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Evolution

Chromosomal speciation and adaptive radiation of mole rats in Asia Minor correlated with increased ecological stress

(recombination/selection/*Spalax leucodon*/*Spalax ehrenbergi*/Turkey)

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ABSTRACT **The evolutionary forces causing chromosomal speciation and adaptation are still enigmatic. Here we tested the Israeli evolutionary model of positive association of diploid chromosome number ($2n$) and genetic diversity with aridity stress in subterranean mole rats, on a 30-times-larger scale in Asia Minor. We analyzed both karyotype and allozyme diversity across Turkey, based on 37 allozymic loci in 20 localities of the *Spalax leucodon* and 4 localities of the *Spalax ehrenbergi* superspecies. We found extensive chromosomal speciation in *S. leucodon* ($2n$ = 38, 40, 50, 54, 60, and 62) and in *S. ehrenbergi* ($2n$ = 52, 56, and 58), presumably representing from 14 to >20 additional biological species. Genetic diversity indices were low, but, like the chromosome number ($2n$), positively correlated with aridity stress, increasing centripetally from the periphery toward geologically young, arid, and climatically unpredictable central Anatolia. Nei's genetic distance *D* across all populations averaged 0.174 (range 0.002–0.422), supporting, combined with $2n$ and ecogeography, the biological species status of most tested populations. Chromosome evolution is the basis of speciation and adaptation in *Spalax*; it provides both postmating reproductive isolation, as well as higher levels of recombination with increased $2n$. A mathematical model shows that a Robertsonian fission of a single metacentric considerably increases haplotype diversity. This haplotype diversity may contribute to population adaptation to climatic stress and ecological unpredictability in space and time. The increase in diversity corroborates the niche-width genetic-variation hypothesis.**

Speciation and adaptation, the major twin processes of evolution, are yet largely mysterious, notwithstanding the great past achievements and the present molecular biological revolution. The unresolved issues involve the relative importance of the relevant evolutionary forces, origin, genetics, mechanisms, modes, dynamics, and abiotic/biotic environmental correlates (1–6). This is true for most catalogued species, which are only a small fraction of the unknown number of living species (7). Likewise, the significance of karyotypic evolution has been extensively debated (6, 8–19). Highlighting these extremely complex problems calls for multidisciplinary studies involving molecular, genetic, chromosomal, organismal, and ecological insights (e.g., ref. 1). Here we explore two major unresolved problems of evolutionary biology in mole rats: (*i*) What is the role of diploid chromosome number ($2n$) values and allozymes in evolution, primarily in speciation and adaptation? and (*ii*) Is the evolutionary process in *Spalax* oriented primarily by stochastic, nonselective, and neutral factors, or, by contrast, is it driven by selective factors? In short, is the origin of species and adaptation chaotic or ordered (20), reflecting adaptive environmental patterns?

The evolutionary model of subterranean mole rats of the *Spalax ehrenbergi* superspecies in Israel (1) supports the environmental theory of genic and chromosomal diversity in both speciation and adaptation. The four chromosomal species ($2n$ = 52, 54, 58, and 60), previously considered one species, represent four biological sibling species at progressive stages of speciation (1). Speciation in Israeli *Spalax* is chromosomal with $2n$ values equal to 52–60 and genic observed heterozygosity (*H*; equal to 0.044–0.088), increasing southward toward the desert. Both $2n$ and *H* positively correlate with aridity stress, climatic unpredictability, and increased steppic conditions. Is the observation in Israel unique, or does it depict a general trend throughout the range of *Spalax*? We have chosen Asia Minor, the center of the *Spalax* range, involving two major superspecies—*S. leucodon* and younger *S. ehrenbergi* (21)—as our ecological large-scale testing grounds. Asia Minor is 30 times larger than Israel (770,000 km^2 versus 26,000 km^2). Furthermore, we tested the longitudinal North–South Israeli model in the Turkish centripetal model, where aridity and climatic unpredictability increase centripetally toward geologically young central Anatolia.

We show that in Turkish *Spalax*, speciation and adaptation, revealed in Israel by $2n$ and *H* (1), positively correlate with aridity stress and climatic unpredictability. $2n$ values and *H* increase toward the ecologically harsh, arid, and climatically unpredictable and geologically young central Anatolian Plateau from the west, north, south, and east, repeating from all directions the pattern seen in Israel. However, while variation in the Israel speciational trend involves only four chromosomal species, ranging from north to south, chromosomal speciation in Turkey is centripetal, involving at least 14, but probably >20, additional biological species defined by a combination of chromosome number, genetic (allozymic) distances, and ecogeography (22).

MATERIALS AND METHODS

We analyzed karyotypes ($2n$) and allozymic diversity (*A*, *P*, *H*, and *He*, see Table 1) at 37 gene loci in 69 subterranean mole rats (Spalacidae, Rodentia) from 24 localities across Turkey collected in four field excursions during 1988–1991. The mole rats belonged to two related superspecies *S. leucodon* superspecies (N = 55; 20 populations) and younger *S. ehrenbergi* superspecies (N = 14; 4 populations) (Table 1). Karyotypes were prepared in the field from bone marrow (23), and frozen tissues were analyzed electrophoretically for protein variation (24). Detailed analysis appears in ref. 22. Climatic and geological data were taken from the Atlas of Turkey (25).

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Abbreviations: *H*, heterozygosity; *He*, genic diversity; $2n$, diploid chromosome number; Δd, increase in haplotype diversity due to one Robertsonian fission; *D*, Nei's genetic distance.

$2n$ values appear in Fig. 1 and are listed in Table 1. When two localities had the same karyotype, but were geographically and genetically distant, they were regarded as different species—designated by E (East), C (central), W (west): 54W, 54E, 52W, 52E, 50W, 50E, 60W, 60C, and 60E, which are considered here as good biological species (see *Discussion*). Climatic data appear in Table 1.

For technical details of chromosomal preparation, allozymic loci, and genetic distance estimation, see ref. 22. The high number of loci analyzed ensured good estimates in spite of the small sample size of some populations. Values of *H*, *He*, and genetic distance (*D*) are therefore reliable with a reasonable margin of precision (27, 29, 30). Notably, *H* and *He*, after conducting Nei's correction (27), are essentially independent of sample size, and only they were used in our analysis. Furthermore, because we originally explored chromosome evolution as the basis of speciation in *Spalax* (21), we preferred sampling more localities across Turkey than larger numbers of animals in each locality.

RESULTS AND DISCUSSION

Six distinct $2n$ groups (equal to 38, 40, 50, 54, 60, and 62; Fig. 1) were identified in the *S. leucodon* superspecies, with fundamental arm numbers ranging from 74 to 92. The *S. ehrenbergi* superspecies in Turkey was characterized by three diploid numbers ($2n$ = 52, 56, and 58) and fundamental arm numbers ranged from 82 to 90. $2n$ numbers are assumed to increase from 38 to 62, mainly by Robertsonian fissions (Fig. 2). The karyotypes described elsewhere (22) consist of different proportions of metacentric, submetacentric, and acrocentric chromosomes. They compose a group of unchanged chromosomes that are shared by all karyotypes and a group of Robertsonian chromosomes, with a maximum presence of 18 pairs of metacentric (submetacentric) chromosomes in $2n$ = 38.

Genetic diversity indices were low, on average, in the *S. leucodon* and *S. ehrenbergi* superspecies: allele diversity A = 1.081 and 1.074; polymorphism P = 0.077 and 0.068; observed H = 0.038 and 0.027; and He = 0.038 and 0.034, respectively (Table 1). The average Nei's genetic distances (D; ref. 27) = 0.174, range 0.002–0.422. Average D values within and between the two superspecies were as follows: within *S. leucodon* (20 populations): D = 0.132 (range, 0.002–0.325); within *S. ehrenbergi* (4 populations), D = 0.145 (range 0.063–0.264); between *S. leucodon* and *S. ehrenbergi*, D = 0.275 (range 0.204–0.422). This superspecies division is also supported by molar teeth (31) and mtDNA and rDNA diversities (unpublished work). Based on our Israeli *Spalax* molecular and organismal evidence, its $2n$ and D estimates as well as ecogeography (1), we consider the different karyotypes, mostly newly described, as representing 14 good biological species (Fig. 1 and Table 1). Furthermore, combining the evidence of $2n$ and D, we suggest that almost each of our populations may represent a different biological species, revealing extensive ecological speciation (22). Critical testing is imperative to validate this claim, involving the search for potential natural hybridization, reproductive isolation, gene flow, and ecological compatibility, as was conducted in the Israeli *Spalax* (1).

Karyotypes represent chromosomal trends of increased $2n$, in both Turkey and Israel, stepwise increasing the number of acrocentrics, through Robertsonian fissions of metacentrics (32, 33), whereas changes in the fundamental numbers derive from centromeric shifts (Fig. 2). Evidence of the direction of karyotype evolution from low to high $2n$ in *Spalax* is derived on multiple grounds involving fossil, geological, biogeographical, and molecular evidence. This is true across small Israel, as well as across the entire large Eurasian family range, where $2n$ increases to 60 or 62 toward the Balkans, Russia, Near East, and North Africa. The same trend is probable in Turkey, the $2n$ = 62 complex being the youngest offshoot. The highest diploid numbers in Israel, North Africa, Balkans, Ukraine, and Russia, as well as in Turkey occupy the most xeric regions. Moreover, in Turkey, central Anatolia is geologically young, consisting extensively of continental Quaternary sediments (25). The arrival of *Spalax* $2n$ = 62 to central Anatolia must have occurred during Pleistocene and Holocene times, representing late colonization and speciation events on the dried-up pluvial plains. Notably, the lowest diploid number in *Spalax* occurs in the center of its range, in mesic environments, where the oldest

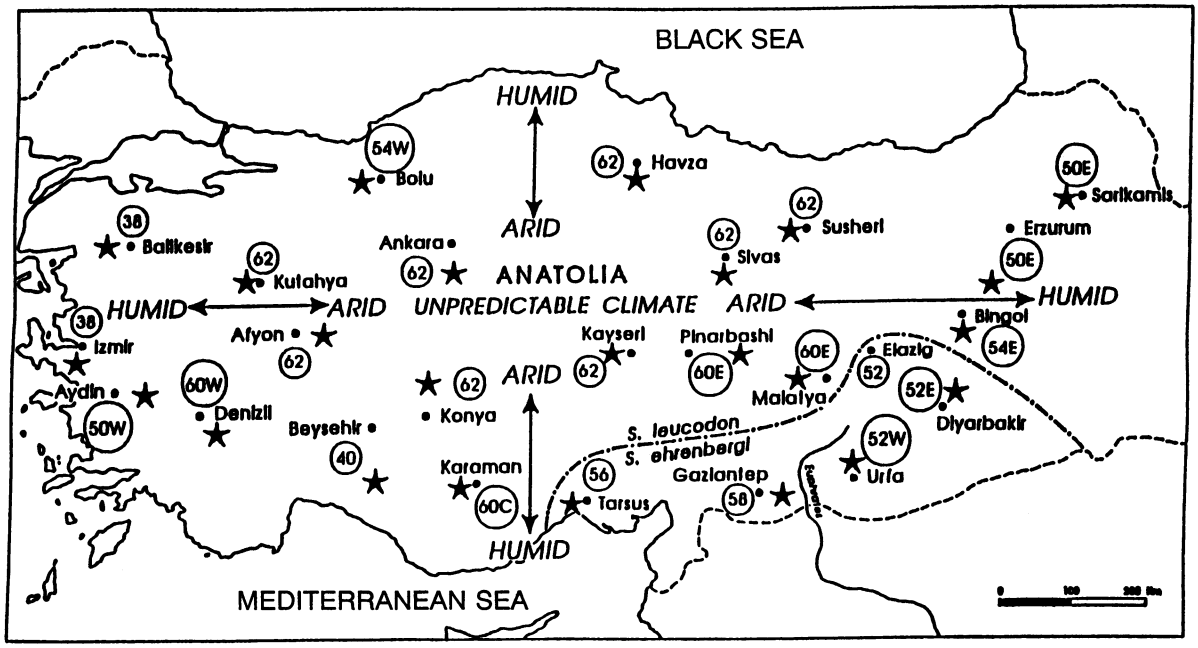

FIG. 1. Geographic distribution and karyotypes of sampling localities (★) of subterranean mole rats in Turkey. Karyotypes are represented by their circled diploid number ($2n$); when two localities have the same karyotype and are geographically and genetically distant, they are designated with E (East), C (Central), or W (West), 54W, 54E, 52W, 52E, 50W, 50E, 60W, 60C, and 60E, which are considered here as good biological species.

Table 1. Geographical and climatological data for 20 samples of *S. leucodon* and for 5 samples of *S. ehrenbergi* in Turkey and summary of genetic variation, based on 37 allozyme loci

Population				Ecogeographical variables											Genetic indices			
Species (2*n*)	No.	Location	*N*	Al	Tm	Tjuac	Td	Rn	Rd	Rnja	Rnju	Th	Ar	Vg	*A*	*P*	*H*	*He**
S. leucodon superspecies																		
38	1	30 km W-Balikesir	1	300	15.1	22.5	20.5	780	80	100	9	5	31	1	1.054	0.054	0.054	0.054
38	2	5 km S-Izmir	2	50	17.6	26.0	19.9	790	76	130	2	−2	28	2	1.000	0.0	0.0	0.0
40	3	60 km S-Beysehir	5	1400	17.6	20.0	21.1	1250	70	125	7	30	50	1	1.027	0.027	0.014	0.012
50W	4	35 km E-Aydin	1	65	18.0	25.5	20.4	780	75	120	8	−5	25	2	1.000	0.0	0.0	0.0
54W	5	Bolu	5	710	13.8	15.5	20.3	580	135	110	28	−2	24	3	1.108	0.081	0.027	0.039
60W	6	25 km SE-Denizli	2	1080	18.0	22.5	21.6	800	65	100	12	−18	25	3	1.054	0.054	0.041	0.032
60C	7	Karaman	1	1014	17.3	21.0	22.5	400	60	50	5	−20	18	4	1.027	0.027	0.027	0.027
62	8	5 km W-Kutahya	1	916	15.5	21.0	21.3	450	105	60	20	5	26	3	1.054	0.054	0.054	0.054
62	9	35 km E-Afyon	5	1007	16.4	20.8	22.5	400	98	50	17	−22	20	3	1.189	0.162	0.043	0.064
62	10	45 km N-Konya	3	1030	16.8	22.0	23.1	380	85	40	7	−22	17	4	1.135	0.108	0.045	0.043
62	11	30 km S-Ankara	4	960	16.1	20.5	23.0	390	85	40	10	−25	19	4	1.243	0.243	0.088	0.088
62	12	20 km W-Kayseri	1	1043	16.0	20.2	23.8	370	90	45	5	−25	18	4	1.027	0.027	0.027	0.027
62	13	10 km S-Havza	3	600	14.5	20.0	21.0	400	110	45	28	−20	25	4	1.108	0.108	0.036	0.043
62	14	10 km S-Sivas	6	1275	14.9	17.0	24.6	400	100	42	9	−20	20	4	1.189	0.189	0.077	0.067
62	15	3 km W-Susheri	4	930	15.2	15.1	24.4	600	92	50	20	0	27	4	1.054	0.054	0.034	0.026
60E	16	58 km E-Pinarbashi	2	1930	17.0	18.0	26.0	470	82	48	5	−22	23	4	1.054	0.054	0.027	0.027
60E	17	30 km W-Malatya	2	900	17.9	26.0	27.8	380	75	50	5	−21	23	3	1.000	0.0	0.0	0.0
54E	18	10 km S-Bingol	2	1100	17.5	25.0	30.2	500	97	48	8	−19	30	3	1.081	0.081	0.054	0.045
50E	19	80 km S-Erzurum	2	1950	16.3	15.0	31.0	800	112	80	30	20	50	3	1.108	0.108	0.068	0.059
50E	20	14 km W-Sarikamis	3	2100	14.0	16.4	30.0	550	125	30	50	20	45	1	1.108	0.108	0.036	0.047
Mean															**1.081**	**0.077**	**0.038**	**0.038**
S. ehrenbergi superspecies																		
52E	21	20 km NE-Diyarbakir	2	850	20.0	30.8	29.8	550	82	80	4	−25	19	4	1.081	0.054	0.041	0.036
52W	22	10 km N-Urfa	3	550	21.0	31.5	26.7	500	68	130	3	−25	18	4	1.108	0.108	0.045	0.052
58	23	10 km E-Gaziantep	2	600	19.4	28.7	24.2	550	70	140	6	−22	24	3	1.027	0.027	0.0	0.018
56	24	5 km W-Tarsus	7	10	18.5	26.0	19.8	770	60	110	2	−22	25	2	1.081	0.081	0.023	0.028
52	25	Elazig†		1020	17.1	21.0	28.8	580	76	50	7	−21	20	3				
Mean															**1.074**	**0.068**	**0.027**	**0.034**

The means are unweighted. Climatic data were deduced from maps in ref. 25. W, west; E, east; N, north; S, south; SE, southeast; NE, northeast; C, central. Symbols of variables are as follows: (*i*) Geographical: Al = altitude (in m); (*ii*) temperature: Tm = mean annual temperature (C°), Tjuac = mean July actual temperature (C°), Td = temperature range (C°); (*iii*) water availability: Rn = mean annual rainfall (in mm), Rnju = mean July rainfall (in mm), Rnja = mean January rainfall (in mm), Rd = mean number of rainy days, Th = Thornthwaite's moisture index, Ar = annual indices of aridity according to De Martonne's formula; (*iv*) biotic: Vg = vegetation: 1 = forest, 2 = Mediterranean, 3 = woodland, bush and steppe, 4 = steppe; (*v*) genetic indices: *A* = mean number of alleles per locus, *P* = mean proportion of loci polymorphic per population, with criterion of the most common allele <0.95, *H* = mean number of loci heterozygous per individual, *He* = genic diversity (= expected heterozygosity under panmixis).

*Unbiased estimate of *He* according to Levene (26) and Nei (27).

†2*n* of *S. ehrenbergi* in Elazig has been reported by Yuksel (28).

spalacid fossil from the early Miocene, *Heramys eviensis*, was found in Greece (34). The highest diploid numbers occur in the steppic peripheries of the family range, except in Turkey, where the most steppic environment is in central Anatolia.

The increase in acrocentrics drastically elevates the level of recombination generating new haplotype diversity. This process could also possibly reduce, according to the minimum-interaction hypothesis, the genetic risk resulting from reciprocal translocation (35).

Robertsonian chromosomal fission can be considered as an important evolutionary adaptation, increasing the potential of haplotype genetic diversity produced by meiotic recombination. The following analysis estimates the expected increase in haplotype diversity in a population per generation due to fission of one metacentric. One possibility to calculate it is to compare a formal measure of haplotype diversity generated by recombination in an initial metacentric and in its two acrocentric derivatives. As an appropriate indicator, we used Shannon's information approach (36). In our case, the formal diversity of haplotypes resulting from meiosis with frequencies p_i is $d = -\Sigma p_i \log_2 p_i$. This measure is nonnegative and additive. Thus, all that we need to estimate the change in potential haplotype diversity is to calculate the frequencies p_i of different haplotypes generated by recombination (in a metacentric bivalent and in its two acrocentric derivatives) and put p_i into the Shannon's measure (*d*).

(*i*) Consider first the metacentric. For simplicity, let us assume that no more than two chiasmata can occur in the metacentric bivalent, one being obligatory. If the probability of the second chiasma is c, then the mean chiasma frequency is $1 + c$. We assume also that N potential points of recombination are evenly distributed along the chromosome with the ratio of these points in the shorter and longer arms being $\alpha:(1 - \alpha)$ (where $0 < \alpha \leq 0.5$; clearly the higher the deviation of α from 0.5, the more asymmetric the metacentric). In cells with one exchange in the considered chromosome, distribution of the exchange is assumed to be even along the bivalent—i.e., the distribution between arms is proportional to the arm-lengths ratio. For cases with two exchanges in the metacentric, one exchange is assumed in each arm, but within the arms the exchanges occur randomly. To calculate the frequencies of different haplotypes arising from crossing-over, we considered the four-chromatid stage with no chromatid interference—i.e., the ratio of two-, three-, and four-strand double exchanges was 1:2:1 (37). On the basis of the

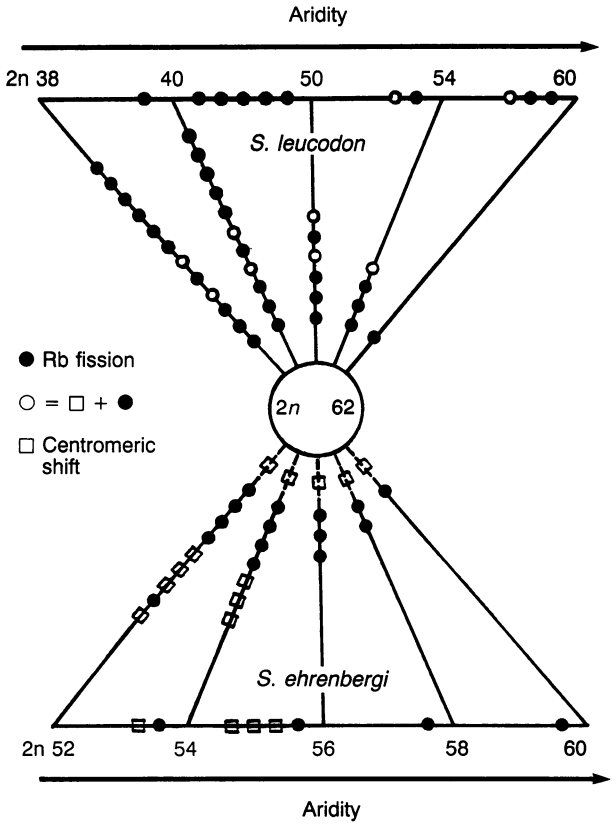

FIG. 2. Karyotypic evolution of all karyotypes of *S. leucodon* superspecies ($2n$ = 38, 40, 50, 54, 60, and 62) and *S. ehrenbergi* superspecies [$2n$ = 52, 54, 56, 58, and 60; the two karyotypes $2n$ = 54 and 60 are from Israel (1)]. The ecological trend across which speciation presumably proceeded in both Israel and Turkey is from mesic to xeric environments, in accordance with increased aridity stress and climatic unpredictability. Rb, Robertsonian.

above assumptions, we can obtain the value of haplotype diversity due to recombination in the metacentric chromosome as follows:

$$dm = 2.5 - 0.5\log(2 - c) + G(c) + 0.25c\log[\alpha(1 - \alpha)] + 0.5(1 + c)\log N - 0.5\alpha F(c, \alpha)\log F(c, \alpha) - 0.5(1 - \alpha)F(c, 1 - \alpha)\log F(c, 1 - \alpha).$$

where $F(c, \alpha) = 1 - c + 0.5c/\alpha$, $G(c) = 0.25\,c\log[(2 - c)/c]$.

(*ii*) Consider now the two derivative acrocentrics. As before, we assume that one chiasma is obligatory in each of the acrocentric bivalents. Let the probabilities of the additional chiasma, to the obligatory one, in the two derivative acrocentrics be c_1 and c_2, respectively. For simplicity, we consider only the situation of the acrocentric bivalents, where each has one or two exchanges. The distribution of exchanges is random, without chromosome interference. Then, haplotype diversity for the acrocentrics is as follows:

$$da_1 = 2.5 - 0.25c_1 - 0.5\log(2 - c_1) + G(c_1) + (1 + 0.5c_1)\log\alpha + 0.5(1 + c_1)\log N$$

$$da_2 = 2.5 - 0.25c_2 - 0.5\log(2 - c_2) + G(c_2) + (1 + 0.5c_2)\log(1 - \alpha) + 0.5(1 + c_2)\log N.$$

Now we can calculate the increase in the potential of population haplotype diversity per generation (Δd) due to one fission:

$$\Delta d = da_1 + da_2 - dm = 2.5 - 0.25(c_1 + c_2) - 0.5\log[(2 - c_1)(2 - c_2)/(2 - c)] + G(c_1) + G(c_2) - G(c) + (1 + 0.5c_1)\log\alpha + (1 + 0.5c_2)\log(1 - \alpha) + 0.5\,\alpha F(c, \alpha)\log F(c, \alpha) + 0.5(1 - \alpha)F(c, 1 - \alpha)\log F(c, 1 - \alpha) + 0.5(1 + c_1 + c_2 - c)\log N.$$

This result can be interpreted as if Δd additional Shannon's information units were introduced into the population. In genetic terms, it means that the number of haplotypes due to Robertsonian fission in the population will be multiplied by a factor $2^{\Delta d}$. Fig. 3 displays the pattern of Δd as a function of the metacentric asymmetry coefficient (α) and the probability of an additional chiasma (to the obligatory one) in the derivative acrocentric, provided that the probability of the additional chiasma is the same in both acrocentrics (i.e., $c_1 = c_2$).

The higher number of recombinants presumably provides adaptive diversity to cope with extensive climatic stress (extremely cold winters and hot summers) and ecological (primarily climatic) unpredictability characterizing central Anatolia.

Karyotypes and allozymes are nonrandomly distributed across Turkey, displaying correlations with climatic and biotic factors. $2n$ increases significantly from the periphery toward central Anatolia (Fig. 1). Both $2n$ and H significantly correlate with the aridity stress and climatic unpredictability (Spearman rank correlation between $2n$ and rainfall, $r_s = -0.74$; $p < 0.001$). Diploid number is negatively and significantly regressed on the distance from central Anatolia ($p < 0.043$ and $p < 0.0027$ if the Beysehir outlier of $2n = 40$ locality is excluded). This *centripetal* pattern over a large-scale subcontinent strongly corroborates the Israeli evolutionary model of *Spalax*. Chromosome number (and therefore the recombinational level) and heterozygosity of subterranean mole rats, both in Israel (northern Negev) and Turkey (central Anatolia), display a striking positive correlation with climatic stress of aridity and unpredictability, supporting the prediction of the niche-width genetic-variation hypothesis

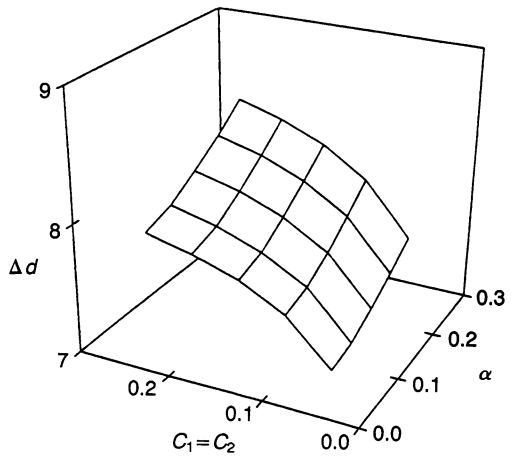

FIG. 3. Increase of haplotype diversity (Δd) due to one Robertsonian fission, as a function of the following recombination parameters: (*i*) probability of an additional chiasma (c_1, c_2 when $c_1 = c_2$), (*ii*) metacentric asymmetry coefficient α (the higher the deviation of α from 0.5, the more asymmetric the metacentric). This example is based on 200 potential recombination points (N) and one additional chiasma in the metacentric chromosome (c = 1). See text for a detailed explanation.

(38). The wide climatic range from extremely cold winters to extremely hot summers in central Anatolia (25) displays the climatically broadest ecological niche over time, as compared with other regions in Turkey. This prediction of positive correlation between niche-width, in space, time, or both, and H has been supported at the allozyme level of multilocus heterozygosity, for plants and animals across phylogeny at local, regional, and global scales (39) and for subterranean mammals that live in a narrower niche underground as compared with above-ground small mammals (40).

Chromosomal speciation may involve 90–98% of all speciation events (6), and Robertsonian rearrangements are the most common cause of karyotype evolution in mammals (41). However, few studies (e.g., refs. 1 and 6) have suggested that directional trends in $2n$ are adaptive. Adaptation may derive from the level of genetic diversity due to recombination (Fig. 3), reduction of genetic risks in meiosis (35), and the contribution of chromosomal difference to reproductive isolation (14, 15, 19). Noteworthy, reproductive isolation may derive from divergence in $2n$ but is independent of the direction of change. Therefore, a consistent trend of $2n$ suggests that additional factors determine the trend. Otherwise, a random pattern of $2n$ would be expected. Remarkably, the Israeli trend of $2n = 52 \rightarrow 60$ and the Turkish trend of $2n = 38 \rightarrow 62$ are also true for the entire family Spalacidae (21). Diploid numbers in Spalacidae increase in three directions: (*i*) Turkey $\rightarrow$ Russian steppes ($2n = 38 \rightarrow 62$); (*ii*) Turkey $\rightarrow$ Balkan steppes ($2n = 38 \rightarrow 62$), and (*iii*) Turkey $\rightarrow$ Near Eastern and North African steppes ($2n = 38 \rightarrow 60$), assuming a Turkish center of origin of the Spalacidae, based on the oldest fossils (34). The trends of chromosome evolution in the Spalacidae involve increase in $2n$ along gradients of increased aridity. These trends generate more haplotype diversity derived from recombination (Fig. 3), complemented by increase in H in Turkey, as well as in Israel (1).

Diversities at the genic and chromosomal levels of *Spalax* evolve in parallel; both are presumably selected by climatic stress and unpredictability, rather than, as is presumed in pocket gophers, by either demographic or historic factors (42). Higher rates of karyotype evolution occur in placental mammals (9), small populations (11, 43), and species-rich genera (13), with limited mobility (8), as in the Spalacidae (32). The canalization model of chromosomal evolution (10) implies that the karyotype represents an "adaptive strategy" (but see ref. 17).

The phylogeny and systematics of Spalacidae need a critical revision (21). The biological, rather than the morphological species concept (1–3), is the only viable species concept in the Spalacidae. Hence, the recent taxonomic revision of the Spalacidae in Turkey, based only on morphology (44), is unrealistic. To date, 30 or more species based mainly on karyotypes have been described in the Spalacidae (21). Our present study almost doubled the number of species to 50 or more. Chromosomal speciation in the Spalacidae was presumably peripatric (1), through fixation of Robertsonian mutations in small populations (11, 45). Speciation was ecological, gradual, and with relatively little genomic changes, rather than revolutionary or punctuational (1). Active speciation, during Pleistocene and Recent times, occurred in Israel (1), as well as in the $2n = 62$ complex in central Anatolia described here and expanded in ref. 22.

Speciation and adaptation in the Spalacidae appear to be ordered and associated with ecological heterogeneity (1, 21). They are not stochastic or neutral (5) but seem adaptive at both the chromosomal and multilocus gene levels (4, 39, 40), in accordance with the niche-width variation hypothesis (38), and appear to correlate with ecological aridity stress and climatic unpredictability.

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