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Evolution Under Environmental Stress at Macro- and Microscales

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Abstract

Environmental stress has played a major role in the evolution of living organisms (Hoffman AA, Parsons PA. 1991. Evolutionary genetics and environmental stress. Oxford: Oxford University Press; Parsons PA. 2005. Environments and evolution: interactions between stress, resource inadequacy, and energetic efficiency. Biol Rev Camb Philos Soc. 80:589–610). This is reflected by the massive and background extinctions in evolutionary time (Nevo E. 1995a. Evolution and extinction. Encyclopedia of Environmental Biology. New York: Academic Press, Inc. 1:717–745). The interaction between organism and environment is central in evolution. Extinction ensues when organisms fail to change and adapt to the constantly altering abiotic and biotic stressful environmental changes as documented in the fossil record. Extreme environmental stress causes extinction but also leads to evolutionary change and the origination of new species adapted to new environments. I will discuss a few of these global, regional, and local stresses based primarily on my own research programs. These examples will include the 1) global regional and local experiment of subterranean mammals; 2) regional experiment of fungal life in the Dead Sea; 3) evolution of wild cereals; 4) "Evolution Canyon"; 5) human brain evolution, and 6) global warming.

Key words: stress, subterranean mammals, Dead Sea, "Evolution Canyon", global warming.

Mosaic Evolution of Subterranean Mammals

Stress is a major driving force of evolution (Hoffman and Parsons 1991; Parsons 2005). Parsons defined stress as an environmental factor causing potential injurious changes to biological systems with the potential for impacts on evolutionary processes. Variation in the approximation fitness is inversely related to stress levels. Here I will not attempt comprehensiveness and a general overview but base the following examples primarily on my own and my colleagues' research programs, unfolding major relationships among environmental stresses as an evolutionary driver. The genomic perspectives are mostly just beginning to assemble but could be dramatically advanced in the next generation sequencing that opens new horizons relating genomes and phenomes, and thus revolutionizing biology (e.g., Biemont and Vieria 2006; Schuster 2008; Atwell et al. 2010; Belyayev et al. 2010; Conrad 2010). The extensive convergent evolution of subterranean mammals across the planet (Nevo 1999; Beagall et al. 2007) began during the global climatic transition from the middle Eocene to the early Oligocene 45–35 million years ago. It involved seasonal climatic stresses proceeding progressively throughout the Cenozoic. The ecological stress of savannoid open biota set the stage for a rapid evolutionary play of recurrent adaptive radiations of unrelated mammals on all continents to the subterranean sheltered ecotope. This transition involved several hundreds of small mammalian species belonging to 50 genera, 11 families, and 3 mammalian orders (fig. 1). The subterranean ecotope provided small mammals shelter from predators and extreme climatic fluctuations and stress of temperature and humidity. However, they had to evolve genomic adaptive complexes (Brodsky et al. 2005) to the immense underground stresses of darkness, solid soil, low productivity, hypoxia, hypercapnia, and high infectivity. All subterranean mammals display convergent molecular and organismal adaptations to life underground, though in different degrees, depending on the degree of their underground confinement. Adaptive convergence of subterranean mammals comprises structural and functional reductions and expansions through tinkering. These involve burrowing, energetics, and respiratory adaptations affecting "genomics," "proteomics," and "phenomics" compared with those of small aboveground mammals (Nevo 1999; Beagall et al. 2007). Life underground triggered the

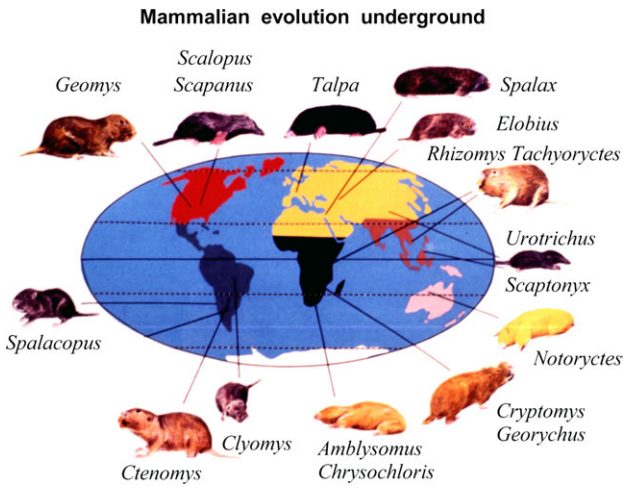

Fig. 1.—Distribution of subterranean mammals across the planet. Palearctic region: *Talpa* (Talpidae, insectivores), *Spalax* (Spalacidae, rodents; SE Europe, Turkey, Near East, N. Africa) and *Ellobius* (Arvicolidae, rodents; Asia); Ethopian: *Chrysochloris* and *Amblysomus* (Chrosochloridae, insectivores; S. Africa), *Tachyoryctes* (Rhizomyidae, rodents: S. Africa); Oriental: *Scaptonyx* and *Urotrichus* (Talpidae, insectivores; E. Asia) and *Rhizomys* (Rhizomyidae, rodents); Australian: *Notoryctes* (Nortorctidae, marsupial moles; Australia); Nearctic: *Scalopus* and *Scapanus* (Talpidae, insectivores) and *Geomys* (Geomyidae, rodents); Neotropical: *Spalacopus* (Octodontidae, rodents), *Ctenomys* (Ctenomyidae, rodents), and *Clyomys* (Echimyidae, rodents). Different symbols mark the different zoogeographical regions. (From Prof Hynek Burda, a personal slide.)

evolution of photoperiodic perception and circadian rhythms through complex mosaic eyes (Cooper et al. 1993) and brain evolution (Rehkamper et al. 1994; Frahm et al. 1997; Mann et al. 1997), as well as the evolution of aggression (Nevo et al. 1975; Nevo, Heth, et al. 1986) and seismic communication (Nevo, Heth, et al. 1991) affecting brain reorganization such as the expansion of the somatosensory cortex into the visual cortex, which is highlighted in extreme cases of the genus *Spalax* (Nevo 1999, 2007; Nevo et al. 2001). Stress underground selected for a suit of progressive adaptive traits to underground life such as seismic, magnetic, auditory, chemical, nasal, and vomeronasal communication systems, compensating for the regressive loss of sight and affecting the entire genome and its regulation (Brodsky et al. 2005). Adaptive convergence of subterranean mammals also generally implicates the following traits: intraspecific territoriality and aggressive competition, circadian rhythmicity, food generalism, equilibrium populations, and K-strategy. Likewise, they display low-allozyme genetic diversity and homoselection, interspecific competitive exclusion, and largely parapatric species distribution between ecologically and genetically similar species. A major reorganization for life underground relates to respiratory adaptations in accordance with the extreme hypoxic–hypercapnic subterranean atmospheres. The dual stresses of hypoxia and hypercapnia increase under flooding, as was demonstrated in Israeli *Spalax*, and climax in the adaptive respiratory system of four species of the *Spalax ehrenbergi* superspecies in Israel (Nevo et al. 2001). *Spalax ehrenbergi* superspecies differentiated allopatrically into four species in Israel following the gradient of increasing aridity stress and decreasing predictability southwards toward the desert: *Spalax galili*→*S. golani*→*S. carmeli*→*S. judaei* ($2n$ = 52→58→60), and eastward *S. galili*→*S. golani*, $2n$ = 52→54 (Supplementary fig. 1a, b, and c). This chromosomal and associated genetic trend of *Spalax* is intimately associated with the regional aridity stress southwards in Israel: budding new species adapted genomically, proteomically, and phenomically (i.e., in morphology, physiology, and behavior) to increasing stresses of higher solar radiation, temperature, and drought southwards (Nevo 1999; Nevo et al. 2001; Nevo list of *Spalax* at http://evolution.haifa.ac.il). *Spalax* share a positive correlation of genetic polymorphisms with aridity stress (Nevo et al. 1996 and supplementary fig. 1d) as well as many other diverse taxa in Israel across phylogeny (Nevo and Beiles 1988 and supplementary fig. 1e); the higher the stress the higher the genetic polymorphism in *S. ehrenbergi* superspecies (in Israel). The chromosomal diploid number increases southwards and is adaptively polymorphic associated with both adaptation and speciation (Nevo, Corti, et al. 1988).

Multiple adaptive systems (genetic, ecological, biochemical, morphological, physiological, and behavioral) characterize each species, adapting it to its unique ecogeographical climatic region and stresses. These adaptations relate primarily to four different climates: cool and humid (*S. galili*, $2n$ = 52), cool and semi-humid (*S. golani*, $2n$ = 54), warm and humid (*S. carmeli*, $2n$ = 58), and warm and dry (*S. judaei*, $2n$ = 60). The combination of aridity and temperature stresses southwards and eastwards determine their necessary genomic adaptive complexes to four climates. Competitive exclusion between neighboring species apparently determines their parapatric distribution, hence mode of peripatric speciation, in accordance with climatic shifts. Intriguingly, speciation proceeded with relatively little genetic divergence (Nevo and Cleve 1978). Henceforward, I will use the diploid chromosomal numbers $2n$ = 52, 54, 58, and 60 instead of the species names to save space.

Morphological Adaptations

Although morphologically indistinguishable, except by detailed multivariate analysis (Nevo 1985; Nevo, Tchernov, et al. 1988), size variation follows as: $2n$ = 52 (54)→58→60 (Nevo, Beiles, et al. 1986). The southward cline in size shows that northern species ($2n$ = 52, 54), living in cooler and more productive mesic environments, are larger than the southern species ($2n$ = 60) living in warmer and less productive xeric environments (supplementary fig. 2, Supplementary Material online). The best predictors of body size, explaining up to 87% of size variation, include

temperature and plant cover variables, thus suggesting that thermoregulation (Bergmann's rule) and productivity (food resources) were the natural selective forces causing size decrease southwards to better thermoregulate and survive in the stressful food scarce desert. Relative brain size and encephalization are highest in *S. judaei*, the desert species, followed by *S. galili*, *S. golani*, and *S. carmeli* ($2n = 60$, 52, 54, and 58). Brain size increases in the *S. ehrenbergi* superspecies with ecological stresses of aridity and climatic unpredictability and is adaptively molded by natural selection. The larger territory of *S. judaei* in the desert selects for a larger brain to cope with the higher burrow complexity and navigation due to food scarcity (Nevo, Pirlot, et al. 1988).

Physiological Adaptations

The physiology of *Spalax* showed strong association with aridity stress southward toward the desert. Mean basal metabolic rates decrease progressively and adaptively southwards $1.03 > 0.86 > 0.85 > 0.62$ for $2n = 52$, (58, 58), and 60, respectively (Nevo and Shkolnik 1974). This pattern was substantiated genomically by the upregulation of brain genes in the desert species (Brodsky et al. 2005). Gross energy intake and apparent dry-matter digestibility was 132.8 and 155.9 kJ/day for the $2n = 52$ and 58 "mesic" species, contrasting with 80.3 and 75.0 kJ/day in the $2n = 54$ and 60 "xeric" species, respectively, reflected genomically in the upregulation of kidney genes including urea cycle, amino acid, and amine metabolism (Brodsky et al. 2005). Under dry conditions, when the food supply is limited, the "xeric" species maintain energy metabolism at a lower level than the "mesic" species (Yahav et al. 1988). Likewise, water turnover was significantly lower in the xeric $2n = 60$ (Yahav et al. 1989). Hyperosmolarity urine concentrating ability under protein and salt load was significantly higher in the "xeric" $2n = 60$ ($P < 0.05$) than in the other three species and was established genomically by amino peptidase activity and arginine metabolism (Brodsky et al. 2005), pushing speciation in the *S. ehrenbergi* superspecies to its limit (Yahav et al. 1990). Adaptive variation with aridity stress was also found in the structure and functions of the kidney (Nevo, Simson, et al. 1989; see genomic correlate in Brodsky et al. 2005). Nevertheless, *Spalax* never colonized the true desert south of the 100 mm isohyet. Finally, adaptive thermoregulatory patterns showed that the xeric $2n = 60$ was a significantly better thermoregulator both in warm and cold environments than the other three species (Haim et al. 1985). Furthermore, in the warm environment, a physiological cline in thermoregulation appeared: $2n = 60 > 58 > 52 > 54$, whereas in the cold environment, the sequence was $2n = 60 > 54 > 52$, 58. Likewise, adaptive variation was also found in nonshivering thermogenesis (Haim et al. 1984). The aforementioned evidence suggests that in the *S. ehrenbergi* superspecies in Israel, physiological correlates are molded by natural selection as a genomic adaptive complex to climatic stresses. The genomic ingredients include an increase northward in metabolic processes, cell growth and maintenance, O_2 and electron transport, ion transport, hemoglobin complex, O_2 transport, mitochondrial electron transport, and oxi-reductase activity (Brodsky et al. 2005). Adaptive climatic respiratory variation in the *S. ehrenbergi* superspecies in Israel dramatically reveals the reaction to hypoxia and hypercapnia stresses and is unfolded by the whole-genome analysis (Brodsky et al. 2005). Oxygen and carbon dioxide pressures in subcutaneous gas pockets were—for O_2: 11.8, 13.6, 16.9, and 17.2 torr, and CO_2 pressures of 84.2, 82.9, 80.1, and 64.1 torr were measured for the species $2n = 52$, 54, 58, and 60, respectively (Arieli et al. 1984 and supplementary fig. 3, Supplementary Material online). Low O_2 and high CO_2 pressures prevail in the flooded and more hypoxic habitats of *S. galili* ($2n = 52$), contrasting with the high O_2 and low CO_2 pressures of xeric *S. judaei* ($2n = 60$). The interspecific variation underlies an important respiratory physiological correlate of ecological speciation in the extremely hypoxic and hypercapnic subterranean environment. Remarkably, both hematocrite (HCT) and hemoglobin (Hb) concentrations were lowest in *S. judaei* ($2n = 60$) and higher in the three northern species (Arieli, Heth, Nevo, and Hoch 1986). Xeric $2n = 60$ lives in a hot climate where a reduced blood viscosity is advantageous for thermoregulation under heat loads when blood is directed peripherally. *S. galili* ($2n = 52$) is better adapted to hypoxia in accordance with its more often winter-flooded habitat than xeric *S. judaei* ($2n = 60$) (Arieli and Nevo 1991). The better hypoxic survival of $2n = 52$ is also reflected in its significantly higher beating and heart frequencies (Arieli, Heth, Nevo, Zamir, et al. 1986), and a higher metabolic network was also unfolded genomically (Brodsky et al. 2005) (fig. 2), and by cytochrome b evolution (Nevo, Beiles, Spradling, 1999).

All *Spalax* globins reflect the adaptive climatic selection revealed in hemoglobin (Hb) (Kleinschmidt et al. 1984, 1985), haptoglobin (Nevo, Ben-Shlomo, et al. 1989), myoglobin (Mb) (Gurnett et al.1984), neuroglobin (Ngb), and cytoglobin (Cygb) (Avivi et al. 2010). *Spalax* hemoglobin (Kleinschmidt et al. 1984, 1985) shows an exchange of 23 amino acid residues in the alpha chains and 26 in the beta chains. *Spalax* blood has a high-oxygen affinity as an adaptation to life underground under hypoxia and hypercapnia (Shams et al. 2005). Mb, Ngb, and Cygb are O_2-binding respiratory proteins contributing to hypoxia adaptation of *Spalax* (Avivi et al. 2010). Messenger RNA (mRNA) and protein levels of *Spalax* brain Ngb are 3-fold higher than in *Rattus norvegicus* under normoxia. Hypoxia induced Cygb transcription in the heart and liver of both mammals, with the most prominent mRNA upregulation (12-fold) in *Spalax* heart. Tissue globins contribute to the remarkable adaptive tolerance of *Spalax* toward environmental hypoxic stress (Avivi et al. 2010). *Spalax* is dramatically superior to *Rattus*

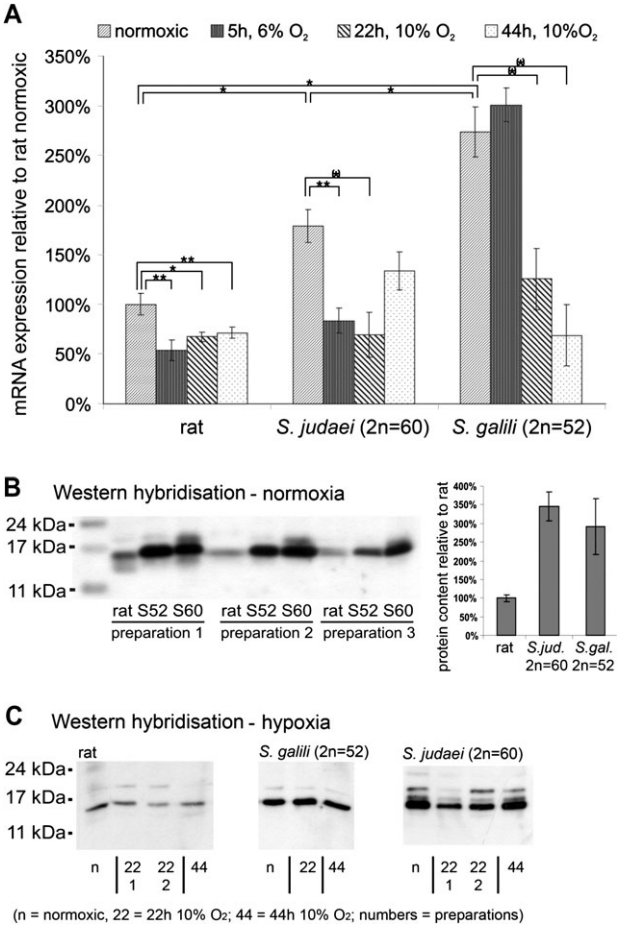

Fig. 2.—Neuroglobin (*Ngb*) expression quantification. (*A*) Ngb mRNA expression in total brain, quantified by quantitative reverse transcriptase polymerase chain reaction. Under normoxia, Ngb expression is 1.8- and 2.8-fold higher, respectively, in *S. judaei* and *S. galili* than in rat. In *S. judaei* and rat, severe short-time hypoxia (4 h, 6% O_2) decreases Ngb mRNA to half of its normoxic value, whereas the amount in *S. galili* is unchanged. Longer term moderate hypoxia (22 and 44 h, 10 O_2) decreases Ngb expression to 40–75% of the normoxic condition in all three species. Significance levels, indicated by asterisk and horizontal brackets, were obtained by the Student's *t*-test: $^{**}P \leq 0.01$, $^{*}P \leq 0.05$, $^{(*)}P \leq 0.1$. (*B*) Western blot analysis of Ngb protein expression in rat, *S. judaei* ($2n = 60$; S60), and *S. galili* ($2n = 52$; S52) normoxic total brain. Three individuals of each species were tested (preparations 1–3). The blot, containing equal amounts of protein per lane, indicates an up to 3.5-fold higher Ngb protein level in the *Spalax* species as compared with rat. (*C*) Western blot analysis of Ngb in hypoxic versus normoxic (n) animals. In rat, we observe a slight downregulation after 22 or 44 h of moderate hypoxic stress (10% O_2). In *S. galili* (S52) and *S. judaei* (S60), protein levels do not proportionately reflect the decreasing mRNA but show that there is no hypoxic upregulation of Ngb (from Avivi et al. 2010).

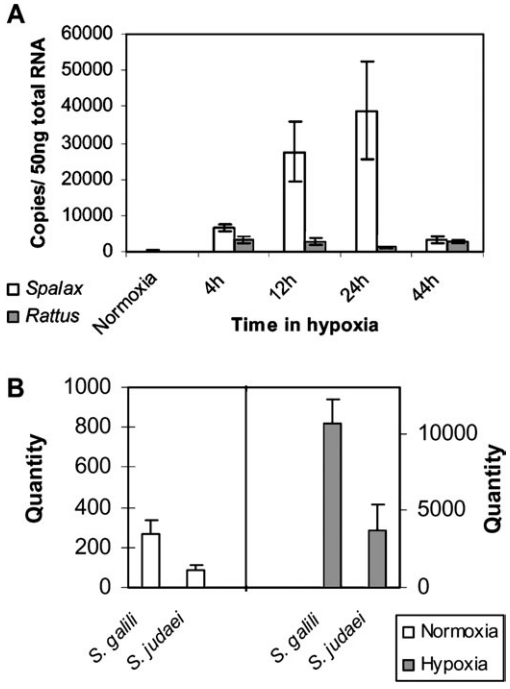

Fig. 3.—(A) Time course of *Epo* gene expression in *Spalax* and *Rattus* kidneys in normoxia and 10% hypoxia. The numbers of copies in 50 ng of total RNA in *Spalax* were 190 ± 57; 6,805 ± 946; 27,485 ± 8,322; 38,898 ± 13,548 and 3,177 ± 877; and the numbers in *Rattus* were 130 ± 53; 3,398 ± 898; 3,040 ± 963; 1,355 ± 209 and 2,691 ± 523 under normoxia and after 4, 12, 24, and 44 h of hypoxia, respectively (From Shams et al. 2004). (B) Comparative *Epo* gene expression in *S. galili* and *S. judaei* under normoxia and 6% O_2 for 10 h. *Spalax galili* values were 269 ± 65 and 10, 687 ± 1,506 and *S. judaei* values were 85 ± 30 and 3,739 ± 1,620, under normoxia and hypoxia, respectively (from Shams et al. 2004).

in vascular endothelial growth factor activity (Avivi et al. 1999), myocardial performance (Edoute et al. 1988), higher capillary and mitochondrial density than *Rattus* (Widmer et al. 1997), and restores blood flow in ischemic mice (Roguin et al. 2003) (fig. 3A and B). Erythropoietin, a key regulator of circulating erythrocytes, and hypoxia-inducible factor 1alpha (*HIF-1a*) *Epo* expression inducer show significantly higher, quicker, and longer responses to different O_2 levels in *Spalax* than in *Rattus* (Shams et al. 2004). After 4-h hypoxia at 6% O_2, *Spalax Epo* levels are 3-fold higher than in *Rattus*. After 24 h of 10% hypoxia, *Spalax Epo* is remarkably 6-fold higher than the maximum of *Rattus* (fig. 3A). *Spalax* HIF-1a achieves maximal expression after 4-h hypoxia at 3% O_2, a 2-fold increase compared with normoxia, whereas no significant change was detected in *Rattus* HIF-1a at any of the studied conditions. At 6% O_2 for 10-h hypoxia, in which *Rattus* cannot survive, *Epo* and *HIF-1a* levels in *Spalax galili*, living in a flooded climatic regime, are higher than in *S. judaei* living in xeric climates (fig. 3B). This, as well as the extracellular region of the *Spalax* erythropoietin receptor maturation (Ravid et al. 2007), provides substantial contributions to the adaptive strategy of hypoxia tolerance in *Spalax*, which evolved during 40 My of evolution to cope with underground hypoxic stress. Remarkably, kidney and brain mRNAs of northern (N) and southern (S) *Spalax* species vary by 20–30% of the expression signal variability. Similar N–S

effects were obtained for all tissues and types of arrays: two affymetrix microarrays using probe oligomer signals and the spotted array. Likewise, an analysis of variance and *t*-test statistics demonstrated significant N–S ecogeographic divergence and region-tissue specificity in gene expression. Analysis of differential gene expression between species corroborates at the whole genomic level of 15,000 genes, with previous results having been deduced by allozyme and DNA molecular polymorphisms. Functional categories show significant N–S ecological putative adaptive divergent upregulation of genes highlighting a higher metabolism in N and kidney urine cycle pathways in S. These results confirm ecologic–physiologic genomic separation of blind mole rats into N and S. Gene expression regulation appears to be central in *Spalax* evolution underlying all physiological N–S divergence (Brodsky et al. 2005). Most remarkably, other important genes, such as those in *Spalax*, mutated P53 similar to mutations in human tumors (Ashur-Fabian et al. 2004; Avivi, Ashur-Fabian, et al. 2005) and a unique alternative splice variant of heparanase inhibiting extracellular matrix degradation, tumor growth, and metastasis (Nasser et al. 2009), which similarly contribute to the adaptive evolution of *Spalax* to underground hypoxic stress. Finally, increased *Spalax* blood vessel density (Avivi et al. 2005) and differential expression profiling, using a mouse cDNA array containing 15,000 gene elements, uncover dramatic species-specific responses to hypoxic stress among numerous genes involved in angiogenesis, apoptosis, and oxidative stress (Avivi et al. 2006). Hypoxic stress clearly, physiologically and adaptively, shapes the *Spalax* genome as revealed by genome-wide gene expression (Brodsky et al. 2005). Underground stress evolution dramatically generated genomic, proteomic, and phenomic adaptive complexes to underground life. This outstanding hypoxic-tolerant genome is now ready for medical utilization to cope with all diseases of the modern world associated with hypoxia, such as cancer, stroke, and cardiovascular diseases.

Adaptive Fungal Life under Hypersaline Stress of the Dead Sea

The Dead Sea is one of the most saline and stressful lakes on earth (salinity is 340 g/l; 10 times saltier than the world's oceans) (see Nevo et al. 2003 on history, geology, limnology, and biology of the Dead Sea). The studies on the biology of the Dead Sea extremophiles revealed a variety of microorganisms including red halophilic Archaea, unicellular green algae (*Dunaliella parva*), different types of bacteria, and possibly even protozoa (Oren 2003). Most remarkably, filamentous fungi were recently isolated from surface water to 300 m down in the Dead Sea (Buchalo et al. 1998; Wasser et al. 2003; Kis-Papo et al. 2003a, fig. 4A). Although the isolated fungi did not grow in undiluted Dead Sea samples, they showed remarkable salt-tolerance and, in many cases, even required high-salt concentrations, making them halophilic. *Eurotium herbariorum* is the most common species isolated from the Dead Sea, from the surface to a depth of 300 m, in all investigated seasons (Kis-Papo et al. 2001; for their physiology and genetics, see Kis-Papo et al. 2003a, 2003b, respectively).

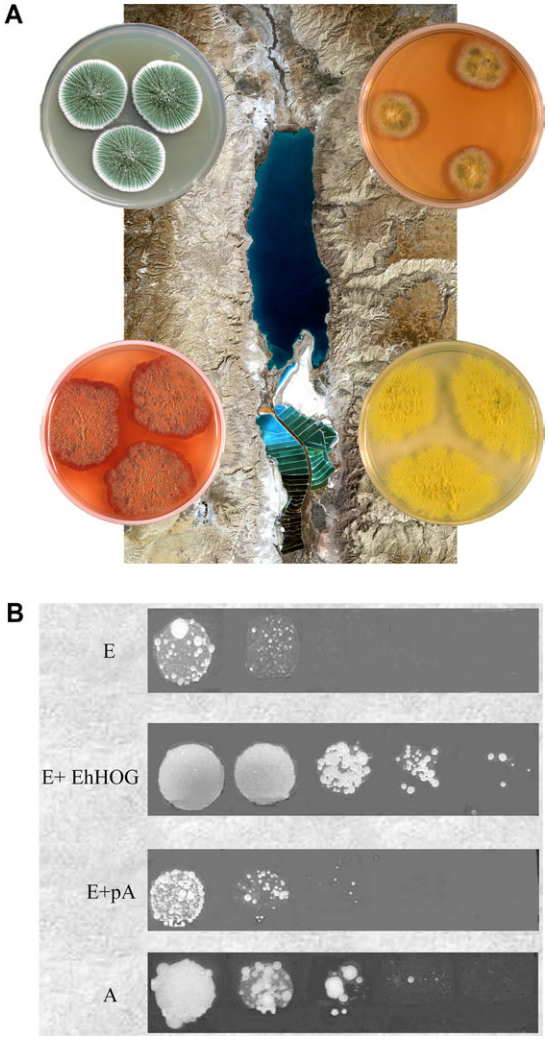

Fig. 4.—(A) Dead Sea with four species of its filamentous fungi: *Penicillium crustosum*, *Aspergillus versicolor*, *Eurotium rubrum*, and *Eurotium anstelodami*. (B) Transformation of the HOG gene into a mutant yeast and growth of the transformant in Dead Sea water with 250 mμ LiCl: (1) E = hog 1△yeast mutant, (2) E + EhHOG: the transformant: hog1 yeast mutant containing HOG gene from the fungus *E. herbariorum*, EhHOG. (3) E + pA : hog1△yeast mutant containing empty plasmid pADNS; (4) A = Wild-type yeast strain. Note that the growth of the transformant with EuHOG is best. (from Jin et al. 2005).

The first whole genomic sequence of *E. herbariorum* is currently underway. All of these extremophile organisms need to adapt to the extremely high salinity of the Dead Sea brines. Exposure to high-environmental osmolarity leads to dehydration in microorganisms—consequently, cell viability decreases. To cope with this challenge, the cells of both prokaryotic and eukaryotic microorganisms have developed

osmoregulatory mechanisms to adapt to severe osmotic stresses in their environments (Jin and Nevo 2003). To adapt to salt stress, microorganisms balance high external osmotic pressure by synthesizing and/or accumulating low-molecular mass compounds, which are compatible with cellular function and do not inhibit enzymes. Increased synthesis and/or accumulation of glycerol and other compatible solutes, mainly polyols, have been the major feature of fungi osmoregulation (fig. 4B).

Genetic Resources of Dead Sea Fungi and Advancing Saline Agriculture

The most common filamentous fungus of the Dead Sea, *E. herbariorum*, was isolated from its water. EhHOG gene, encoding a mitogen-activated protein kinase (MAPK) that plays an essential role in the osmoregulatory pathway in yeast and many other eukaryotes, was isolated from *E. herbariorum* (Jin et al. 2005). The deduced amino acid sequences of EhHOG indicated high similarity with homologous genes from *Aspergillus nidulans*, *Saccharomyces cerevisiae*, and *Schizosaccharomyces pombe* and contained a TGY motif for phosphorylation by MAPK kinase. When EhHOG was expressed in *S. cerevisiae hog1_* mutant, the growth and aberrant morphology of *hog1_* mutant was restored under high-osmotic stress conditions. Moreover, intracellular glycerol content in the transformant increased to a much higher level than that in the mutant during salt-stress situations. *hog1_* mutant complemented by EhHOG outperformed the wild type or had higher genetic fitness under high LiCl and freezing–thawing conditions (see all figures in Jin et al. 2005). Thus, the putative presence of a high-osmolarity glycerol response pathway in *E. herbariorum* and the significance of *EhHOG* in multiple-stress resistance of osmotic regulation, heat stress, freeze stress, and oxidative stress were revealed. EhHog transformed *Arabidopsis* also showed increased salt tolerance. The Dead Sea is becoming increasingly and rapidly more saline, while the fungi living in it evolutionarily strongly adapt to its high-saline environment, particularly with the extraordinarily high LiCl concentration. The Dead Sea is potentially an excellent model for studies of active adaptive evolution under extreme stressful environments and is an important gene pool for future genetic improvement of crops and the advancement of saline agriculture.

Genome Evolution across Microscale Divergent Ecologies

What are the evolutionary driving forces of genomic adaptation and speciation processes under microgeographic divergent ecologies? Ecological contrasts of a microsite 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 (Nevo 1995b). The latter involve mutation (in the broadest sense, including recombination), migration, chance, and selection. At a microsite, a mutation, which is usually considered a clockwise neutral process, is expected to be similar across the microsite. Migration, operating for any organism at the microsite (even for 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 (Nevo et al. 1984; Nevo 2011). At the Institute of Evolution, University of Haifa, Israel, in 1977, 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., Nevo et al. 1977); aridity index (high vs. low in wild cereals; e.g., Nevo et al. 1981, 1983; Li, Roder, et al. 2000; Li, Fahima, Korol, et al. 2000; Li et al. 2003); lithology (igneous, volcanic, and sedimentary rocks, e.g., Li, Fahima, Peng, et al. 2000); soil types (terra rossa, rendzina, and basalt in wild cereals; e.g., Li, Fahima, Korol, et al. 2000; Li, Fahima, Peng, et al. 2000); topography (Nevo, Noy-Meir, et al. 1991; Li, Roder, et al. 2000); 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., Nevo 1986). The aforementioned studies demonstrated differential viability of allozyme and DNA genotypes where allozyme and DNA diversity and divergence were selected either at a microscale or under critical, empirically stressful, contrasting conditions, and ecologies. Remarkably, the noncoding genome also displays ecological correlates at regional and local scales, as in outbreeding mammals (e.g., Nevo et al. 1996) and inbreeding wild cereals (e.g., Li, Roder, et al. 2000; Li, Fahima, Korol, et al. 2000; Li, Fahima, Peng, et al. 2000; Li, Fahima, Krugman, et al. 2000; Li et al. 2002; Li et al. 2003). The following examples of wild cereals at ecologically divergent microsites dramatically demonstrate the effects of stressful environments on genetic evolution of both noncoding and coding genomic regions.

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 (simple sequence repeat, 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, which displayed topographic, edaphic, and climatic ecological contrasts, respectively (Li et al. 1999; Li et al. 2000; Li, Fahima, Korol, et al. 2000; Li, Fahima, Peng, et al. 2000; Li, Fahima, Krugman, et al. 2000; Li et al. 2002;

Li, Korol, et al. 2002; Li et al. 2003, 2004) (fig. 5). Likewise, we examined molecular diversity with RAPD and SSR markers in wild barley, *Hordeum 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 combinations of these. 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 (Li et al. 1999; Li et al. 2000; Li, Fahima, Korol, et al. 2000; Li, Fahima, Peng, et al. 2000; Li, Fahima, Krugman, et al. 2000; Li, Roder, et al. 2002; Li, Korol, et al. 2002; Li et al. 2003, fig. 5). Remarkably, the three marker systems (allozyme, RAPD, and SSR) at the Ammiad microsite revealed similar trends of diversity and divergence. All three molecular markers displayed *nonrandom* allele distributions, habitat-specific and habitat-unique alleles, and linkage disequilibria. The subpopulations in the drier habitats showed higher genetic diversities in the three marker systems (Li, Fahima, Krugman, et al. 2000). These results may suggest that ecological selection, probably through aridity stress, acts both on structural protein coding and presumably on partially regulatory noncoding DNA regions (SSR and RAPD), resulting in microscale adaptive divergent patterns. Similar microscale molecular (allozymes, RAPDs, and SSRs) divergence was found in two populations of wild barley (*H. spontaneum*) at Tabigha and Neve Yaar (Owuor et al. 1999; Huang et al. 2002).

Evolution in Action Across Life at "Evolution Canyons," Israel

The "Evolution Canyon" model reveals "evolution in action across life" at a microscale involving biodiversity divergence, adaptation, and incipient sympatric ecological speciation in diverse organisms from viruses and bacteria through fungi and animals, up to mammals (Nevo 1995b, 1997, 2001, 2006, 2009). The model highlights the interslope divergence of 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 stresses, tropical and temperate microclimates on a *xeric*, tropical "African" (AS) south-facing slope (SFS) abutting with a mesic, temperate, "European" (ES) north-facing slope (NFS) separated by 200 m on average. Four "Evolution

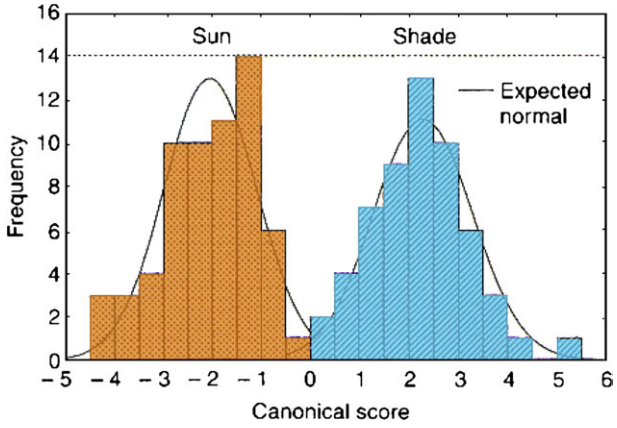

Fig. 5.—Microclimatic stress and adaptive RAPD DNA differentiation in wild emmer wheat, *Triticum dicoccoides*, from the Yehudiyya microsite, Golan. The test involved two climatic microniches in the open oak-park forest of *Quercus ithaburensis* (1) sunny between trees and (2) shady under the trees' canopies. The histograms of frequencies of canonical scores show the difference between shady and sunny niches according to 25 polymorphic RAPD loci (from Li et al. 1999)

Canyons" (EC) are currently being investigated in the Carmel, Galilee, Negev, and Golan Mountains (EC I–IV) in Israel, respectively (fig. 6). The SFSs, in canyons north of the equator, receive higher solar radiation (200–800% at EC I) than on the opposite NFS slopes (Pavlicek et al. 2003). This solar radiation is associated with higher temperature and drought on the AS = SFS compared to the low-light, shadier, more humid, and cooler ES = NFS causing dramatic abiotic and biotic interslope divergence, which may have originated by water erosion several million years ago after mountain uplifts (fig. 6). This model facilitates tracking divergent adaptive and speciational evolutions and the identification and ranking of driving forces of evolution, for example, mutation, genetic drift, gene flow, and natural selection. Even strongly sedentary organisms, for example, lichens and cyanobacteria, can "migrate" between slopes. Thus, migration is basically excluded from the evolutionary scenario as a determinant interslope divergent force (see Nevo 2011). These canyons are extraordinary natural microscale evolutionary laboratories. If rocks, soils, and topography are similar on the opposite slopes (Nevo et al. 1998), 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, "African" open, dry, and "European" covered 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 adaptive ecological speciation (Nevo 2006). We identified 2,500 species in ECI (Mount Carmel)

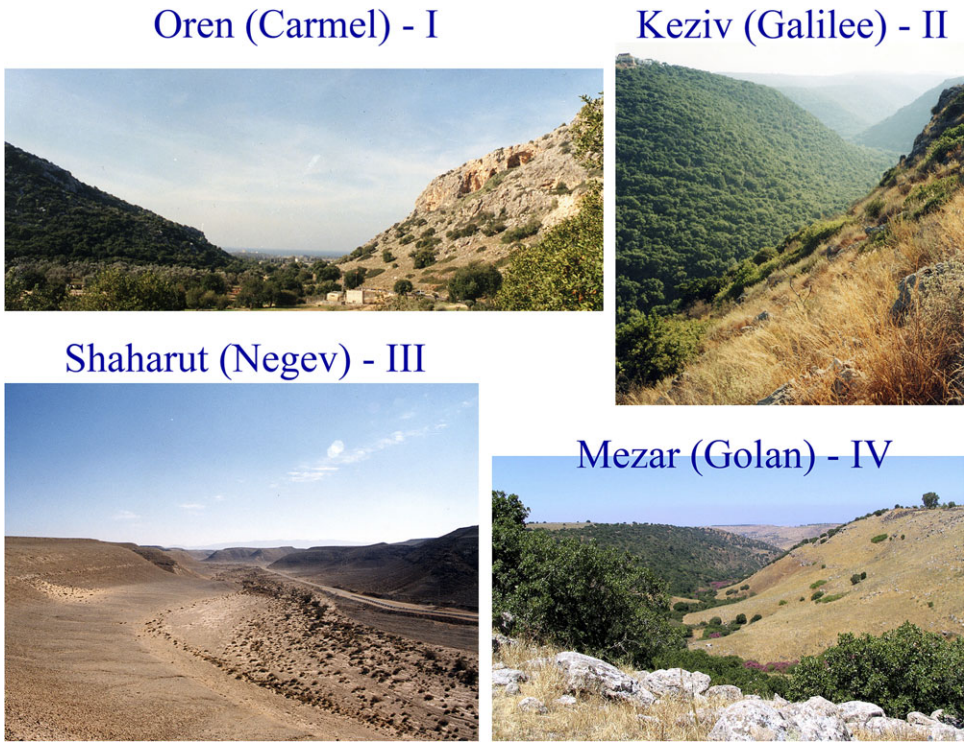

Fig. 6.—The four "Evolution Canyons" in Israel (EC I–IV). Note the plant formation on opposite slopes. The green lush, "European," temperate, cool-mesic, north-facing slope (NFS) sharply contrasts with the open-park forest of the warm-xeric, tropical, "African-Asian" savanna on the south-facing slope (SFS). Note the interslope divergence in vegetation, even in EC III in the Negev desert where the SFS is covered by cyanobacteria and the NSF by lichens (from Nevo 2009).

from bacteria to mammals in an area of 7,000 m^2. These include bacteria (100 species), protozoa (5), fungi (500), plants (340), invertebrates, primarily insects (1,500), and vertebrates (55). Local biodiversity patterns parallel global patterns (Nevo 1995b). Higher terrestrial species richness was found on the AS. Aquatic species richness prevails on the ES (Nevo 1995b). In 9 of 14 (64%) model organisms across life, we identified a significantly higher genetic polymorphism on the more stressful AS (Nevo 2006, 2009; Nevo et al. 1997). Likewise, in some model taxa, we found largely higher levels of mutation rates, gene conversion, recombination, DNA repair, genome size, SSRs, single nucleotide polymorphisms, retrotransposons, transposons, candidate gene diversity, and genome-wide gene expression and regulation on the more stressful AS (Nevo 2009; Kossover et al. 2009). Remarkably, interslope incipient sympatric adaptive ecological speciation was found across life from bacteria to mammals (Michalak et al. 2001; Nevo 2006; Sikorski and Nevo 2005; Korol et al. 2006). The "Evolution Canyon" model represents the Israeli ecological analogue of the Galapagos Islands. Microclimatic selection overrides gene flow (Pavlicek et al. 2008; Nevo 2011) and drift and drives both interslope adaptive divergence and incipient sympatric adaptive ecological speciation at a microscale (Nevo 2006, 2009, 2011).

The "Evolution Canyon" Model: Prospects

What is next? Biodiversity evolution across life needs expansion at the EC by adding diverse taxa and embracing all categories (individuals, genomes, populations, species, communities, and biota) and life cycles. Importantly, a 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 (Grishkan and Nevo 2008). 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 also through genomic-biochemical networks related to individual and collective environmental stresses, temperature, drought, photosynthetic deprivations, biotic stresses, etc., focusing on *regulatory noncoding* elements. 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 (Nevo 2009, and references therein). 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. These future studies should be conducted across their entire genomes by new generation sequencing (NGS) novel sequence methodologies to unravel structural and expressional adaptive complexes, speciation genes, and regulators (Brodsky et al. 2010). Mate and habitat choice and transplant experiments and comparisons 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 across life and is appropriate for testing many mysteries of Evolutionary Biology caused by interslope divergent stresses at a microsite (fig. 6).

Human Brain Evolution through RNA Editing

The following discussion is based on the paper of Paz-Yaacov et al. (2010) led by Professor Gidi Rechavi at Chaim Sheba Center at Tel Hashomer, Israel.

Adenosine-to-inosine (A-to-I) RNA editing is a widespread posttranscriptional modification, altering the sequence of RNA from that encoded in the DNA. It is catalyzed by the double-stranded RNA-specific adenosine deaminase acting on the RNA (ADAR) enzyme family and appears to be tissue-specific with the brain being the most edited (Paull and Bass 1998) by widespread modification of the transcriptome (Gott and Emerson 2000). The splicing and translational machineries recognize inosine (I) as guanosine (G). Many of the RNA editing targets play a central role in neurogenesis. RNA editing in humans occurs predominantly within primate-specific *Alu* repetitive elements affecting thousands of genes in tens of thousands of sites primarily in noncoding sequences (Levanon et al. 2004). Remarkably, the level of RNA editing in humans is more than an order of magnitude higher than that in mouse, rat, chicken, and fly (Eisenberg et al. 2005). *Alu* sequences present in more than a million copies per primate genome are frequently found in gene-rich regions, generally within noncoding segments. Several studies showed that A-to-I RNA editing of *Alu* elements can affect gene expression through a variety of mechanisms including alternative splicing, mRNA stability, nuclear retention, and microRNA biogenesis and targeting (Chen and Carmichael 2003, 2009; Chen et al. 2008; Nishikura 2006; Prasanth et al. 2005). These findings led the group of Professor Rechavi to explore the level of RNA editing in primate evolution. The Rechavi group first found that the editing level in humans is significantly higher compared with nonprimates (fig. 7A) due to exceptional editing within the primate-specific *Alu* sequences (fig. 8). They found that, on average, the editing level in transcripts analyzed was higher in human brains compared with nonhuman primates (chimpanzees and rhesus), even where the genomic *Alu* structure is unmodified (fig. 7). Remarkably, new editable species-specific *Alu* insertions, subsequent to the human–chimpanzee split, are significantly enriched in genes related to neuronal functions and neurological diseases. The enhanced editing level in the human brain and the association with neuronal functions both suggest the possible contribution of A-to-I editing to the development of higher human brain function. Paz-Yaacov et al. (2010) showed that combinatorial editing is the most significant contributor to shaping the richer diversity of the human transcriptome repertoire and suggested that *Alu* editing adapted by natural selection may therefore serve as an alternate information mechanism based on the binary A/I code. This natural selection might have been reinforced after apes descended from the rain forest to the dry and stressful savanna (Mayr 2004; Pruetz and Bertolani 2007). They speculated that the more abundant RNA editing found in the human brain may contribute to more advanced human capabilities such as memory, learning, and cognition (figs. 7–9). Their findings reinforce the hypothesis that a digital programming system based on noncoding RNA signaling is a driving force for adaptive complex evolution (Mattick 2009). The combinatorial posttranscriptional RNA editing of noncoding sequences may therefore contribute to higher brain function. It may drive human cultural evolution as the most important human adaptive complex in the stressful African savanna followed by later scenarios of terrestrial human evolution (Roffman and Nevo 2010). Remarkably, the relatively higher brain size in the blind mole rat complex in Israel is also associated with the stressful desert conditions (Nevo, Tchernov, et al. 1988). Environmental stress seems to be a major evolutionary driving force in physical, biological, and cultural evolutions in increasing brain complexity.

Global Climatic Change and Stress

Global climatic change is proceeding at an unprecedented rate with dire ecological consequences (Parmesan 2006). Diverse analyses of more than 1,700 species showed that recent biological trends match climate change predictions in affecting living systems (Parmesan and Yohe 2003; Root et al. 2003). Climatic change induces phenotypic (e.g., phenological) and range shifts, but genetic changes are less documented. A major question is whether species can adapt fast enough to a changing and increasingly stressful world (Visser et al. 2010) before becoming extinct (Nevo 1995a) due to ecological stresses (e.g., Nevo 1995b). Here I briefly describe phenotypic and genotypic changes caused by climatic changes of global warming stress on the wild progenitors of wheat and barley, *Triticum dicoccoides* (Nevo et al. 2002) and *H. spontaneum* (Nevo 1992), which originated and diversified in the Near East Fertile Crescent. Both are of paramount importance as major sources of genetic

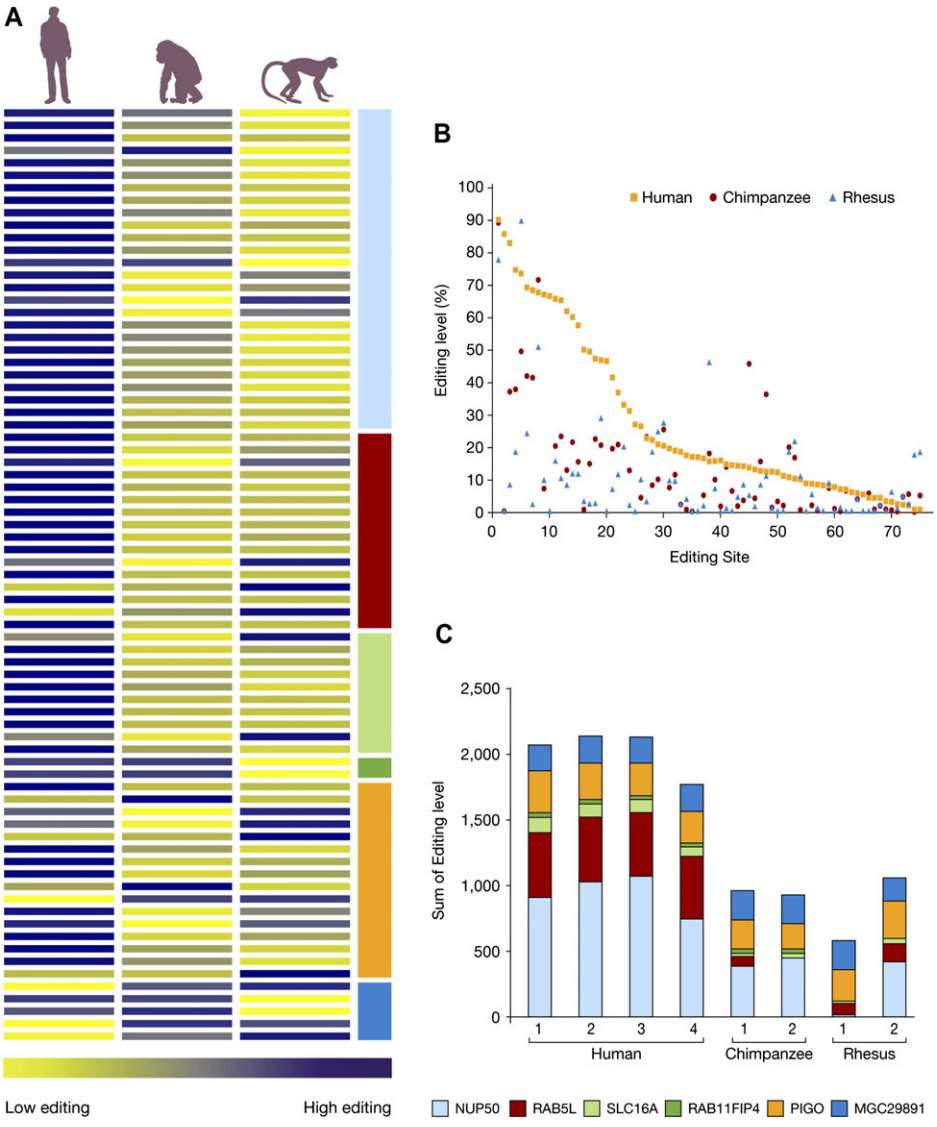

Fig. 7.—Higher RNA editing level in human versus nonhuman primates. (A) RNA editing levels of 75 sites in six transcripts originating from cerebellum tissues of four humans, two chimpanzees, and two rhesus monkeys were quantified after polymerase chain reaction amplification using the DS gene program. Average editing values were normalized (*Z*-score) and colored accordingly with blue-yellow gradient using the Spotfire program (Tibco). (B) RNA editing levels per site for humans, chimpanzees, and rhesus monkeys. The human editing sites are ordered in decreasing editing levels, and the nonhuman primate editing sites are aligned accordingly. (C) RNA editing levels in cerebellum tissues of eight individual primates: a total of the resulting editing level quantification in the six tested transcripts are plotted in four human, two chimpanzees, and two rhesus individuals where the bar size is proportional to the total of the editing levels in all tested sites (from Paz-Yaacov et al. 2010).

resources for improving cultivated wheat and barley, as human and animal food, respectively. They have been major model organisms for studying their evolutionary trajectory and genetic resources for crop improvement at the Institute of Evolution, University of Haifa, since 1975 (see Nevo list of wild cereals at http://evolution.haifa.ac.il). We collected and studied genetic resources from natural populations of wild barley and wild emmer wheat across Israel and the Near East Fertile Crescent in an attempt to provide genetic resistances against abiotic and biotic stresses for crop improvement (Nevo list of wild cereals linked to http://evolution.haifa.ac.il).

In 2009, we conducted a common garden experiment in a large greenhouse at the Aaronsohn agricultural station near Atlit (Nevo et al. unpublished data). We planted ten populations of wild barley and ten populations of wild emmer wheat collected in 1980 and 2008. The results, to be published elsewhere, demonstrated dramatic earliness of all 20 populations in 2008, in comparison with the 1980 collection, on average, by 10.94 days in wild barley and 8.53 in wild emmer wheat. Likewise, depletion and turnover of SSR genetic diversity, elite drought-resistant populations, and genotypes were identified in terms of yield under a drought

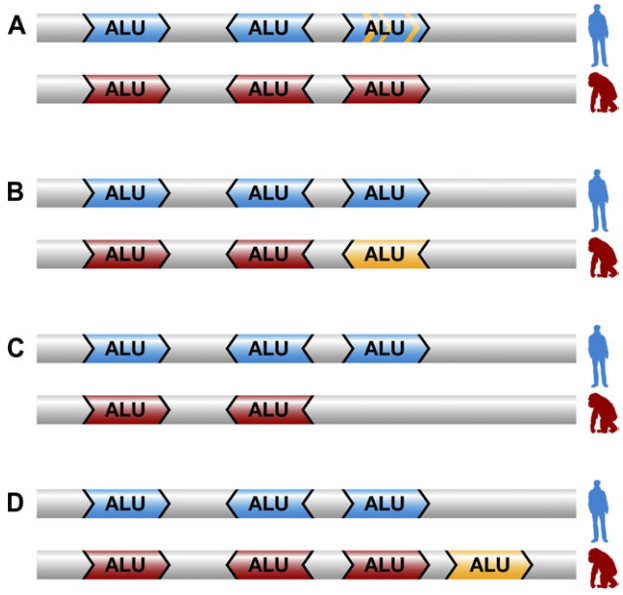

Fig. 8.—Possible effects of *Alu* architecture alterations on RNA editing. Schematic representation of the genomic *Alu* elements' location and orientation: *Alu* elements are marked as arrow-shaped boxes in the human (blue) and monkey (red) genomes. Alterations between the species are indicated in orange. (A) Minor alteration in *Alu* sequence between the species. (B) Inversion of one of the *Alu* sequences along primate evolution. (C) Deletion of *Alu* element along evolution. (*D*) Insertion of additional *Alu* sequence along evolution (from Paz-Yaacov et al. 2010).

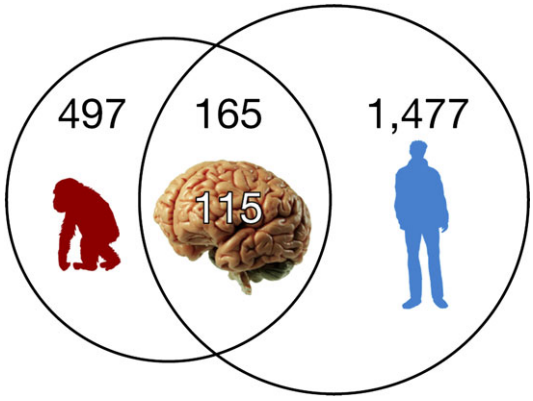

Fig. 9.—Analysis of newly inserted *Alu*s. Among the 165 shared genes representing new (independent) *Alu* insertions in the human and the chimpanzee, 115 are neurological function and neurological-associated genes (from Paz-Yaacov et al. 2010). Upper right: Number of common human and chimpanzee genes showing new (independent) *Alu* element insertions. Among the 165 shared genes representing new independent *Alu* insertions in the human and chimpanzee, 115 are neurological function and neurological disease-associated genes (from Paz-Yaacov et al. 2010).

irrigation regime of 300 mm. Because the population samples were the same, we interpreted both the phenotypic earliness in flowering, yield, and genetic changes during the 28 years, separating both collections (1980–2008) as the result of global warming stress (Nevo et al. 2011).

Conclusions and Prospects

The evidence presented above in multiple model organisms under environmental stress clearly indicates the importance of environmental stress as a major driving force of evolution on genomes, proteomes, and phenomes of diverse phylogenetically distant organisms across life on global, regional, and local scales. Evolutionary change occurs under environmental stresses on natural populations and species in which there is a premium on energetically efficient exploitation of resources in a resource-inadequate world (Parsons 2005). The building up of adaptive complexes are expedited under environmental stress, either underground (subterranean mammals) or aboveground (humans), in water (filamentous fungi in the Dead Sea), or on land (wild cereals). At all organizational levels of genes, genomes, phenomes, and biomes, abiotic (thermal, chemical, climatic) and biotic (parasites and pathogens) environmental stresses usually drive genetic and genomic diversity higher. A high degree of genomic diversity in nature is adaptive as structural, expressional, or regulatory polymorphisms all coping with diverse stresses. Natural selection seems to be a dominant determinant in driving adaptive polymorphisms of diversity and divergence at spatiotemporal environmental variables at micro- and macroscales. Reassuringly, protein and DNA, in both coding and noncoding genomes, are subjected adaptively to the editing of natural selection. The organization and evolution of molecular and organismal diversity in nature on all scales are *nonrandom and structured* driven by environmental stress. They display patterns and 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 (Nevo 2011). In the genomic era with its current dramatic tools of NGS, the mysteries of genome structure, expression, and regulation under environmental stress can be elucidated and highlight the driving forces of the twin evolutionary processes of genome adaptation and speciation. We need to strongly advance functional and regulatory ecological genomics in the future in order to underline and substantiate the paramount importance of environmental stress as a major driving force of active evolution.

Supplementary Material

Supplementary figures 1–3 are available at *Genome Biology and Evolution online* (http://gbe.oxfordjournals.org/).

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