as they do in the hormonally altered ground substance of the ligament of the pregnant mouse (*2*).

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Mole Rat Spalax: Evolutionary Significance of Chromosome Variation

Abstract. *Four forms of mole rats with diploid numbers of chromosomes of 52, 54, 58, and 60, respectively, were found in Israel and the vicinity. The differences between the chromosome sets are due to whole-arm (Robertsonian) changes and pericentric inversions. The geographic distribution of the different forms is contiguous. Only a few hybrid individuals have been discovered. These chromosome forms are tentatively considered as sibling species, almost completely isolated by cytogenetic and possibly ethological mechanisms. The weak dispersal powers of mole rats may have contributed to a rapid fixation of adaptive homozygous chromosomal changes.*

Mole rats from different parts of Israel and the vicinity appear morphologically quite uniform; individuals from all over the Middle East are called *Spalax ehrenbergi* Nehring 1898 (*1*). We have found, however, that the chromosome complement of these subterranean rodents varies from region to region. Four major types have been detected so far.

The diploid chromosome numbers ($2n$) of these forms are 52, 54, 58, and 60. The chromosome sets consist of metacentric and acrocentric chromosomes in different proportions. Three groups of chromosomes were distinguished by the type of chromosome changes involved (Table 1 and Fig. 1). Group A is composed of the unchanged chromosomes which are shared by all members of this series. Group B (Robertsonian chromosomes) and group C (inverted chromosomes), defined by their postulated history of rearrangement, contribute to the differences observed between the various chromosome sets.

All metacentric chromosomes, including the X chromosome, can be individually characterized and have been given designations (Fig. 1). Only a few of the acrocentric chromosomes, however, can be identified individually. The assignment of particular acrocentric chromosomes to the three groups, as well as their matching in pairs, is thus largely arbitrary. The Y chromosome is one of the smallest acrocentric chromosomes.

There are 18 pairs of unchanged chromosomes, including the X and Y chromosomes. Seven pairs of the shared autosomes are metacentric, and ten are acrocentric. The Robertsonian chromosomes consist of four pairs of metacentric chromosomes at one end of the series ($2n = 52$) and of eight pairs of acrocentric chromosomes at the other end ($2n = 60$). The two other karyotypes ($2n = 54$ and $2n = 58$) possess three pairs and one pair of metacentric chromosomes, respectively. The acrocentric chromosomes of this group are considered equal in genetic content to the corresponding arms of the metacentric chromosomes. The inverted chromosomes are related presumably through pericentric inversions. In group C the metacentric chromosomes are confined to the two lower numbered karyotypes.

The numerical relationships of the karyotypes are summarized in Table 1. Assuming that the homologous chromosomes of group C have the same length, regardless of the position of the centromere, one arrives at one and the same corrected figure of "arms" for all chromosome forms. The karyotypes in Fig. 1 were arranged so as to bring out the similarity in the quantity of chromosome material in the four forms.

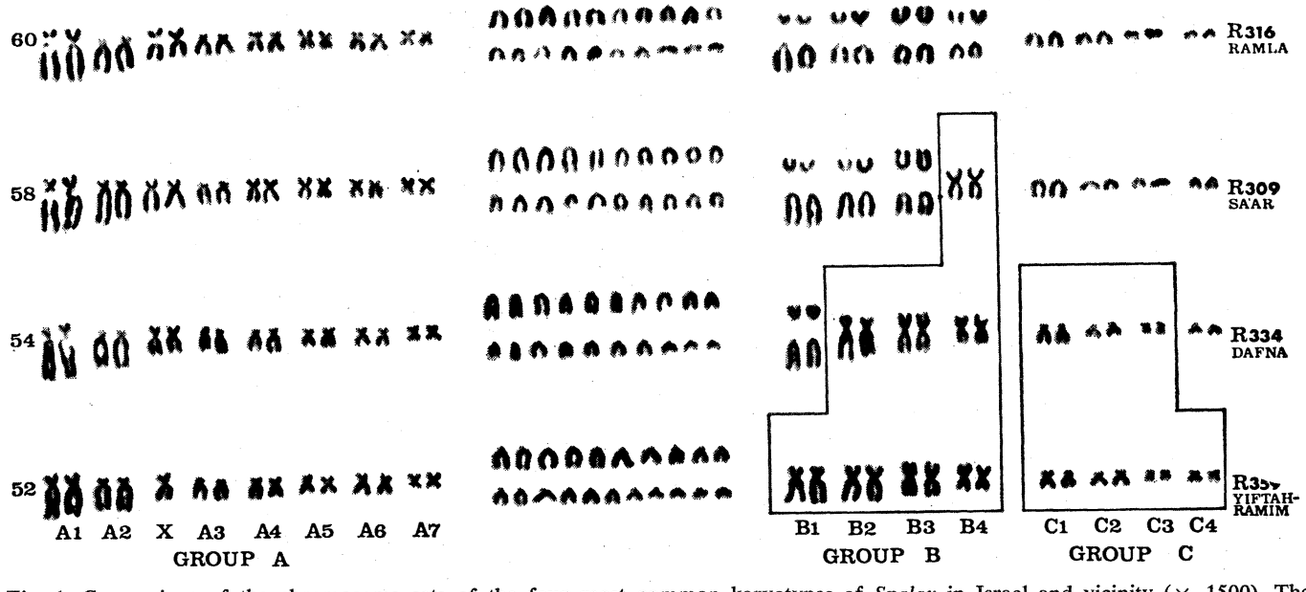

Fig. 1. Comparison of the chromosome sets of the four most common karyotypes of *Spalax* in Israel and vicinity (× 1500). The metacentric chromosomes of groups B and C are in boxes. The serial numbers and the localities of the animals utilized are at the right-hand side. The $2n$ numbers are on the left-hand side. Karyotypes were analyzed in hypotonically treated dividing bone marrow cells. The $2n = 52$ karyotype belongs to a male; the others are from females.

The area sampled extended from Mount Hermon in the northeast to the deserts of the Negev in the south (Fig. 2). The four chromosome forms are, in the main, mutually exclusive but have a contiguous distribution. Five hybrid animals with 59 chromosomes have been discovered between the ranges of animals with 58 and 60 chromosomes. Chromosome B4, the only Robertsonian metacentric chromosome of the $2n = 58$ form, is heterozygous in the hybrid individuals. All other metacentric chromosomes can be paired in the hybrids. Two hybrid animals with 53 chromosomes have been found in a contact region between animals with 52 and 54 chromosomes. In these hybrids chromosome B1 is heterozygous. Repeated sampling in other contact areas so far has yielded no hybrids.

It is interesting to note that the chromosome numbers increase gradually from 52 and 54 in the north to 60 in the marginal populations of the south. The present distribution of the four forms roughly coincides with biogeographical regions characterized by increasing degrees of aridity. Form $2n = 52$ is found in the mountains of Upper Galilee, which have a humid to subhumid climate. Form $2n = 54$ extends over Mount Hermon and the Golan region, which is a subhumid to humid area with lower mean annual temperatures than in the $2n = 52$ region. Form $2n = 58$ ranges in Lower Galilee and Central Israel in a subhumid to semiarid climatic regime. Finally, $2n = 60$ is found in Samaria and Judea extending toward the southern and eastern deserts of Israel, a semiarid to arid region with some subhumid mountainous enclaves. Mole rats do not penetrate into the true desert area. They reach the limit of their range at a line which coincides approximately with the 100 mm isohyet (*2*). Usually small, semi-isolated populations are found at the southern border of the range.

We propose that the four forms described here should be considered as sibling species. This interpretation is supported by the achievement of homozygous fixation in most populations and by the rarity of natural hybrids. Further corroboration is provided by possible mating preferences among the different forms (*3*). In 77 crossing tests, copulations were more frequent in pairs with the same chromosome constitutions than in pairs with dissimilar chromosomes. Aggressive behavior preceding copulation, measured by frequency of biting, was also less pronounced in the former pairs than in the latter. These observations indicate that ethological barriers to reproduction may complement cytological barriers, in preventing widespread hybridization.

Table 1. The number of metacentric (M) and acrocentric (A) chromosomes among the unchanged chromosomes (group A), the Robertsonian chromosomes (group B) and the inversion chromosomes (group C) of *Spalax*. The chromosome classification refers to females. The names of the regions given are for general orientation only.

Location	2n	Chromosomes (No.)						Total arms (No.)	
		Group A		Group B		Group C		Actual	Corrected*
		M	A	M	A	M	A		
Upper Galilee	52	16	20	8		8		84	76
Mount Hermon, Golan	54	16	20	6	4	6	2	82	76
Lower Galilee-Central Israel	58	16	20	2	12		8	76	76
Samaria, Judea, Negev	60	16	20		16		8	76	76

* Metacentric chromosomes of group C are counted as if they were one-armed elements.

Our working hypothesis for the evolution of this complex of sibling species assumes that ancestral populations of *Spalax* inhabited an unknown center, which could have been somewhere in Asia Minor or Southeastern Europe. A progressive colonization from this center outward was initiated including an extension southward to increasingly arid areas. In this process, new, selectively superior homozygous karyotypes became fixed. This fixation was possible even in the absence of geographic isolation, in the classic sense, inasmuch as mole rats in peripheral situations live in small, somewhat isolated populations, and their mobility, in general, is very restricted (*2, 4*). Such populations probably are highly inbred, and gene exchange is restricted between them and more central populations. The achievement of relatively rapid fixations of new karyotypes hampers further hybridizations, and ethological barriers leading to a reinforcement of reproductive isolation may have evolved. Ultimately the gene pools became more or less separated and the species level was practically reached, despite some hybridization along borderlines. It follows from this hypothesis that chromosome rearrangements primarily led to the formation of acrocentric elements from metacentric ones.

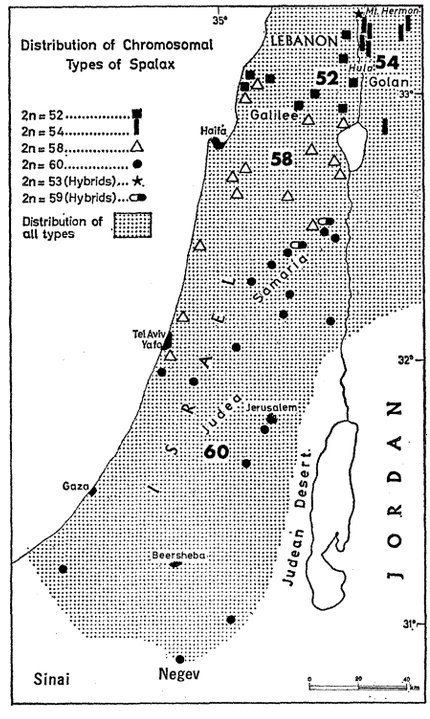

Fig. 2. Distribution map of the four chromosomal sibling forms of *Spalax* in Israel and vicinity. The map is based on the examination of about 100 individuals from 50 localities.

The correspondence between the distribution of the chromosome forms and the biogeographical zones supports the hypothesis that the different karyotypes are adaptive genetic systems. The specialized subterranean environment might account for the striking morphological similarities of the four sibling species.

Several chromosome forms have been described in another "species" of *Spalax* (*5*). A comparison of all known chromosome sets of *Spalax* suggests, in fact, that they are molded, presumably by natural selection, of essentially the same initial chromosome material into differently numbered and shaped linkage groups. It is not unlikely that *Spalax* is an example of a series of sibling species whose separate existence depends upon chromosomal reproductive barriers reinforced by ethological mechanisms (*6*).

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Honey Bee Recruitment to Food Sources: Olfaction or Language?

Abstract. *Honey bee recruits locate food sources by olfaction and not by use of distance and direction information contained in the recruitment dance. Recruitment efficiency increases as odor of the food source accumulates in the hive, from hour to hour and from day to day. Flight patterns, landing patterns, bee odor, and Nassanoff secretion apparently do not aid in recruitment of bees.*

When von Frisch generated the "dance language" hypothesis of honey bee recruitment (*Apis mellifera* L.) (*1*), it was based on the results of elegantly simple experiments and withstood the tests of repeatability and "proof" by verification (*2*). Inadequacies in this explanation have been revealed (*3*) through studying the nature of correlations between behavioral and environmental parameters. Wenner and Johnson (*4*) documented the existence and relevance of simple conditioning during recruitment, just as Lopatina (*5*) had earlier.

This demonstration led to a questioning of the assumption that the "language" was an "instinctive" act. Challenging a basic assumption (*6*), Wells and Giacchino (*7*) found that altering the sugar concentration did not alter the amount of solution ingested by foragers.

Furthermore, the language hypothesis has failed the more critical test of refutation (*8*) in that experimentation with controls not incorporated in the early experiments yielded results other than those predicted by the hypothesis (*9*). In the later experiments, recruited bees arrived at sites in the field in apparent disregard of any dance information that they could have acquired before leaving the hive.

Such data are not only incompatible with a language hypothesis but also provide a basis for the a posteriori generation of an alternative hypothesis (*10*): Potential recruits stimulated to leave the hive search the field for the odor (or odors) carried into the colony by successful foragers.

That bees locate a food source by olfaction is especially possible in view of the extremely low recruitment rate of regular foragers collecting unscented sucrose at an unscented site. On 25 July 1968, for instance, in the absence of a major nectar source for the colony, we received only five recruits from a hive of approximately 60,000 bees after ten bees had foraged at each of four stations for a total of 1374 round trips during a 3-hour period.

Although the olfaction hypothesis can explain most (if not all) of these results, no a priori experimental design has contrasted the two hypotheses. We felt that such a test was necessary and should be possible with the use of a single hive.

Despite the difficulties in designing such an experiment (*10*), some unexpected results obtained during the summer of 1967 provided the basis for just such a test. In the experimental series of 1967, ten individually marked bees routinely visited each of two clove-scented sources (0 to 0.26 ml of oil of clove per liter of 1.5 molal sucrose solution), 200 m in opposite directions from the hive. Each new recruit landing at a dish was killed in a covered jar of alcohol.

We had expected a constant number of recruits per unit time, but an increasing number of new bees arrived and were killed as the experiment progressed (Fig. 1). Since the number of new arrivals reflected the cumulative number of trips by experienced foragers, we concluded that a recruit more readily locates a site in the field as a direct consequence of odor in the hive. Furthermore, the data gathered on 1 day were not independent of the previous day's manipulations.

If odor accumulates in the hive and contributes to the relative success of a recruit searching in the field, a rationale exists for designing an experiment. Bees visiting certain scented sources in the field for 1, 2, or 3 days accumulate odor in the hive and continue to visit

Table 1. Total number of recruits received per day and the experimental procedure at the three sites. Foragers never visited the control site (No. 2), and ten bees made a relatively constant number of trips per unit time to the experimental sites (Nos. 1 and 3). On day 7 only five of the regular foragers arrived at site No. 3. On day 16 a second scent (0.13 ml of oil of peppermint per liter of 1.5 molal sucrose solution) was used at each experimental site (no peppermint scent had accumulated in the hive previous to this time). The number of times the Nassanoff gland was exposed is the average for sites 1 and 3.

Day	Procedure	Recruitment (No. at each site)			Nassanoff exposure
		No. 1	No. 2	No. 3	
1	Scent at 1 and 3	42		71	31.0
2	No scent at 1 and 3, scent at 2	15	38	3	134.5
3	Scent at 1 and 3	89		76	71.5
4	No scent at 1 and 3	20		7	182.0
5	Scent at 1 and 3	87		90	94.5
6	Scent at 1 and 3	70		55	82.0
7	No scent at 1 and 3, scent at 2	4	51	0	139.5
8	Scent at 1 and 3	111		101	136.5
9	No scent at 1, 2, or 3	0	3	17	223.0
10	Scent at 1 and 3	44		90	149.0
11	Scent at 1 and 3	159		89	160.0
12	No scent at 1 and 3, scent at 2	4	91	5	253.0
13	Scent at 1 and 3	102		61	92.0
14	No scent at 1, 2, or 3	6	2	5	161.5
15	Scent at 1 and 3	93		87	87.5
16	2nd scent at 1 and 3, 1st scent at 2	2	44	0	82.0
17	Scent at 1 and 3	71		29	55.5
18–22	[Separate experimental series, scent at 1 and (or) 3]				
23	Scent at 1 and 3	68		32	168.5
24	Scent, but no bees at 1, 2, and 3	1	0	0	0.0