominance of *Fusarium* species and frequent occurrence of adaptive dark-colored melanic micromycetes characterized sunny soils.[15,62] *Penicillum* species dominated the mesic, forested ES and shady soils of the AS (Figure 5). Seasonally, winter slope communities were less heterogeneous. Forest localities were dominated by mesophilic *Penicillium species*. Remarkably, the sunny localities on the AS were dominated by melanin-containing micromycetes primarily pronounced in the summer and by high occurrence and abundance of thermotolerant and thermophilic *Aspergillus* and *Fusarium* species, and sexual ascomycetes that are stress-selected fungi. Notably, they were more than ten times more abundant on the AS than on the ES, peaking in the sunny summer community. The results strongly suggest a microscale adaptive spatiotemporal inter- and intraslope divergence in soil mycobiota composition. Clearly, microclimatic and edaphic natural selection determine soil fungal diversity patterns locally and regionally (Figure 5).

In the extreme Negev Desert at EC III, Nahal Shaharut, stress-selected, slow-reproducing, melanic species with large multi-celled conidia were dominant (see below). By contrast, in the forest localities of the northern canyons (EC I and II) and in EC IV, where the AS is agriculturally disturbed and predominantly ruderal-selected, fast-reproducing *Penicillium* species prevail. Climatic and edaphic natural selection appeared to be the major factors affecting adaptive diversity patterns of soil microfungi.[15] Adaptive differential growth rates of fungi from the *Aspergillus niger* group in contrasting osmostress environments of the Dead Sea (DS) and AS and ES of EC I show decreasing salt tolerance adaptively, as expected DS>AS>ES.[67]

An extraordinary discovery of soil fungi at "Evolution Canyon" III, extreme southern Negev Desert

An *extraordinary* and *unexpected* 223 species representing 80 genera were found at

EC III, Nahal Shaharut (Figure 2), an extreme southern site in the Negev Desert.[65] Superdominance of dark-colored thermotolerant species with large multi-celled conidia characterized this unique mycobiota. Density increased in the shady valley bottom. The lowest density was found in the most stressful summer and spring. Only harsh desert climatic and edaphic strong selection can explain these results. The mycobiota display a remarkable adaptive strategy to harsh, hot, and dry desert stress reflected by: (i) superdominance of melanic species; (ii) Aspergilli (mainly *A. fumigatus* and *sexual* ascomycetes) comprise a basic part of the thermotolerant mycobiota; (iii) small spatiotemporal variation in community structure but drastic fluctuations in microfaunal densities with high-positive dependence on organic matter content; and (iv) decreasing biodiversity levels in microclimatically extreme localities and climatically extreme seasons. The most remarkable phenomenon of the desert mycobiota is its unique *adaptive complex of melanism, sexuality, drought and UV-resistant morphology*.

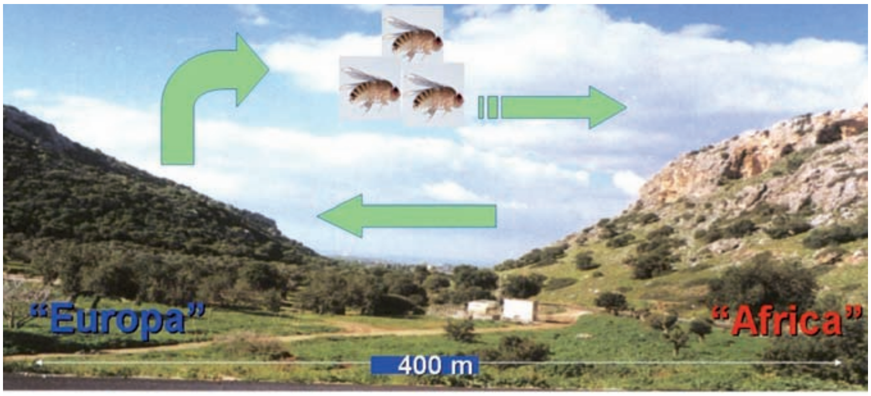

Figure 6. *Drosophila melanogaster* at "Evolutionary Canyon" I: interslope migration.[128]

Adaptive mutation frequencies in soil fungi at "Evolution Canyon" I

Will mutation frequencies differ on the opposite slopes of EC I? We first found that the frequencies of new spontaneous mutations expressed in a common mild lab environment differed between strains of the fungus *Sordaria fimicola* according to the level of stress in their sites of origin.[68] Strains isolated from the harsher, drier AS of EC I had higher inherited spontaneous mutation frequencies (2.3-4.4% for ascospore color mutations, summed up over all relevant loci) than those (0.9-1.3%) isolated from the milder, moister, and lusher ES; *S. fimicola* has no asexual spores and reproduces sexually by ascospores.

To see whether natural genetic variation for spontaneous mutation frequencies is a more general phenomenon and is subject to stressful environment-related natural selection, we examined spontaneous mutation frequencies in two other filamentous fungi from EC I, *Penicillium lanosum* and *Aspergillus niger*.[69] In contrast to *Sordaria fimicola*, these two species have abundant asexual conidia but no sexual cycle. All three ascomycetes species live on dung and plant remains and are vegetative haploids.[15,63]

We found remarkable, even higher, spontaneous mutation frequencies in the AS strains than in the ES strains in these two additional non-sexual soil fungi, primarily interslope but also within slopes. In *Penicillium lanosum*, spontaneous mutation frequencies ranged from 6.51-11.56% for the AS strains, compared with 0.29-2.38% for the ES strains, with no overlap between ranges. In *Aspergillus niger's* total mutation frequencies in AS, strains ranged from 2.30% to 4.89% compared with only 0.42-1.50% for ES strains, again with no overlap between the ranges. In the pooled values, the mutation frequencies for conidial color mutants, morphological mutants, and total mutants were 4.27-, 8.86-, and 5.68-fold higher, respectively, in AS strains than in ES strains for *P. lanosum*, and corresponding values of 2.44-, 5.36-, and 3.87-fold in *A. niger*. All differences were highly significant with extremely low *p* values. This *non-random higher mutation rate* on AS seems evolutionarily adaptive and important, reflecting a positive correlation between mutation diversity and stress. The harsher and more fluctuating AS selects for higher mutation rates, as is generally true for genetic polymorphism;[3,5,56,57] [Figure 4] *locally, regionally, and globally*.[70]

Inherited differences in crossing over and gene conversion frequencies between wild strains of *Sordaria fimicola* from "Evolution Canyon" I

Recombination generates new combinations of existing genetic variation and is, therefore, important in adaptative evolution. We investigated whether there was natural genetic variation for recombination frequencies and whether any such variation was environment-related and possibly adaptive.[71] Crossing over and gene conversion frequencies often differed significantly in a consistent direction between wild strains of the fungus *Sordaria fimicola* isolated from the harsher AS than on the milder ES at EC I. First and second generation descendants from the AS had higher frequencies of crossing over in locus-centromere intervals and of gene conversion compared to those of ES strains. There were some significant differences between strains within slopes, but these were less marked than between slopes. Such inherited variation could provide a basis for natural selection for optimum recombination frequencies in each environment. The conversion analysis was made using only conversions to wild type. Of the 20 recombination comparisons between AS and ES strains, 16 gave highly significant differences, always with higher values on the more stressful AS, *which selects for higher recombination*.

Natural selection causes microscale allozyme diversity in the lichen *Caloplaca aurantia* at "Evolution Canyon" I

Will symbiotic lichens follow the genetic patterns of soil fungi and cyanobacteria? We studied allozymic diversity in proteins encoded by 13 putative allozyme loci in 78 different thalli of the lichen *Caloplaca aurantia* from EC I.[72] Out of 13 putative genetic loci tested, 12 (92%) were polymorphic, more than in *H. spontaneum* (71.4%) at the same site. Only Sod was monomorphic. In total, the 13 putative genetic loci comprised 31 alleles, 30 and 29 alleles on the AS and ES, respectively. Allozymic variation of *C. aurantia* paralleled that of *H. spontaneum* at the same microsite in displaying higher levels of genetic polymorphism on the AS, climaxing uphill. Lichens follow the general trend across life at EC of higher polymorphism on the AS (Figure 4).

Wild barley, *Hordeum spontaneum*, in "Evolution Canyon" I: A Major Model Organism at "EC" I

Wild barley, *Hordeum spontaneum*

I have previously described the origin, evolution, population genetics, and resources for breeding of wild barley, *Hordeum spontaneum*, in the Fertile Crescent.[6] *Hordeum spontaneum* is the wild ancestor of cultivated barley, a good biological species[73] and an excellent annual diploid evolutionary model. Extensive genomic studies have been conducted on wild barley at the Institute of Evolution, University of Haifa.[12] The center of origin and diversity of wild barley is the Near East Fertile Crescent, where it displays regionally rich *non-random adaptive* diversity to climatic and edaphic diversity in both *coding* and *non-coding genomes*. I will now review the locally extraordinary adaptive radiation and incipient sympatric ecological speciation of *H. spontaneum* at the EC I microsite.

Phenotypic adaptive radiation of wild barley in "Evolution Canyon" I colon germination patterns

Ecological-genetic differentiation and dry storage temperatures regulate the germination of caryopses of *Hordeum spontaneum*. After dry storage for more than 68 days at high temperatures, caryopses germinated in their dispersal units.[74] This dormancy is clearly adaptive in the Israeli dry summer and breaks before the winter rains. The degree of after-ripening (dormancy) was found to differ in three local populations tested in wild barley in EC I: "European" slope (ES), valley bottom (VB), "African" slope (AS), and the outgroup of the northern Negev Desert population of Sede Boker (SB). Germination declined adaptively following decreasing humidity: (ES>AS>SB). AS caryopses had significantly deeper dormancy than ES caryopses in follow-up studies.[75-77] Likewise, the seedling revivability after periods of drought was also significantly higher on the AS than on the ES. Dormancy and seedling revivability at the canyon bottom (VB) was intermediate. Clearly, the EC I microsite demonstrates locally adaptive germination and revivability patterns, imitating regional patterns across Israel. In the germination stage, natural selection adapted wild barley to xeric micro- (e.g., AS) and macro- (e.g., Israeli deserts) environments by increasing the diversity of after-ripening, enhancing desiccation tolerance[4] and improving root-length growth.[75] AS populations of wild barley are more drought-tolerant than ES populations.[78] Transplant experiments support the slope-divergent patterns of wild barley.[79]

Genotypic patterns of wild barley in "Evolution Canyon" I

A series of ecological-genomic studies in wild barley indicate generally higher polymorphism on the xeric-tropical AS slope. This was shown for allozymes[72] based on 28 putative loci in 170 plants where polymorphism, heterozygosity, and allele and gene diversity were higher on the more stressful and variable upper station of the AS, as expected by the niche width variation hypothesis.[80] The AS represents a broader niche and the environmental theory of genetic diversity,[3,5,56,57] predicts positive correlation between genetic polymorphism and ecological diversity. Diversifying natural (microclimatic) selection appears to be the major evolutionary driving force causing interslope genomic divergence and gametic phase disequilibria in wild barley. ADH-3 diversity[81] and RAPD DNA[82] were also higher on the AS.

Adaptive climatic molecular evolution in wild barley at the ISA defense locus

Wild barley, *Hordeum spontaneum,* represents a significant genetic resource for crop improvement in barley, *Hordeum vulgare*, and for evolution and domestication studies. The *Isa* gene from barley has a putative role in plant defense. This gene encodes a bi-functional α-amylase/subtilisin inhibitor that inhibits the bacterial serine protease subtilisin, fungal xylanase, and the plant's own α-amylases suggesting that this protein may also be important for grain quality from a human perspective. We identified 16 single nucleotide polymorphisms (SNPs) in the coding region of the *Isa* locus of 178 wild barley genotypes from eight climatically divergent sites across Israel.[19] The pattern of SNPs suggested a large number of recombination events within this gene, indicating that the low-outcrossing rate of wild barley is not a barrier to recombinant haplotypes becoming established in the population. Seven amino acid substitutions were present in the coding region. Highly significant correlations were found between diversity at the *Isa* locus and key water variables, evaporation, rainfall, humidity and latitude. In addition, there are also local differences between *Isa* diversity at EC I and Tabigha microsites. Natural selection is the most plausible explanation for the observed gradient in diversity between the AS and ES at EC I. The regional pattern of association across Israel, as well as the interslope divergence at EC I, suggest selective sweeps and directional selection in the wetter climates with resulting low diversity in northern Israel and the ES at EC I. By contrast, balancing or diversifying selection occurs in the drier climates in southern Israel and locally at the AS of EC I, resulting in much higher diversity.

rDNA polymorphism in wild barley at "Evolution Canyon" I

Are intergenic spacers subjected to natural selection? The variation in length of the intergenic spacer (IGS) region of the ribosomal DNA repeat unit was examined in 63 accessions of wild barley, *Hordeum spontaneum*, and seven accessions of cultivated barley, *Hordeum vulgare*.[83] Sixteen spacer-length variants (SLVs) observed in this study presumably belonged to two known rDNA loci (Rrn1 and Rrn2). Each accession had one or more variants, which together represented the rDNA phenotype. The rDNA phenotypes of wild barley were widely diverse and differed substantially from those of cultivated barley. The SLV phenotypes and the corresponding alleles were shown to be largely correlated with different *climatic, edaphic,* and *ecogeographical* variables and niches at both EC I and Upper Galilee Tabigha microsites. A particular rDNA phenotype of a genotype could be used to predict the climate and soil to which the genotype belonged. This very sharp microsite *ecogeographic* variation in ribosomal DNA appears adaptive in nature and is determined by climatic and edaphic natural selection. In contrast to common beliefs, intergenic rDNA spacers are subject to natural selection even at microscales and apparently regardless of population size, as was shown earlier in subterranean mole rats.[84] Natural selection is also the major architect of rDNA intergenic spacer polymorphism in wild emmer wheat.[85]

Genome size evolution of wild barley, *Hordeum spontaneum*, by BARE-1 retrotransposon dynamics in response to sharp microclimatic divergence at "Evolution Canyon" I

Can genome size vary due to environmental stress? Complementarily, are retrotransposons "junk DNA"? The replicative spread of retrotransposons in the genome creates new insertional polymorphisms, increasing retrotransposon numbers and, potentially, their share of the genome and genome size. The *BARE*-1 retrotransposon constitutes a major dispersed active component of *Hordeum* genomes, and *BARE*-1 number is positively correlated with genome size. We examined interslope genome size and *BARE*-1 insertion patterns and numbers in wild barley, *Hordeum spontaneum,* in EC I across a microclimatic gradient.[86] A simultaneous increase in the *BARE*-1 copy occurs upslope with increasing dryness on both slopes at EC I but especially on the drier AS. The simultaneous lower recombinational loss would favor maintenance of more full-length copies, enhancing the ability of the *BARE*-1 family to contribute to genome size growth. These local data parallel regional trends for *BARE*-1 in *H. spontaneum* across Israel[87] and, therefore, seem to reflect adaptive selection for *increasing genome* size through retrotransposon activity. Similar trends of larger genome size in the xeric "African" slope of EC I have also been found in the carob tree *Ceratonia siliqua*,[88] the annual legume *Lotus peregrinus*,[89] Cyclamen[90] and in females of the beetle *Oryzaephilus surinamensis*.[31] Genome size is dynamically fluctuating and seems to positively and adaptively correlate with stress.

Genomic microsatellite adaptive divergence of wild barley by microclimatic stress in "Evolution Canyon" I

Are microsatellites (SSRs) neutral "junk DNA"? We examined diversity levels and patterns of 19 nuclear microsatellites and 4 chloroplast microsatellites in 275 genotypes of wild barley, *Hordeum spontaneum*, in seven stations at the EC I microsite.[91] We found strikingly significant inter- and intraslope diversities based on the 19 nDNA SSR alleles, climaxing with a remarkable genetic distance between mid-slope stations (D_A = 0.481) across a distance of 200 m. This genetic distance is as large as that found between *H. spontaneum* populations of Jerusalem and Sede Boker, separated by 100 km (500-fold more distant than at EC I). nuDNA SSR genic differentiation was very high between opposite slopes, with *Gst* = 0.187; for chDNA SSR this value was 0.127. Our results are inexplicable by stochastic processes and indicate that microclimatic diversifying selection is the major evolutionary fast-acting interslope force, suggesting that natural selection is a major differentiating force of both *coding* and *noncoding* SSRs, linking *micro*- and *macro*evolutionary processes.[92] Far from being neutral "junk DNA", SSRs appear to be subjected to natural selection forming adaptive functional patterns.[93,94]

Adaptive structural and expressional dehydrin evolution in wild barley at "Evolution Canyon" I

The dehydrin gene family in plants is cardinal in drought resistance.[95] We showed dramatic differential expression of dehydrins in response to water stress in resistant and sensitive wild barley.[96] At EC I we examined single nucleotide polymorphism (SNP) sequences of dehydrin 1 (*Dhn* 1) in 47 plants (genotypes) from the opposite slopes.[97] Out of 29 haplotypes, derived from 45 SNPs in a total of 708 bp sites, only a single haplotype was common to both slopes. Genetic divergence was significantly different between populations, particularly between the slopes. These results clearly indicate adaptive natural microclimatic selection as the major evolutionary divergent driving force in dehydrin drought resistance evolution.[97]

Evidence for postzygotic incipient sympatric speciation in wild barley, *Hordeum spontaneum*, based on hybridization estimates at "Evolution Canyon" I

If the interslope genetic distance is so big, is it conceivable that wild barley at EC I initiated *incipient sympatric ecological speciation*? Indeed, preliminary evidence was presented for postzygotic incipient sympatric ecological speciation in wild barley, *Hordeum spontaneum*, at EC I.[4,29,30] The evidence is based on 40 intraslope and 50 interslope crosses. The F_1 hybrids obtained from these crosses were examined for 13 vegetative and reproductive traits. The results indicated that in 3 out of 13 analyzed traits in all crosses, the *interslope* crossbred plants were significantly inferior to *intraslope* hybridizations. The results for an additional 6 out of 13 traits (69%) supported the hypothesis of *interslope* hybrid inferiority[30] (see also Table 5 in Parnas, 2006[29]). If only crosses confirmed by microsatellites are considered, 12 out of 13 traits (92%) showed *interslope* hybrid inferiority[30] (see also Table 7 in Parnas, 2006[29,30]). If substantiated, especially in forthcoming generations, then wild barley in EC I will have started on a course of incipient ecological sympatric speciation initiating postzygotic isolation. Preliminary unpublished results from the second generation conducted in 2007 points in the same direction. This preliminary conclusion is also supported by the large interslope genetic distance[91] as well as by drastic interslope SSR physiological divergences in germination patterns[74] and drought resistance.[52,76,77,98] If slope selection overruns migration (gene flow), as in several model taxa in EC I, this process may progress (see below).

Biodiversity and interslope divergence of vascular plants caused by microclimatic differences at "Evolution Canyon" I

What does the interslope divergence in vegetation patterns tell us? Species diversity of plants was recorded in 1992 and 1993 at seven stations of the "Evolution Canyon" I microsite.[99] Higher solar radiation on the AS causes warm, xeric, savannoid plant formation versus temperate, cool, mesic, dense forest maquis on the "European" slope (ES), and riverine, segetal plant formations on the valley bottom (VB) (Figure 1A and B). In an area of 7,000 m², we recorded 320 vascular plant species in 217 genera and 59 families. Plant cover varied from 35% (AS) to 150% (ES). Annuals predominated (61.3%) among all species. Remarkably, the AS and ES varied in species content, *sharing only 31-48% of species*. The interslope plant divergence at EC II (Galilee) is even stronger; 255 plants grew on the AS and only 54 species on the ES.[100] At EC II only 27 species are found on both slopes (11.6%), separated by only 50 meters at the bottom. The contrast of *slope-unique* species at EC II is dramatic; 178 versus 22 on the AS and ES, respectively ($p<0.0001$). At both EC I and II phytogeographical types and species composition varied drastically among the two slopes and valley bottom. Both ECs demonstrate adaptive contrasting ecosystem complexes, savannoid (AS) and forested (ES), indicating natural selection in action at a microsite, mirroring regional and global distributional patterns caused by differential climatic stresses.[1,13]

Drought and light anatomical adaptive leaf strategies in three woody species caused by microclimatic selection at "Evolution Canyon" I

Is the interslope divergence reflected also by woody long-lived species? We quantified seven structural and morphological characteristics of leaves from three woody species: olive, carob, and pistacia (*Olea europaea, Ceratonia siliqua, and Pistacia lentiscus*, respectively).[101a] Our results indicate leaves of these three species on the AS were smaller and *xeromorphic* compared to the more *mesomorphic* leaves of the ES. Discriminant analysis succeeded in significantly differentiating between leaves collected from seven stations for the three species. The results suggest leaf xeromorphic drought adaptations on the arid AS, caused presumably by xeric climatic selection. By contrast, light stress may be an important determinant of the thicker palisade layers in *P. lentiscus* growing in shaded understory niches on the ES, where, at least in some places, light is the limiting factor for optimal photosynthesis. This, of course, is not a crucial factor in trees, such as olives and carobs. Notably, this interslope ecological contrast was also identified by allozymes in *Lotus peregrinus*[102] and by full genome tiling arrays in the annual *Ricotia lunaria*.[21,103]

Phytohormonal adaptations in woody plants at "Evolution Canyon" I

Are the interslope differences of woody plants primarily anatomical or also physiological? We conducted preliminary estimations of phytohormone levels of indolacetic acid (IAA), abscisic acid (ABA) and cytokinins in leaves of three woody species distributed at the EC I on the opposite slopes of olive, *Olea europaea*, carob, *Ceratonia siliqua* and the live oak *Quercus calliprinos.*[101b] IAA and cytokinins were largely higher in ES trees, adaptively promoting growth primarily in *Q. calliprinos* and *O. europaea*. By contrast, the highest free ABA content was recorded in AS samples of *O. europaea* and *Q. calliprinos* adaptively promoting drought resistance. These preliminary adaptive physiological results complement the anatomical findings but await further critical tests. Our results indicate that perennial bushes and long-living trees evolve drought resistance and light-trapping anatomical and physiological slope-specific fitness adaptive strategies similar to those of annual plants (e.g., the legume *Lotus peregrinus*,[102] the crucifer *Ricotia lunaris*[103]) over very short but contrasting ecosystems.

In summary, adaptive strategies for drought resistance appear to be of prime importance on the warm and xeric AS. By contrast, light-trapping adaptive strategies appear important on the more humid and shadier ES. This has also been found by genome-wide analysis of the annual crucifer *Ricotia lunaria.*[21,22,103] These interslope divergences appear to involve coupled genetic-environmental, anatomical and physiological adaptations, as is clearly indicated by transplant experiments in wild barley from the opposite slopes.[79] The AS *xeromorphic* and ES *mesomorphic* adaptations all have a genetic basis, but they can vary phenotypically following their normal reactions of shape and form in the same genotype.

Developmental instability and stress in eight plant species caused by microclimatic selection at "Evolution Canyon" I

Does fluctuating asymmetry (FA), presumably a stress signature, indicate a higher drought stress on AS? At EC I we studied the developmental instability of eight perennial plant species (*Ceratonia siliqua, Pistacia lentiscus, Salvia fruticosa, Cyclamen persicum, Olea europaea, Stachys palestina, Quercus calliprinos,* and *Rhamnus palestinus*) by measuring the fluctuating asymmetry of their leaf margins reflecting stress effects.[104]. Seven out of eight plant species (*O. europaea* was an exception) and the shrub *Calycotome villosa* displayed higher *fluctuating asymmetry* and *xeromorphic* leaves on the AS than on the ES, and all the studied species displayed higher mesomorphic leaf area on the ES. Higher FA on the AS is presumably due to the combined stress effect of higher illuminance, higher temperature and lower humidity on the AS than on the ES.[104,105] In the live oak *Quercus calliprinos*, secondary leaf veins displayed higher FA on the AS.[105] After correction for negative size scaling, only five species were more asymetric on the AS (work in progress, unpublished data).

Biodiversity, genetic diversity and spatiotemporal adaptive radiation in invertebrates at "Evolution Canyon" I

Do invertebrates follow the general interslope pattern at EC? We analyzed biodiversity of invertebrates at EC I taxonomically, genetically, morphologically and physiologically. The analysis included diplopods, grasshoppers, scorpions, earthworms and gastropods[106] (see also below). Species richness was significantly higher on the more stressful, xeric and fluctuating tropical AS than on the mesic, milder and temperate ES. Levantine species comprised 42% on the AS and only 18% on the ES. We found interslope drought-adapted divergence in body size. Summer species, e.g., grasshoppers, active in the daytime, were larger on average on the AS, possibly providing better drought resistance due to optimal surface-volume ratio. By contrast, spring beetles and gastropod landsnails were larger on the ES, corroborating the Bergman ecological rule. The Bergman Rule, in warm-blooded vertebrates, states that the small-sized geographical populations of a species are found in the warmer part of the range, the larger-sized populations in the cooler regions. This has been shown here in beetles and landsnails. Significantly higher genetic diversity was found in AS populations in six taxons of earthworms, grasshoppers, and beetles, as was also found in other model organisms at EC I (Figure 4). The average interslope distance was Wright's F_{st}= 0.034. In terrestrial invertebrates, as elsewhere, *selection overrides migration* and leads to higher biodiversity and genetic diversity on the AS, and ecological rules can be unraveled between the opposite slopes at a microsite.

Biodiversity patterns and densities of invertebrates on the opposite slopes of "Evolution Canyon" I

Scorpion biodiversity and interslope divergence at "Evolution Canyon" supported a major general hypothesis that the homogenizing effect of migration and stochasticity are not able to eliminate the interslope species differences in biodiversity despite a valley bottom distance of only 100 meters.[107] A total of 195 scorpion specimens representing six species were recorded at EC: *Androctonus crassicaudata, Hottentotta judaicus, Compsobuthus carmelitis, Scorpio maurus fuscus, and Nebo hierochunticus*. Scorpions, like reptiles, butterflies, darkling and skin beetles, and grasshoppers, are richer in species on the "African" slope. In the richness and abundance of scorpions reveal the overruling of climatic selection caused by higher insolence on the AS leading to higher ecological heterogeneity of the AS savanna-type ecosystem which selects for thermophilic species.

Coleoptera in "Evolution Canyons" I and II

At EC I we described 633 beetle species representing 47 families in an area of 7,000 m^2, with about 30% of all described species in EC I.[108,109] Significantly more species have been identified on the broad-niche *tropical* AS than on the narrower niche *temperate* ES (412 vs. 346; p<0.05). We identified one species new to science, 10 new species to Israel, and 30 species new to Mount Carmel. At EC II[100] we identified 513 beetle species representing 50 families in an area of 7,000 m^2,[100] significantly more species found on the AS than on the ES (307 vs. 198; p<0.001). Slope-*unique* species were significantly higher on the AS than on the ES (171 and 62, respectively, p<0.001). One species was new to science, 19 species (3.7%) new to Israel, and 136 species (26.6%) new to the Upper Galilee region. The repetitive pattern in both EC I and EC II is consistent with the niche width variation hypothesis[80] supporting higher biodiversity on the tropical broader-niche AS.

We found higher representation of local arid Levantine species on the AS than on the ES due to their speciation and adaptation to the trend of long-term aridization of the Near East region. Thus, microsite in-depth analysis unravels numerous *non-randomly* distributed species, as is highlighted in 90 chrysomelid species,[110] 39 buprestid species[111] and other beetle species (leaf, tenebrionid, sap beetles, dermestid beetles, ants and grasshoppers).[112,113] The local interslope mesic-xeric microscale transect at EC (ES→AS) parallels the regional Israeli macroscale aridity transect from the Galilee to the Negev. Importantly, new species to science are constantly accumulating as a by-product in Israel, e.g. Mount Carmel and Galilee Mountains.

Genetic divergence in populations of the beetle *Carabus hemprichi* from "Evolution Canyon" I

What is the genetic pattern of the beetle at EC I? Interslope differences in genetic diversity were tested in ground beetles, *Carabus hemprichi* (Coleoptera, Carabidae), from EC I.[114,115] Twenty individuals from the "African" (AS), 16 from the "European" (ES), and 12 from the valley bottom (VB) were tested for variation in 22 putative allozymic loci. Numbers of alleles per locus (A), allelic polymorphism (P), observed heterozygosity (H), and gene diversity (H_e) were higher on the AS as expected; the ratios were A = 2.2/2.0, p= 0.91/0.82, H = 0.179/0.163, and H_e = 0.399/0.369. Although the differences were not statistically significant in multilocus comparison, we found significant deviations from zero in genetic distance between the slopes in four loci. Similar interslope genetic divergence at EC I was found in the scarabeid beetle *Oxythrea noemi*, n=88; 51 from the AS, 16 from the ES and 22 from the VB were tested for 22 allozyme loci,[116] supporting the niche width variation hypothesis[80] which is broader on the AS, or more generally the environmental theory of genetic diversity predicting positive correlation between stress and genetic diversity[3,5,56,57] (Figure 4).

Seasonal and spatial distribution of butterflies (*Lepidoptera-rhopalocera*) in "Evolution Canyon" I

Do *flying* butterflies mirror *sedentary* beetle species distribution patterns? Twenty-five species recorded from EC I represent 18% of the 138 butterfly species known to Israel.[117] Most EC species are widely distributed in the Palearctic region or even beyond its border. Only the distribution of *Pseudotergumia pisidice* and *Thymelicus hyrax*, and six subspecies is limited to the East Mediterranean sub-region at EC I. A significantly higher number of species was recorded on the AS than on the ES, the same as for beetles. Butterfly distribution was significantly and negatively correlated with the tree and bush cover and differences in host-plant accessibility and quality. Remarkably, flying butterflies mirrored interslope divergence of sedentary beetles. Habitat choice prevails and selects for the local adaptive behavioral complex. The interslope ecological divergence prevails over the higher migration potential of flying butterflies than that of sedentary beetles.

Biodiversity and microhabitat distribution of earthworms at "Evolution Canyon" I

Seven species of earthworms were unequally distributed in EC I[118]: *Bimastos syriacus, Dendrobaena semitica, D. orientalis, D. veneta, D. samarigera samarigera, Microscolex dubius,* and *Allolobophora* sp. *B. syriacus* was abundant in all three habitats (AS, VB, ES) together with 3-4 smaller *Dendrobaena* or *Allolobophra* species. *D. semitica* was prevalent on the bottom, while *D. orientalis* and *M. dubius* were found only at the VB. *D. veneta* and *D. samarigera* were more abundant on the ES and AS, respectively. *Allolobophora* sp. was found on both slopes in very low numbers. Earthworm density on the ecologically warmer, drier and more heterogeneous AS was significantly higher in the shade under tree canopies and bushes than in open sunny microhabitats, whereas on the ES no significant differences were found between shady and sunny microhabitats. In the decomposition of organic material in Mediterranean ecosystems, earthworms are regarded as the most important group of soil animals since they constitute most of the soil-fauna biomass.[119]

Gene diversity in an earthworm, *Bimastos syriacus*, from "Evolution Canyon" I

Will soil-living earthworms reflect the same interslope divergent genetic patterns as aboveground organisms? Gene diversity encoded by 16 putative allozyme loci was analyzed in the East Mediterranean earthworm *Bimastos syriacus* at EC I.[120] Thirty-four specimens and 22 specimens were examined on the AS and ES, respectively. Gene diversity indices, gene diversity (*He*), heterozygosity (*H*), number of alleles per locus (*A*) and allelic polymorphism (*P*) (1%), on the AS/VB/ES stations were as follows: 0.17/0.162/0.133; 0.068/0.068/0.039; 1.81/1.81/1.56 and 0.69/0.63/0.50, respectively. Our results indicated, firstly, lower allozyme diversity in earthworms, generally predicted for subterranean animals, compared with aboveground animals,[121] and secondly, higher diversity on the more heterogeneous and stressful AS than on the ES, as predicted by the niche width variation hypothesis.[80]

Biodiversity of ants at "Evolution Canyon" I

How do ants diverge on the opposite slopes? The biodiversity and differentiation of ants into microclimatically contrasting habitats were investigated at EC I.[122] The ant fauna at EC I consists of 17 species: 10 species occur on the AS, 10 on the ES, and 6 at the valley bottom (VB). The two slopes differ by 30% of their species, out of which 3 species are *unique* to the ES and 2 species are *unique* to the AS and VB. Four species were unique to the VB. Ants also diverge at EC I microecologically following climatic and biotic stresses.

Gastropod biodiversity at the "Evolution Canyon" I

Do highly sedentary gastropods mirror migratory taxa? Twenty-six species of sedentary gastropods (terrestrial, sedentary shell-bearing snails and slugs) were recorded at EC I.[40] Twenty-five species were recorded at the ES and 20 species at the AS. Six species were ES-specific and one species was AS-specific. The interslope difference was probably partly due to missing forest species on the AS in comparison with the ES. Twenty-two species were Levantine endemics (84.6%), and 4 species were more widely distributed in the Palaearctic region (15.4%), contrasting flying butterflies. The Levantine species are inhabitants of the mesic and mainly mountainous regions, penetrating the deserts. Seven species were significantly more abundant on the AS than on the ES. The local physical microclimatic sharp divergence leads to sedentary gastropod adaptive interslope biotic divergence as in other taxa caused by natural selection.

Genetic and physiological adaptations of the prosobranch landsnail *Pomatias olivieri* to microclimatic stresses in "Evolution Canyon" I

Can differential interslope drought stress at a microsite affect gastropod genetics and physiology? Resistance to desiccation and genetic diversity of the north-eastern Mediterranean prosobranch landsnail *Pomatias olivieri*, whose southern border is on Mount Carmel, was examined to elucidate potential physiological and genetic adaptations to the contrasting microclimatic conditions at EC.[123] The body water content and the extra-pallial fluid content were significantly higher in fully hydrated xeric AS snails than in mesic ES snails. Similar rates of water loss were found during normothermic and hyperthermic desiccation experiments in AS snails, whereas in ES snails water loss increased in hyperthermic conditions. Thus, the snails on the opposite slopes differ adaptively in their physiological properties. We genetically examined 59 individuals of *P. olivieri* from the AS and 78 from the ES in five populations, and compared them with a sample of 12 specimens from the Galilee Mountains in northern Israel. The polymorphism of the AS populations at 20 putative allozymic loci was 50%, whereas on the ES it was 45%. Heterozygosity was 0.088 on the stressful AS and 0.067 on the mild ES. Genetic distances among all Carmel populations at *EC* were very small, but there was a conspicuous difference between the Carmel and Galilee populations, which indicated a long isolation of the Carmel population, as was also shown in *Drosophila*.[32] The population of the mid-ES (station 6) contained 11 private alleles, whereas at most 2 private alleles were found in other populations. This fact indicates a patch of unfavorable conditions within the habitat, and thus we suggested that the preferred habitat of *P. olivieri* is at the edge of the woodland or in cleared spaces on the ES. Finally, snails of the AS and ES differed in their susceptibility to hyperthermic desiccations. Genetic diversity increases with environmental stress, as is true regionally and globally.[3,5,56,57,70]

Fine-scale biodiversity of Drosophilidae in "Evolution Canyon" I: major model organisms at EC I

What is the biodiversity and spatiotemporal distribution of fruit flies at EC I? Does flying potential lead to slope similarity? In all, 2 genera and 9 (nearly all cosmopolitan or subcosmopolitans, and colonizing) species were found at the microsite.[124] Species richness per sample and total sample abundance were higher in 5 species on the tropical AS than on the temperate ES. Two sibling species, *Drosophila simulans* and *D. melanogaster*, were found temporally quasi-exclusive, but a few hybrid males were also found. Significant interslope gene differences were found in *D. simulans* but most remarkably in *Zaprionus tuberculatus*,[125] a new colonizing species that arrived in Israel in the early 1980s; it was absent on Mount Carmel in 1976.[126] Data on drosophilid species showed significant interslope differences in mutation and recombination rate, behavioral variability, adaptive complexes, fluctuating asymmetry and mate choice; however, it was outstanding to see a similar phenomenon in a recent arrival, *Z. tuberculatus*, suggesting *rapid evolution by strong interslope differential selection*.[127-129] We demonstrated in drosophilid the complex pattern of differences on taxonomic, genetic, morphological and behavioral levels of biodiversity at a distance of only 100 meters. The main differentiating cause seems to be microclimate selection that overcomes the homogenizing effect of migration or gene flow of a good flier.[90] There must be selection against migrants contributing to the rapid evolution of ecological speciation of *Drosophila* described later.[129] *Zaprionus tuberculatus* indicated significantly higher allozyme diversity on the AS (=SFS) as compared to ES (=NFS): *H*=0.101 versus 0.089; and *P*=36.1 versus 31.5, respectively, also displaying by a recent colonizer, the general rule of higher genetic diversity on the AS at EC (Figure 4). This indicates that climatic selection overrides migration in fruitflies at EC I despite their interslope migration,[128] and that the speed of interslope genetic divergence is high. *D. simulans* displayed an opposite trend with higher genetic indices on the ES (=NFS).

A complex adaptive syndrome in *Drosophila* caused by microclimatic contrasts

How does microscale selection affect flying *Drosophila* in sympatric (100-400 meters apart!) but sharply subdivided ecologies? Temperature and humidity are among the most important environmental factors affecting insect adaptive strategies and evolution. We reported that populations of *Drosophila* have multiple-adaptive differences on the opposite slopes of EC I.[130] The multivariate fitness complex analyzed in *D. melanogaster* included oviposition temperature preferences, viability, and longevity changes caused by short-term and lifetime temperature treatments, and resistance to drought stress at different temperatures. Some of these measures were obtained for the sibling species, *D. simulans*, and gave results that parallel those of *D. melanogaster*. Further examination of fitness-related traits[32,39,131] included fecundity, fertility, viability, developmental time, habitat choice, longevity, thermotolerance, sequence polymorphism and survival in two temperature regimes (24°C and 29°C) in two seasons, spring and fall. Significant interslope divergence was found, except in fertility. A higher variance of traits contributing to survival was found on the AS which is more climatically stressful and variable. AS flies develop significantly and adaptively slower than ES flies and respond even stronger at higher temperatures. The interslope divergences seem to be adaptive. They were discovered after years in the mild lab but displayed original field, temperature and drought adaptations. In further experiments we discovered adaptive interslope differentiation of thermotolerance in *Drosophila*.[32,39] We examined whether the physiological evolution of two *Drosophila melanogaster* populations, separated by only 100-400 m, was idiosyncratic and temporary or persisted over multiple years. We explored the high temperature tolerance of *Drosophila* descended from populations of the ES and AS of EC I, which were collected in 1997, 1999 and 2000. Results for *Drosophila* uniformly resembled other studies. We found an inverse relationship between survival and heat-shock temperature, male-female differences in thermotolerance and inducible thermotolerance. Importantly, for all the years of collection, AS flies (isofemale lines, synthetic populations, or inbred lines) consistently exceeded ES flies in basal and inducible thermotolerance after diverse heat shocks, with and without thermal pre-treatment. Inbred lines had lower themotolerance than outbred lines. Several non-exclusive processes may explain the evolution of such physiological differentiation.[39] In *D. melanogaster*, fluctuating asymmetry, mutation rates[132,133] and recombination rates[134] are higher on the more stressful AS, paralleling other organisms at EC, exposing slope-convergent regularities across life.

We concluded that strong microclimatic *natural selection overrides migration* in *Drosophila* establishing interslope and complex adaptive syndromes of fitness-related traits of *D. melanogaster* to the opposite "African" (AS) and "European" (ES) slopes. This is an outstanding result because *Drosophila* can fly more than 10 km between sites,[135] and consistent interslope divergence of adaptive complexes, separated by only 200 meters, clearly demonstrates that *selection overrides gene flow* and possibly involves selection against migrants.[129]

Substantial new information was recently provided by Pechkovsky[32] on: (i) adaptively significant traits; (ii) interslope differentiation of candidate genes (*mei-9, Dmp53,* and *Ptc*); and (iii) incipient postzygotic isolation. Seasonal variations were recorded for starvation stress: AS flies displayed higher starvation tolerance in the spring. Fall flies were more drought-tolerant than spring flies, and AS flies were more tolerant to desiccation than ES flies. Generally, AS flies proved more tolerant to high temperature, starvation and desiccation as adaptively expected.[136-138] Significant interslope differences were found for three candidate genes: *mei-9, Dmp 53,* and *Ptc* generally more polymorphic on the AS, as was true for clock genes in the cyanobacterium *Nostoc linckia* (Figure 4). Remarkably, the microscale responses to stress pattern mirror global patterns.[138]

Incipient sympatric ecological speciation in *Drosophila* at "Evolution Canyon" I

Tests for *D. melanogaster* and *D. simulans* showed that adaptation to contrasting ecological conditions of the opposite slopes had resulted in strong genetic divergence for habitat choice apparently as a by-product[130] leading towards incipient ecological sympatric speciation. The direction of the revealed interslope differences in oviposition-preferred temperatures, viability, longevity and resistance to drought stress[32,130] is exactly that expected in accordance with *habitat selection* and *habitat choice* models. Furthermore, we have recently shown that despite existing asymmetric interslope migration, *selection overrides migration*.[128] Habitat choice can also develop positive assortative mating evolving reproductive isolation and leading to incipient sympatric ecological speciation.[130] Our continued experiments on mate choice,[140,141,33,142-145] interslope candidate gene divergence, and postzygotic reproductive isolation[32] indeed support the hypothesis of incipient sympatric ecological speciation of *Drosophila* at the EC I microsite.

Prezygotic sexual and reproductive behavior, isolation and initiation of postzygotic isolation of *Drosophila melanogaster* at "Evolution Canyon" I

The strong microscale interslope environmental differences in "Evolution Canyon" I provide an excellent natural model of sympatric speciation, i.e., without geographical spatial isolation and within a deme. Our previous studies revealed significant *slope-specific* differences for an adaptive fitness complex of *Drosophila*. This complex involved either adaptation traits (tolerance to high temperature, different viability, and longevity pattern) or behavioral differentiation manifested in habitat choice[146] and *non-random* mating.[33,142,143] This remarkable differentiation has evolved despite a very small interslope distance (200 meters on average, while *Drosophila* can fly 10 km or more!). Our hypothesis is that strong interslope microclimatic divergence accentuated sexual isolation as a by-product of slope-specific adaptations initiating incipient sympatric speciation following the Darwin-Muller-Mayr model.[147] The evidence indicates that: (i) mate choice derived from differences in mating propensity and discrimination; (ii) females from the ES discriminated strongly against males of the opposite slope; (iii) both sexes of the AS displayed distinct reproductive and behavioral patterns with females showing increased fecundity, shorter time before re-mating, and relatively higher receptivity; and (iv) males showed higher mating propensity. These patterns represent adaptive life-strategies contributing to higher fitness.[46]

Incipient postzygotic isolation has been recently found in *D. melanogaster* at EC by Pechkovsky.[32] Interslope hybrid populations displayed lower fitness than slope-specific hybrid populations in some traits, e.g. biomass, fecundity and viability. This suggests that the generalist *D. melanogaster* diverged into opposite slope specialist populations, incipiently sympatrically speciating like bacteria, wild barley and spiny mice at EC I.[4]

Drosophila flies in "Evolution Canyon" as a model for incipient sympatric ecological speciation: general overview

The genetic basis of population divergence leading to adaptive radiation and speciation is a major unresolved problem of evolutionary biology. Molecular elucidation of "speciation genes", i.e., genes restricting gene flow between the incipient species and related taxa,[148,149] advanced recently.[150,151] Almost totally missing are the gene complexes and regulatory elements, participating in reproductive isolation between natural populations, particularly in sympatry. Interslope migration of flies is easy at EC I and has been verified[128] (Figure 6). Nevertheless, significant interslope *D. melanogaster* populations' divergence was established in EC I involving habitat choice, mate choice, reproductive activity, courtship song patterns, significant positive assortative mating,[144] thermal and drought tolerances, adaptive genes, and mobile elements as well as various aspects of induced changes in viability and longevity caused by short-term and lifetime high temperature treatments.[33,142,143] Remarkably, parallel patterns of stress tolerance, habitat choice, and mate choice were also demonstrated in *Drosophila simulans* at *EC* although on a smaller scale. The combined prezygotic and postzygotic evidence suggest that *D. melanogaster* at EC incipiently speciates sympatrically as was shown also in *Bacillus simplex*, wild barley *Hordeum spontaneum*, and spiny mice *Acomys cahirinus*.[4] Sexual isolation and patterns of speciation in *Drosophila* were revisited by Coyne and Orr[148-150] including hybrid inviability, sterility and speciation genes.[139,151] The first step towards speciation of genetically differentiated sympatric populations of *Drosophila* in Brazzaville, Congo, were described by Capy *et al.*[152] Malet[153] critically analyzed *Drosophila* speciation compared to speciation in general "*in less tractable but more charismatic organisms, such as flowering plants, vertebrates, and larger insects. Work with these organisms complemented Drosophila studies of hybrid unfitness to provide a more complete understanding of speciation*". Notably, tests for interslope genetic differentiation in *Drosophila*, carried out in a number of laboratories gave somewhat conflicting results regarding sexual behavior and genetic differentiation of accessory gland proteins and sexual isolation,[154] and lack of interslope microsatellite substructuring.[155,156] A possible explanation to the seemingly missing evidence of SSR population substructuring was provided by Korol *et al.*[142,143] and Pechkovsky.[32] Adaptive differentiation can withstand destructive effects of interslope migration, but it should not necessarily be accompanied by differentiation for microsatellites, unless the latter will be in linkage disequilibrium with selected loci. The last condition can also be maintained despite migration but only under tight linkage and strong selection. For some *Drosophila* genes, linkage disequilibrium is known to decay within just a few kilobases. Thus, differentiation for adaptive trait complexes and relevant candidate genes[32,38] seems to offer much better evidence for interslope divergent selection than that displayed by genetic distances estimated using SSR molecular markers. We presented new empirical data on interslope genetic divergence of *Drosophila* at EC and summarized previous supporting and controversial results.[4,32,142,143] We suggested that *Drosophila* populations at EC demonstrate how *selection overrides migration*, and proposed an ecological model of incipient sympatric divergence based on the dramatic interslope abiotic and biotic divergence encompassing bacteria, fungi, plants, and animals,[4] i.e., *sympatric speciation across life*.

Does the grain beetle O*ryzaephilus surinamensis* incipiently speciate at Evolution Canyon I?

Our new study of the grain beetle *Oryzaephilus surinamensis* in EC I[31] showed interslope differences in distribution and abundance. Significant morphological and genetic differences were found between the natural populations at EC I and the indoor silo pest populations. Likewise, female genome size at EC I was significantly larger on the AS than the ES. Preliminary beetle crossing experiments within and between the AS and ES slopes indicated inferiority of the interlope as compared to each of the slope crossings in fecundity. If these preliminary results are supported by the current larger crossing program, *O. surinamensis* in nature (EC I) is not only significantly different from the domesticated silo populations, but *incipiently sympatric speciation* may be ongoing on the opposite EC I slopes.

Biodiversity patterns of vertebrates on the opposite slopes of "Evolution Canyon" I

Reptile biodiversity at "Evolution Canyon" I

During the period from October 1993 to September 1994, we recorded 308 individuals in 20 excursions, representing 14 reptilian species at seven stations (three stations on the AS, three on the ES, and one on the valley bottom).[157] Reptiles were more active on the AS on 11 days, whereas on three days, higher activity was recorded on the ES. The AS exhibited higher species richness than the ES, 13 versus 8 species, respectively. Four reptilian species were abundant: *Laudakia stellio* (*Agama stellio*, 140 individuals), *Lacerta laevis* (72), *Ptyodactyllus guttatus* (29), and *Mabuya vittata* (30). Three of these were mainly on the AS, and *Lacerta laevis* occurred mainly on the ES. The difference is highly significant, except for *M. vittata*. Altogether, 2 species (*L. laevis* and *Ablepharus kitaibelii*) were more frequent on the ES, whereas 10 species were observed either exclusively or chiefly on the AS. Of 18 reptilian (4 species found out in the comparative study) species, those occurring primarily on the ES, derive from southeastern European origins (e.g., *Lacerta laevis, Ablepharus kitaibelii,* and the now extinct *Lacerta trilineata*). Some of the species on the AS derive from north-eastern Saharo-Arabian origins (e.g., L*audakia stellio, Ptyodactyllus guttatus, Mabuya vittata, Chalcides ocellatus*). Species interslope divergence is also reflected quantitatively (see Table 1 in Nevo *et al.,* 1996 [157]). Parallel interslope biotic divergence patterns to those described here for reptiles have been observed in many other taxa of EC I. Behavioral effects of rodent predation,[158] fitness studies in wild barley[79] and germination contrasts,[74] and many other genetic studies described in this review reinforce interslope adaptive divergence.

Genetic divergence and incipient sympatric ecological speciation in common spiny mice, *Acomys cahirinus*, at "Evolution Canyon" I

Genetic divergence in *Acomys cahirinus*

Do vertebrate rodents diverge interslopes as bacteria, fungi, plants, and invertebrates? Genetic allozyme and RAPD diversities were examined for ecological-genetic patterns in 2 rodents, the spiny-mouse *Acomys cahirinus*[159,160] and the woodmouse *Apodemus mystacinus*,[161] from the ecologically contrasting opposite slopes of EC I, Mount Carmel.[162] Likewise, morphological measurements were compared. Samples of both rodents were collected from six stations: three on the "tropical" xeric AS and three on the opposite "temperate" mesic ES. *Inter*- and *intraslope* allozymes, RAPD, and morphological divergence were found in both rodents. Local microclimatic variation stress caused *interslope* and intraslope adaptive genotypic (proteins and DNA) and phenotypic (morphological, physiological, and behavioral) differences paralleling regional patterns across Israel in *Acomys*, and in northern and central Israel in *Apodemus.* This variation suggests that, at both *micro*- and *macroscales*, diversifying microclimate natural selection appears to be the major driving evolutionary force of phenotypic divergence. EC I proved, in small rodents as in other organisms, an optimal model for unraveling *evolution in action across life and organization.*

Non-random mate choice in *Acomys cahirinus*

Recently, we tested estral females of *A. cahirinus* for *mate choice* between alternative "African" and "European" males in a Y-maze design (*and unpublished data*). If substantiated in future experiments, the identified *non-random mating* trend may suggest incipient sympatric ecological speciation of *A. cahirinus* on the opposite slopes. This will complement the lack of interslope *Acomys* migration[163] and the remarkable interslope genomic and phenomic divergences,[162] including a dramatic interslope divergence of 20% in basic metabolic rates (BMR),[164] similar to that found between Galilee and Eilat populations that are 500 km apart.

Incipient sympatric speciation at "Evolution Canyon" I

In conclusion, preliminary evidence from diverse taxa across life (bacteria, fungi, flowering plants, and invertebrate and vertebrate animals) suggests that *incipient sympatric ecological speciation* may follow interslope adaptive phenomic divergence in morphology, physiology, and behavior at EC I as a by-product. Thus, the "Evolution Canyons" may be embryonic cradles of the twin evolutionary processes of *adaptation and sympatric ecologi-*

cal speciation across life and extensive and intensive future research based on whole genome analyses is justified to establish the *genetic basis of adaptation and active sympatric speciation*.[4]

Genetic diversity patterns across life at "Evolution Canyon" I: summary

Genetic diversity (both allozymes and DNA) was generally higher on the more heterogeneous and stressful AS in 11 out of 16 test organisms (involving 14 species) in EC I (69%), equal on both slopes in one taxon (6%), and higher in four taxa on the ES (25%) (Figure 4). Remarkably, heritable mutation rates in the coprophilous fungus *Sordaria fimicola*[68] were 3-fold higher and up to 11.56 times higher on the AS in 2 additional soil fungi.[165] Likewise, male recombination in *D. melanogaster* was 4-fold higher on the stressful AS than on the milder ES.[134] In *S. fimicola* the estimates of crossing over and gene conversion frequencies were also higher on the AS[71] as was Amplified Fragment Length Polymorphism (AFLP) diversity.[166] As mentioned earlier, AS populations facing dry tropical microclimate display transposon density, genome size, DNA repair, fluctuating asymmetry and genetic polymorphism that are higher on the AS than on the ES in several model organisms. These complex adaptive strategies resist higher UV radiation, temperature and drought on the AS than on the ES. By contrast, ES populations facing temperate conditions and light stress evolved better photosynthetic machinery, anatomy (mesophylic), physiology (phytohormones), and generally lower genetic polymorphism adapted to the cooler, shadier and milder ES.[103]

In EC I we tested genotypically and phenotypically, 2 phylogenetically and biologically very distant organisms: the sessile, predominantly inbreeding plant, wild barley *H. spontaneum*, and the vagile and outbreeding drosophilid fruit fly, *Zaprionus tuberculatus*, a very recent colonizer of Israel.[125] The genomes of these extremely different organisms were tested by AFLP for genetic diversity at 357 and 345 genetic markers (presumed gene loci), respectively.[52] In both organisms we found parallel genetic and phenotypic patterns reflecting the opposite canyon slopes based on AFLP genetic distances, with significantly higher AFLP genetic polymorphism in populations on the ecologically more stressful, warmer and drier AS. Likewise, both organisms displayed higher viability in response to severe drought stress on the more arid and climatically fluctuating AS. Our results suggest the following: (i) microclimatic selection is the major evolutionary interslope, fast-acting, diverging ecological force on genotypes and phenotypes, *overriding migration and genetic drift*; (ii) ecological stress can generate global-scale, adaptive, evolutionary genome, and phenome strategies at microscales and macroscales reinforcing homeostasis and fitness, and suggesting continuity between microevolution and macroevolution.[92] In the following, I will discuss theoretically the EC evidence as a microscale natural laboratory of *evolution in action* caused by drought (AS) and light (ES) stresses.

Theory

Adaptation

The twin evolutionary processes of adaptation and speciation

The major hypothesis supported in this review is that *climatic ecological* stress at a microsite generates slope convergence (*adaptation*) and interslope divergence (*speciation*) across life, from bacteria to mammals, demonstrating *evolution in action*.

Adaptation and speciation, the two central issues of biodiversity evolution, have been studied by researchers of the Institute of Evolution at all geographical scales: *global*, *regional*, and *local*.[12] The local research program at "Evolution Canyon" I (and EC II-IV) described here unravels dramatic *evolution in action* in diverse taxa across life from bacteria to fungi, plants, and animals. From the biodiversity record of 2,500 species and 14 model organisms studied at EC I distributed on the opposite slopes, we identified extensive and intensive active evolutionary processes displaying both slope adaptive *convergence* and interslope *divergence leading to incipient sympatric ecological speciation*. The multiple-adaptive complexes relate to multiple responses to climatic abiotic stresses of UV, warm temperature and drought on the AS, and contrasting light stresses, constrained photosynthesis and response to low-light stimulus, cooler temperature and humidity on the ES.

The interslope adaptive complexes are coupled with by-products of *incipient sympatric ecological speciation* displaying differential initial speciation stages. Remarkably, even the drosophilid *Zaprionus tuberculatus*, which colonized Israel and Mount Carmel during the last three or four decades,[125] indicates an interslope genetic differential at EC I as the model organism most examined (Figure 4). In our massive *Drosophila* collections across Israel from 1976 to 1977,[126] no *Z. tuberculatus* was caught in Israel at large and Mount Carmel in particular. Assuming several generations per year, *Z. tuberculatus* developed interslope genetic divergence and possibly initial mate choice and habitat selection, i.e., a very fast, active *adaptive evolution and incipient sympatric ecological speciation* over some 30 years or approximately 100 generations (assuming its post-1977 colonization). (For theoretical and empirical perspectives of the speed of ecological speciation in dozens to hundreds of generations in plants and animals see Hendry *et al.* [127]).

Interslope adaptive complexes

This review highlighted significant interslope differences for a complex of adaptive fitness stress response traits (genetic, genomic, morphological, physiological, and behavioral) in diverse organisms from bacteria to soil fungi, plants, and animals.[12] These fitness traits respond to local opposite interslope stresses: "*African*" (high UV, temperature, and increased drought) and "*European*" (low UV, temperature and lower drought stress, light restriction for photosynthesis in plants) and cool temperature for tropical or Mediterranean organisms in both plants and animals. Recently, we found dramatic genome wide divergent interslope patterns in the annual crucifer *Ricotia lunaria* transcriptome and methylation regulation on the opposite slopes of EC I revealed by full genome tiling array hybridizations.[21,22] This novel methodology allows the sensitive identification of upregulated genome fragments even for cross species hybridizations. *Ricotia* RNA samples taken under normal conditions and temperature stress were contrasted with RNA samples from adult and seedling *Arabidopsis* plants.

Several gene categories are specifically *upregulated in Ricotia on the stressful EC I AS* in contrast to the ES and *Arabidopsis*: blue light signaling pathway, circadian rhythm, ethylene-mediated genes, leaf development, protein amino acid phosphorylation, RNA splicing, and flower development. The Gene Onthology (GO) categories at the AS involve the *phosphate transport, phosphorus uptake and phosphorylation, abscisic acid mediated signaling, multiple responses to stresses, heat response genes and multiple-transcribed activated transposons*. The more shaded ES demonstrates *upregulation of chlorophyll-related processes, photosynthesis and carbon utilization GO categories*. The advanced analysis of tiling array hybridizations reveals many genome-wide transcriptional events including activation of pseudogenes in the CG-rich area of chromosome 2, expression activity of multiplicity intergenic regions, and transcription of putative silencing RNAs. Interestingly, the zone of actively expressed pseudogenes in the CG-rich area of chromosome 2 is flanked by the transposon-rich genome regions. The revealed interslope divergence between AS and ES appears to reflect the genome-wide adaptations to the interslope microclimatic stresses mediated by natural selection.[21] The "divide-and-conquer" algorithm-based analysis and integration of *Arabidopsis* whole genome tiling array data[22] appears to overcome current challenging problems of expression under different conditions, expression regulators and the genome structural organization. Utilizing the accuracy of this algorithm, the whole genome enrichment SEPALLATA 3 binding sites were constructed and compared with the similarly constructed landscapes of DNA methylation histone modification, exosome substrate expression, and transcriptome expression according to public domain data of *Arabidopsis*. The analysis demonstrates the parallelism in landscapes based on data from different projects and shows the putatively important interplay between chromatin remodeling and TF binding sites in pericentromeric areas of *Arabdiopsis* chromosomes.[22] This analysis contributed also to the *Ricotia* study at EC and will be important for future genome-wide comparisons related to both *adaptation* and *speciation* at the EC model and elsewhere.

The organism-environment response of organisms distributed on the opposite slopes is to *xeric-tropical* versus *mesic-temperate* conditions. These opposite slopes are dramatically, microclimatically divergent[13] and ecologically selecting for contrasting adaptive complexes. A major by-product of the interslope evolving adapting complexes is *mate choice* and *habitat choice in animals* such as the *Drosophila* fruit fly, beetles such as *Oryzaephilus surinamensis*,[31] and the *Acomys* rodents.[4] These by-products, following the Darwin-Muller-Mayr model,[147] generate behavioral components of pre-mating reproductive isolation mechanisms following the evolution of gradual interslope adaptive complexes.

The relative importance of evolutionary forces

Evolutionary change in organisms is affected by the interaction of several major forces including mutation (broadly conceived), recombination, inbreeding, migration, natural selection, and genetic drift presently operating on past evolutionary constraints of individuals and higher levels of organization. However, the relative importance of these evolutionary forces in the processes of adaptation and speciation of natural populations remains as enigmatic now as ever.[4] The following discussion will focus on natural selection, which seems to be the predominant evolutionary force underlying (directly and indirectly) the twin evolutionary processes of adaptation and speciation. In 1859 Darwin and Wallace suggested that the major cause of evolutionary change is natural selection. Despite the passage of 150 years, the exact nature and relative importance of diverse mechanisms of natural selection (e.g. stabilizing, diversifying, balancing, frequency-dependent, cyclical, etc.) in evolutionary change at single loci and multi-locus structures, coding and non-coding genomic elements, and the applicability to both genotypic and phenotypic levels and the entire genome are still the topics of much debate.[35, 36, 121, 167] The analysis of microarray data in ecological settings is discussed by Li *et al.*[93,94]

Evolutionary forces and adaptive complexes

How can the foregone conclusions derived from "Evolution Canyon" be theoretically explained? What is the relative importance of mutation, migration, stochasticity and natural selection in microgeographical genetic divergence at *coding* and *non-coding* DNA levels and proteins? The evidence of genetic structure and interslope divergence of populations and species from EC I-IV[1-4] and overviewed here at the local scale, indicates that molecular polymorphisms and heterozygosity of proteins and DNA, and genome profile in general, are *non-randomly* structured *across life* on a massive and parallel scale. Notably, the EC studies involve many individuals, populations and species across phylogeny including bacteria, fungi, plants and animals influenced by sharply diversified ecologies at a microsite due to contrasting microclimatic stress. The analysis involves biodiversity patterns of 2,500 species and molecular markers in model organisms (allozymes, RAPDs, AFLP, SSR, SNP) single genes, multi-locus structures, candidate genes, and the entire genome organization and sequencing in natural populations at a microscale[1,2] as was studied earlier on regional and global scales.[1,5,56,57,70]

Genetic diversity at all levels (SSR, AFLP and SNP), as well as in retrotransposons and transposons, is partly correlated and predictable by a combination of a few variables primarily involving climatic ecological factors, solar radiation, temperature, drought on the AS, and poor light, cooler temperatures and higher humidity on the ES. The patterns and correlates of interslope genetic diversity at both the protein and DNA levels, including tiling genome-wide expression levels in *coding* and *non-coding* genomes of *Ricotia*,[103] and over many unrelated species, subdivided into microclimatic ecological contrasts, strongly implicate natural selection in population and species differentiation.[35,36] Various forms of selection, primarily through the mechanisms of diversifying, balancing, and directional selection regimes are massively involved, singly or in combination, affecting genetic structure and differentiation of populations at various life-cycle stages of organisms. Other evolutionary forces including mutation, migration and genetic drift interact with natural selection, either directly or indirectly, but appear secondary in importance. Natural selection does appear, according to our results at the "Evolution Canyon" model, to overrule migration and stochasticity in the dynamic evolution of population genetic structure in diverse taxa across life from bacteria to mammals challenging Wright's[168] promotion of genetic drift as a major evolutionary factor even in very small populations.[84] The parallel patterns described rule out stochasticity as a major evolutionary driving force at the EC model. This conclusion may be relevant to life in general if the EC model mirrors global patterns, as it apparently does.

Natural selection and evolution

Theoretically, spatial and temporal variations of selection ('diversifying selection') could maintain genetic polymorphisms. Spatial variation appeared more effective than temporal variation, though their coupled action could reinforce the maintenance of polymorphism. Most results related to selection variation in time analyzed the one locus case. However, polymorphism maintenance may be reinforced in the case of two loci or multi-locus structures,[121] as well as in entire genomes.[21] The selective mechanism is much more effective in promoting genetic diversity if carriers of the alternative alleles are able to *select the niche in which their fitness is greatest*, as is choosing between opposite slopes, xeric versus mesic in "Evolution Canyon", displayed by *Drosophila*. Future quantification is needed of natural selection in adaptive ecological radiations[25,27,35,36,169-173] at the ECs.

Ecological-genetic diversity and stress

Several explanations are suggested for the maintenance of genetic diversity subjected to ecologically contrasting environmental stress. Spatial and temporal ecological variations, which predominate in nature, are of prime importance in maintaining genetic diversity in natural populations. This may be true because different genotypes display varying fitness in variable environments and stresses. Genetic polymorphism, recombination frequencies, and mutation rates tend to increase under stressful conditions[3,5,57,70,137,171,174-177] as was clearly shown in EC I.[4,68,71,165] Rates of evolutionary change are therefore enhanced in adverse environments, as we showed under controlled laboratory experiments in the case of mercury pollution.[176] Likewise, *regionally* (under aridity stress across the Israeli stressful environment) and *locally* at EC I because of higher solar radiation temperature and drought on the xeric, tropical, "African" south-facing slope and light deprivation on the "European" north-facing slope (Figure 4). The evidence for the adaptive evolution of mutation rates has been reviewed by Metzger and Wills[177] hypothesizing that "*it would be highly adaptive for organisms inhabiting variable environments to modulate mutational dynamics in ways likely to produce necessary adaptive mutations in a timely fashion while limiting the generation of other probably deleterious mutations*". This hypothesis has been profitably explored in EC III[54] inviting continuation.

Stable equilibria for multiple alleles will best be explained by multiple-niche selection.[178] Ecological heterogeneity and stress appear to cultivate genetic polymorphisms,[3,5,56,57] particularly in contrasting and dynamically cycling environments that can generate complex supercycles.[179] This supercycle mode of multi-locus dynamics far exceeds the potential for maintaining genetic polymorphism attainable under ordinary selection models. It may represent a novel evolutionary mechanism increasing genetic polymorphism over long-term time periods. Models of sexual reproduction such as an adaptation to resist parasites[180] may also contribute to sex evolution,[181] recombination and polymorphism. Finally, our model[182] of genetic interaction between multiple species governed by abiotic and biotic selection for multi-locus quantitative traits opens wide horizons for the evolution of genetic diversity due to the species dynamic interactions in nature.

Genetic diversity at local, regional, and global scales: from structural to functional genomics

The enigma of genetic diversity and genome organization and evolution in nature has been fruitfully explored using modern molecular techniques and is now evolving from structural genomics to functional genomics. Genetic diversity is found in all species at both the protein and DNA and whole genome levels. Its organization in nature, which is also clearly demonstrated at the EC microsite, *is non-random, heavily structured, and correlated with abiotic and biotic ecological diversity and stress*.[3-5,56,57] Deciphering the origin and maintenance of genetic diversity in nature will be enhanced if investigations focus on the interface between ecology and genetics, utilizing *critical tests and strong inferences in nature of abiotic and biotic factors*. Experimentation could include transplant experiments, particularly *at microscales*, to unravel genome organization, *function*, and *fitness* in contrasting stressful and changing environments. Molecular ecology now has modern tools with increasingly diverse genetic markers and sequence potentials using high throughput structural genomics at the population level.[17,21,22] These tools hold a promise for the future to explain the origin and maintenance of the abundant genetic polymorphism in nature. Reassuringly, DNA polymorphisms (RFLP, AFLP, RAPD, SSR and SNP) largely mirror protein (isozyme) polymorphisms and can be utilized to highlight genome structure and evolution caused by environmental stress, as clearly and unambiguously shown at ECs by genome-wide gene expression[20] and tiling arrays of both coding and non-coding parts of genomes in the crucifer *Ricotia* and *Arabidopsis*.[21,22,103]

The following is a short list of genome analyses to assess evolutionary dynamics.

(1) *Probing genomic architecture and dynamics of genes* and intergenic spacers is facilitated by applying novel polymorphic molecular markers (RAPD-PCR, RFLP, AFLP, ISTR mini- and microsatellites, SSR, and SNP). These techniques probe the entire genome, both *coding* and *non-coding* regions.

(2) *Sequence and marker polymorphism of stress activated genes* (i.e. alleles of candidate genes associated with specific stresses such as temperature, drought, salinity, chemical pollution, and resistances to pathogens and parasites) could be probed, and their biochemical networks and physiology deciphered at EC.[19,32,38,183]

(3) *Testing biomolecular sequences* such as the relative abundance or "Genomic Signature" of oligonucleotides; analyzing sequence compositional spectra and distribution heterogeneity of specific signals-methylase targets, telomeric repeats, micro- and minisatellites, palindromes, recombinational "hot spots", mobile elements, and codon usage bias,[184,185] SSRs,[91,186] and genome-wide analysis[21,22,103] provide powerful tools for comparative and functional genomic evolutionary analysis. These techniques have already contributed to evaluating stress-activated genes and their control elements in large genomic stretches at EC I.[21,22,38]

From DNA sequencing to biological function: systematic genome sequencing of both *coding* and *non-coding* regions[172,187] by high throughput novel technologies, such as 454 and Illumina/Solexa,[17] provides biology with enormous novel insights into genome dynamics, transcriptomes and evolution.[18] Significant progress has been made in sequencing the genomes of many viruses and plastids, chloroplasts and mitochondria of model organisms, numerous bacteria, and increasing numbers of eukaryotes such as budding yeast, the nematode, *Caenorhbdites elegans*, fruit flies, *Drosophila*, humans, chimps, dog, rat, mouse, and the higher plants *Vitis vinifera, Populus, Oryza*, and *Arabidopsis thaliana* amounting (in December 2007) to completely sequenced genomes of 3,300 species.[24] We are now planning the full sequencing of the cyanobacterium *Nostoc linckia* at EC I. The era of comparative genomics and post genomics will be pushed dramatically forward by novel high-throughput revolutionary technologies, the second sequencing fast generation after the Sanger's classic method.[17] These are expected to unravel in depth, *adaptation, speciation,* and *regulation* across the genome.[151,188] These forthcoming discoveries at microsites like EC will provide a substantial quantum leap for unraveling mysteries of Evolutionary Biology.

Origin of species: species and speciation

The problem of the origin of new species is fundamental and persistent in evolutionary biology.[25-27,53,147-149,153,189-199] Darwin's hypothesis was that speciation was gradual and driven by natural selection. Others argued that speciation is divorced from selection and involves non-adaptive and macromutational leaps. Bateson[200] expressed a mutationist view: "*to me it seems that the origin of variation, whatever it is, is the only true origin of species*". Thus, gradualism and mutationism opened a long-lasting and endless debate on speciation, continuing to the present, about the nature of species and speciation. For Darwin, the *Origin of Species*[53] was identical to the origin of adaptation within a species, though he did appreciate organic discontinuity, regarding them as artificial human constructs rather than discontinuous realities of nature. The last major treatise on speciation[149] overviews the history and current state-of-the-art on speciation with its recent dynamic burst on speciation studies, including the genetics of speciation, its driving forces, and the relative importance of speciation modes.[199] Likewise, Dieckman *et al.*[27] examined the history, theories, dynamics, ecology, experimentation, and patterns of speciation.

Mayr considered the origin of new species the most important single event in evolution. He identified species as "*groups of interbreeding populations that are reproductively isolated from other groups, thus representing independent units of evolution*" (see his analysis of the species problem in Chapter 10 of his last book published at the age of 100[194]). Mayr also argued that species arise only from populations that are allopatric, though he became much more responsive to sympatric speciation towards the end of his life.[4,14] Mayr wrote "*there is now no longer any doubt about the frequency of sympatric speciation*".[194]

The major problem of speciation is that of the origin of discrete groups of organisms living together (in sympatry) in nature, which adopts Mayr's biological species concept, and assumes that species are real. Finding out why organisms form discrete clusters instead of an organic continuum is certainly the most intriguing, unsolved problem of evolutionary biology.[149] The second major problem of speciation is whether species require complete geographical isolation between populations, or whether they can emerge in the face of gene flow.[167]

The following problems still await resolution. How does reproductive isolation arise? What are the roles of selection and drift in speciation? Can species evolve sympatrically? Clearly, species vary in kind and in the evolution of reproductive isolation.[189] Reproductive isolation may evolve as a consequence of genetic drift[197] or as a by-product of adaptive divergence in ecological speciation (the Darwin-Muller-Mayr model)[139,147,192,201] or by direct selection.[189] Reproductive isolation may also evolve in sympatry resulting from disruptive or divergent resource-based selection;[201-202] (examples in Refs. 25,149,170,199; theoretical perspective in Refs. 198, 203, 204) when premating reproductive isolation arises without geographical isolation, as well as in plant polyploidy. The major challenge is to document between the alternative hypotheses of diversification in natural populations and to determine the relative proportions of selection and drift in speciation. Allopatric speciation is well substantiated[199] but sympatric speciation is, by far, less supported, more controversial and needs additional evidence. It may prove far more common than allopatric speciation if it becomes substantiated in insects and other groups exposed to sharply divergent ecologies at a microscale.[25,129,170,195]

Sympatric speciation (SS)

No aspect of speciation is as controversial as the view that new species can arise sympatrically, that is, within a *freely interbreeding population*.[27,149] Darwin[53] initiated the idea of sympatric speciation (SS) and considered it important, though not an exclusive model of speciation,[198,203-205] arguing that new species arose in sympatry to fill empty niches in nature. Numerous theoretical, experimental, and field works[195,206] support sympatric speciation,[4,14,32,33,142,144,149] but the relative proportion in nature of SS is still unknown. While theory [198,203-205] suggests that sympatric speciation can occur (if disruptive selection and/or assortative mating and habitat choice are strong enough to overrule gene flow), it is dependent upon diverse assumptions whose reality is unknown. Theory must overcome the problems arising from recombination and competitive exclusion. The concern is that if SS occurs, it must involve resource differentiation, i.e. depending on ecological divergence, which may initiate reproductive isolation by niche selection and/or assortative mating (as is presumably the case at EC I). The final conclusion about SS must be derived from nature. Such work encounters the problem of allopatry or reinforcement as alternative models; *Rhagolites pomonella* was a paradigm of sympatric host-race formation[206] until recent work showed that critical evolutionary changes might have occurred in allopatry.[149] But the case for EC is decisive: no geographical barriers separate the 200 m interslope average distance and potentially free interbreeding is possible between opposite slope populations. Disruptive natural selection involving alternative competition for resources apparently overcomes gene flow in EC.

The three alleged cases of SS (tilapine cichlids in Cameroon, Arctic char in Iceland, and parasitic fig wasps[149]) require documentation. While other cases of SS were suggested, only polyploidy is certainly a frequent mode of speciation in plants. However, while theory supports the occurrence of SS, the evidence in nature is sparse. Coyne and Orr [149] suspect that theory has provided an overly optimistic view of SS. Moreover, they conclude that there is no strong support of SS in nature and trust that *the data at hand cannot justify the current enthusiastic wave for sympatric speciation*.[199] This seems an overly pessimistic view, though the evidence of SS is still scant and requires extensive and intensive future research, as in the "Evolution Canyon" model.

Sympatric ecological speciation at "Evolution Canyon" I

Our studies at "Evolution Canyon" I support the hypothesis of ongoing parallel divergence in similar settings of incipient sympatric ecological speciation in bacteria,[16] wild barley *Hordeum spontaneum*,[4,29,30] *Drosophila melonogaster* and *D. simulans*,[32,33,140-143,144,146] in the beetle *Oryzaephilus surinamensis*,[31] and spiny mice, *Acomys cahirinus*.[4] The EC is certainly, empirically and theoretically, a sympatric model since genes of all organisms (including sedentary and moving/flying organisms) can easily flow between the 50-100 meters of interslope distance. Moreover, our results indicate parallel differential interslope divergence across *diverse taxa across life*, indicating that the interslope xeric-mesic divergence occurs *in situ*, rather than by repeated invasions from the far away Israeli deserts (hundreds of kilometers south and east from this generally Mediterranean territory). African representatives live mostly on the AS, but only a few are found on the ES, such as spiny mice, *Acomys cahirinus*.[162] By contrast, European representatives live mostly on the ES and only a few on the AS, as in the woodmouse, *Apodemus mystacinus*.[207] Finally, transplant experiments[79] and the inferiority of interslope hybrids in wild barley[4,29,30] and *Drosophila*[32] reveal slope-specific fitness adaptive complexes that characterize parallel evolutionary patterns. The latter is evidently common in adaptive divergence and competition for resources, which are important processes in many adaptive radiations[170] including variation in the adaptation to stress.[137]

In the soil bacterium *Bacillus simplex*, we identified slope-specific parallel ecotypes in EC I and EC II, identical in their 16S sequences, suggesting high-genetic similarity and monophyletic origin but demonstrating parallel divergent ecotypes in each canyon, separated by 38 km,[16,43] regardless of geographical distance. The interslope divergence of DNA repair, wild barley,[46] and *Drosophila*[45] relate to higher ecological stress on the AS. The bacterial ecotypes suggest not only interslope adaptive divergence at a microscale but also demonstrate sympatric ecological speciation in soil bacteria.

We concluded that, despite different biology, prokaryotes, like sexually reproducing organisms, may consist of true species and may display parallel ecological speciation as in eukaryotes. We also identified the fundamental units of diversity among *Bacillus* isolates from "Evolution Canyon" III[49] (*J. Sikorski, E. Perry, A. Koeppel, D. Krizanc, A. Rooney, R. Pukall, M. Roberts, N. Field, J. Francisco, S. Vergarg, N. Connor, E. Nevo, F. Cohan, "Incorporating Ecology and Evolution into the Classification of Species: Proposal of Bacillus negevensis sp. nov.", in preparation*) and developed an algorithm to compare simulations of bacterial sequence evolution within and between clades. We could estimate lineage-specific rates of evolution within and between clades, and identify ecotypes strongly associated with different microhabitats confirming their ecological distinctness. In a further study[50] we suggested a paradigm shift to integrate ecological diversification into bacterial evolution, and identifying ecotypes within a natural community while focusing on two *Bacillus* clades from "Evolution Canyons" I and II. We identified multiple ecotypes within traditional species, each ecotype being an ecologically distinct lineage with specialization to different canyon slopes with different solar exposures. Ecotype simulation provides a long-needed natural foundation for microbial ecological speciation and may highlight confusing kinship.[42]

Remarkably, cosmopolitan *Drosophila melanogaster* and *D. simulans* fruit flies also demonstrate a model for incipient sympatric ecological speciation in "Evolution Canyon".[32,33,141-144] Interslope migration of flies at EC I has been demonstrated experimentally.[41] Despite migration, significant interslope *Drosophila melanogaster* population divergence was established at EC I involving *habitat choice* and *mate choice*, thermal and drought tolerances, viability and longevity, slope adaptive candidate genes, and mobile elements. The most exciting findings related to sexual behavior are: interslope differences in mating propensity, sexual discrimination, reproductive activity, courtship songs, significant positive assortative mating[144-146] and, recently, initials of postzygotic isolation.[32] Parallel patterns of stress tolerance, habitat choice and mate-choice were demonstrated in *Drosophila simulans* at EC slopes although on a smaller scale. *Drosophila* populations at EC represent an example demonstrating how *strong selection overrides migration*, and we proposed an *ad hoc* ecological model of incipient sympatric speciation. Possibly, selection against migrants occurs, contributing to the rapid evolution of ecologically-dependent reproductive isolation;[129] but there is still no evidence for this. Earlier, we presented preliminary evidence that incipient speciation is also ongoing in the flowering plant, wild barley *Hordeum spontaneum*[4,29,30] and in the rodent *Acomys cahirinus*[4] that colonized Israel from Africa some 20,000-30,000 years ago.[162] Thus, "Evolution Canyon" I represents an "Israeli Galapagos" island situation *but is sympatric, not allopatric, across all life from bacteria through plants, insects, and Drosophila to mammals*. This seems to be a world-unique demonstration of adaptive incipient sympatric speciation across life at a microsite.

Our results in "Evolution Canyon" I *across life* appear to derive from sharp interslope ecological divergence and the emerging evolution of reproductive divergence. This follows the hypothesis of ecology of adaptive radiation.[170,171,201] The "ecological theory" proposes that "*phenotypic divergence and rapid speciation in adaptive radiation are ultimately the outcome of divergent natural selection, arising from differences between environments and competition for resources*".[170,171] This is true of bacterial ecotypes that presumably represent ecospecies[16,43,49,50] to soil fungi,[15] flowering plants, such as *Ricotia lunaria, Hordeum spontaneum*, and *Lotus pereginus*, to the flies *Drosophila melanogaster* and *D. simulans*, the beetle *Oryzaephilus surinamensis*, and the rodent *Acomys cahirinus* representing differential degrees of reproduction isolation that emerged at EC I.[4]

Strong selection appears in all of the aforementioned cases to *override gene flow*, despite existing interslope migration in parallel incipient sympatric ecological speciation of diverse taxa across life. The discovery of interslope divergence of candidate genes, such as "period" in *Drosophila*,[38] and *mei*-9, *Dmp53*, and *pts*[32] supports the hypothesis of interslope adaptive divergence and the presumable by-product evolution of reproductive isolation. The latter evidence leads to incipient sympatric ecological speciation in model organisms across life rather than to a zone of secondary contact and introgression. This is displayed by interslope hybrid inferiority in wild barley *Hordeum spontaneum*[4,29,30] and *Drosophila*.[32] The parallel patterns in "Evolution Canyon" I and II, separated by 38 km, substantiate the idea of local sympatric ecological speciation caused by microclimatic selection. The full genome tiling array of *Ricotia lunaria* sharply demonstrates interslope divergent patterns of genome transcription and methylation regulation.[21,103] The fates of the emerging new species either terminating speciation or extinction are unknown and depend on future climatic evolution.

Conclusions and prospects

The "Evolution Canyon" model is uniquely suitable to follow *evolution in action across life* both in terms of *adaptive interslope divergence*, due to the sharp ecological microclimatic contrasts at a microscale, and of *incipient sympatric ecological speciation*. Species richness of terrestrial taxa is largely higher on the xeric slope (AS) displaying xeric-tropical species richness. Aquatic-related species prevail on the mesic-temperate ES. Adaptations of populations within a species to the tropical AS display largely higher resistance to solar radiation, DNA destruction, temperature, and drought; all increasing tolerance against the "African" slope stresses. Moreover, and very importantly, higher genetic polymorphism, mutation and recombination rates, gene convergence, DNA repair, lateral transfers, splice variations, genome wide transcription and genome size are all areas of genomic variation coping with higher stresses on the AS. By contrast, adaptations to the temperate-mesic but forested, overshaded, cooler, and temperate ES largely involve opposite traits than those in the AS, which are climaxed by the upregulation of chlorophyll-related processes and photosynthesis in a light-deprived environment.[103]

Clearly, the interslope microscale adaptive radiation selects for slope-specific adaptive complexes. Existing gene flow, even if high (as in *Drosophila*, up to 10% from the "African" to the "European" slope[128]), cannot break the strong adaptive complexes through recombinational reshuffling. The preservation of the *slope-specific adaptive complexes* is enhanced by the evolution of ecological and behavioral prezygotic and sometimes postzygotic (wild barley and *Drosophila*) reproductive isolation probably as by-products of adaptive differentiation following the Darwin-Muller-Mayr model and possibly by selection against migrants. Thus, deviation from panmixia by strong disruptive selection accompanied by positive assortative mate choice and habitat/niche choice, as in *Drosophila*[33,142] and *Acomys*,[4] may promote incipient sympatric ecological speciation by *strong selection that overrides gene flow*, and thus adaptive divergence is preserved against the destructive effects of recombination. This model starts with the evolution of adaptive complexes against the slope-specific stresses through strong natural ecological selection followed by the evolution of behavioral traits, habitat choice, and positive assortative mating that restrict interslope gene flow, despite high-migration rates, which substantiate sympatry.[143] Possibly, selection against hybrids and migrants proceeds from *slope adaptation to interslope incipient sympatric ecological speciation across life*. Much future research is needed to substantiate this model.

The theories of ecology of adaptive divergence and sympatric speciation need further substantiation in nature. What is next? Biodiversity evolution across life needs expansion at EC by adding diverse taxa and embracing all categories (individuals, genomes, populations, species, communities and biota). Importantly, comparative analysis of the four "Evolution Canyons" (EC I, II, III and IV) should be extended to all major taxa from bacteria to mammals following the exemplary study in soil fungi.[15] The major focus should be on *population functional ecological genomics* coupled with *proteomics, phenomics, metabolomics* and *ecological speciation*. A major future perspective should try to analyze the effect of stresses, not only through individual genes but through genomic-biochemical *networks* related to individual and collective *environmental stresses* (solar radiation, temperature, drought, photosynthetic deprivations, biotic stresses, etc). Metagenomics could and should be developed as well as the in depth analysis of DNA methylation histone modification and the regulatory effects of small RNA, and *transposon dynamics in adaptation and speciation* to evaluate genome-wide adaptive divergence.[172,187,208,209] Comparisons should be made with the ecology of adaptive radiation and ecological speciation across Israel as a regional genetic laboratory and *the entire globe as a genetic laboratory* using representative populations and species of the model organisms studied at ECs across their entire genomes by novel sequence methodologies to unravel *structural and expressional adaptive complexes, speciation genes and regulators*.

Mate and habitat choice transplant experiments and comparison of interslope and intraslope crosses could highlight the stages of *ecological adaptive radiation and incipient sympatric speciation*, as well as comparisons with populations outside the canyons. The "Evolution Canyon" model is a 'hot spot' of evolution in action of *biodiversity, adaptation,* and *speciation* and is appropriate for testing many mysteries of Evolutionary Biology.

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