Geographic dialects in blind mole rats: Role of vocal communication in active speciation

(premating reproductive isolation/chromosomal speciation)

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ABSTRACT We compared and contrasted the physical structure of male "courtship" calls of 59 subterranean mole rats belonging to the *Spalax ehrenbergi* superspecies in Israel, comprising 11 populations of four chromosomal species ($2N$ = 52, 54, 58, and 60). We also conducted behavioral auditory discrimination tests of 144 females of the four species in the laboratory. The results indicate that each chromosomal species has a vocal dialect significantly different from all others, although the call of $2N$ = 60, the last derivative of speciation, is not yet fully differentiated. Females of $2N$ = 52, 54, and 58 preferred their homospecific mates' calls, whereas females of $2N$ = 60 did not. We conclude that call differentiation builds up gradually and provides an efficient ethological reproductive premating isolation mechanism between the emerging species in the active speciation of mole rats in Israel.

Speciation is a key problem of evolution (1, 2). Yet, despite numerous studies (reviewed in refs. 1–7), its modes, genetics, and mechanisms remain little known and highly hypothetical (e.g., refs. 7 and 8). To unveil the course of speciation events, studies must be conducted in cases of active speciation (9). The crux of speciation is the development of reproductive isolation between populations, followed by ecological compatibility of the emerging species. Reproductive isolation may be attained by either premating or postmating isolation mechanisms, or both (1–7). The role of vocal communication as a premating isolation mechanism in animal speciation has been reviewed (1, 2). Specific examples are *Drosophila* (10), arthropods (11), orthopterans (12), spiders (13), frogs (14, 15), birds (16, 17), and mammals (18).

We analyzed the existence of geographic dialects in the "courtship" calls of blind subterranean mammals of the *Spalax ehrenbergi* superspecies in Israel. We also tested, in the laboratory by female discrimination experiments, the function of the vocal dialects as a premating ethological isolation mechanism. This was done in an attempt to highlight the evolutionary significance and rate of the build-up of reproductive isolation, and hence of speciation, in this case of active speciation (19–23).

Subterranean mole rats in Israel, belonging to the *S. ehrenbergi* superspecies, involve four morphologically indistinguishable, homozygous chromosomal species (19) with $2N$ = 52, 54, 58, and 60. Multidisciplinary studies (reviewed in refs. 20–23) provide telling evidence of both the evolutionary dynamics and the various stages of speciation of the complex. The evolution of *S. ehrenbergi* is intimately associated with the climatic and biotic differentiation of Israel. The chromosomal species inhabit extensive regions, which are distributed clinally and parapatrically along a southward gradient of increasing aridity. Their distribution is correlated with four climatic regimes: $2N$ = 52, cool and humid; $2N$ = 54, cool and

dry; $2N$ = 58, warm and humid; and $2N$ = 60, warm and dry (see figure 1 in ref. 21).

The "courtship" call of *S. ehrenbergi* is a weak purring sound emitted primarily by males, but sometimes also by females, during the entire lengthy mating procedure (24). Sometimes this call is also uttered in the nonbreeding season as well; it is uttered primarily by the submissive but also by the dominant partner during fights (E.N. and G.H., unpublished results). Hence, it may function also as an appeasement call. Therefore, it seems to have a multipurpose function. The courtship call is characterized by a main frequency of 568.0 ± 35.6 Hz, and the pulse repetition rate is 23.7 ± 2.8 per second. Calls are noisy, and the energy is spread from 0.5 to 4.5 kHz (39). The frequency range of the mole rat's cochlear hearing sensitivity is 0.1–10 kHz, with maximal sensitivity between 0.5 and 1.0 kHz. Recordings of evoked potentials from the inferior colliculus of the mole rat midbrain revealed a maximum sensitivity around 0.5 kHz (V. Bruns, M. Muller, W. Hofer, G.H., and E.N., unpublished data). We have experimentally substantiated that the mole rat's low-frequency calls, at around 500 Hz, are transmitted underground better than higher frequencies (25).

Here we present evidence that the four chromosomal species of *S. ehrenbergi* have physically different courtship dialects that contribute substantially to the selective and positive homospecific, assortative mating of the females. Dialect differentiation, therefore, appears to provide a significant ethological reproductive isolation mechanism among the emerging species of blind mole rats, in which visual communication has been eliminated during their evolutionary history (26).

MATERIALS AND METHODS

We recorded and analyzed the courtship calls of 59 adult mole rats representing 11 populations of the four chromosomal species. Animals were kept under standardized conditions ($22° \pm 1°$C; relative humidity $70 \pm 5\%$; same vegetable food; 12 hr light/12 hr dark photoperiod). Estrous females were placed together with males in an aquarium ($60 \times 60 \times 60$ cm) that had a thin layer of sawdust. Recordings were conducted only if the mating process started. Most estrous females were receptive to the male's courtship behavior, but in cases of aggression between the two, they were separated and another estrous female was introduced to the male. Mating of the blind mole rat is a lengthy process that can continue for about an hour (24). During this time we recorded the male's courtship calls with a microphone held a few centimeters in front of his mouth. Acoustic distortions were minimized by coating the aquarium walls with sponge. The ambient temperature in the recording room was $20° \pm 2°$C. Recordings were made during the breeding season (November–March, 1979–1982) with a Nagra IV D tape recorder (Kudelski, Switzerland) and a D24B microphone (AKG, Austria), which has an upper frequency response of 16 kHz.

Table 1. Mean and standard errors of the five call parameters of the four chromosomal species of *S. ehrenbergi*

	Value, mean ± SEM			
Call variable	$2N = 54$ (n = 10)	$2N = 52$ (n = 19)	$2N = 58$ (n = 16)	$2N = 60$ (n = 14)
Pulse rate, no. per sec	21.0 ± 0.66	25.3 ± 0.49	23.9 ± 0.63	23.2 ± 0.74
Main frequency, kHz	595 ± 6.1	555 ± 6.8	583 ± 7.8	562 ± 10.9
Lower value of main frequency, kHz	521 ± 3.3	487 ± 6.7	506 ± 10.3	505 ± 11.8
No. of harmonics	6.12 ± 0.28	5.79 ± 0.21	9.58 ± 0.31	8.41 ± 0.25
Amplitude, dB	16.7 ± 0.88	16.0 ± 0.68	15.4 ± 0.97	17.5 ± 0.82

n, Number of individuals.

Call analysis was conducted with a Kay Sonagraph (Kay Elemetrics, Pine Brook, NJ) (model 7030A Kay Vibralizer). Two modes of graphic displays were used: (*i*) sonogram (sound spectrogram), which displays sound frequency distribution over time, with a wide-band filter; and (*ii*) section, which shows energy distribution over the frequency range at a certain point of time within a call. The following call parameters were measured from the graphic displays: (*a*) main frequency according to peaks of sections (kHz), (*b*) lowest value and bandwidth of the main sound energy (kHz), (*c*) pulse repetition rate of repeating notes in a sequence (pulses per sec), (*d*) number of apparent harmonics, and (*e*) relative amplitude, in decibels (dB), at 500-Hz intervals from 0.5 to 4.5 kHz, of the frequency components at each of nine sampling frequencies. We averaged the call parameters of each tested individual by randomly choosing three regions across the entire call.

We also tested the behavioral auditory discrimination of 144 estrous females representing all four chromosomal species. Female discrimination between homospecific and heterospecific courtship calls was tested in a room temperature of 20° ± 2°C. The two calls were broadcast simultaneously from two opposite loudspeakers (Philips, AD 7081/M8) situated 100 cm apart. The intensity of broadcasting was 46 dB, which is the average intensity of the mole rat's courtship call. Females were put midway between the two loudspeakers, and they subsequently moved along the Perspex tube connecting the loudspeakers. Positive phonotactic response was scored when the female stayed near the loudspeaker broadcasting the courtship call of her homospecific male more than 50% of the time spent near both loudspeakers; sometimes, females displayed lordosis. near the preferred loudspeaker.

RESULTS

The comparison and testing between the call structures of the four chromosomal species are presented in Tables 1–3 and Fig. 1. The spectral peaks largely increase, and the pulse rates decrease; eastward, from $2N = 52$ toward $2N = 54$ on

the one hand, and southward, from $2N = 52$ toward $2N = 58$ and 60 on the other hand.

The analysis of variance (ANOVA) (27) of the five call parameters of the four chromosomal species is presented in Table 2. The means of the four call parameters (excluding amplitude) differ significantly among the species. To separate the four vocal dialects, we used stepwise discriminant analysis (27). This technique aims to unravel the best multivariate combination that discriminates between groups, (i.e., the four chromosomal species we studied). Based on three out of the four call parameters, including the number of harmonics, pulse rate, and main frequency, the computer program significantly separated the courtship dialects of the four chromosomal species (Fig. 1). While the courtship dialects of $2N = 52$, 54, and 58 are clearly spatially separated in the diagram, that of $2N = 60$ partly overlaps with the call of $2N = 58$. All pairwise multivariate call differences between the species in the discriminant analysis are highly significant (*P* < 0.001, except between $2N = 58$ and $2N = 60$, where *P* < 0.05; Table 3). The mean percentage of "grouped" cases correctly classified was 79% ($2N = 52$, 84%; $2N = 54$, 100%; $2N = 58$, 75%; but $2N = 60$, only 58%).

The results of female auditory discrimination are given in Table 4. Clearly, females of the three phylogenetically older species ($2N = 52$, 54, and 58; ref. 28) displayed distinct call differentiation (Fig. 1). They largely discriminated positively between the courtship calls of their homospecific males and those of heterospecific males. In contrast, $2N = 60$ females, whose males' calls are not yet sharply differentiated, did not discriminate between the alternative calls. Thus, the female preferences are in line with the degree of physical call differentiation discussed earlier.

DISCUSSION

Positive mate preference provides the basis for sexual isolation and assortative mating in the *S. ehrenbergi* complex (24, 29, 30). Most estrous females significantly preferred their chromosomal mates in two choice experiments where the female choice was made between two alternative chromo-

Table 2. Analysis of variance (ANOVA) of five call parameters of male mole rats, by nested ANOVA

	Mean square					Mean square			
Call variable	Within animals*	$df^†$	Between animals	df	F, an/w‡	P	Between species	df	F, sp/an§	P
Pulse rate	4.093	115	16.758	54	4.09	<0.001	178.04	3	10.62	<0.001
Main frequency	755.9	118	2986.0	55	3.95	<0.001	15,137.1	3	5.069	<0.004
Lower value of main frequency	261.8	147	4632.5	54	17.69	<0.001	19,442.6	3	4.197	<0.010
No. of harmonics	1.048	118	2.921	54	2.79	<0.001	154.96	3	53.05	<0.001
Amplitude	7.695	118	31.414	55	4.08	<0.001	35.249	3	1.122	0.348

*Variance among repetitive measurements within calls of one individual.
†The variation in degrees of freedoms (*df*) is caused by a few cases in which variables could not be measured and from more repetitions in some animals, especially in the variable "lower value of main frequency."
‡an/w is the ratio of call variances between and within animals.
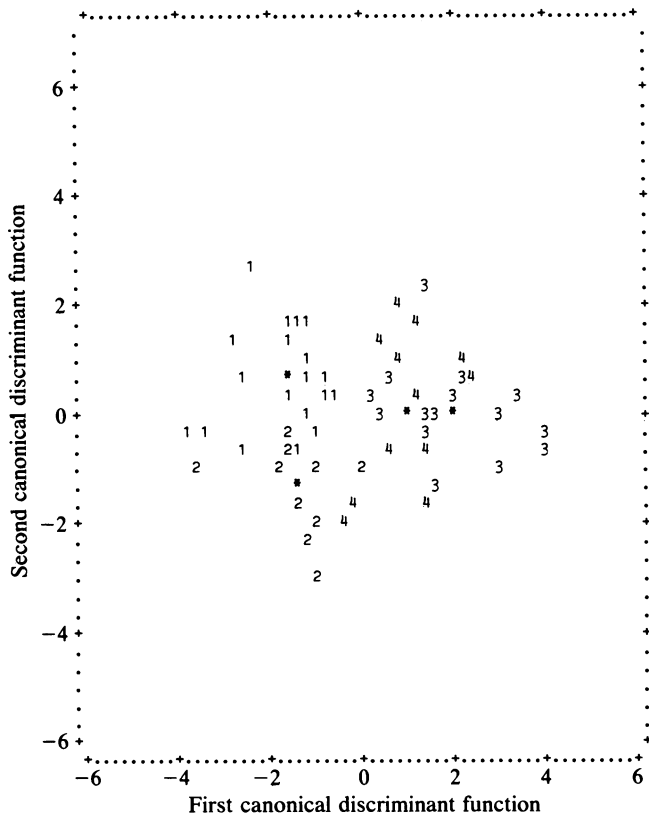§sp/an is the ratio of call variances between and within species.

FIG. 1. Stepwise discriminant analysis of the courtship calls of the four chromosomal species of *S. ehrenbergi*, based on three call parameters: (*i*) number of harmonics; (*ii*) pulse rate; (*iii*) and main frequency, chosen by the computer program. The program maximizes the overall F statistics among the four species and reveals the discriminatory power of the variables used. *, Group centroid; 1, $2N = 52$; 2, $2N = 54$; 3, $2N = 58$; 4, $2N = 60$.

somal live males (29, 30). However, females of the older species ($2N = 52, 54$, and 58) select their mates much better than females of the recent speciation $2N = 60$, and $2N = 52$ and $2N = 54$ females also select better than $2N = 58$ females when tested against males of their $2N = 60$ derivative (30, 31). The vocal preference tests reported here perfectly match the aforementioned mate choice experiments conducted with live males.

The differentiation of the physical structure of the courtship call of $2N = 60$ and its $2N = 58$ ancestor is less distinct than call differentiation among the other species. This is in line with the results of a previous study on the origin and evolution of assortative mating in the *S. ehrenbergi* complex (31). Estrous females of the recent derivative of speciation ($2N = 60$) showed trimodal mate-preference behavioral phenotypes comprising negative, low positive, and high positive preference for homospecific males. By contrast, encounters of ancestral species ($2N = 52, 54$, and 58) showed

Table 3. Multivariate pairwise comparisons among species, based on discriminant analysis of three call parameters: no. of harmonics, pulse rate, and main frequency

	$2N = 52$		$2N = 54$		$2N = 58$	
	F	P	F	P	F	P
$2N = 54$	9.264	0.0001				
$2N = 58$	39.033	<0.0001	27.195	<0.0001		
$2N = 60$	18.137	<0.0001	14.225	<0.0001	3.519	0.0216

The table presents the F statistics and significances between pairs of species. Each F statistic has 3 and 50 degrees of freedom.

Table 4. Female auditory discrimination

Species	No. of individuals	% preferring homospecific call	χ^2	P
$2N = 52$	53	79	15.868	<0.001
$2N = 54$	14	77	4.571	<0.05
$2N = 58$	43	77	12.302	<0.001
$2N = 60$	34	44	0.470	>0.250

The χ^2 shows the level of female preference of the call of the homospecific male, for each chromosomal species (the χ^2 compares the deviation in numbers of females showing positive phonotaxis for the homospecific call, as described in *Materials and Methods*, vs. other tested females).

a prevalence of a positive homospecific mate preference. Thus, all three behavioral criteria—i.e., male call differentiation, female mate choice, and female call choice—are the least developed in $2N = 60$. We suggest, therefore, that the premating vocal isolation capacity of $2N = 60$, which is possibly 70,000 or more years old (28), is *in statu nascendi*. This contrasts the premating vocal isolation capacity of the older species (28) $2N = 52, 54$, and 58, which are well established. The evolution and establishment of premating isolation mechanisms in mole rats may be a long process, lasting tens to hundreds of thousands of years; this is also evidenced by the slow rate of closure of the hybrid zones in nature (30, 32).

Our results highlight vocalization as an important premating isolation mechanism in blind mole rats, where visual cues do not operate (26). Vocalization complements olfaction (33) and aggression (34, 35) in substantiating premating reproductive isolation between the emerging species. The premating mechanisms complement and slowly perfect the initial chromosomal postmating reproductive isolation (36), which apparently started speciation (21). This is indicated by the multiple evidences of natural hybridization, the evolution of assortative matings, and the vocal mate choices discussed here. Thus, although speciation in mole rats may be geologically rapid, it appears to be genetically gradual and does not support the punctuated-equilibrium theory of speciation (37). Speciation in mole rats corroborates the neo-Darwinian evolutionary theory, which envisages, from gradual to punctuated speciation, the entire spectrum of all evolutionary rates (38).

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