nected with reproductive fitness ...'. Presumably this is because for characters concerned with reproductive fitness, species can only tolerate a limited amount of inherited variation. In natural populations of tsetse flies there is selection against certain size classes[2,3] and as GLASGOW[2] (on p. 167) concluded 'unidentified agents tend to remove extremes of size' from tsetse populations. In view of these field observations it is not surprizing that heritability of adult weight is low in tsetse flies. However, our knowledge of the genetics of adult weight and of the biology of strains of flies in the high and low weight range is not sufficient to permit reasonable speculation on the usefulness, in tsetse control, of weight variations as conditional lethals.

Assortative Mating Between Chromosome Forms of the Mole Rat, *Spalax ehrenbergi*

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Summary. Females of two parapatric chromosomal forms (2n = 52 and 2n = 58) of the fossorial mole rat, *Spalax ehrenbergi*, in Israel, were tested for mate selection between two alternative, a homo- and a heterochromosomal, males. Estrous females significantly preferred the male of their own chromosomal form, on the basis of several behavioural criteria. The evolutionary significance of the positive assortative mating found, lies presumably in reinforcing reproductive isolation between the chromosome forms, thereby contributing to finalize speciation.

Speciation depends on the evolution of effective premating and/or postmating isolating mechnisms[2]. Most analyzed cases involve well established reproductively isolated sympatric species pairs. Yet little detailed information is known about isolating mechanisms during the final stages of speciation. Our objective was to explore premating sexual isolation among the actively speciating complex of mole rats, *Spalax*, in Israel.

Spalax ehrenbergi, is a subterranean rodent displaying extensive chromosomal speciation ranging from 2n = 48 to 2n = 62 in the eastern Mediterranean region[3]. In Israel, four chromosome forms (2n = 52, 54, 58 and 60) inhabit extensive parapatric regions and are distributed clinally from north to south along an ecological gradient of increasing aridity[4]. They display progressive final stages of speciation, as evidenced by the increasingly narrower hybrid zones separating them[5]. The existant hybrid zones indicate that chromosomal incompatibility is still incomplete, and suggest that behavioural premating isolating mechanisms would be at a selective premium to prevent mismating. Preliminary studies on mating behaviour of *Spalax*[6] have suggested that homogametic mate selection operates between karyotypes. The present study was designed to test the hypothesis that assortative (nonrandom) mating between the chromosomal forms 2n = 52 and 2n = 58 operates, thereby reinforcing reproductive isolation of the newly emerging species.

Materials and methods. Laboratory female discrimination tests were conducted in the winters of 1969 and 1970 on animals collected 1–8 weeks prior to testing. Experimental animals included 83 breeding adults composed of 40 individuals of 2n = 52 (24 females; 16 males), and 43 individuals of 2n = 58 (23 females; 20 males). The total number of tests performed in 1969 and 1970 was 262, comprizing 140 tests of 2n = 52 females, and 122 tests of 2n = 58 females (table). Each chromosome form included animals collected across the range excluding contact zones[5]. Sampling was done in extensively karyotyped areas previously shown to be karyotypically homozygous[7]. All animals were kept in tin cages with wood shavings and received the same diet of carrots, onions and potatoes.

The testing apparatus consisted of three tin cages (each 25 × 10 × 10 cm), interconnected by means of Y-shaped interchangeable glass tubes 50 cm long and 7 cm wide. One cage included the tested female, the other two the alternative males. Males were kept in their respective cages while the female was allowed free movement in tubes and free contact with the male's screen divider, but not allowed entrance into his cage. Testing was conducted during daytime and sometimes during the night under fully lit and warmed (25 °C) conditions.

Each test involved a pair of alternative males, one 2n = 52, the other 2n = 58. The males were randomly placed in the left or right position. Experiments lasted 30 min in 1969 and 90 min in 1970. Each female was tested once in 3 days. If females proved receptive, they were retested within several hours on another pair of males after switching their positions. Only sexually active males displaying external testes were used. Vaginal smears were taken from each tested female at the end of the test to estimate her estrous phase. Females were considered estrous when vaginal smears contained above 80% cornified epithelial cells.

Observations were recorded manually every 30 sec throughout the experiments. Thus each test resulted in a behavioural profile of the test female in both space and time. The following 18 behavioural variables were recorded: *resting, presenting, biting male's screen, sniffing, grooming, licking-genitals, defecating, urinating, bulldozing, scraping, beating tube with head, running forward, running backwards, turning, approaching male, retreating slowly, vocalizing, teeth chattering*. The following data were calculated: a) number of acts in males' tubes, in the junction, and in the female's tube; b) time spent in each region of the apparatus; c) number of acts near screen divider of each male; d) number of entries into each male's tube;

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Mate discrimination by females of two chromosome forms (2n = 52 and 2n = 58)[a].

Criterion	Test female[b] (2n)		Total number of acts and time of all females in all tests near either male: 2n = 58	2n = 52	χ^2 of WILKS[8]	p
1. Acts in male's tube	I	58	3566	1955	20.1	<0.001
		52	2460	5007		
	II	58	3976	1643	6.8	<0.01
		52	1046	1760		
2. Acts near male	I	58	2697	1448	21.2	<0.001
		52	1904	4110		
	II	58	3568	1370	6.8	<0.01
		52	858	1428		
3. Time in male's tube	I	58	904	450	31.1	<0.001
		52	515	1340		
	II	58	1509	432	9.2	<0.005
		52	253	405		
4. Entries into male's tube	I	58	815	728	1.2	>0.10
		52	1167	1450		
	II	58	880	759	0.1	>0.90
		52	676	787		
5. Contacts with male's screen divider	I	58	748	630	4.9	<0.03
		52	959	1300		
	II	58	738	686	0.1	>0.90
		52	622	659		

[a] I: 201 tests of 30 min each, performed in 1969. II: 61 tests of 90 min each, performed in 1970. [b] The localities sampled, accompanied by the number of individuals tested in each, are as follows: a) *2n = 52:* Saar (3), Bezet (1), Ma'alot (13), Peqiin (1), Rami (1), Meiron (4), Sasa (1), Kerem Ben-Zimra (13), Ein-Zeitim (3); b) *2n = 58:* Bet-Lid (16), Check-Post (6), Elroi (6), Ibelin (1), Bet-Alpha (6) and Mghar (8).

e) number of contacts with screen divider of each male. These five criteria for assessing preference were chosen a priori but are obviously correlated and are not mutually exclusive.

The statistical test used to assess female discrimination between the two alternative males was Wilk's χ^2 [8], which is more sensitive than the usual χ^2 test. The null hypothesis tested was that no correlation existed between female preference and chromosome form of the chosen male. Preference was assumed when the female spent at least twice as much time in the homochromosomal male tube (T_1) as compared to time spent in the heterochromosomal one (T_2); in other words, when $T_1/T_1 + T_2 \geqslant 0.66$. The parametric relevariance test[9] was used to rank the five preference criteria as to their relative efficiency in characterizing sexual selection.

Results and discussion. A representative test of an estrous female consisted sequentially of 3 typical stages: a) *adaptation* b) *exploration* and c) *discrimination.* The adaptation stage involved both agonistic and motivational conflict behaviour. Agonistic elements included attack postures with exposed incisors, defensive postures, and biting the apparatus. Motivational conflict involved plugging the male entrance with wood shavings, transfer of shavings between males, bulldozing, scraping, urinating, and defecating. The exploratory stage involved unplugging the male entrance, sniffing, grooming and biting the male's screen. In the discrimination stage, the female moved slowly with arched back and rested for long periods of time near the male's screen. Occasionally, the female deserted the preferred male, sniffed at the alternative male, then returned to the one initially preferred, sometimes with distinct lordosis. In nonreceptive (diestrous) females, both stages b) and c) are missing; females usually roamed swiftly throughout the apparatus, transferring shavings, or stayed primarily in the female's cage or in the Y junction.

The results of the 1969 and 1970 tests, involving both estrous and diestrous females, are given in the table. The following conclusions are drawn: a) Females of both 2n = 52 and 2n = 58, significantly preferred homochromosomal males on each of the following correlated criteria: acts in male's tube; number of acts near male's screen; and time spent in male's tube. The latter proved the best criterion of the 5 tested. b) Mate selection was practiced only by estrous females as defined above: $\chi^2_{(1)} \leq 13.2$ in 1969 and $\chi^2_{(1)} \leq 17.7$ in 1970; in both years $p < 0.001$. Diestrous females selected mates at random. The behaviour of estrous females was different from that of diestrous ones. Their adjustment stage was short, they showed little motivational conflict behaviour, and moved with arched-backs, showing lordosis in front of the male's cage. c) The efficiency of the preference criteria in mate selection was similar, but not identical, between years, the ranking being $3 > 2 > 1 > 5 > 4$ in 1969 but $3 > 2 > 1 > 4 > 5$ in 1970. The higher efficiency of criterion 3 and also 2 and 1 reflect primarily the behaviour of the test female at the discriminatory phase.

Assortative mating has important evolutionary consequences on the genetic structure of populations[10] and the process of speciation[2]. Nonrandom mating, either positive[11], or negative[12], is well documented in many animal populations[13]. In the house mouse, positive assortative mating has been recorded between subspecies

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reinforcing sexual isolation[14], whereas negative assortative mating has been found in different strains within subspecies[15–17] reeinforcing outbreeding and thereby increasing heterozygosity.

In a twin-study[18], olfactory discrimination proved at least one of the mechanisms contributing to positive assortative mating in mole rats. Additional behavior such as vocal[6,19] and/or tactile[6] cues may complement olfaction, and they are currently being investigated in our laboratory.

In speciating mole rats[4,5] positive assortative mating together with other species-specific signals such as aggression patterns[20], may act as an important premating isolating mechanism. The latter presumably complement chromosomal incompatibility, thereby contributing to finalize the process of species formation.

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Olfactory Discrimination as an Isolating Mechanism in Speciating Mole Rats

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Summary. Olfactory discrimination was tested in two chromosome forms of the speciating fossorial rodent, *Spalax ehrenbergi*, in Israel. Females of the chromosome forms 2n = 52 and 2n = 58 were tested for male odour discrimination, the source of odour being either cage litter or urine. Estrous females of both forms preferred homochromosomal odours, whereas diestrous females showed no discrimination. These results suggest that olfactory discrimination may serve as a reproductive isolating mechanism in the speciation of mole rats.

Fossorial mole rats of the *Spalax ehrenbergi* superspecies complex in Israel involve four main chromosome forms (2n = 52, 54, 58, and 60) representing four closely related species at final stages of speciation[2–4]. The four karyotypes inhabit vast parapatric regions and are distributed clinally, from north to south Israel (see distribution map in [17]). Selective matings between the karyotypes serve as premating reproductive isolating machanisms providing species-specific recognition signals[5,6]. However, the nature and operation of the communication signals by which females discriminate between males have not hitherto been elucidated. The objective of the present study was to test olfactory discrimination as a potential mechanism in sexual preference and isolation between the karyotypes.

Materials and methods. Based on the results of previously conducted mating experiments[5] and female discrimination tests[6], this study involved both estrous and diestrous females of two karyotypes. Experimental animals were sampled at two northwestern Israeli populations: Ma'alot (2n = 52) and Kabri (2n = 58). These collecting sites lie, at the closest point, approximately 3 km apart, and just on opposite sides of the 300 m hybrid zone between the 2n = 52 and 2n = 58 karyotypes[4]. The sexually adult animals were live-trapped in the field during November–December 1974 and 1975, caged individually in the laboratory, and tested during the December 1975–January 1976 breeding season[7].

The testing apparatus was a two-choice olfactorium comprising a square perspex box (40 × 40 × 20 cm), with two short (11 cm) removable tunnels protruding on opposite sides. Six volt light bulbs and corresponding photocells were connected across the tunnels. The amount of time a test animal spent in each tunnel was automatically recorded. At the end of each tunnel there was a small odour stimulus receptacle, and odours diffused into the rest of the apparatus via a perforated perspex plate. When the test female was placed into the olfactorium, the odour stimuli were already in position, no stimulus-free period of adaptation being allowed prior to testing. Test length was standardized to 1 h. The initial orientation of homo- and heterochromosomal stimuli (i.e., of the same, or different chromosomal form as the tested female) was arbitrarily decided, but in order to eliminate possible bias due to a directional preference of the test females, runs were generally repeated in clean apparatus using fresh stimuli in the reversed positions. After each test the olfactorium was dismantled and thoroughly washed with hot water and detergent.

Estrus was artifically induced. About 44 h before testing, females were injected 0.1 mg estradiol benzoate (suspended in olive oil), followed by 0.8 mg progesterone 6–8 h before the run. Females normally came into estrus 42–48 h after the initial injection of estrogen. The high doses were found to be necessary for consistent induction of estrus. Estrous state was determined by taking samples of vaginal fluid prior to testing and assessing the relative amounts of leucocytes, epithelial cells and cornified epithelium present. Females were considered estrous when their vaginal smears included more than 80% cornified epithelial cells, and diestrous when the smears consisted primarily of leucocytes.

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