
Genome coding and noncoding structure function and evolution in *Spalax ehrenbergi* superspecies and *Hordeum spontaneum* in Israel: a review (Rodentia: Spalacidae)

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Abstract. The structure and function of the intergenic noncoding genome (repeatome) whether “selfish” and “junk” or “biochemically functional and regulatory” is still hotly debated, despite the mounting empirical and theoretical evidence supporting the regulatory role, provided by transposable elements, and the ENCODE results. In our studies in Israel on sympatric speciation (SS) in two evolutionary speciation models of abutting divergent ecologies, microclimatic at “Evolution Canyon” and geologic-edaphic at “Evolution Plateau”, we showed genomically that SS is a common speciation model across life from bacteria to mammals. Since such contrasting and abutting ecologies, climatic, geologic, edaphic, abiotic and biotic abound globally, SS might be a common speciation model across the planet. During our SS studies we discovered an additional remarkable result supporting the regulatory role of the repeatome. We found that the noncoding genome, in both subterranean mammals and wild barley at “Evolution Plateau” present a genomic mirror image of the coding genome supporting the regulatory hypothesis of the repeatome. Moreover, the repeatome also succeeded to identify the four subterranean chromosomal climatically adaptive mole rat species in Israel. This genomic mirror imaging and repeatome function is now open to genomic exploration across life.

Key words. Blind subterranean mammals, *Spalax ehrenbergi*, wild barley.

The structure and function of the genome have been and are still hotly debated (SHAPIRO 2022). McCLINTOCK (1956) first discovered the moving, transposable elements in her paper “controlling elements and the gene”. The (re)discovery of BRITTEN & KHONE (1968) of repetitive DNA distributed inter-genically in hundreds of thousands copies of DNA sequences that have been incorporated across the genomes of higher organisms revealed that most of the genome involves repeats interpreted by ORGEL & CRICK (1980), and many others, as “selfish” and “junk”. Remarkably, the idea that most of the genome is “selfish” and “junk” was transferred with the ENCODE (Encyclopedia of DNA Elements) results in humans (DUNHAM & KUNDAJE 2012). ENCODE revealed that the human genome is copied into RNA, functioning as interactive regulatory proteins, and are compacted into chromatin, organizing the genome for cellular divergence. Remarkably, ENCODE revealed that 80% of the human genome contained biochemically functional elements invalidating the view that the human genome consists mostly of “junk DNA” and “selfish genes”. By contrast, intergenic spaces are filled with DNA regulatory elements, like enhancers, promoters, and RNA transcripts that are not

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translated into proteins but might have a regulatory role (JACOB & MONOD 1961). Regulation, rather than "junk" and "selfishness" is the role of the noncoding and repetitive DNA. The early discovery of McCLINTOCK (1956) of mobile elements, jumping genes, that affect the genome structure and function in maize, may be "controlling elements", substantially transforming the "junk" genome hypothesis by the ENCODE results. The debate between "junk DNA" and the regulatory noncoding genome is still ongoing, but seems to be gradually challenged by the view that most of the genome is biochemically functional, though functionality has been challenged (see discussion). Noteworthy, SHAPIRO (1979) had a great interest in the repeatome since up to two-thirds of the human genome is composed of mobile genetic elements, and he first discovered

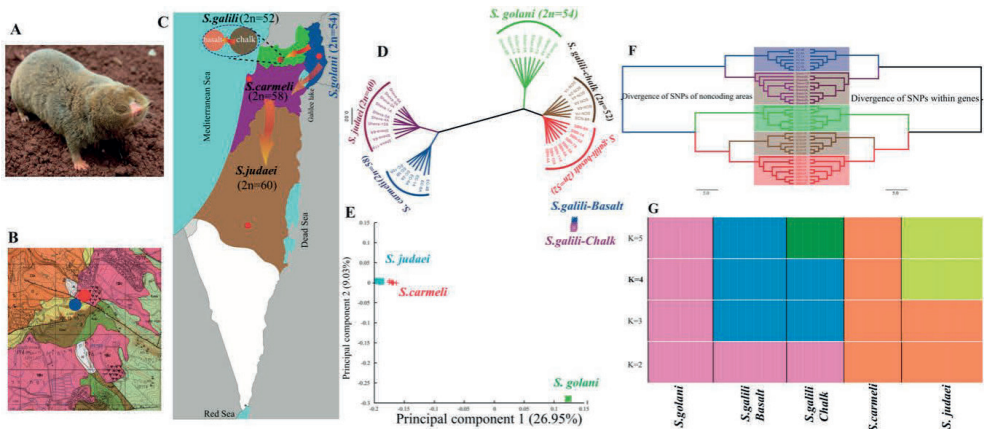


Fig. 1. The ecogeographic distribution and the genomic divergence among *Spalax* species in Israel. (A) Blind mole rat, *Spalax*. (B) Geological map of east Upper Galilee including the Evolution plateau and the two sympatric species, the ancestor on Senonian chalk marked in yellow and blue circle; the derivative sympatric new species on basalt marked in pink and red circle across a geological fault marked by a black line. (C) Ecogeographic map of species distribution (from north to south marked in different colors) and sampling sites (red dots) of the four climatic and chromosomal peripatric species from which one species, *S. galili* ($2n=52$) marked in green, diverged into geologically-edaphically and genically sympatric species derivative *S. galili* basalt ($2n=52$) marked with a brown smaller circle and its ancestor *S. galili* chalk ($2n=52$) marked with a bigger gray circle; the four chromosomal species forming a southward climatic cline of increasing aridity starting from *S. golani* ($2n=54$) marked in blue, and *S. galili* marked in green, *S. carmeli* ($2n=58$) marked in violet, and *S. judaei* ($2n=60$) marked in brown. (D) A neighbor-joining tree was reconstructed with the allele shared matrix of SNPs of the five blind mole rat species populations, and the scale bar represents the p distance. (E) Genetic clusters of the five species showed by PCA based on SNPs, only principal component 1 (26.95%) and principal component 2 (9.03%) are displayed. (F) Neighbor-joining tree based on the SNPs located in the mirroring coding and noncoding genomes, respectively (G) Structure: genetic bar plots of the five *Spalax* species. The number of putatively genetic populations (K) was defined from K=2 to K=5, each column denotes one individual (from Li et al. 2020a, b; all figures are copied by permission).

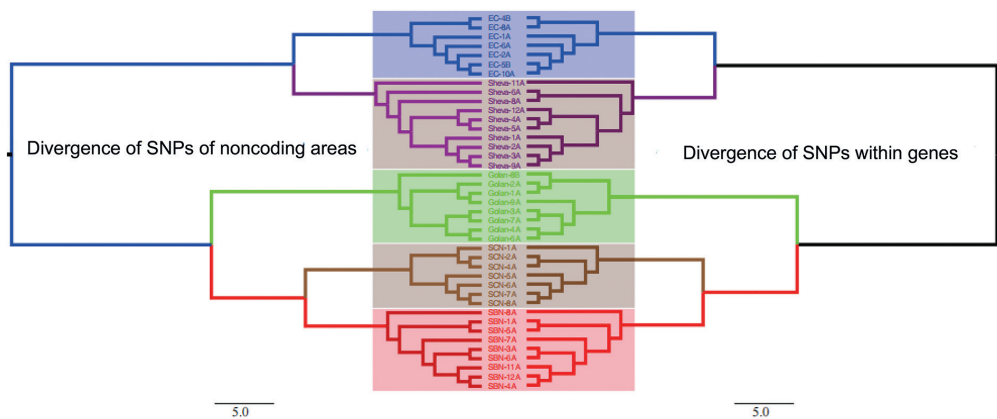


Fig. 2. The genomic divergence among *Spalax ehrenbergi* superspecies five species in Israel. Neighbor-joining tree based on the SNPs in *coding* (genes, right side) and *noncoding* (left side) *genomic regions*. Note the remarkable mirror image of both genome regions (from LI et al. 2020a, b).

them in bacteria. They were also discovered by BUKHARI et al. (1977), in a volume describing DNA insertion elements, plasmids, and episomes in the genomes. SHAPIRO & VON STERNBERG (2005) anticipated the recent ENCODE results by DUNHAM & KUNDAJE (2012). Its basic idea was that the genome is a highly sophisticated information storage organelle, greatly formatted by generic (i.e. repeated) signals enabling it to access the stored information, when and where it will be useful (see also elaborated discussion in SHAPIRO 2022). The main message indicates that there are clear theoretical reasons and examples showing that repetitive DNA acts as essential genome functional elements. Our recent work on blind mole rats and wild barley described below, supports the important functionality of the repeatome, the ensemble of divergent repeats in the genome. We show that the repeatome is selected by ecological stresses precisely like the coding genome region as indicated by their mirror image in the phylogenetic trees of blind subterranean mole rats and wild barley genomes (Figs. 1–12).

Our last 30 years' studies on the role of sympatric speciation (SS) in evolution (NEVO 2021) reinforced the idea that human genome results may be true in other eukaryotes besides humans. Our results demonstrated that SS appears to be a common speciation model in nature since there are numerous “Evolution Canyons”, “Evolution Plateaus”, and “Evolution Slopes” microsites divergent ecologically across the planet with reproductive isolation in taxa sharing opposite slopes or abutting divergent ecologies. They involve climatic, geologic, edaphic, abiotic, and biotic ecological contrasts causing SS involving viruses, bacteria, fungi, plants, and animals (NEVO 2021). Our genomic studies on SS revealed dramatic results concerning the *coding and noncoding genomes* in two of our model SS organisms, blind subterranean mole rats, *Spalax ehrenbergi* superspecies in Israel (LI et al. 2020a), and wild barley, *Hordeum spontaneum* (LI et al. 2020b), the progenitor of cultivated barley, both in eastern Upper Galilee, Israel in the “Evolution Plateau” microsite (Figs. 1–12).

Speciation mechanisms remain controversial since two speciation models occur in Israeli subterranean mole rats of the genus *Spalax*: a *regional* speciation cline southward of four peripatric, climatically adaptive, chromosomal species and a *local*, geologic-edaphic, genic, and sympatric speciation (Fig. 1A–G). Li et al. (2020a) highlighted their genome evolution. The five blind mole rat species we discovered in Israel and studied genomically were separated into five genetic clusters by *single nucleotide polymorphisms*, *copy number variations (CNVs)*, *repeatome*, and *methylome in sympatry* (Fig. 1A–E, Figs. 2–10). The regional interspecific divergence corresponds to Pleistocene climatic cycles. Climate warmings caused *chromosomal speciation in four species: Spalax golani*, $2n=54$; *S. galili*, $2n=52$; *S. carmeli*, $2n=58$; *S. judaei*, $2n=60$, and in one *sympatric species* in the *S. galili* complex, where both sympatric species are $2n=52$ and are divergent geologically by abutting Senonian chalk and Pleistocene basalt (HADID et al. 2013, LI et al. 2015, 2016, LÖVY et al. 2015, 2017, ŠKLÍBA et al. 2016, ZHAO et al. 2016). Triple effective population size, N_e , declines match glacial cold cycles. Adaptive genes evolved under positive selection to underground stresses and to divergent climates, involving *interspecies reproductive isolation*. Genomic islands evolved mainly due to adaptive evolution involving ancient polymorphisms. *Repeatome*, including both *CNV* and *LINE1* repetitive elements, separated signifi-

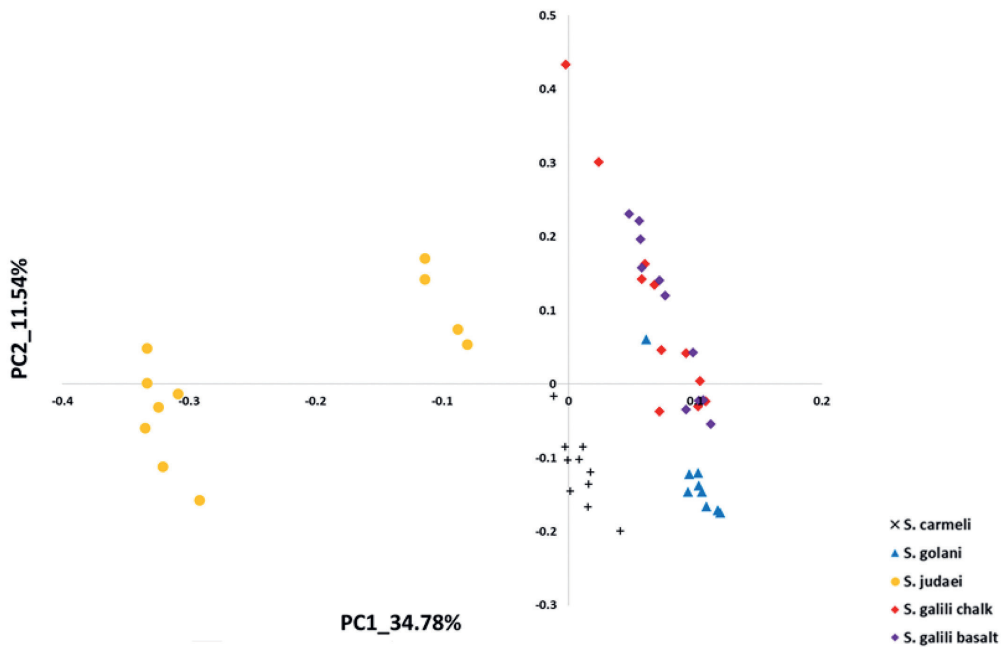


Fig. 3. The two-dimensional plot of five *Spalax* species in Israel shows the projection of abundance data of well-annotated 300 RepBase repetitive elements on the first two PCs. The samples are colored according to *Spalax ehrenbergi* superspecies (from LI et al. 2020a: Fig. S15A).

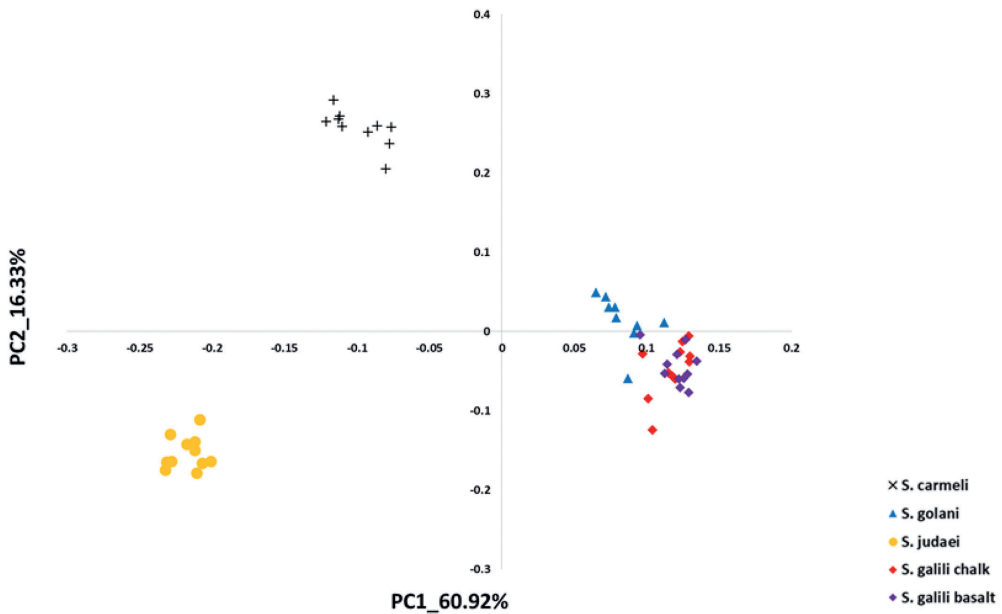


Fig. 4. The two-dimensional plot shows the projection of k-Chain abundance data on the first two PCs. The samples are colored according to *Spalax ehrenbergi* superspecies. Note that the *Spalax galili* chalk and *S. galili* basalt are still overlapping (from Li et al. 2020a: Fig. S15B).

cantly the five *Spalax* species (Figs. 1–10, 12). Methylation in sympatry identified geologically chalk-basalt *Spalax galili* two sympatric species that differentially affect thermoregulation, hypoxia tolerance, DNA repair, P53, and other pathways. Genome adaptive evolution highlights speciation across climatic aridity cline southwards, and geologic-edaphic stress unfolding, thus the two speciation models, peripatric and sympatric. Remarkably, noncoding genome mirrors completely the coding genome in subterranean mole rats in the four chromosomal species of the subterranean mole rats of the genus *Spalax* (Figs. 2–10, 12; all figures are from Li et al. 2020a) and in wild barley from the same microsite of “Evolution Plateau” (Figs. 11 and 12). The mirror image of the coding and noncoding genomes (Fig. 1F) clearly indicates that both genome regions are selected equally by the same ecological stresses, hence their biochemical functionality is demonstrated not only in humans but first also in other eukaryotes.

Incipient sympatric speciation in wild barley, *Hordeum spontaneum*, caused by geological-edaphic divergence, highlights mirroring image of the coding and noncoding genome. Sympatric speciation has been contentious since the idea was suggested by Darwin. We proposed SS in wild barley due to geologic and edaphic divergence in “Evolution Plateau”, Upper Galilee, Israel, at the same microsite where *Spalax galili* basalt speciated sympatrically (Li et al. 2020a). Our

whole-genome resequencing data in wild barley showed SS separating between the progenitor old Senonian chalk and abutting derivative young Pleistocene basalt wild barley populations. The basalt wild barley species unfolds larger effective population size, lower recombination rates, and larger genetic diversity. Both species populations show a similar descending trend ~200,000 yaers ago associated with the last glacial maximum. Coalescent demography analysis indicates that SS was *local, primary, in situ, and not due to a secondary contact from an ex-situ allopatric population*. Adaptive divergent putatively selected genes were identified in both the chalk and basalt populations. Remarkably, disease-resistant genes were selected in the wet basalt population, and genes related to flowering time, *leading to temporal reproductive isolation, were selected in the chalk population*. The evidence substantiates adaptive ecological SS in wild barley, highlighting the genome landscape during SS with gene flow, due to geolo-

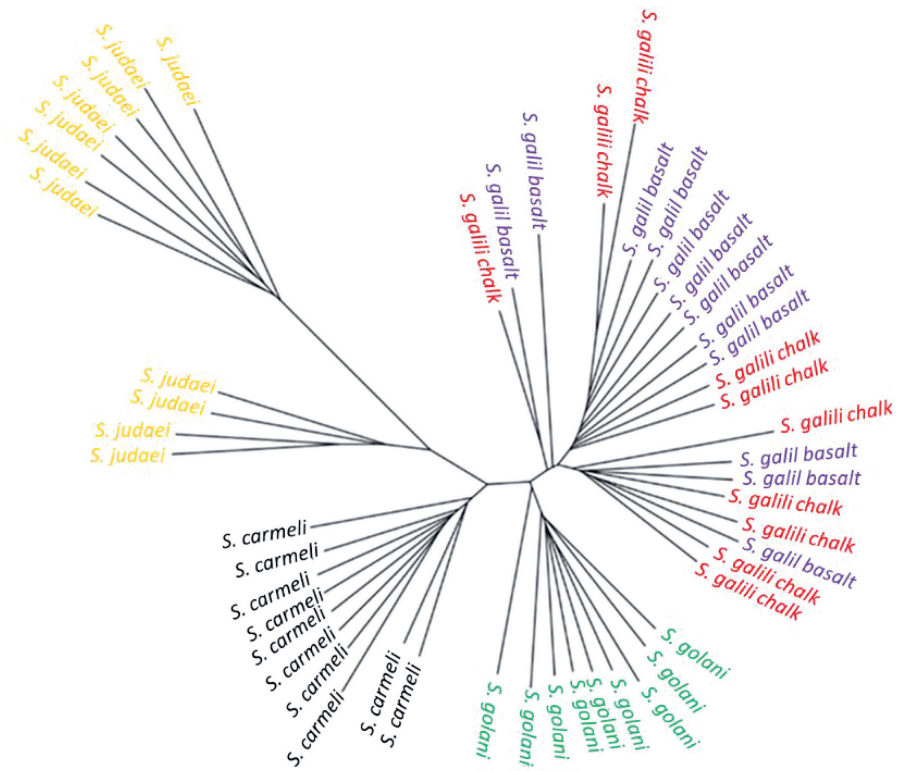


Fig. 5. Unrooted phylogenetic tree of *Spalax ehrenbergi* superspecies based on the abundance of RepBase annotated repetitive elements. The tree was prepared with Ape package with neighbor-joining method. Note that the sympatric species pair of *Spalax galili* chalk-basalt sympatric pair is largely mixed (from Li et al. 2020a: Fig. S15C).

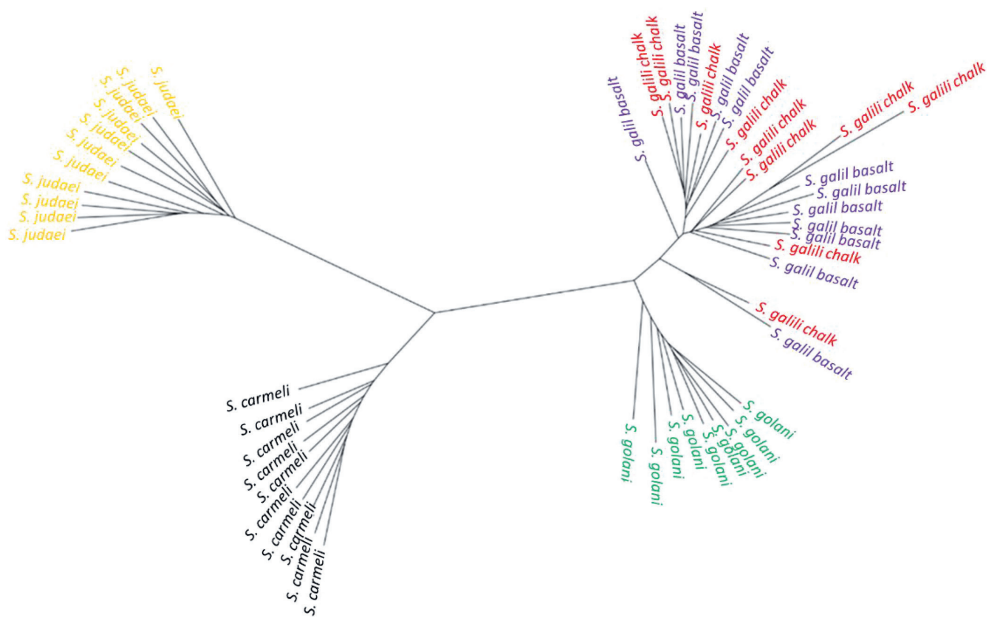


Fig. 6. Unrooted phylogenetic tree of *Spalax ehrenbergi superspecies* based on the abundance of k-Chains. The tree was prepared with Ape package with neighbor-joining method. Note that the sympatric complex pair of *Spalax galili* is largely mixed (from Li et al. 2020a: Fig. S15D).

gic-edaphic divergence. The dramatic discovery is that in both *Spalax* five species (Figs. 1F, 2) and the taxonomically distant wild barley, *Hordeum spontaneum*, the noncoding genome (repeatome) mirrored the coding genome (Figs. 2 and 11; Fig. 12 for comparison between *Spalax* and *Hordeum*) suggesting that both genomes responded to, and were selected by, the same ecological stresses.

It was exciting to find cases of SS and the mirroring image of coding and noncoding genomes in two dramatically distant eukaryote taxa as blind subterranean mammals of the *Spalax ehrenbergi* superspecies and wild barley, *Hordeum spontaneum*, the progenitor of cultivated barley. Both SS events and the nature of the noncoding genome are still contentious, but are gradually realized by both empirical and theoretical studies to be realistic adaptive phenomena (for SS see NEVO 2021; for the noncoding genome see MCCLINTOCK 1956, BRITTEN & KOHNE 1968, SHAPIRO 2022). The repeatome is spread intergenically across the genome in hundreds of thousands divergent repeats (BRITTEN & KOHNE 1968), many of them jumping within and between chromosomes hence called transposable elements, first described by MCCLINTOCK (1956), who dubbed them “controlling” elements of genes and considered them important in adaptive evolution. Sympatric speciation, the origin of new species in the same population or meta-population, where interbreeding and gene flow are operating, and seemingly preventing

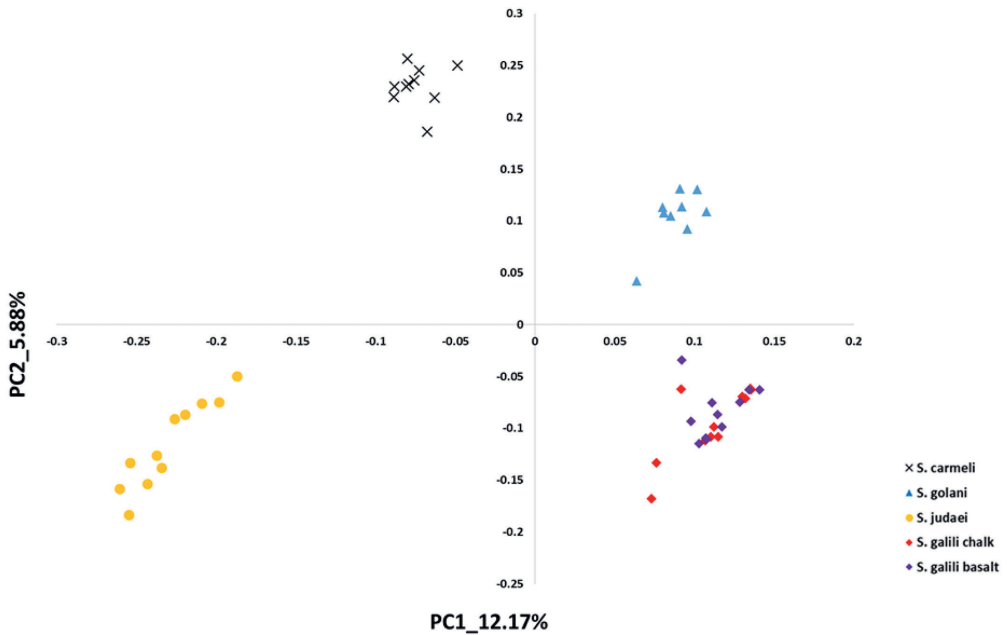


Fig. 7. The two-dimensional plot shows the projection of mutability data of *Spalax ehrenbergi* superspecies of well annotated 300 RepBase repetitive elements on the first two PCs. The samples are colored according to *Spalax ehrenbergi* superspecies. Note that the *Spalax galili* sympatric complex is largely mixed (from Li et al. 2020a: Fig. S16A).

speciation, is highly debated since hypothesized by DARWIN (1859). The SS model contrasts the *allopatric model of speciation*, based on *geographic isolation* that safeguards organisms from interbreeding and gene flow, hence preventing mixture, and leads to speciation, the origin of biodiversity. By contrast, the sympatric model is seemingly preventing speciation, and increasing homogeneity by gene flow. DARWIN (1859), hypothesized that SS may be a common speciation model (though he phrased it differently) basing his hypothesis on one species with multiple persistent variants. The Appendix presents 72 cases of SS supported both theoretically and empirically. However, even the citations in Appendix restrict SS to one or several species in a site. Our two speciation models, first “Evolution Canyon” (EC), unfolding *microclimatic* ecologically divergent microsities; and second, “Evolution Plateau” (EP), consisting of abutting geologic-edaphic ecologically divergent microsities, demonstrate SS, across life, from viruses and bacteria to mammals, including human domestication (NEVO 2014, 2021). The EC model (Fig. 14) comprises four ecogeographic repeats of tropical hot-dry savannoid “African” vs temperate cool-humid forested “European” abutting slopes in Carmel, Upper Galilee, Negev, and Golan Mountains (EC I-IV). The distance between the opposite slopes is between 0 m to

hundreds of meters (Figs. 13 and 14). Fig. 13 shows EC I, in Mount Carmel, with the 7 distant taxa from bacteria to mammals that speciated sympatrically. Fig. 14 shows the four Evolution Canyons. Since ecologically divergent microsites abound globally (climatic, geologic, edaphic, abiotic, and biotic), we suggested that SS is a *common speciation model* (NEVO 2021).

Our second finding in both species that speciated sympatrically at “Evolution Plateau”, blind subterranean mole rats, the genus *Spalax*, plus all additional four chromosomal species that speciated climatically across Israel (LI et al. 2020a), and wild barley, the genus *Hordeum* (LI et al. 2020b), highlight the nature of the noncoding genome, or repeatome, the complement of repeated sequences in a genome (e.g., MENEVERI et al. 1985). Hundreds of thousands of repeated elements are distributed intergenically across the genomes of living organisms (BRITTEN & KOHNE 1968). The non-coding genome has been described as “junk” and “selfish” genome (ORGEL & CRICK 1980). By contrast, McCLINTOCK (1956), who first discovered the jumping repeated genes or transposable elements, suggested that they are “controlling elements”, regulating the

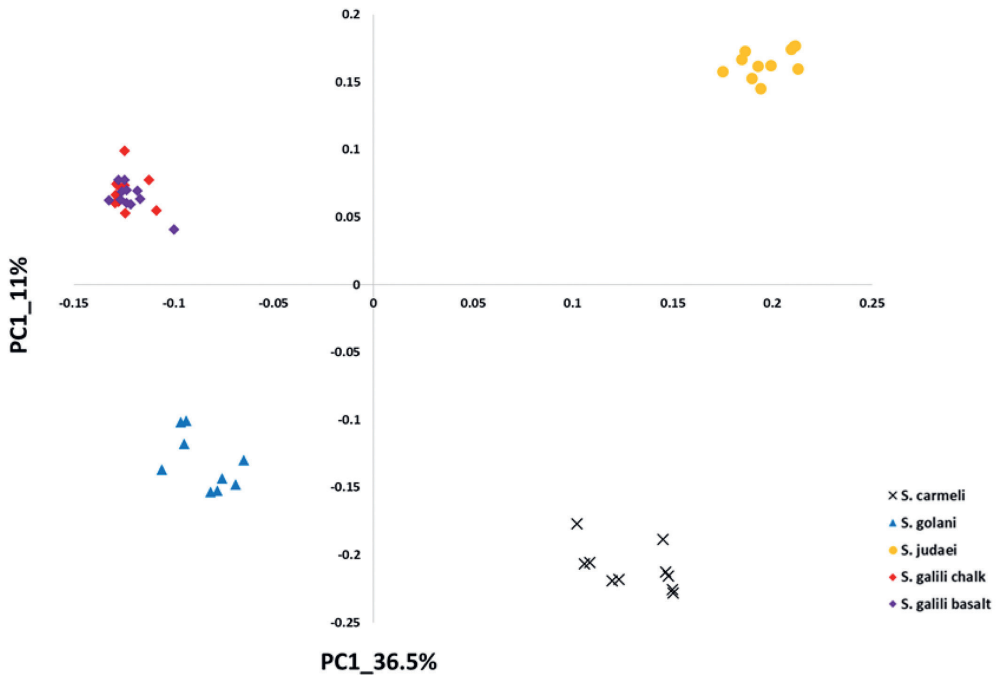


Fig. 8. The two-dimensional plot of *Spalax ehrenbergi* superspecies shows the projection of k-Chain mutability data on the first two PCs. The samples are colored according to *Spalax ehrenbergi* superspecies in Israel. Note that *Spalax galili* sympatric complex of chalk-basalt is mixed (from LI et al. 2020a: Fig. S16B).

genes (MCCLINTOCK 1956), later discovered in bacteria (SHAPIRO 1979) and other organisms (BUKHARI et al. 1977). Moreover, the ENCODE results (DUNHAM & KUNDAJE 2012) revealed in humans that 80% of the genome is biochemically functional. The nature of the noncoding genome, whether “junk” and “selfish” or biochemically functional and regulatory, is still hotly debated (e.g., PONTING & HARDISON 2011, GRAUR et al. 2013, KELLIS et al. 2014, RANDS et al. 2014, GIUDICELLI & ROEST CROLLIUS 2021). Similar findings in two distant eukaryotic taxa, one animal, the blind subterranean mole rat *Spalax*, representing five species of the *Spalax ehrenbergi* superspecies in Israel, the second plant, wild barley, *Hordeum spontaneum*, studied genomically demonstrate the stunning figure of mirror image between their coding and non-coding genomes. This pattern (Figs. 1, 11, and 12) strongly suggests that both are challenged by the same ecological stresses and are equally selected adaptively across the genome. This result strongly supports the view that the noncoding genome, or repeatome, is significant evolutionarily (SHAPIRO & VON STERNBERG 2005, DUNHAM & KUNDAJE 2012, LAUKIEN 2022, SHAPIRO 2022). The big mystery of the noncoding genome and its regulatory functions are now open to intensive and extensive investigations.

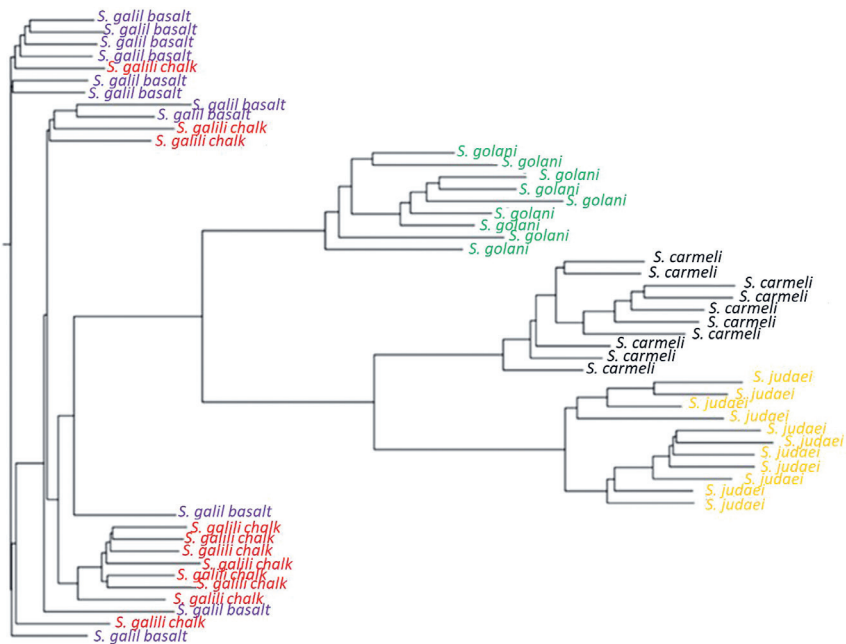


Fig. 9. Unrooted phylogenetic tree of *Spalax ehrenbergi* superspecies prepared with Ape package based on mutability of k-Chains. Note that the *Spalax galili* sympatric complex of chalk-basalt is still not fully separated (from LI et al. 2020a: Fig. S16B).

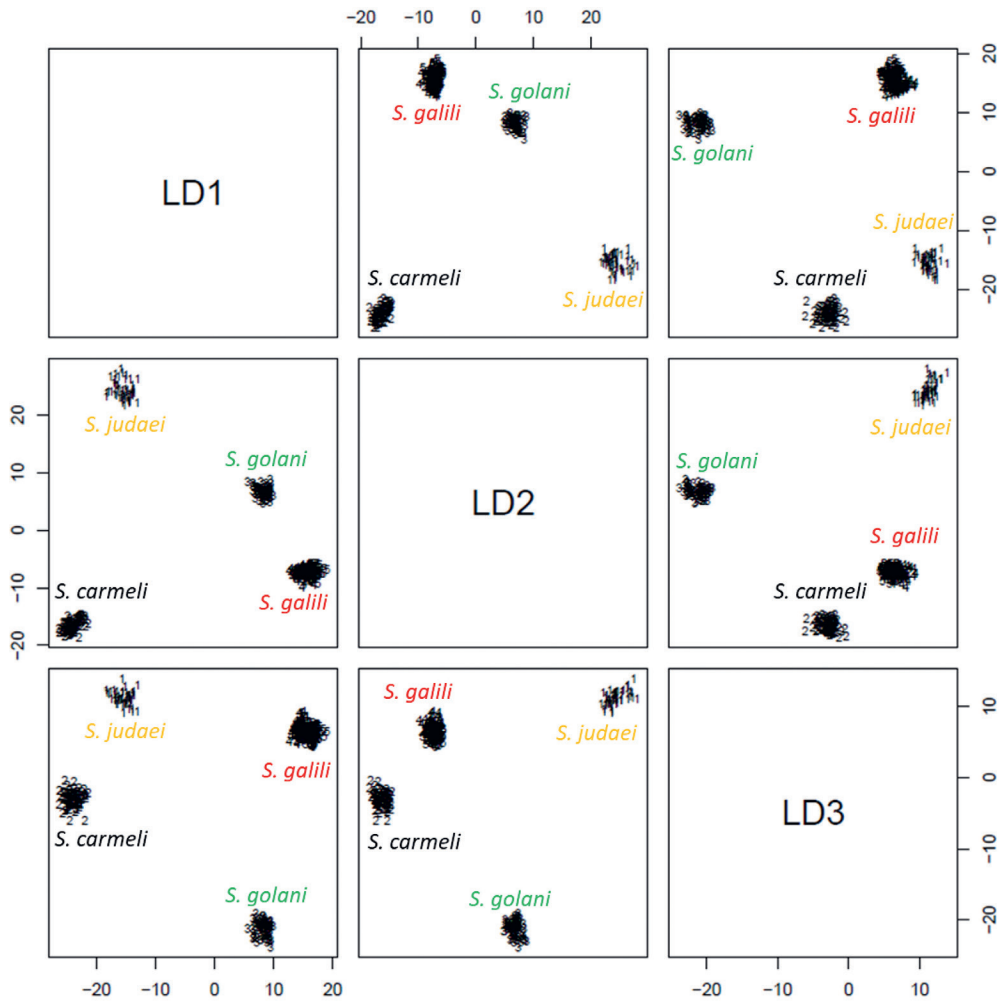


Fig. 10A. Putative repetitive elements (k-Chains) identified as differentiating between *Spalax ehrenbergi* superspecies four chromosomal species (*S. galili* is not divided in this diagram to the sympatric complex of chalk-basalt (from Li et al. 2020a: Fig. S17A).

Acknowledgements

This review paper is warmly dedicated to the 70th birthday anniversary of my dear friend and colleague Hynek BURDA co-researching with me the underground life mysteries of subterranean mammals.

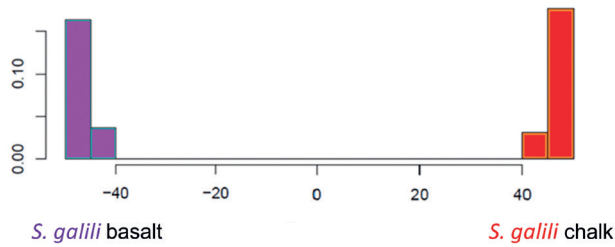


Fig. 10B. The two-dimensional plot (A) above shows the LD1-LD2-LD3 projections of abundances of 300 k-Chains identified as differentiating between four chromosomal species of *Spalax ehrenbergi* superspecies by Random Forest analysis. The two-dimensional plot (B) shows that the LD1-LD2 projection of abundance of 150 k-Chains differentiated between two *Spalax galili* species sympatric complex (basalt and chalk) indicating that the Random Forest analysis can separate the chalk progenitor and the basalt derivative sympatric new species (from Li et al. 2020a: Fig. S17B).

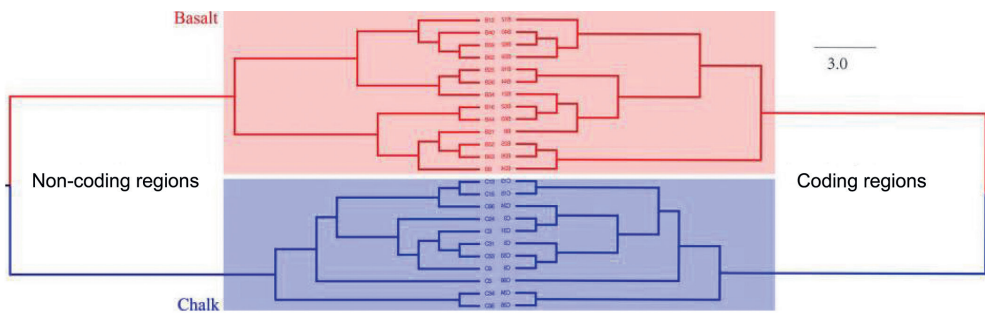


Fig. 11. Neighbor-joining tree based on the SNPs from coding and non-coding genomic regions in wild barley, *Hordeum spontaneum*, the progenitor of cultivated barley, at “Evolution Plateau”(right coding region and left the noncoding region or repeatome) in the same microsite of *Spalax galili* basalt. The mirroring effect of the coding and noncoding genomes demonstrates that both are subjected to the same ecological stresses and are selected accordingly (from Li et al. 2020b: Fig. S2).

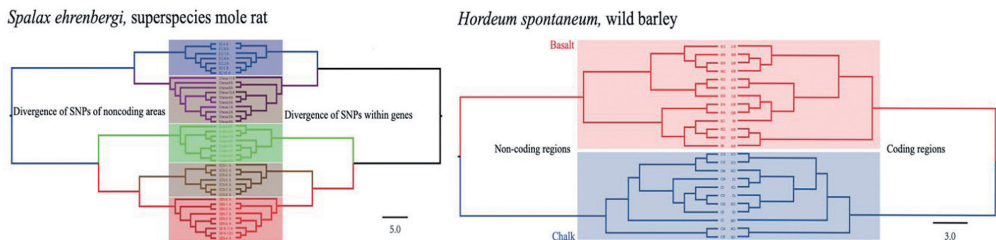


Fig. 12. Phylogenetic tree based on SNPs from coding and the mirroring noncoding genome regions. Five species of the *Spalax ehrenbergi* superspecies (left) and wild barley on the right. The mirroring image in subterranean *Spalax* mole rats and plants suggests the commonality of the phenomenon of functionality of both genomes due to the same ecological stresses.

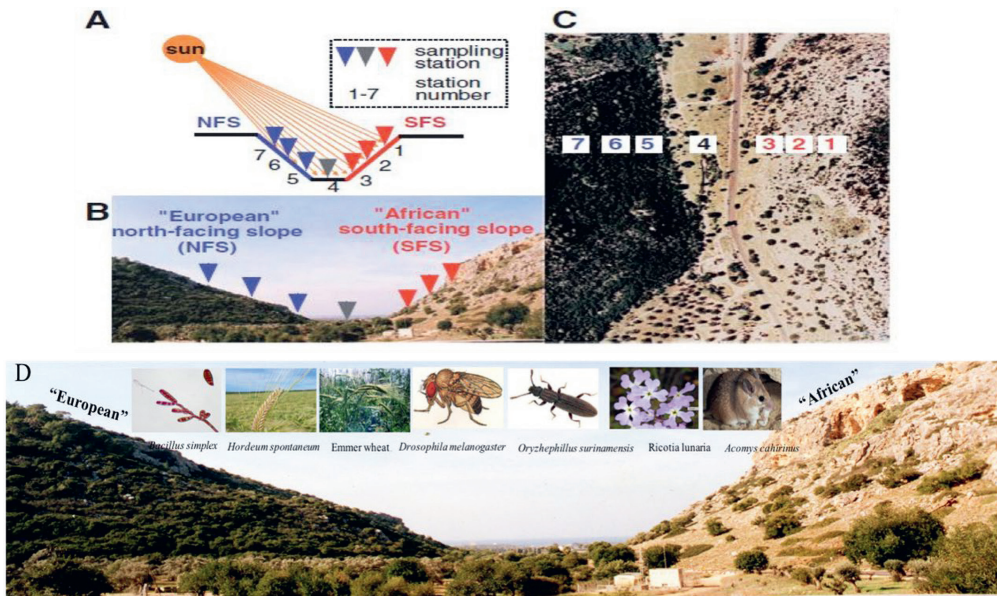


Fig. 13. “Evolution Canyon” I (EC I) at Mount Carmel, Israel. (A) The model of Evolution Canyon I (EC I): Microclimatic interslope divergence: stations (populations) #1–3 are on the tropical, savannoid, South facing slope (SFS), also dubbed the “African” slope (AS; red triangles in A and B, and red numbers in C, SFS=AS), characterized by high solar radiation, temperature, and drought; station 4, in the crick (gray triangle), and stations # 5–7 (blue triangles in B, NSF, and blue numbers in C NSF) are on the temperate, forested, North Facing Slope (NFS), also dubbed the “European” slope (ES, blue triangles in B, and blue numbers in C), characterized by low solar radiation and temperature, high humidity, and forested. AS is distant 250 m from ES. (B) Cross section of Evolution Canyon I (EC I), covered, on the left hand side, by a green forest with live oaks, *Alon* in Hebrew, *Quercus calliprinos*, and *Pistacia palaestina*, *elah* in Hebrew, on the “European” slope (ES=NFS), versus the savannoid “African” slope (AS=SFS). The blue triangles represent experimental stations (populations) colored as in A. (C) Air view of Evolution Canyon I (EC I), Mount Carmel, showing the forested slope (ES=NFS) versus the savannoid African slope, with open park forest (AS=SFS), with carob trees, *Ceratonia silqua* and *Pistacia lentiscus* bushes, and African grasses, *Hyparrhenia hirta*, *Andropogon distachion*, and *Pennisetum asperifolium*. (D) Cross section of Evolution Canyon I (EC I), Mount Carmel, with the forested slope (ES=NFS), and opposite, abutting savannoid, park-forest (AS=SFS), with five distant organisms from bacteria to mammals, that represent incipient adaptive sympatric ecological speciation across life: soil bacterium, *Bacillus simplex*; wild barley, *Hordeum spontaneum*; fruit-flies, *Drosophila melanogaster*; saw-toothed grain beetle, *Oryzaephilus surinamensis*; and spiny mouse, *Acomys cahirinus*.

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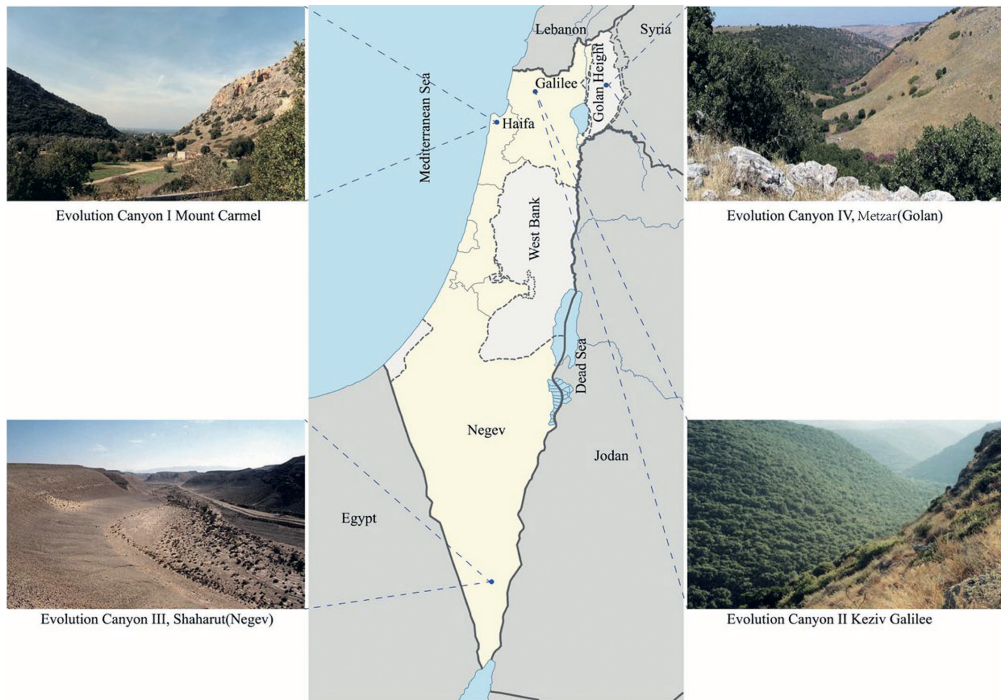


Fig. 14. The four “Evolution Canyons” studied in Israel: lower Nahal Oren (Mount Carmel) – EC I; Keziv (Galilee) – EC II; Shaharut (south Negev desert) – EC III, and Metzar (Golan) – IV. Note that in ECs I, II, IV, the North Facing Slope (NFS), also dubbed the European slope (ES), is on the left-hand-side, representing temperate, cool, humid, and forested biome. The opposite, abutting slope, on the right-hand side, is tropical, hot, dry, and savannoid. By contrast, in Shaharut – EC III, the slope orientation in the picture is reversed: the SFS is on the left, covered by cyanobacteria, and the NFS is on the right, darker in color, covered by lichens, with angiosperm bushes only in the creek.

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APPENDIX

Sympatric speciation, by definition, is the origin of new species within a freely interbreeding population. Recently, sympatric speciation has received widespread attention in the scientific community and has been the subject of heated debate. The following studies focused on sympatric speciation:

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