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Mammalian microevolution in action: adaptive edaphic genomic divergence in blind subterranean mole-rats

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Genomic diversity of anonymous regions across the genome, most probably including *coding* and *non-coding* amplified fragment length polymorphisms (AFLPs), was examined in 20 individuals of the blind mole-rat, *Spalax galili*, one of four allospecies of the *Spalax ehrenbergi* superspecies of blind subterranean mole-rats in Israel. We compared 10 individuals from two nearby populations in Upper Galilee, separated by only a few dozen to hundreds of metres and living in two sharply contrasting ecologies: white chalk and rendzina soil with *Sarcopterium spinosum* and *Majorana syriaca* versus black volcanic basalt soil with *Carlina hispanica–Psorelea bitominosa* and *Alhagi graecorum* plant formations. The microsite tested ranged in an area of less than 10 000 m^2. Out of 729 AFLP loci, 433 (59.4%) were polymorphic, with 211 soil *unique* alleles. Genetic polymorphism was significantly higher on the ecologically more xeric and stressful chalky rendzina soil than on the neighbouring mesic basalt soil. This is a remarkable pattern for a mammal that can disperse each generation between tens to hundreds of metres. These results cannot be explained by migration (which causes homogenization) or by chance (which will exclude sharp genomic *soil divergence*). Natural selection is the only evolutionary adaptive force that can cause genetic divergence across the genome matching the sharp microscale ecological contrast.

Keywords: adaptive radiation; genome; microscale ecology; *Spalax ehrenbergi* superspecies

1. INTRODUCTION

(a) *Microgeographical 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 (Nevo 2001). These forces involve mutation (in the broadest sense, including recombination), migration, chance and selection. Mutation—usually considered to be a clockwise neutral process—is expected to be similar across the entire site. 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 repeated divergent ecologically correlated patterns (Nevo 2001).

In 1977, at the Institute of Evolution, we initiated a series of microsite studies to compare sharply contrasting ecological alternatives. All are cited numerically in the following parentheses according to the full publication list of Nevo (see http://evolution.haifa.ac.il; cold versus hot in balanids, sessile crustaceans; ref. 75); aridity index (high versus low in wild cereals; refs 105, 128, 189 and 230); lithology and soil types (terra rossa, rendzina and basalt in wild cereals; refs 248, 685 and 709); topography (refs 352, 447, 557 and 681); and chemical (non-polluted versus polluted) environments with inorganic heavy metals (Hg, Cd, Zn, Pb and Fe) and organic (detergents and oil) pollutants in marine organisms (review in ref. 197). The aforementioned studies demonstrated differential viability of allozyme and/or DNA genotypes where allozyme diversity and divergence were selected at a microscale or under critical contrasting ecological conditions.

Will the *noncoding* genome also display ecological correlates at regional and local levels? The answer is emphatically 'yes' for outbreeding mammals (e.g. ref. 483) and inbreeding wild cereals (e.g. refs 639, 681, 685, 709, 742 and 748).

(b) *The* Spalax ehrenbergi *superspecies in Israel*

(i) *Species, climates and parapatric distribution*

Blind subterranean mole-rats of the *Spalax ehrenbergi* superspecies in Israel, previously recognized as one classical species, represent an evolutionary model of adaptive speciation in action (Nevo 1999; Nevo *et al.* 2001). The superspecies involves four morphologically chromosomally divergent allospecies: *Spalax galili*, *Spalax golani*, *Spalax carmeli* and *Spalax judaei* with diploid numbers $2n = 52$, 54, 58 and 60, respectively. Their distribution is correlated with four climatic regimes: *S. galili* ($2n = 52$, cool–humid); *S. golani* ($2n = 54$, cool–semi-dry); *S. carmeli* ($2n = 58$, warm–humid); and *S. judaei* ($2n = 60$, warm–dry). Increasingly narrowed hybrid zones northwards separating the species characterize the complex indicating Pleistocene speciation at the final stage of species formation (Nevo *et al.* 2001).

Previously, in mole-rats we have shown regional gradients in allozymes (Nevo *et al.* 1994) and DNA diversities (Ben-Shlomo *et al.* 1996; Nevo *et al.* 1996) associated with increasing aridity southwards. However, we have never attempted a microgeographical genomic divergent analysis in mole-rats as was accomplished in wild cereals (Nevo *et al.* 2002). Here, we demonstrate in *S. galili* ($2n = 52$) a dramatic microscale intersoil genomic divergence of DNA amplified fragment length polymorphism (AFLP) markers.

2. MATERIAL AND METHODS

(a) *Species and populations tested*

The test was conducted in an area of less than 10 000 m^2 in the eastern Upper Galilee Mountains at a microsite sharply divergent between white chalky rendzina (Kerem-Ben-Zimra) and black volcanic basalt (Dalton) soil types (figure 1). The rendzina and basalt soils sharply contrast both physically and biotically. The drier and stressful rendzina is covered by the plants *Sarcopterium spinosum* and *Majorana syriaca*, whereas the clayey wetter and milder basalt soil is covered by *Carlina hispanica–Psorelea bitominosa* and *Alhagi graecorum* plant formation (Rabinovitch-Vin 1986). AFLP analysis was carried out on 20 subterranean mole-rats, 10 individuals sampled randomly from each soil type.

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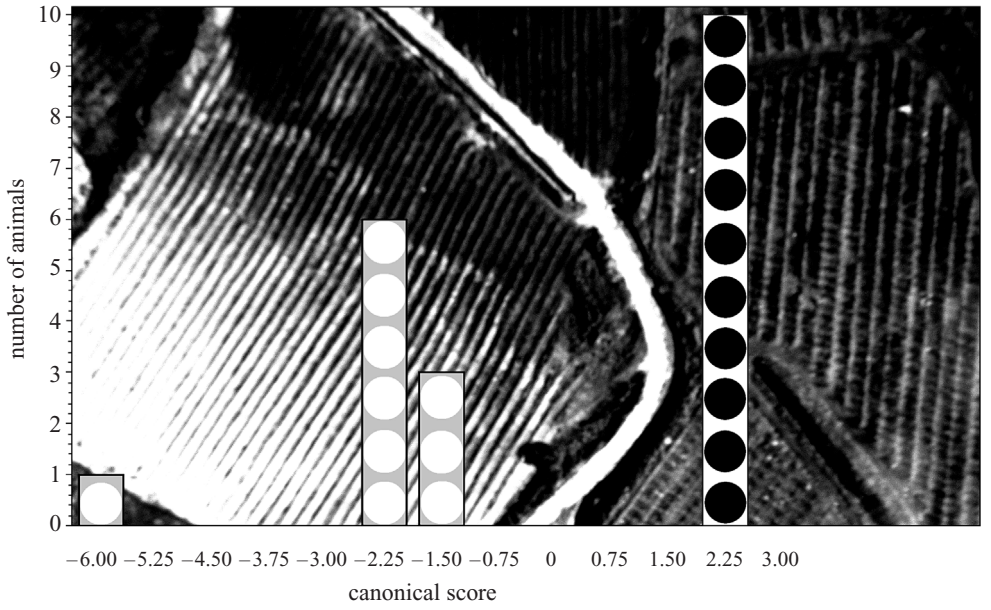

Figure 1. The ecological microscale theatre of white chalky rendzina in Kerem-Ben-Zimra sharply contrasting the black volcanic basalt in Dalton, eastern Upper Galilee, Israel. Each circle in the histograms represents the canonical score of a subterranean mole-rat, *Spalax galili*, from chalk (white) or basalt (black), derived from discriminant analysis based on the best two differentiating AFLP loci. Complete (100%) discrimination of animals was achieved according to soil origins.

(**b**) ***The amplified fragment length polymorphism procedure***

The AFLP procedure followed Vos *et al.* (1995). *Eco*RI (having a 6 bp recognition site) and *Mse*I (having a 4 bp recognition site) restriction endonucleases were used to digest the genomic DNA of the mole-rats. The specific recognition sites of these enzymes are scattered through the whole genome every 2 kb for *Eco*RI and every 300–400 bp for *Mse*I. Sequences from anonymous regions of the genome, most probably including *coding* and *noncoding* DNA regions, were then analysed after digestion. To reduce the number of sequences analysed and increase their concentration by means of PCR amplification double-stranded oligonucleotide adapters were next ligated to the sticky ends of the pools of restricted DNA fragments.

Primers complementary to *EcoRI* and *MesI* adapters with one extra-nucleotide each (A for *EcoRI* adapter and C for *MesI* adapter) were used for the first and second analytic amplification. Ten combinations of nine primers were used with two or more extra nucleotides. Non-radioactive visualization and analysis of PCR products after electrophoresis in 5% polyacrylamide gel was conducted by ALFexpress II DNA automated sequencer (Pharmacia, Sweden). The bands' size was defined relatively to the size marker ladder that contained 40, 100, 200, 300, 400 and 500 bp marker sequences.

(**c**) ***Data scoring and statistical analysis***

Only precisely and evenly expressed electrophoresis bands were chosen for manual analysis. We regarded each band as a locus with two alternative alleles: present (1) or absent (0). The genetic data was analysed by POPGENE program v. 1.31 (Yeh *et al.* 1999) as a dominant mode of inheritance in a diploid organism, assuming Hardy–Weinberg equilibrium. Discriminant analysis was conducted by use of the SAS software (SAS Institute 1996).

3. RESULTS

(**a**) ***Pattern of amplified fragment length polymorphism diversity***

Out of 729 AFLP loci, 433 loci (59.4%) showed polymorphism among the 20 *S. galili* animals collected from the two different soil types, while 296 loci (40.6%) were monomorphic in all 20 individuals.

For the two populations, we calculated the percentage of polymorphic loci (*P*) and the gene diversity (*He*; Nei 1973). Higher values of diversity were obtained in the stressful chalk soil ($P = 52.9\%$, $He = 0.159$) compared to those found in the nearby basalt ($P = 26.7\%$, $He = 0.097$). Both *P* (χ^2-test: $\chi^2_1 = 104.4$, $p < 0.0001$) and *He* (Wilcoxon two-sample test: $Z = 8.39$, $p < 0.0001$) were significantly different between the soils. Out of all 211 *unique* alleles (e.g. restricted only to one soil type), 192 (91%) characterized the chalk population (table 1) and only 19 (9%) alleles characterized the basalt population (binomial test: $p \leqslant 0.0001$).

(**b**) ***Genetic differentiation (G_{ST}) within and among populations***

The total gene diversity (H_T) of a subdivided population can be partitioned into the mean gene diversity within the populations (H_S), the average gene diversity among populations ($D_{ST} = H_T - H_S$) and the gene diversity between the populations, relative to H_T ($G_{ST} = D_{ST}/H_T$) (Nei 1973). The G_{ST} partitioning of the two soil populations averaged (overall loci) a high value of 0.1008. To test the significance of these results, a permutation test was applied, by producing 1000 randomized datasets by random permutations of the individual animal data into the various populations. The G_{ST} obtained from the 1000 randomized datasets averaged 0.0638 (range of 0.0427–0.1088). G_{ST} was lower for 997 randomized datasets ($p = 0.003$) when compared with that of the real data (0.1008). Thus, we demonstrated that the partition of AFLP genetic diversity in the present study showed very high interpopulation soil divergence.

(**c**) ***Genetic distance***

Notably, unbiased genetic distance (*D*) (Nei 1978) was 0.025 between the chalk and basalt mole-rat populations, separated only by a few dozens to hundreds of metres. By contrast, $D = 0.018$, based on 426 AFLP loci (A. Polyakov, T. Krugman, A. Beharav, A. Avivi, A. Z. Fahoum and E. Nevo, unpublished data) diverged Mt

Table 1. Number of unique AFLP alleles in two Israeli populations of *Spalax galili* ($2n = 52$) from the Kerem-Ben-Zimra (chalk) and Dalton (basalt) microsites.

	frequency (f)[a]						
soil population	0.051	0.106	0.163	0.225	0.293	0.452	total
chalk	39	46	99	7	1	0	192
basalt	14	1	2	0	1	1	19
total	53	47	101	7	2	1	211

[a] Six categories based on the AFLP allele frequencies are distinguished.

Hermon and Quneitra populations in the Golan Heights, separated by 22 km.

(d) *Discriminant analysis*

Discriminant analysis was performed and the SAS procedure STEPDISC, using the STEPWISE option, chose the suitable loci as the best differentiating factors between the two soil mole-rat populations. Using the SAS procedure PROC NEIGHBOUR (which is suitable for classification when the classes have radically non-normal distribution), the level of correct classification of individual animals into their respective soil origin by only one, the best differentiating locus, was 85% (expectation by chance: $100/2 = 50\%$). One hundred per cent correct intersoil classification of the 20 animals to their soil origins (figure 1) was obtained, based on the best two differentiating loci. To test the significance of these results, a permutation test was applied, running 10 randomized datasets (Beharav & Nevo 2003). A lower estimate of correctly classified per cent (average of 77.7%, range of 75–80% for one best differentiating locus; average of 87%, range of 80–90% for two best differentiating locus) was obtained for each randomized set compared to that of the real data (sign test: $p < 0.001$). Thus, the significant AFLP intersoil differences unequivocally distinguish between the two soil populations of *S. galili*.

4. DISCUSSION

The dramatic genetic divergence of *S. galili* by the chalk–basalt sharp microscale contrast is clearly intriguing in a highly *outbreeding* mammal that despite its limited migration can *migrate* during young dispersal tens to hundreds of metres (Nevo 1979, p. 285). Exploratory patterns are higher in 'mesic' *S. galili* than in 'xeric' *S. judaei* (Nevo 1999, p. 146) and species-specific habitat selection was demonstrated (Nevo 1999, pp. 146–147). The territorial size deduced by radiotracking in *S. galili* females was 63 m^2 (Kushnirov *et al.* (1998) in Nevo 1999, p. 97). No evidence is currently available on either intersoil dispersal distances or non-random mating and the emergence of pre- or post-mating reproduction isolation between the two nearby soil populations. It is, however, noteworthy that even between the major four species in Israel, natural hybridization extends between 300 and 3000 m and differential levels of pre- and post-mating isolating mechanisms were described between the main four species (Nevo *et al.* 2001, pp. 95–130, 155–174). So it is inconceivable that *complete* reproductive isolation occurs in the midst of *S. galili*. Future studies will investigate the levels of migration and reproductive isolation between the two genetically divergent soil populations. Remarkably, the two soils are sharply divergent ecologically. The calcareous rendzina soil is, by far, drier hence more stressful than the volcanic basalt (Rabinovitch-Vin 1986) in terms of water availability and plant food resources for mole-rats. Notably, the chalk population was significantly more polymorphic than that of the basalt population. This follows the general pattern that stressful environments select for higher protein and DNA genomic diversity in all organisms tested at all *local*, *regional* and *global* geographical scales (Nevo 2001). The relatively low value of $N = 20$ is compensated by $N = 729$ AFLP loci tested (Nei 1978).

Our present results cannot be explained by migration, which causes homogenization, or by chance, which will exclude sharp *Spalax* soil divergence. No population bottlenecking can affect a population differentially across a sharp boundary in a microscale, although the evolutionary history of these local soil populations is unknown. Both populations should be equally affected, especially in view of effective migration. The only evolutionary divergent driving force is diversifying natural selection that probably causes genomic divergence in both *coding* and *noncoding* regions. Selection can operate either directly on AFLP loci and/or indirectly on linked genes. The result is intersoil alternative *adaptive genomic structures* associated with divergent environmental stresses of both soil and vegetation, i.e. food resources, affecting the energetic balance (Nevo 1999). Importantly, our recent reviews of microsatellites both between and within genes suggest that they are involved in regulation and gene expression (Li *et al.* 2002, 2003). This may also partly be the function of AFLP loci.

This microscale divergent genomic pattern is reminiscent of plants (wild cereals) that demonstrate striking topographic, lithologic, soil and microclimatic divergence at several microsites in North Israel (Nevo *et al.* 2002). Remarkably, however, interslope divergence across a few hundreds of metres has been demonstrated not only in plants but also across life in the 'Evolution Canyon' model (Nevo 2001 and refs 447 and 557 in the full list in http://evolution.haifa.ac.il). We have demonstrated in the ecologically outstanding model 'Evolution Canyon' not only interslope adaptive divergence but also sympatric incipient speciation in the cosmopolitan *Drosophila melanogaster* that otherwise can migrate up to 10 km (ref. 767). Likewise, dramatic interslope genetic and physiological divergence was found in spiny mice, *Acomys cahirinus* (refs 539 and 615), as well as non-random, slope-specific mate choice (E. Nevo, unpublished data).

We conclude that subterranean mole-rats, and probably many other mammals, can adaptively radiate genomically

at a microscale sharply divergent ecologies by natural selection, which reinforces adaptive habitat selection, philopatry, and eventually advanced genomic divergence that might lead over time towards incipient sympatric speciation even without geographical barriers.

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