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Zoology

Aggression patterns and speciation

(natural selection/interspecific aggression/fossorial mammals)

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ABSTRACT The evolutionary significance of interspecific aggression as a factor in speciation was tested among three chromosome forms of the actively speciating fossorial rodent *Spalax ehrenbergi* in Israel. Laboratory experiments testing intra- and interspecific aggression were conducted on 48 adult animals from 10 populations comprising three chromosome forms with 2n = 52, 58, and 60. Twelve agonistic, motivational-conflict, and territorial behavioral variables were recorded during 72 combats involving homo- and heterogametic encounters between opponents. Analysis of the data matrix was carried out by the nonmetric multivariate Smallest Space Analysis (SSA-II). The results indicate that (*a*) aggression patterns, involving agonistic conflict and territorial variables, are higher in heterogametic encounters than in homogametic ones; and (*b*) aggression is higher between contiguous chromosome forms (2n = 58–60, and 2n = 52–58) than between noncontiguous ones (2n = 52–60). Both *a* and *b* suggest that high interspecific aggression appears to be adaptively selected at final stages of speciation in mole rats as a premating isolating mechanism which reinforces species identification and establishes parapatric distributions between the evolving species.

Successful speciation requires both reproductive isolation and ecological compatibility (1). Yet, while species specific behavioral signals are well-known reinforcers of reproductive isolation, the role of aggression as a factor in species formation is poorly known. Aggressive behavior is common and adaptive *within* many animal species chiefly in spacing out individuals (2), but its evolutionary significance *between* species is known primarily as an ecological rather than as a speciational determinant (3, 4). The objective of the present study was to evaluate the evolutionary significance of interspecific aggression as a factor during final stages of speciation. Can species recognition be reinforced by natural selection through high levels of aggression at a stage when both reproductive isolation and ecological compatibility are still incomplete? Likewise, is the parapatric distribution between the sibling species due to aggressive behavior? To solve these problems we followed earlier suggestions (5) and investigated mole rats belonging to the *Spalax ehrenbergi* complex as a model of active and prolific speciation (6).

Four main chromosome forms (2n = 52, 54, 58, and 60) of the fossorial rodent *Spalax ehrenbergi* are distributed clinally and parapatrically in Israel (ref. 7, and Fig. 1). They appear to represent four young sibling species at final stages of speciation (8). In order to assess the evolutionary significance of aggression during active speciation we compared intra- and interspecific aggression among three of the chromosome forms of *Spalax ehrenbergi*.

MATERIALS AND METHODS

Laboratory experiments were conducted from June to December 1969 with animals collected from June to November 1969. Experimental animals included 48 adults from 10 populations (Fig. 1). These comprised 17 individuals of 2n = 52 (8 females and 9 males); 9 individuals of 2n = 58 (all females); and 22 individuals of 2n = 60 (10 females and 12 males). Each chromosome form included animals collected across the range, excluding contact zones where narrow hybrid zones occur (E. Nevo and H. Bar-El, submitted for publication). Sampling was done in areas previously karyotyped extensively and shown to be homozygous. All animals were

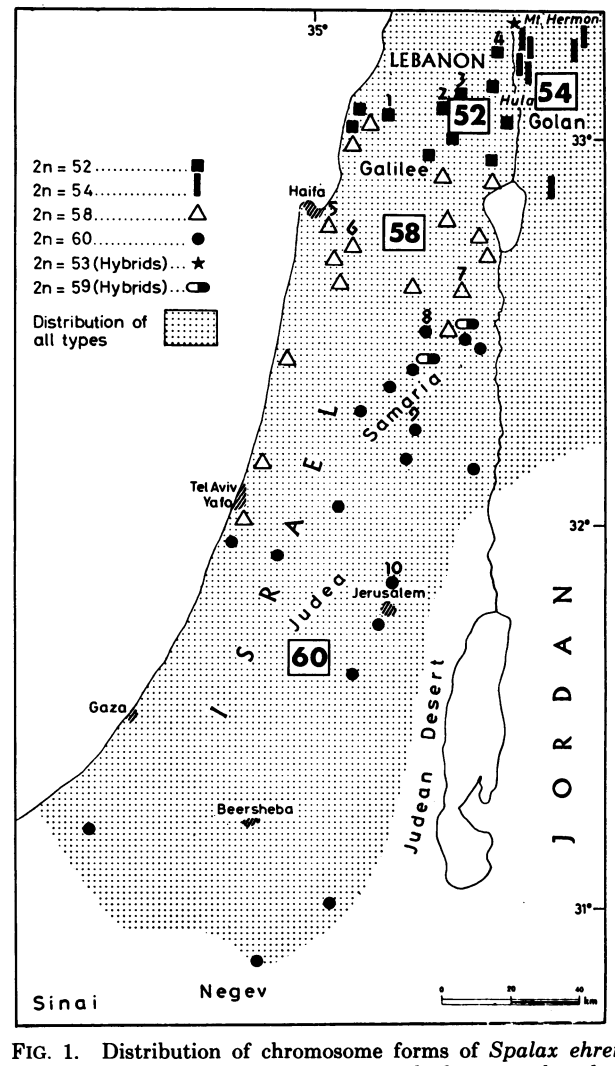

FIG. 1. Distribution of chromosome forms of *Spalax ehrenbergi*. Localities of the 10 populations studied are numbered sequentially on this map. The number of animals studied in each locality appears in parentheses. 2n = 52: 1 = Maalot*(6); 2 = Sasa(3); 3 = Kerem Zimra(6); 4 = Kiryat Shemona(2). 2n = 58: 5 = Check Post(3); 6 = Ramat David(2); 7 = Bet-Alpha*(4). 2n = 60: 8 = Jenin*(6); 9 = Shechem(5); 10 = Jerusalem(11).
Populations marked with asterisks occur near contact zones.

Abbreviation: SSA, smallest space analysis.

FIG. 2. First two dimensions of three-dimensional SSA-II space diagram of 27 behavioral categories in the testing apparatus. For further explanations see *text*.

kept in tin cages with sawdust bedding and received the same diet of carrots, onions, and potatoes.

The test apparatus represented an attempt to simulate natural conditions. It consisted of two "territorial" cages each 60 × 40 × 30 cm, connected by a glass tube 120 cm in length and 9.5 cm in diameter. The cages in which the animals had been kept since capture were used as territorial cages. The connecting glass tube represented a neutral zone for both animals. Each experiment lasted 30 min and consisted of two stages. In stage A, to insure similar interaction periods to all experimentals, animals were allowed free movements across the entire testing apparatus. Animals were weighed after testing, and connecting tubes were washed thoroughly before the next experiment to remove previous odors. Three tests were conducted daily. Minimal time between tests of each animal was 24 hr. Each animal was tested three times with animals of its own sex, first *always* with a homogametic partner, the other two times with random heterogametic ones. Since aggression in mole rats is correlated with weight, attempts were made to pair opponents of similar weight; hence, encounters of the two sexes were avoided, males being usually heavier than females. Altogether the 48 experimental animals yielded 144 observations (48 × 3) consisting of 72 combats (144/2).

The behavior of each of the two experimental animals was recorded separately by a different experienced observer. Sequential recordings per sec were registered on a 20 channel Angus Event Recorder (Easterline Angus Series "S" 620 T). The analysis involved five background and 12 behavioral variables. The behavioral variables are classified and described below and categorized in Table 2. Variables were defined as follows:

(A) Background variables: (*1*) sex; (*2*) chromosome form (2n = 52, 58, and 60); (*3*) type of encounter (homogametic = within chromosome form; heterogametic = among chromosome forms); (*4*) outcome of encounter (win, lose, draw; winner stays longer in opponent's territory); (*5*) weight.

(B) Behavioral variables (Table 2):

(I) *Agonistic behavior:* (*6*) latency (time in sec until interaction starts); (*7*) *attack posture* (animal either advancing forward or exposing lower incisors ready to bite; number of such postures in stage A); (*8*) retreat (animal retreats backwards assuming defensive posture; number of retreats in stage A); (*9*) "head on" position (heads of animals touch, each being in a defensive posture; duration in stage B, in sec); (*10*) bulldozing (animal retreats, collects and pushes sawdust forward with nose; duration in stage B, in sec); (*11*) freezing (animal is motionless above or below sawdust, either prostrate or ball-shaped; presence compared with absence). (Variables *6–9* involve explicit fighting behavior; variables *10–11* involve avoidance behavior).

(II) *Motivational conflict:* (a) *Displacement behavior:* (*12*) "displaced" bulldozing (animal lowers its head, touching and/or pushing sawdust forward with nose by abrupt, short movements; number of acts in stage A); (*13*) grooming (involves licking body, washing, passing licked forefeet over head and nose; number of acts in stage A); (*14*) eating (no eating; eating its own food; eating opponent's food); (*15*) hoarding (no hoarding; hoarding its own food; hoarding opponent's food and transferring it to its own cage). (b) *Terri-*

Table 1. Eating and hoarding behavior of *Spalax ehrenbergi* in heterogametic as compared with homogametic encounters

Type of encounter	Eating behavior		
	None	Own food	Opponent's food
Homogametic	38	17	6
Heterogametic	37	11	21

$\chi^2_{(2)} = 10.64; P < 0.01$.

torial behavior: (*16*) time spent in opponent's territory (in stage B, in sec); (*17*) time spent in glass tube (in stage B, in sec).

Analysis of the data matrix was carried out by *Smallest Space Analysis*, specifically SSA-II (for details of this technique see refs. 9–11). SSA-II is one of a family of nonmetric multivariate computer programs which portrays the original observations with the smallest possible number of dimensions displaying *relative* distances within a set of points. Nonmetric techniques involve no linear or distributional assumptions. The data can represent frequencies, probabilities, likelihoods, or correlations. SSA-II is particularly suitable for analyzing conditional joint occurrences of many qualitative variables simultaneously. In the present case, the raw data matrix consisted of the conditional frequency of occurrence of each category of each variable for every other category of every variable [see Guttman *et al.* (12)]. For the purpose of the SSA analysis all variables were ordered into two or three categories, such as presence:absence or high:medium:low. The latter was accomplished by dividing the populations scores into three classes of equal frequencies and categorizing each individual score in accordance with the class to which it belonged. The categories are listed in Table 2.

The computer plots the relative distances between the categories of the variables in a Euclidean space. These distances express the similarity among categories of different variables: the larger the coefficients of liklihood between them, the smaller will be the Euclidean distance separating them. The goodness of fit of the space obtained with a given number of dimensions is expressed by a coefficient of alienation. The distance d_{ij} between any two categories i and j may be calculated from the coordinates obtained when coordinate 1 = x, 2 = y, 3 = z, by the use of the Euclidean formula:

$$d_{ij} = \sqrt{(x_i - x_j)^2 + (y_i - y_j)^2 + (z_i - z_j)^2}.$$

Since only relative distance between points is of concern to SSA, coordinate systems for the smallest space need have no intrinsic interest. Hence, we have omitted labeling the coordinates in Fig. 2. The distances between points do not at all depend on choice of a particular coordinate system. The SSA-II program selects the principal axes of the space for final output purposes.

RESULTS

Heterogametic encounters differ considerably from homogametic ones in their behavioral patterns. Fig. 2 is a partial portrayal of two dimensions of the three-dimensional space as given by the SSA-II analysis made of all 48 categories of the 17 variables included in the original data matrix. There is a clear separation in the space with regard to hetero- and homogametic encounters. This we have indicated with a vertical line. Whereas, two dimensions are quite sufficient for the separation, the "heterogametic" and "homogametic" points are even further separated on the third dimension. This is indicated in Fig. 2 by an arrow pointing up for hetero- and down for homogametic encounters. The heterogametic region is characterized by high levels of attack posture, bulldozing, grooming, and time spent in the tube and in the opponent's area. This region also contains high likelihoods of hoarding and of eating the opponent's food; 53% of the dominant animals (defined as animals that stay longer in the opponent's territory in stage B) either ate or hoarded the opponent's food as compared with only 10% that did so in homogametic encounters ($\chi^2 = 12.0$; $P < 0.001$) (Table 1). In other words, the heterogametic encounters are character-

Table 2. Euclidean distances of aggression and conflict categories in homogametic and heterogametic encounters of *Spalax ehrenbergi*

Variable	Category	Distance: Type of encounter	
		Homogametic	Heterogametic
Stage A: First 10 min of encounter			
Attack posture (no. of postures)	1 = 0	0.770	1.474
	2 = 1-7	1.172	1.273
	3 = 8-11	1.662	0.844
Retreat (no. of retreats)	1 = 0	1.199	1.229
	2 = 1-4	1.712	0.509
	3 = 5+	0.969	1.647
Latency (sec)	1 = 1-18	1.694	0.568
	2 = 19-40	1.127	0.927
	3 = 41-330	0.520	1.701
Grooming (no. of acts)	1 = 0	0.363	1.632
	2 = 1-3	1.480	0.896
	3 = 4+	1.704	0.612
"Displaced" bulldozing (no. of acts)	1 = 0	0.534	1.712
	2 = 1-22	1.171	1.163
	3 = 23-71	1.544	0.823
Stage B: Last 20 min of encounter			
"Head on" (sec)	1 = 0	1.429	0.846
	2 = 10-50	1.511	1.091
	3 = 51-1000	0.759	1.517
Bulldozing (sec)	1 = 0	0.998	1.474
	2 = 1-248	0.874	0.993
	3 = 249-830	1.581	0.972
Freezing	1 = no	1.389	0.952
	2 = yes	1.168	1.295
Eating	1 = none	1.133	1.369
	2 = own food	0.876	1.456
	3 = opponent's food	1.682	0.619
Hoarding	1 = none	0.609	1.657
	2 = own food	1.752	0.291
	3 = opponent's food	1.655	0.743
Time (sec) spent in opponent's area	1 = 0	1.142	1.415
	2 = 1-314	0.789	1.335
	3 = 315-1200	1.514	0.921
Time (sec) spent in tube	1 = 0	1.412	1.245
	2 = 61-489	0.865	1.490
	3 = 490-1200	1.265	1.042

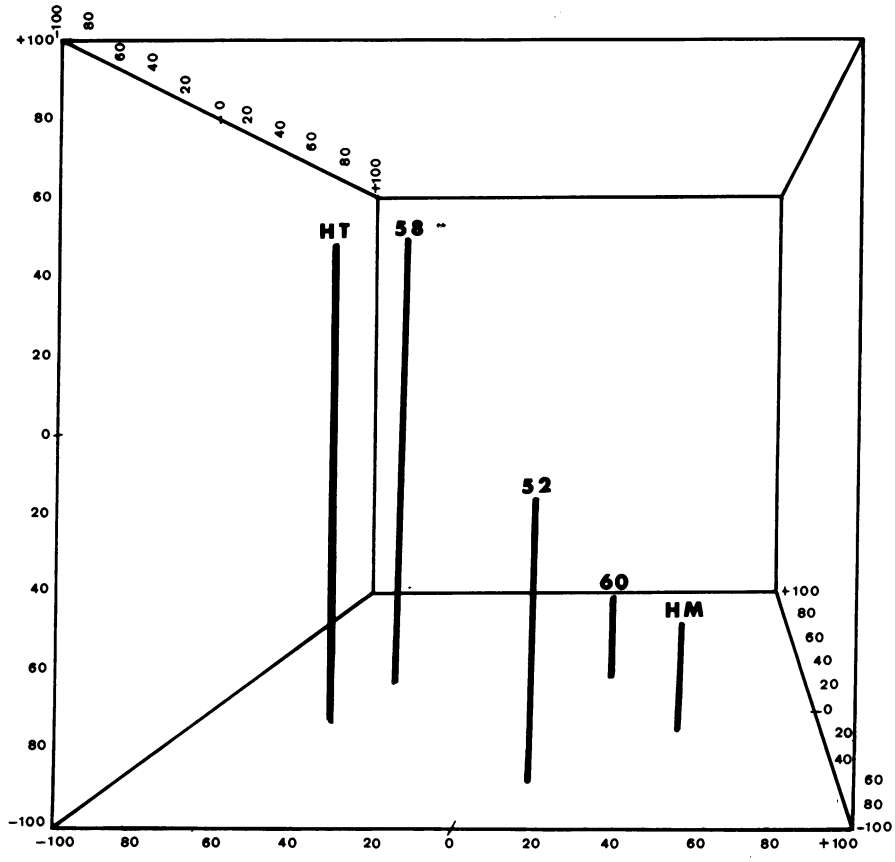

FIG. 3. Ethological distance among three chromosome forms of *Spalax ehrenbergi* (2n = 52, 58, and 60) and between homogametic (HM) and heterogametic (HT) encounters, as determined by the three-dimensional SSA-II analysis of 48 categories of 17 variables.

ized by liklihoods for high aggression, high conflict, and high territoriality.

In contrast, the homogametic region is characterized by low likelihoods for attack posture, bulldozing, grooming, and time spent in the tube and in the opponent's area. In the homogametic encounters animals are more likely to eat their own food, or no food at all. Thus, homogametic encounters involve lower levels of aggression, conflict, and territoriality as well as eating and hoarding the opponent's food, as compared with heterogametic encounters. This is portrayed by the Euclidean distances (Table 2), where a relatively larger distance indicates minimal likelihoods between any two categories in question. One should keep in mind that *all* animals participated in both types of encounters and contributed equally to the structure of the space. We may thus conclude that it is the *type of encounter* an animal finds itself in that determines the nature of the behavior pattern.

Overall ethological distances obtained between each of the chromosome forms, based on simultaneous considerations of all variables studied, are given in Fig. 3 in a three-dimensional space. The greatest ethological distance is between the pair 2n = 58–60 (d_{ij} = 1.596); next comes the pair 2n = 52–58 (d_{ij} = 1.420); and the smallest ethological distance is between the pair 2n = 52–60 (d_{ij} = 0.963). Since greater ethological distance reflects higher levels of aggression, it appears that interspecific aggression is higher between contiguous species as compared to noncontiguous ones.

DISCUSSION

The following evidence from earlier studies suggests that the four chromosome forms of *Spalax ehrenbergi* are sibling species at progressive stages of species formation. They are distributed parapatrically with progressively narrower hybrid zones (between 2n = 58–60, 2.8 km; between 2n = 54–58, 0.50 km; and between 2n = 52–58, 0.32 km) separating extensive karyotypically homozygous regions (E. Nevo and H. Bar-El, submitted for publication). Selective mating and interspecific aggression probably reduces hybridization between contiguous forms as is suggested by mating experiments (5). The four karyotypes seem to be closely related genetically on both electrophoretical and immunological evidence. They differ in only 4%, on the average, of electrophoretically tested proteins controlled by 25 gene loci (ref. 13; E. Nevo and H. Cleve, in preparation). No diagnostic allele characterizes the 2n = 52, 58, and 60 karyotypes and only one locus, transferrin, has a nearly diagnostic allele which characterizes 2n = 54 (E. Nevo and H. Cleve, in preparation). Immunological distances between the four karyotypes are also very small (0–5 units), suggesting close genetic similarity and a recent origin (14). Morphologically, the four chromosome forms are very similar, differing, on the average, by 2.86 Mahalanobis distances, based on 40 skull and other variables (E. Nevo and E. Tchernov, in preparation). The southward decrease in basic metabolic rates, BMR (15), suggests that the four species represent adaptive systems to increasingly arid environments. Finally, in Israel, *Spalax* fossils become increasingly abundant in late Pleistocene and Holocene deposits (16).

The results obtained in this and a previous study (5) suggest that interspecific aggression reinforces both species identity and parapatric distributions between the young sibling species of *Spalax ehrenbergi*. At final stages of speciation, if secondary intergradation of semispecies takes place,

natural selection would favor the evolution of species specific signals in each of the sexes that insure the integrity of the young species. Such signals are particularly important if and when reproductive isolation and/or ecological compatibility between young congeners are still incomplete, as in the present *S. ehrenbergi* case, thereby protecting the young species from breakdown by hybridization. Species identity may be sharpened by overt fighting which causes reduced successive interactions between congeners. Likewise, fighting is certainly a very effective mechanism leading to competitive exclusion between aggressive species, such as mole rats, thus establishing parapatric boundaries. Our conclusions are supported by the following findings. (*a*) Aggression patterns are higher in heterogametic encounters than in homogametic ones. This has been suggested previously also in heterosexual encounters in mating experiments (5). (*b*) Aggression is higher between contiguous forms (58–60, and 52–58) than between geographically distant and noncontiguous forms (52–60) which are also more remote phylogenetically. Both *a* and *b* suggest that species recognition signals have evolved in the *S ehrenbergi* karyotypes to insure species identification and that parapatric distribution is mediated through interspecific aggression. The species specific signals may involve auditory (17) and/or olfactory and tactile cues.

Interspecific competition among rodents is widespread, both in the wild and in the laboratory, and is frequently mediated by aggressive interaction (18). Laboratory studies reflect, however imperfectly, behavior exhibited in the wild. Interspecific aggression may cause competitive exclusion between species, and appears to vary in degree in relation to ecological and evolutionary correlates. Whereas, in many cases of regular, well-established, species, aggression within species exceeds aggression between species (18), the reverse may be true for young sibling species with little ecological divergence and recent origin. Evolution of high interspecific aggression was described and predicted in bird species living in structurally simple environments, such as grasslands, to cope with little divergence in modes of exploitation owing to recency of origin (20, 21).

We suggest that the high interspecific aggression operating between *contiguous* sibling species of *Spalax ehrenbergi* appears to be adaptively selected at final stages of speciation as an important mechanism that reinforces species identification and establishes parapatric distributions through competitive exclusion. Before speciation is completed, when gene pools may be very similar (13), ecological differentiation small, and primary isolating mechanisms, i.e., chromosomal incompatibility, as yet incomplete, interspecific aggression may complement mate selection as an ethological premating isolating mechanism (5).

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