

Evolution of genome–phenome diversity under environmental stress

Eviatar Nevo*

Institute of Evolution, University of Haifa, Haifa 31905, Israel

This contribution is part of the special series of Inaugural Articles by members of the National Academy of Sciences elected on May 2, 2000.

Contributed by Eviatar Nevo, March 5, 2001

The genomic era revolutionized evolutionary biology. The enigma of genotypic–phenotypic diversity and biodiversity evolution of genes, genomes, phenomes, and biomes, reviewed here, was central in the research program of the Institute of Evolution, University of Haifa, since 1975. We explored the following questions. (i) How much of the genomic and phenomic diversity in nature is adaptive and processed by natural selection? (ii) What is the origin and evolution of adaptation and speciation processes under spatiotemporal variables and stressful macrogeographic and microgeographic environments? We advanced ecological genetics into ecological genomics and analyzed globally ecological, demographic, and life history variables in 1,200 diverse species across life, thousands of populations, and tens of thousands of individuals tested mostly for allozyme and partly for DNA diversity. Likewise, we tested thermal, chemical, climatic, and biotic stresses in several model organisms. Recently, we introduced genetic maps and quantitative trait loci to elucidate the genetic basis of adaptation and speciation. The genome–phenome holistic model was deciphered by the global regressive, progressive, and convergent evolution of subterranean mammals. Our results indicate abundant genotypic and phenotypic diversity in nature. The organization and evolution of molecular and organismal diversity in nature at global, regional, and local scales are nonrandom and structured; display regularities across life; and are positively correlated with, and partly predictable by, abiotic and biotic environmental heterogeneity and stress. Biodiversity evolution, even in small isolated populations, is primarily driven by natural selection, including diversifying, balancing, cyclical, and purifying selective regimes, interacting with, but ultimately overriding, the effects of mutation, migration, and stochasticity.

Ecological Genomics: Extension of Ecological Genetics

Long-Term Research Program in the Institute of Evolution. The genomic revolution has dramatically opened wide horizons for evolutionary studies, linking molecular and organismal organizational levels. Here, I primarily review the 1975–2000 research program in the Institute of Evolution, University of Haifa. This program involves biodiversity evolution, from bacteria to mammals, of genes, genomes, phenomes, populations, species, ecosystems, and biota as well as their intimate reciprocal interaction with spatiotemporal variables and the stressful physical and biotic environments resulting in adaptation and speciation.

Our model organisms represent diverse taxa across life, from bacteria to mammals, in an attempt to unravel regularity, convergence, and divergence of genotypic and phenotypic patterns: *Nostoc linckia* (cyanobacterium); *Sordaria fimicola* (coprophilous fungus); *Triticum dicoccoides* and *Hordeum spontaneum* (wild cereals); *Drosophila melanogaster* (fruit fly); *Spalax ehrenbergi* superspecies (blind subterranean mole rats); *Acomys cahirinus* and *Apodemus mystacinus* (aboveground rodents). We also studied globally and regionally the population genetics of 1,200 species from bacteria to mammals, primarily based on literature data and species richness of 3,000 species in the two “evolution canyons” (ECs).

The aforementioned model organisms comprise haploids and

diploids, inbreeders and outbreeders, as well as sedentary and migratory organisms. Our ecological theaters include global (the entire planet), regional (Israel and the Near East Fertile Crescent), and local (several microsites in the Galilee Mountains Neve-Yaar, Yehudiyya, Tabigha, and Ammiad) studies, and the model system in the Carmel and Galilee Mountains.

The environmental stresses analyzed include thermal, chemical, climatic, and biotic, studied in the field and under critical laboratory conditions. The biological systems include phenotypes (morphological, physiological, and behavioral) and genotypes (proteins and DNA, coding and noncoding levels). We have attempted a holistic, integrative analysis of the organism–environment interaction in the twin evolutionary processes of adaptation and speciation.

Problems

Major problems of evolutionary biology await solutions. They can be resolved in a genomic era, when complete prokaryote and eukaryote genomes are available for comparative analysis (1). A major unresolved question is how much of the coding and noncoding genome diversity, the latter comprising >95% in eukaryotes, affects the twin evolutionary processes of adaptation and speciation. Furthermore, how much of this diversity in coding and particularly noncoding genomes, often called “junk” DNA, contributes to regulation and differential fitness of organisms and is subjected to natural selection? What proportion of observed genic and nongenic diversity is maintained by selection? How much of the diversity in noncoding DNA is adaptive and regulates gene expression, transcription, translation, recombination, and repair? The adaptive nature of the noncoding genome now turns out to be one of the most intriguing questions in evolutionary genetics, as was the case with the coding genome during the last decades of the 20th century.

Methods and Ecological Testing Theaters. To answer the problems of diversity in the coding and noncoding genome, it is not sufficient to measure their genetic diversity. The estimates of molecular diversity derived from PCR-based techniques—such as amplified fragment length polymorphism (AFLP), microsatellites (short sequence repeats or SSR), single nucleotide polymorphism (SNP) and sequence comparisons—are several-fold higher than enzymatic diversity. The elucidation and significance of molecular diversity need critical examination, experiments, and mapping that relate to coded allozymes but particularly to noncoding DNA diversity and to explanatory attempts of DNA linguistics (ref. 2; V. M. Kirzhner, A. B. Korol, A. Bolshoy, and E.N., unpublished work).

Essentially, we are attempting to elucidate the reciprocal relationships between organism and environment. This strategy has been adopted by the school of ecological genetics (4). This

Abbreviations: EC, evolution canyon; AFLP, amplified fragment length polymorphism; SNP, single nucleotide polymorphism; SSR, short sequence repeats; RAPD, randomly amplified polymorphic DNA; SFS, south-facing slope; NFS, north-facing slope.

*E-mail: nevo@research.haifa.ac.il.

strategy guiding the research program of the Institute of Evolution (1975–2000) across life and diverse ecogeographical theaters, coupled with observations and critical experiments, proved successful at global (5), regional (6), and local (7, 8) scales (reviewed in refs. 9–12) at the allozyme diversity level. The strategy has now been extended into the DNA coding and noncoding genomes, thereby advancing the science of ecological genomics. The major questions for allozymes, and now for the DNA coding and noncoding genome, relate to the organism–environment interaction. Is the diversity random or nonrandom? How does differential stress and niche-width affect genomic and phenomic diversity? What are the genome–phenome relationships? Does speciation necessarily entail large genomic changes?

Evidence

Allozyme Diversity and Evolution. Darwin introduced natural selection as the major mechanism of evolution. But what proportion of all genomic and phenomic evolutionary change results from natural selection? The most striking population-genetic feature is the great and widespread allozyme diversity in nature across unrelated organisms from bacteria to mammals (9–12, 16–18). Can the study of origin and dynamics of genotypic and phenotypic diversity, within and between populations, demonstrate that natural selection is indeed the mechanism underlying the genetic and organismal basis of evolutionary change?

What is the proportion of adaptive coding genetic diversity in nature? Does most genetic diversity originate by random transitory lethal mutations constantly removed by purifying directional selection, as proposed by the neutral theory of molecular evolution (13) accentuating the Wrightian idea (14) of random genetic drift in small populations? Are most genetic polymorphisms neutral, linked (that is, “hitchhiked”) to only a few selected loci across the genome? Or, by contrast, does spatiotemporal balancing natural selection maintain diversity within populations and diversify it between populations, thereby causing adaptive evolution and speciation (9–12, 16–18)? Or is the theory of nearly-neutral alleles (19) the satisfactory solution?

These questions remained largely controversial in the classical review of Lewontin (15). Our evidence (reviewed in refs. 5–12) and that of others (16–18) suggest that most isozyme and allozyme diversity in natural populations is apparently maintained by some kind of natural selection. The observation of significant linkage disequilibrium within and between populations reinforces the importance of natural selection in the maintenance of genetic polymorphisms in nature, at single, double, and multilocus structures. Does the adaptive nature of the large proportion of allozyme polymorphisms represent only small genome fractions, or is it relevant to the >95% of the noncoding genome? This question now becomes a major, still largely unresolved issue of biological evolutionary theory. I first briefly review the allozymic evidence, before focusing on the current debate about the noncoding genome, still called unjustifiably by many “junk” DNA, divided into regulatory and random regions.

Allozymic Diversity Patterns at Global, Regional, and Local Geographic Scales. We analyzed 1,200 species, thousands of populations, and tens of thousands of individuals for allozymic diversity encoded by 20–50 enzymatic loci in three natural genetic laboratories: (i) global, across the planet (1,100 species); (ii) regional, Near East (33 species) and Israel (38 species); and (iii) local, four microsites in Israel (29 species).

Genetic diversity varied by different degrees between and within the numerous species tested at both the protein and DNA levels. Genetic organization in nature, at all descending scales, global, regional, and local, is nonrandom and heavily structured. Genetic organization frequently displays parallel and repetitive trends in unrelated taxa, positively correlated with, and predict-

able by, abiotic and biotic ecological heterogeneity (that is, spatiotemporal variation in niche-width) and environmental stress, and is often negatively correlated with population size (11). These results are inconsistent with the predictions of the neutral theory of molecular evolution (13) and seem to be primarily driven by natural selection. Critical laboratory experiments with marine organisms subjected to pollution cause fast differential mortality of allozyme genotypes, indicating that they are selected by the environment (20).

The Adaptive Evolution of Enzyme Kinetic Diversity. Kinetic studies of hemoglobin, haptoglobin, transferrin proteins, and at least a dozen enzyme polymorphisms typically reveal biochemical kinetic differences among the gene products of alternative genotypes at a locus (16). These include single gene effects, such as lactate dehydrogenase in killifish, leucine aminopeptidase in blue mussel, and phosphoglucose isomerase in *Colias* butterflies. Similar results were obtained with alcohol dehydrogenase in fruit flies, salamanders, and barley; glutamate-pyruvate transaminase in copepods; as well as esterase, glucose-6-phosphate, 6-phosphogluconate dehydrogenase, and superoxide dismutase in fruit flies (16). In all these cases, biochemical-kinetic studies reveal differences among phenotypes, either within or across species, that have measurable effects of alternative genotypes on the physiology of whole individuals. These genotypes result in differential fitness distinguished by natural selection in keeping with their alternative spatiotemporal environments (16).

Genome and Phenome Evolution of Subterranean Mammals at Global, Regional, and Local Scales. I exemplify genome and phenome evolution in one of nature’s best studied long-term (about 45 million years old), global evolutionary experiment of mammals adapting to life underground (see figure 2.1 in ref. 21). The global adaptive convergence of subterranean mammals currently involves three orders: rodents, insectivores, and marsupials, which include 11 families, 50 genera, and several hundreds of species. This global evolutionary process followed the stepwise climatic cooling and drought, which in turn was followed by biotic extinction in the transition from the middle Eocene to the early Oligocene. This period witnessed 10 million years (45–35 Ma = million years ago) of profound change in earth’s geology, climate, and biota. The earth changed from the Mesozoic “hot house”—i.e., from a warm, equable, mostly subtropical world that persisted from the Mesozoic to the early Cenozoic—to the Neogene (Miocene to present) “cold house.” The ecological theater of open country biotas that opened up progressively in the Cenozoic, following the Eocene–Oligocene transition, was associated with increasing aridity, colder climate, and terrestriality. This climatic change set the stage for a rapid evolutionary play of recurrent Neogene adaptive radiations of unrelated mammals on all continents into the subterranean ecotope.

Subterranean Ecotope. The subterranean ecotope is relatively simple, stable, specialized, low or medium in productivity, predictable, and discontinuous. Its major evolutionary determinants are specialization, competition, and isolation (21). This ecotope involves the herbivorous (rodents) and insectivorous (insectivores and marsupials) niches. All subterranean mammals share molecular and organismal convergent adaptations to their common unique ecology. By contrast, they display divergent adaptations to their separated niches of herbivory and insectivory and to their different phylogenies. The remarkable adaptive evolution of subterranean mammals involves structural and functional regression and progression changes caused by colonizing the underground ecotope. It is a triumphant example of the comparative method in evolutionary biology demonstrating global convergent evolution caused by similar underground

ecological constraints and stresses at both the molecular and organismal levels, causing distant organisms to converge adaptively (21).

Adaptationist Program. The evidence derived from the global convergence of subterranean mammals (21) and regional divergence (22, 23) corroborates evolutionary theory. Convergent evolution and adaptations to life underground remarkably substantiate a critical adaptationist program, which is the only viable program to explain biological evolution. No alternative model can explain the evolution of subterranean mammals with the stresses of life underground. The descriptive evidence and analytical results across subterranean mammals indicate a massive global genotypic and phenotypic convergence. The adaptive mosaic evolution of the *Spalax* eye presents an outstanding example of coupled regression (reduction) and progression (expansion) caused by molecular and organismal selective evolutionary tinkering underground. Remarkably, the genetic basis of eyes and brains seems to be conservative across the animal kingdom, generated by *Pax-6* homologues and a cascade of homeotic genes. Similar morphological, physiological, and behavioral adaptive regressions and progressions are demonstrated by the auditory system and the massive brain reorganization. Notably, both the cerebellum, underlying digging, and the neocortex, underlying the extensive, unique physiology and behavior of subterranean mammals, relate to brain reorganization through neuroanatomical evolutionary tinkering (21).

Allozymic Diversity. The controversy about allozyme heterozygosity in subterranean mammals was first reviewed across 243 small mammals (111 aboveground and 132 subterranean); the conclusion was that subterranean mammals are indeed less heterozygous presumably because of the relatively narrow microclimatic niche underground (21). This conclusion is supported by a broader perspective at global, regional, and local scales in more than 1,200 species analyzed allozymically at the interface between genetics and ecology (11). The levels of genetic diversity in nature, including the narrow niche subterranean ecotope, are positively correlated with niche-width and often negatively correlated with effective population size, negating neutrality and substantiating selection as the best explanatory mechanism responsible for both the protein and DNA levels of molecular evolution. This phenomenon holds true in subterranean mammals and elsewhere (11).

Microgeographic Critical Tests in Nature

Microsite ecological contrasts are excellent critical tests for evaluating the dynamics of genome and phenome evolution and assessing the relative importance for adaptation and speciation of the evolutionary forces causing differentiation (7, 8). The latter involve mutation (in the broadest sense, including recombination), migration, chance, and selection. At a microsite, mutation, which is usually considered a clockwise neutral process, is expected to be similar across the microsite. Migration, which operates for any organism at the microsite, even sessile organisms, is expected to homogenize allele frequencies. Stochasticity is not expected to result in repetitive, ecologically correlated patterns. Selection seems to be the only evolutionary force expected to result in repeated ecologically correlated patterns (5).

In 1977, at the Institute of Evolution, we embarked on a series of microsite studies comparing sharply contrasting ecological alternative patterns of temperatures (cold vs. hot in balanids, sessile crustaceans; e.g., ref. 24); aridity index (high vs. low in wild cereals; e.g., refs. 8, 11, 25, and 31); lithology (igneous, volcanic, and sedimentary rocks; e.g., refs. 26, 31, and 32); soil types (terra rossa, rendzina, and basalt in wild cereals; e.g., refs. 26, 31, and 32); topography (27); and chemical [nonpolluted vs.

polluted environments with inorganic heavy metals (Hg, Cd, Zn, Pb, Fe) and organic (detergents and oil) pollutants in marine organisms; e.g., ref. 20]. The aforementioned studies demonstrated differential viability of allozyme genotypes where allozyme diversity and divergence were selected at a microscale or under critical empirically contrasting conditions and ecologies.

Will the noncoding genome also display ecological correlates at regional and local scales? The answer is emphatically yes for outbreeding mammals (e.g., ref. 28) and inbreeding wild cereals (e.g., refs. 29–33).

Microscale Molecular Population Genetics of Wild Cereals at Four Israeli Microsites.

We used three molecular marker systems that included allozymes, randomly amplified polymorphic DNAs (RAPD), and microsatellites (SSR) to detect molecular diversity and divergence in three populations of wild emmer wheat (*T. dicoccoides*); these populations were from Ammiad, Tabigha, and Yehudiyya microsites in northern Israel, and these microsites displayed topographic, edaphic, and climatic ecological contrasts, respectively (29–33). Likewise, we examined molecular diversity with RAPD and SSR markers in wild barley, *H. spontaneum*, in the Tabigha microsite north of the Sea of Galilee, and in Neve-Yaar, Lower Galilee; the latter microsite represented a mosaic of microniches of sun, shade, rock, deep soil, and their combinations. The three marker systems represented protein-coding (allozyme) regions and noncoding (most of RAPDs) and short repetitive DNA elements (most of SSRs), hence providing comprehensive coverage of the wild wheat and barley genomes. At each microsite, we identified nonrandom divergence of allozyme, RAPD, and SSR diversities. Significant niche-specific (high frequency in niche type) and niche-unique (limited to a niche type) alleles and linkage disequilibria abounded, allowing classification into niches of either coded or noncoded markers (29–33).

At Ammiad, the three marker systems used in wild wheat showed dramatically different levels of gene diversity (*He*) and genetic distance: SSR > RAPD > allozymes. The gene differentiation (*Gst*) order was allozymes > SSR > RAPD. Remarkably, the three marker systems revealed similar trends of diversity and divergence. All three molecular markers displayed nonrandom allele distributions, habitat-specific and habitat-unique alleles, and linkage disequilibria (29–33). The subpopulations in the drier habitats showed higher genetic diversities in the three marker systems (33). The genetic distances among the four subpopulations tended to increase with the difference of soil moisture after the early rain of the growing season. These results may suggest that ecological selection, probably through aridity stress, acts both on structural protein coding and on presumably partially regulatory noncoding DNA regions (SSR and RAPD), resulting in microscale adaptive patterns. Similar microscale molecular (allozymes, RAPDs, SSRs) divergence was found in two populations of wild barley (*H. spontaneum*) at Tabigha and Neve-Yaar (ref. 11; E. D. Owvov, A. Beharav, T. Fahima, V. M. Kirzhner, A. B. Korol, and E.N., unpublished work; and Q. Huang, Y. C. Li, and E.N., unpublished work).

Regional Edaphic Selection. The three wild emmer microsites can be classified into two groups according to their soil types: the terra rossa group (Ammiad + the terra rossa part of Tabigha) and the basalt group (Yehudiyya + basalt part of Tabigha). Significant SSR diversity was found between the two edaphic groups at the regional scale (31). In particular, soil-specific and soil-unique alleles were observed across the Tabigha microgeographic site and the Ammiad and Yehudiyya regional microsites, 6–8 km away. Permutation tests suggested that the observed soil-specific and soil-unique alleles were unlikely to occur by chance. The results indicate that edaphic selection may cause the SSR divergence in *T. dicoccoides* between the two edaphic

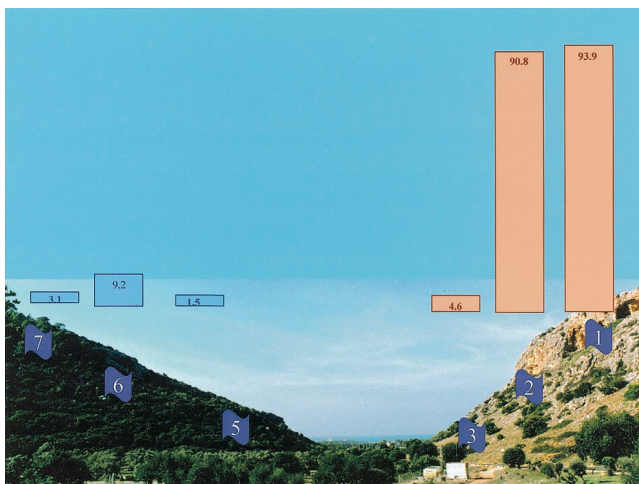


Fig. 1. A cross section of EC I, Lower Nahal Oren, Mount Carmel, Israel. Note the plant formation on opposite slopes. The green lush European temperate cool-mesic NFS, sharply contrasts with the open park forest of warm-xeric, tropical African-Asian savanna on the SFS. The histograms show the levels of HIP1 genetic polymorphism in *N. linckia* in each of six stations at EC: stations 1–3 on the SFS and 5–7 on the NFS. Note the extraordinary divergence among stations (T. Krugman, N. Satish, O. N. Vinogradova, A. Beharav, Y. Kashi, and E.N., unpublished work; see also Fig. 4, which is published as supplemental data on the PNAS web site, www.pnas.org).

groups over the entire regional analysis. One of the SSR loci studied (GWM601) maintained the same allele in the three populations of *T. dicoccoides* and its descendant cultivated wheat *Triticum aestivum*, suggesting that balancing selection may protect this locus from any change (31).

Ecological-Genetic Perspective of SSR Evolution. We reviewed the evidence from a literature survey related to the importance of functional SSRs, particularly in regulation of gene activity (transcription and translation), chromatin organization, genome size, recombination, DNA replication, cell cycle, etc. We also assembled the ecological-genetic perspectives of SSR evolution based on other studies besides our wild cereal studies (30–33). We argued that some SSRs may be of great importance in the process of population adaptation to environmental stress and that random SSR size expansions or compressions are selected against. We suggested that balancing and diversifying ecological selection seem to shape allele-size frequency distribution and constrain repeat sizes at both the upper and lower thresholds of SSR (Y. C. Li, A. B. Korol, T. Fahima, A. Beiles, and E.N., unpublished work; see also ref. 2).

The Evolutionary Model of EC: Life's Microcosm. Local, microcosm, and natural laboratories, designated by us as the EC model, reinforce studies of regional and global macrocosm ecological theaters across life (7, 8). They present sharp ecological contrasts at a microscale, permitting the pursuit of observations and experiments across diverse prokaryote and eukaryote taxa sharing a sharp microecological subdivision. Likewise, they generate theoretical, testable, and predictable models of biodiversity and genome evolution and permit the examination of the mode and tempo of adaptation and speciation. The south-facing slopes (SFS) in canyons north of the equator receive higher solar radiation than on the nearby north-facing slopes (NFS; ref. 34). This solar radiation is associated with higher temperature and drought on the more stressful SFS, causing dramatic physical and biotic interslope divergence, which may have originated several million years ago, after mountain uplifts (Fig. 1; see also supplemental Fig. 5; refs. 7 and 8). These canyons are extraor-

dinary natural evolutionary laboratories. If rocks, soils, and topography are similar on the opposite slopes (50–100 m apart at bottom), microclimate remains the major interslope divergent factor. Even strongly sedentary organisms can migrate between slopes. The interslope divergence of biodiversity (i.e., genes, sequences, genomes, populations, species, ecosystems, and biota) can be examined within each species distributed on both slopes. This intraspecific interslope divergence can be compared in many species across life from prokaryotic bacteria through eukaryotic lower and higher plants, fungi, and animals (7, 8).

These genomic and phenomic multiple taxa interslope comparisons permit slope-convergent and interslope-divergent generalizations of organism–environment relationships across life and of the relative importance of evolutionary forces operating in adaptation and speciation. In a structural and functional genomic era, all available complete genomes or those partially sequenced, including stress genes, are comparable by microarray technology on both slopes along with their proteomes and phenomes, i.e., at the interrelated molecular and organismal levels. These long-lived natural evolutionary laboratories permit in-depth stress studies of genome evolution in adaptation and speciation in close sympatry and under critical tests of past, present, and future divergence. Moreover, future critical tests are feasible by transplant experiments assessing interslope differential fitness and the dissection of quantitative traits loci by genetic mapping of families whose parents originated from the opposite slopes. These tests permit us to focus directly on sets of naturally occurring candidates and variant fitness genes and traits, which could also be tested directly by site-specific mutagenesis to assess the importance of specific amino acids in the function of particular proteins. Finally, diverse problems like sex and social evolution, among others, as well as the similar and/or different adaptive strategies between prokaryotes and eukaryotes can be critically studied at a microsite.

ECs I and II: Carmel and Galilee. We opened two long-term research projects in EC I in Lower Nahal Oren, Mt. Carmel (refs. 7 and 8; Fig. 1; supplemental Fig. 4), and EC II in Lower Nahal Keziv, Upper Western Galilee (ref. 35; supplemental Fig. 5). EC II is 38 km northeast of EC I. In both canyons, we seek local, regional, and global generalizations across life and organizational molecular (proteins and DNA) and organismal (morphological, physiological, and behavioral) levels. Our work at EC I started in 1991 (7, 8) and at EC II in 1998 (35). I summarize our findings below.

Species richness. To date, we have identified more than 2,000 species in EC I and more than 1,000 species in EC II, in an area of 7,000 m² in each. The SFS is significantly richer in species (in both canyons) of “terrestrial” taxa, and the NFS is richer in “humid” taxa, reflecting locally global patterns (refs. 7 and 8; supplemental Fig. 5; M. Finkel, O. Fragman, and E.N., unpublished work).

Genetic diversity. Genetic diversity (both of allozymes and DNA) was higher on the more heterogeneous and stressful SFS in 11 of 14 model organisms (Fig. 2; refs. 7 and 8). Remarkably, heritable mutation rates in the coprophilous fungus *S. fimicola* were 3-fold higher, and male recombination in *D. melanogaster* was 4-fold higher on the stressful SFS than on the milder NFS (8).

In EC I, we tested, genotypically and phenotypically, two phylogenetically and biologically very distant organisms, the sessile, predominantly inbreeding plant wild barley, *H. spontaneum*, and the vagile and outbreeding drosophilid fruit fly, *Z. tuberculatus*, a very recent colonizer of Israel. The genomes of these extremely different organisms were tested by AFLP for genetic diversity at 357 and 345 genetic markers (presumed gene loci), respectively (E.N., Z. Lu, and T. Pavlicek, unpublished work). We found in both organisms parallel genetic patterns reflecting the opposite canyon slopes (Fig. 3) with significantly

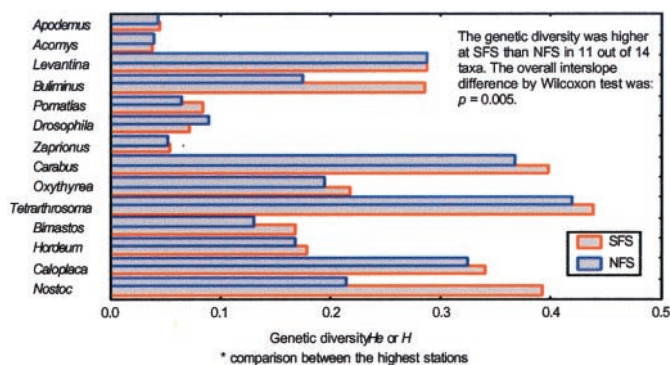


Fig. 2. Genetic (primarily allozyme) diversity in major plant and animal taxa on the opposite slopes of EC I, Lower Nahal Oren, Mount Carmel, Israel. The mesic, temperate, and mild NFS and the xeric, tropical, and stressful SFS. *Caloplaca aurantia* (lichen); *H. spontaneum* (wild barley); *Bimastos syriacum* (earthworm); *Buliminus labrosus*, *Pomatias olivieri*, and *Levantina caesareana* (land snails); *Drosophila simulans* and *Zaprionus tuberculatus* (fruit flies); *Tetraarthrosoma syriacum* (diplopod); *Carabus hemprichi* and *Oxythyrea noemi* (beetles); and *A. cahirinus* and *A. mystacinus* (rodents) are presented. The genetic diversities represent allozyme heterozygosity observed (H) or expected (H_e). Genetic diversity of *Nostoc* is based on AFLP gene diversity.

higher AFLP genetic polymorphism in subpopulations on the ecologically more stressful, warmer, and drier SFS. Likewise, both organisms displayed higher viability in response to severe drought stress on the more arid and climatically fluctuating SFS. Our results suggest the following. (i) Microclimatic selection is the major evolutionary interslope fast-acting diverging force on genotypes and phenotypes, overriding migration and genetic drift. (ii) Ecological stress can generate global-scale adaptive evolutionary genome and phenome strategies at both micro-scales and macro-scales, reinforcing homeostasis and fitness and suggesting continuity between microevolution and macroevolution.

Extraordinary HIP1 Genome Diversity in a Cyanobacterium *N. linckia*. We demonstrated (36) in the cyanobacterium *N. linckia* from EC I a distinct interslope divergence in AFLP (36) and a dramatic interslope intergenomic genetic divergence (Fig. 1) of a highly iterated decameric palindrome (HIP1; refs. 37–41). The highly stressful subpopulations 1 and 2, representing the climax of solar radiation, high temperature, and lowest humidity on SFS, also represent the climax of size-intergenic HIP1 diversity, (i.e., polymorphism in the size of intergenomic spacing between HIP1 decamers, whose average distance is about 1.2 kbp (39). Hypothetically, HIP1 may be involved in stability, gene regulation, bacterial chromosome organization, and genomic rearrangements (37–40), although these assignments await future demonstration. HIP1 may enhance genome plasticity, increase

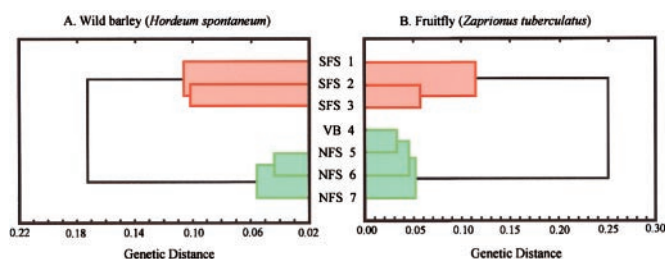


Fig. 3. Dendrograms derived from Nei's genetic distances between the subpopulations of wild barley (*H. spontaneum*; A) and fruit fly (*Z. tuberculatus*; B) at EC, based on 357 and 345 AFLP gene loci (E.N., Z. Lu, and T. Pavlicek, unpublished work).

genetic diversity, and increase gene rearrangements (40) by intrachromosomal and interchromosomal recombination caused by replication slippage. We hypothesized, but still seek experimental evidence, that HIP1 size-intergenic polymorphism is dramatically selected by spatiotemporal variable climatic stresses at a microscale as an adaptive strategy to cope with fluctuations of desiccation, temperature, and solar radiation. The HIP1 size-intergenic polymorphism may reorganize and increase the genome diversity of *N. linckia* and other cyanobacteria, thereby reinforcing their adaptation to multiple ecological stresses. Haploid sedentary *N. linckia* exemplifies the extreme selection of higher genetic polymorphism caused by microclimatic stress in EC I. Is the extreme divergence related to the lack of meiotic recombination and absolute sedentariness in *Nostoc*?

Genome Evolution of Wild Barley by BARE-1 Retrotransposons in EC I.

A critical test of the “junk” DNA hypothesis was conducted by tracking BARE-1 retrotransposon dynamics in wild barley, *H. spontaneum*, in EC I (42). Genomes abound with “selfish,” self-replicating retrotransposons “parasitizing” the host DNA. The BARE-1 retrotransposon constitutes a major, dispersed, active component of *Hordeum* genomes, and the BARE-1 copy number is positively correlated with genome size (43). The number of full-length BARE-1 retrotransposon copies in individuals of *H. spontaneum* in EC I ranges from 8.3×10^3 to 22.1×10^3 per haploid genome (i.e., 3-fold variation at a microsite!). What drives this astonishing local variation? Is it randomly distributed or ecologically structured? The replicative spread of retrotransposons in the genome creates new insertion polymorphisms, increasing retrotransposon numbers and potentially both their share of the genome and genome size.

We examined genome size and BARE-1 insertion patterns and copy number in wild barley, *H. spontaneum*, in EC I (42). On both slopes, but especially on the drier SFS, a simultaneous increase in the BARE-1 copy number and a decrease in the relative number lost through recombination, as measured by the abundance of solo long terminal repeats, seem to have driven the BARE-1 share of the genome upward with the height and dryness of the slope (42). The 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 are consistent with regional trends for BARE-1 in *H. spontaneum* across Israel along a southward transect of increasing aridity (43). This local and regional pattern of BARE-1 may reflect adaptive selection for increasing genome size through retrotransposon activity, because larger genomes may cope more effectively with aridity stress (42). Thus, transposable elements may assume new host mutualistic functions (44).

Genome Evolution: Adaptive Strategies Against Physical Stresses.

Can microscale patterns reflect macroscale adaptive patterns? We tested interslope genotypic and phenotypic adaptive complexes in diverse organisms in EC I, from cyanobacteria to mammals. Here, I outline the adaptive complexes of several model organisms.

Morphophysiological, behavioral, and life-history drought-resistant complex adaptive strategies to the xeric SFS, as compared with those to the mesic NFS, have been described for wild barley *H. spontaneum* (45, 46), three woody species (*Olea europaea*, *Ceratonia siliqua*, and *Pistacia lentiscus*; ref. 47), land snails (48, 49), *D. melanogaster* (50), and mammalian rodents (*A. cahirinus* and *A. mystacinus*; ref. 51). Preliminary findings in spiny mice, *A. cahirinus*, showed a desert pattern of basic metabolic rate lower by 20% on the xeric SFS than on the mesic

NFS.[†] These xeric adaptations involve genetic (described earlier) anatomical, ecophysiological, and behavioral traits to cope with the higher drought on the SFS. Phenotypic adaptations are intimately coupled with genotypic adaptations as complex adaptive strategies against single or combinatorial physical ecological stresses of higher solar radiation, temperature, and drought on the SFS.

Incipient Sympatric Speciation. Interslope adaptive complexes are prerequisites for speciation. Indeed, we have preliminary evidence for the fruit fly *D. melanogaster* (52) and the soil fungus *S. fimicola* (53) of incipient interslope sympatric speciation.

Drosophila. Adaptive ecological differentiation of natural populations of *D. melanogaster*, *D. simulans*, and another drosophilid, *Z. tuberculatus*, in EC I is well established (3). The fitness complex of *D. melanogaster* includes oviposition temperature preferences, tolerance to high temperature, drought stress and starvation, and different longevity patterns (50). This remarkable differentiation has evolved despite short interslope distances (only 100–400 m), enabling easy dispersal. We hypothesized that interslope microclimatic differences caused strong diversifying selection for stress tolerance, accompanied by behavioral differentiation (habitat choice and reduced migration rate), reinforced by sexual isolation. We found highly significant mate choice by flies from different slopes of the canyon, with preference for sexual partners originating from the same slope (52). Preliminary unpublished results (E.N. and T. Pavlicek, unpublished work) suggest no or low interslope migration as found also in rodents in EC I (11).

S. fimicola. Remarkably, we found similar hints of incipient sympatric speciation in the soil fungus *S. fimicola* (53). Fertility diminished slightly for interslope strains compared with intraslope crosses. There was no crossfertility between strains from widely separated areas in Israel, America, and Canada (53). Crossfertility declines with ecogeographic distance. We are currently testing other taxa between the slopes for incipient sympatric speciation (*Nostoc*, *Lotus*, etc.). If substantiated, the EC model may be embryonic evolutionary cradles of the twin evolutionary processes of adaptation and speciation across life.

Theory

Summary of Evidence. The evidence across life, involving numerous species, populations, and individuals, displays massive genetic polymorphic correlations and parallelisms to environmental heterogeneity and stress. Generally, genome and phenome diversity are nonrandom and correlated with stress and higher environmental heterogeneity or niche width (54). Genetic diversity is partly correlated with, and predictable by, a few ecological (primarily climatic) variables. These correlations largely characterize global, regional, and local scales (11).

Evolutionary Forces and Adaptive Complexes. The aforementioned genomic and phenomic patterns over many unrelated species, subdivided into ecological contrasts at macroscales and microscales, strongly implicate natural selection in population and species differentiation. Various forms of selection, primarily diversifying, balancing, and cyclical selection regimes are massively involved, singly or in combination, in genotypic and phenotypic structure and differentiation of populations and species, at various life cycle stages of organisms. Natural selection seems to overrule mutation, migration, and stochasticity in orienting evolution.

Natural Selection. Theoretically, spatiotemporal variations of diversifying selection could maintain genetic polymorphism (55–67). Polymorphism maintenance may be reinforced by two loci and multilocus structures (61–64) and by habitat selection (67). Heterosis cannot explain multiallele polymorphism (68).

Stabilizing Cyclical Selection. Stabilizing selection with a cyclically moving optimum may efficiently protect polymorphism for linked loci, additively affecting the selected trait (refs. 61–64 and references therein). Unequal gene action and/or dominance effects for one or both loci may lead to local polymorphism stability with substantial polymorphism attracting domain. “Supercycles,” with a period comprising hundreds of forced oscillation periods, could substantiate polymorphism and increase the range of temporal variation of allele frequencies. A multilocus system subjected to stabilizing and cycling selection represents a previously uncharacterized evolutionary mechanism that may increase genetic diversity over long-term periods (63) and contribute to overcoming massive extinctions.

Selection vs. Random Drift. Appreciable polymorphism can be preserved in small, long-isolated shrinking populations consisting of several dozens or a hundred individuals, such as in the blind subterranean mole rat, *Spalax* (66). Current theoretical models predict fast gene fixation in small panmictic populations without selection, mutation, or gene inflow. Using simple multilocus models, we demonstrated that moderate stabilizing selection (with stable or fluctuating optimum) for traits controlled by additive genes could oppose random fixation in such isolates for thousands of generations (66). We also showed that in selection-free models, our multichromosome models challenge the hitchhiking hypothesis (69) of polymorphism maintenance for many neutral loci because of close linkage with a few selected loci.

The major conclusion resulting from this modeling is that both mechanisms (stabilizing selection or cyclical selection) can maintain polymorphism for many semidominant loci for thousands of generations, despite a small population size. This negates both Wrightian prediction on the role of genetic drift in small populations (14) and the even more extreme predictions of the neutral theory (13) about random fixation and low H in small populations. These genetic drift and neutral scenarios ignore the role of ecological selection in maintaining genetic polymorphism and predict an unrealistic straightforward demographic positive correlation between effective population size and H , regardless of ecological factors. Our conclusion about the selection in small populations (66) is quite robust in respect to concrete configurations of the selected loci within and between chromosomes.

Strong selection (more than 10% per trait) may be a common phenomenon in nature, as first emphasized by Ford (4). This type of selection could have important evolutionary consequences in the case of small peripheral populations (66). Our modeling clearly demonstrates that moderate or strong selection in a small population can oppose random drift and maintain polymorphism for thousands of generations. These results are significant for the theories of adaptation and speciation, particularly for peripatric speciation (70).

Genetic Interaction Between Species: Biotic Drive of Polymorphism. A simple model of genetic interaction between multiple species governed by abiotic and biotic selection for multilocus quantitative traits was recently suggested. The maintenance of polymorphism may result not only from abiotic, but most importantly, from biotic genetic interaction of species (host–pathogen, symbionts, competitors) that are governed by mutual selection for additively controlled quantitative traits (71). Polymorphism seems to be more naturally promoted by unequal gene effects

[†]Haim, A., Keshet-Siton, A., Blaustein, L., Afiq, D., Neuman, A. & Nevo, E., Annual Meeting of the Israeli Society of Ecology and Environmental Quality, June 16–17, 1997, Haifa, Israel, p. 104 (abstr.).

than by equal gene effects and deviation from purely additive within-locus schemes of gene action.

This new model of species coevolution is based on selection for quantitative traits, complementing the gene-for-gene concept by a multilocus trait-for-trait analogue, based on Mendelian formulation (ref. 71 and references therein). Trait-for-trait interspecies interaction can promote polymorphism in a species experiencing selection pressures from other members of the community if at least one of the following conditions holds: (i) nonequal effects of the additive genes controlling the selected trait; (ii) dominance deviation from the purely intralocus additivity scheme, preserving additivity across loci; and (iii) disturbance of the log concavity/log convexity of the fitness function of the considered species. These results may be considered an extension of the idea of coevolution mediated by selection for qualitative traits, which was first used in the framework of Lande's quantitative genetic model (72).

Genetic Diversity Promoted by Ecological Diversity and Stress. The foregoing discussion on both coding and noncoding sequences suggests several explanations for the maintenance of genetic diversity subjected to environmental diversity and stress. Spatial and temporal variation, which predominate in nature, are of prime importance in maintaining genetic diversity in natural populations. This ecological-genetic pattern is true, because different genotypes display varying fitnesses in variable environments and stresses. Recombination frequencies and mutation rates tend to increase under stressful conditions (73, 74). Rates of evolutionary change are therefore enhanced in adverse environments. We also showed this environmental-genetic relationship under controlled laboratory experiments in the case of mercury pollution (75), under regional aridity stress across the physically stressful environment in Israel (10–12), and locally in EC because of high solar radiation, heat, and drought on the SFS (7, 8).

Genetic polymorphisms at the protein and DNA levels are enhanced under both environmental stress and genomic stress, as was extensively documented above. This ecological-genomics pattern could increase diversity under stressful conditions (74). Heterosis may increase diversity with stress up to high but not extreme levels (75). Environmental heterogeneity and stress cultivate genetic polymorphisms, particularly in dynamically cycling environments that can generate complex supercycles, T cycles, and chaotic-like behavior. This mode of multilocus dynamics far exceeds the potential for maintaining genetic polymorphism attainable under ordinary selection models, including heterosis. It may represent a previously uncharacterized evolutionary mechanism that can assist, in combination with mutation, in maintaining and increasing genetic polymorphism in single species over long periods of time, without frequency- and/or density-dependent selection (63). Models of sexual reproduction as an adaptation to resist parasites may contribute to sex evolution, recombination, and polymorphism (76). Finally, our model of genetic interaction among multiple species governed by abiotic and biotic selection for multilocus quantitative traits (71), provides additional biotic mechanisms for the promotion of genetic diversity in nature caused by species' dynamic interactions.

Conclusions

The enigma of genetic diversity and genome-phenome organization and evolution in nature has been fruitfully explored by using modern molecular techniques. Genotypic and phenotypic diversity has been found in all species at the protein, DNA, and organismal levels. Genome-phenome organization in nature is nonrandom, heavily structured, and correlated with abiotic and environmental diversity and stress. Deciphering the origin and maintenance of genetic diversity will be enhanced through

investigations focusing on the interface between ecology and genomics. Critical tests and strong inferences in nature of abiotic and biotic factors include transplant experiments at microscales (46, 77) and macroscales, to unravel genome organization and fitness in contrasting and changing environments and to relate genomics to phenomics.

Reassuringly, DNA polymorphisms (RAPDs, AFLPs, SSRs, and SNPs) and the noncoding genome largely mirror protein (isozyme) polymorphisms are subjected to natural selection and can be used to highlight genome structure and evolution. The focus of evolutionary biology is the organism-environment interaction involving genomes, phenomes, and biomes, across the tree of life. The noncoding and regulatory genome should become a central target in understanding evolution.

Prospects. What next? Modern molecular techniques, bioinformatics, and computational techniques make detailed structural-functional genome analysis possible. The following aspects could be advanced.

(i) Probing genomic architecture and dynamics of genes and intergenic spacers can be facilitated by applying novel DNA polymorphic molecular markers (RAPD-PCR, AFLP, SSR, or SNP) and sequence polymorphism, elucidating cell cycling and the evolutionary history of life as well as bridging genotypes and phenotypes. These techniques can probe the entire genome, both coding and noncoding regions, especially in the era of complete comparative and functional genomics.

(ii) Genome sequence variation and marker polymorphism of stress alleles (i.e., alleles correlated with specific stresses, such as solar radiation, temperature, drought, salinity, chemical pollution, and resistances to pathogens and parasites) and SNPs could be probed, to decipher their biochemical physiology, "chromosome ecology" (65), and regulation.

(iii) Testing biomolecular signatures, such as the relative abundance or "genomic signature" of oligonucleotides, and analyzing sequence compositional spectra and distribution heterogeneity of specific signals (methylase targets, telomeric repeats, microsatellites and minisatellites, palindromes, recombinational hot spots, mobile elements, and codon usage bias; e.g., ref. 1) provide powerful tools for comparative ecological-genomic and evolutionary analysis in diverse taxa across life sharing ecological stresses. These techniques coupled with transgenics could contribute to evaluating the function of stress alleles and their control elements under the same and different stresses.

(iv) From DNA sequencing or structural genomics to functional genomics: systematic genome mapping, sequencing, functioning, and experimentation by RNA and DNA microarray chip technologies offer biology with enormous opportunities and permit identification and genotyping of mutations and polymorphisms, allowing better insight into structure-function interaction of genome complexity under differential stresses (78). The completion of sequencing genomes of many viruses, chloroplasts, mitochondria prokaryotic bacteria, eukaryotic budding yeast, the nematode *Caenorhabditis elegans*, the fruit fly *Drosophila*, the higher plant *Arabidopsis thaliana*, the important crop rice, *Oryza sativa*, and the human genome has permitted the understanding of biodiversity in molecular terms (e.g., ref. 79 in mammals). Ecological genetics advanced by Ford (4) could now develop into the new science of ecological genomics, interacting with comparative structural and functional genomics.

What physiological challenges need to be analyzed to reveal the functions of uncharacterized genes uncovered by sequencing? A promising approach is to reveal the role of abiotic and biotic ecological factors in the primary organization and diversity of genomes and their phenomic-biomic interactions. (v) Higher resolution physical maps of chromosomes and genomes of model organisms, including expressed sequence tags and sequence tagged sites, derived from genetic mapping of quantitative trait

loci by using the new quantitative trait loci mapping strategy of correlated multitrait complexes (80, 81), and identification of candidate genes, can help unravel the genetic basis of complex patterns of adaptation and speciation and substantiate biotechnology.

(vi) Identifying, and estimating the kind, degree, and stage of operation of the following evolutionary forces: natural selection, migration, mutation, recombination, DNA repair, and mobile elements operating on the coding and noncoding genome. Molecular diversity studied under ecological stress allows sub-

stantial advances in understanding genome–proteome–phenome and biome structure, function, dynamics, and evolution, thereby highlighting the molecular-genetic basis of adaptation and speciation, i.e., of life’s evolution.

I thank Sam Karlin, Abraham Korol, and Avigdor Beiles for their comments on this paper; the University of Haifa for enabling me and my colleagues to conduct these studies; and all of the many foundations that financed our efforts to understand genome and phenome diversity and differentiation in nature.

1. Karlin, S., Campbell, A. M. & Mrazek, J. (1998) *Annu. Rev. Genet.* **32**, 185–225.
2. Trifonov, E. N. (2001) *Gene*, in press.
3. Harry, M. E., Rashkovetsky, E., Pavlicek, T., Baker, S., Derzhavets, E. M., Capy, P., Cariou, M. L., Lachaise, D., Asada, N. & Nevo, E. (1999) *Biologia (Bratisl.)* **54**, 685–705.
4. Ford, E. (1971) *Ecological Genetics* (Chapman & Hall, London).
5. Nevo, E., Beiles, A. & Ben-Shlomo, R. (1984) *Lecture Notes in Biomathematics* **53**, 13–213.
6. Nevo, E. & Beiles, A. (1988) *Biol. J. Linn. Soc.* **35**, 229–245.
7. Nevo, E. (1995) *Proc. R. Soc. London Ser. B* **262**, 149–155.
8. Nevo, E. (1997) *Theor. Popul. Biol.* **52**, 231–243.
9. Nevo, E. (1978) *Theor. Popul. Biol.* **13**, 121–177.
10. Nevo, E. (1988) *Evol. Biol.* **23**, 217–247.
11. Nevo, E. (1998) *J. Exp. Zool.* **282**, 95–119.
12. Nevo, E. (2000) in *Encyclopedia of Life Sciences* (Macmillan, London), pp. 1–11.
13. Kimura, M. (1983) *The Neutral Theory of Molecular Evolution* (Cambridge Univ. Press, Cambridge, U.K.).
14. Wright, S. (1931) *Genetics* **16**, 97–159.
15. Lewontin, R. C. (1974) *The Genetic Basis of Evolutionary Change* (Columbia Univ. Press, New York).
16. Mitton, J. B. (1997) *Selection in Natural Populations* (Oxford Univ. Press, Oxford).
17. Avise, J. C. (1994) *Molecular Markers, Natural History, and Evolution* (Chapman & Hall, London).
18. Hamrick, J. L., Linhart, Y. B. & Mitton, J. B. (1979) *Annu. Rev. Ecol. Syst.* **10**, 173–200.
19. Ohta, T. & Gillespie, J. H. (1996) *Theor. Popul. Biol.* **49**, 128–142.
20. Nevo, E. (1986) In *Environmental Quality and Ecosystem Stability*, eds. Dubinsky, Z. & Steinberger, Y. (Bar-Ilan Univ. Press, Ramat Gan, Israel), Vol. III, pp. 841–848.
21. Nevo, E. (1999) *Mosaic Evolution of Subterranean Mammals: Regression, Progression and Global Convergence* (Oxford Univ. Press, Oxford).
22. Nevo, E. (1991) *Evol. Biol.* **25**, 1–125.
23. Nevo, E., Ivanitskaya, E. & Beiles, A. (2001) *Adaptive Radiation of Blind Subterranean Mole Rats: Naming and Revisiting the Four Sibling Species of the *Spalax ehrenbergi* Superspecies in Israel* (Backhuys, Leiden, The Netherlands), in press.
24. Nevo, E., Shimony, T. & Libni, M. (1977) *Nature (London)* **267**, 699–701.
25. Nevo, E., Beiles, A. & Krugman, T. (1988). *Theor. Appl. Genet.* **75**, 529–538.
26. Nevo, E., Beiles, A. & Krugman, T. (1988). *Theor. Appl. Genet.* **76**, 737–752.
27. Nevo, E., Noy-Meir, I., Beiles, A., Krugman, T. & Agami, M. (1991) *Isr. J. Botany* **40**, 419–449.
28. Nevo, E., Ben-Shlomo, R., Beiles, A., Ronin, Y. I., Blum, S. & Hillel, J. (1996) in *Gene Families: Structure, Function, Genetics and Evolution*, eds. Holmes, R. S. & Lim, H. A. (World Scientific, Singapore), pp. 55–70.
29. Li, Y. C., Fahima, T., Beiles, A., Korol, A. B. & Nevo, E. (1999) *Theor. Appl. Genet.* **99**, 873–883.
30. Li, Y. C., Röder, M. S., Fahima, T., Kirzhner, V. M., Beiles, A., Korol, A. B. & Nevo, E. (2000) *Theor. Appl. Genet.* **100**, 985–999.
31. Li, Y. C., Fahima, T., Korol, A. B., Peng, J. H., Röder, M. S., Kirzhner, V. M., Beiles, A. & Nevo, E. (2000) *Mol. Biol. Evol.* **17**, 851–862.
32. Li, Y. C., Fahima, T., Peng, J. H., Röder, M. S., Kirzhner, V. M., Beiles, A., Korol, A. B. & Nevo, E. (2000) *Theor. Appl. Genet.* **101**, 1029–1038.
33. Li, Y. C., Fahima, T., Krugman, T., Beiles, A., Röder, M. S., Korol, A. B. & Nevo, E. (2000) *Conserv. Genet.*, in press.
34. Shreve, F. (1922) *Ecology* **3**, 269–274.
35. Finkel, M., Fragman, O. & Nevo, E. (2001) *Isr. J. Plant Sci.* **46**, 169.
36. Satish, N., Krugman, T., Vinogradova, O. N., Nevo, E. & Kashi, Y. (2001) *Microb. Ecol.*, in press.
37. Robinson, P. J., Craneburgh, R. M., Head, I. M. & Robinson, N. J. (1997) *Mol. Microbiol.* **24**, 181–189.
38. Smith, J. K. (1998) *Mol. Microbiol.* **144**, 2791–2801.
39. Bhaya, D., Vault, D., Amin, P., Takahashi, A. W. & Grossman, A. R. (2000) *J. Bacteriol.* **182**, 5692–5699.
40. Kotani, H. & Tabata, S. (1998) *Annu. Rev. Plant Physiol. Plant Mol. Biol.* **49**, 151–171.
41. Mrazek, J., Bhaya, D., Grossman, A. P. & Karlin, S. (2001) *Nucleic Acid Res.*, in press.
42. Kalendar, R., Tanskanen, J., Immonen, S., Nevo, E. & Schulman, A. H. (2000) *Proc. Natl. Acad. Sci. USA* **97**, 6603–6607 (First Published May 23, 2000; 10.1073/pnas.110587497)
43. Vincient, C. M., Suoniemi, A., Anamthawat-Jonsson, K., Tanskanen, J., Beharav, A., Nevo, E. & Schulman, A. H. (1999) *Plant Cell* **11**, 1769–1784.
44. Kidwell, M. G. & Lisch, D. R. (2001) *Evolution (Lawrence, Kans.)* **55**, 1–24.
45. Gutterman, Y. & Nevo, E. (1994) *Isr. J. Plant Sci.* **42**, 183–195.
46. Lavie, B., Stow, V., Krugman, T., Beiles, A. & Nevo, E. (1993) *Barley Genet. Newsletter* **23**, 12–14.
47. Nevo, E., Bolshakova, M. A., Martyn, G. I., Musatenko, L. I., Sytnik, K. M., Pavlicek, T. & Beharav, A. (2000) *Isr. J. Plant Sci.* **48**, 33–46.
48. Rankevich, D., Lavie, B., Nevo, E., Beiles, A. & Arad, Z. (1996) *Isr. J. Zool.* **42**, 425–442.
49. Rankevich, D., Lavie, B., Nevo, E. & Arad, Z. (2001) *J. Mollusc Stud.*, in press.
50. Nevo, E., Rashkovetsky, E., Pavlicek, T. & Korol, A. B. (1998) *Heredity* **80**, 9–16.
51. Nevo, E., Filippucci, G. M., Pavlicek, T., Gorlova, O., Shenbrot, G., Ivanitskaya, E. & Beiles, A. (1998) *Acta Theriologica* **5**, 9–34.
52. Korol, A. B., Rashkovetsky, K., Iliadi, P., Michalak, P., Ronin, Y. I. & Nevo, E. (2000) *Proc. Natl. Acad. Sci. USA* **97**, 12637–12642 (First Published October 24, 2000; 10.1073/pnas.220041397)
53. Lamb, B. C., Kozlakidis, Z. & Saleem, M. (2000) *Fungal Genet. Newsletter* **47**, 69–71.
54. Van Valen, L. (1965) *Am. Nat.* **99**, 377–390.
55. Levene, H. (1953) *Am. Nat.* **87**, 331–333.
56. Felsenstein, J. (1976) *Annu. Rev. Genet.* **10**, 253–280.
57. Karlin, S. (1979) *Proc. Natl. Acad. Sci. USA* **76**, 541–545.
58. Ewens, W. J. (1979) *Mathematical Population Genetics* (Springer, New York).
59. Hedrick, P. W. (1986) *Annu. Rev. Ecol. Syst.* **17**, 535–566.
60. Gillespie, J. H. (1991) *The Causes of Molecular Evolution* (Oxford Univ. Press, Oxford).
61. Kirzhner, V. M., Korol, A. B., Ronin, Y. I. & Nevo, E. (1994) *Proc. Natl. Acad. Sci. USA* **91**, 11432–11436.
62. Kirzhner, V. M., Korol, A. B. & Ronin, Y. I. (1995) *J. Evol. Biol.* **8**, 93–120.
63. Kirzhner, V. M., Korol, A. B. & Nevo, E. (1996) *Proc. Natl. Acad. Sci. USA* **93**, 6532–6535.
64. Korol, A. B., Kirzhner, V. M., Ronin, Y. I. & Nevo, E. (1996) *Evolution (Lawrence, Kans.)* **50**, 1432–1441.
65. Korol, A. B., Preygel, I. A. & Preygel, S. I. (1994) *Recombination Variability and Evolution: Algorithms of Estimation and Population Genetics Models* (Chapman & Hall, London).
66. Nevo, E., Kirzhner, V. M., Beiles, A. & Korol, A. B. (1997) *Philos. Trans. R. Soc. London B* **352**, 381–389.
67. Nevo, E., Beiles, A., Korol, A. B., Ronin, Y. I., Pavlicek, T. & Hamilton, W. D. (2000) *Evolution (Lawrence, Kans.)* **54**, 586–605.
68. Lewontin, R. C., Ginzburg, L. & Tuljapurkar, S. D. (1978) *Genetics* **88**, 149–170.
69. Hedrick, P. W. (1982) *Bioscience* **32**, 845–853.
70. Mayr, E. (1954) in *Evolution as a Process*, eds. Huxley, J., Hardy, A. & Ford, E. (Allen & Unwin, London), pp. 157–180.
71. Kirzhner, V. M., Korol, A. B. & Nevo, E. (1999) *J. Theor. Biol.* **198**, 61–70.
72. Kiestler, R. A., Lande, R. & Schemske, D. W. (1984) *Am. Nat.* **124**, 220–243.
73. Hoffmann, A. A. & Parsons, P. A. (1991) *Evolutionary Genetics and Environmental Stress* (Oxford Univ. Press, Oxford).
74. Korol, A. B. (1999) in *Evolutionary Theory and Processes: Modern Perspectives, Papers in Honour of Eviatar Nevo*, ed. Wasser, S. P. (Kluwer, Dordrecht, The Netherlands).
75. Nevo, E., Perl, T., Beiles, A. & Wool, D. (1981) *Experientia* **37**, 1152–1154.
76. Hamilton, W. D., Axelrod, R. & Tanese, R. (1990) *Proc. Natl. Acad. Sci. USA* **87**, 3566–3573.
77. Linhart, Y. B. & Grant, M. C. (1996) *Annu. Rev. Ecol. Syst.* **27**, 237–277.
78. Wakefield, M. J. & Graves, J. A. M. (1996) *Mamm. Genome* **10**, 715–716.
79. O’Brien, S. J., Menotti-Raymond, M., Murphy, W. J., Nash, W. G., Weinberg, J., Stanyon, R., Copeland, N. G., Jenkins, N. A., Womack, J. E. & Graves, J. A. M. (1999) *Science* **286**, 458–481.
80. Korol, A. B., Ronin, Y. I. & Nevo, E. (1998) *Genetics* **148**, 2015–2028.
81. Korol, A. B., Ronin, Y. I., Itzcovich, A. & Nevo, E. (2001) *Genetics*, in press.