Quantities of ecdysones in various animals from natural waters

Whole animal	Stage	Weight extracted (kg)	Ecdysone	Ecdysone concentration (mg/kg)
Homarus americanus[14]	Postmolt	5	Ecdysterone	0.006
Jasus lalandei[2]	Intermolt	1,000	Ecdysterone	0.002
	Intermolt	3,000	2-Deoxycrust-ecdysone	0.00007
Callinectes sapidus[3]	Premolt 'Green'	25	Inokosterone	0.005
	Premolt 'Peeler'	25	Inokosterone	0.020
			Ecdysterone	0.004
	Postmolt 'Soft Shell'	25	Ecdysterone	0.280
			Makisterone A	0.024
Mytilus edulis[15]	—	—	N.I.[a]	—
Carcinus maenas[15]	Intermolt	—	N.I.	—
Crangon vulgaris[16]	Intermolt	3,000	N.I.	—

[a] Not identified.

were separated from the shell and extracted 3 times with *n*-butanol in a blender. The combined butanol extracts were concentrated at 55 °C under vacuum, yielding a red oil. The latter was taken up in ethyl acetate and extracted 3 times with water. The water extracts were backwashed 3 times with ethyl acetate and concentrated to dryness under an N_2 stream. The residue of the aqueous extract was stirred first with cold petroleum ether, then with cold acetone. The acetone extract was filtered and evaporated to dryness.

Thin layer chromatography (TLC) of a small portion of this material on silica gel with chloroform/methanol/acetone (6:2:1) as eluent gave 3 radioactive spots; one of which corresponded exactly with the retention time of an ecdysterone standard. This fraction was subsequently isolated by preparative TLC to give the crude ecdysterone. Structural identification was completed by adding 1 mg of ecdysterone standard to the crude ecdysterone mixture and acetylating with acetic acid – pyridine at room temperature for 2 h[11]. Radio TLC of the resulting mixture demonstrated that it contained 4 radioactive products which co-chromatographed with the 4 products produced on acetylation of authentic ecdysterone. These results were further verified by scintillation counting of the active fractions on the TLC plate.

Recently it has been reported that injection of ecdysterone into both normal and destalked intermolt lobsters induced precocious molting[12]. This work supports our results that ecdysterone, by itself, or in combination with other ecdysones, is playing a major role in the molting process of the lobster.

Injection of ecdysterone into other arthropods such as horseshoe crabs, barnacles, scorpions, and spiders also initiates molting[13]. These results and those of the extraction and identification of ecdysterone from both insects[9] and crustaceans (Table), strengthen the hypothesis that ecdysterone is a general arthropod molting hormone[17].

Résumé. L'ecdystérone, hormone de mue des insectes, a été extraite de homards femelles (*Homarus americanus*) venant de muer. Sa concentration moyenne est de 6µg/kg de homard vivant. L'assimilation de cholestérol (4-^{14}C) par des homards femelles avant la mue et la biosynthèse d'ecdystérone à partir de ce précurseur est démontrée.

R. B. GAGOSIAN[18,19], R. A. BOURBONNIERE[18], W. B. SMITH[20], E. F. COUCH[20], C. BLANTON[20] and W. NOVAK[20,21]

Department of Chemistry, Woods Hole Oceanographic Institution, Woods Hole (Massachusetts 02543, USA); and Departments of Chemistry and Biology, Texas Christian University, Forth Worth (Texas 76129, USA),

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[18] Woods Hole Oceanographic Institution.

[19] To whom correspondence should be addressed.

[20] Texas Christian University.

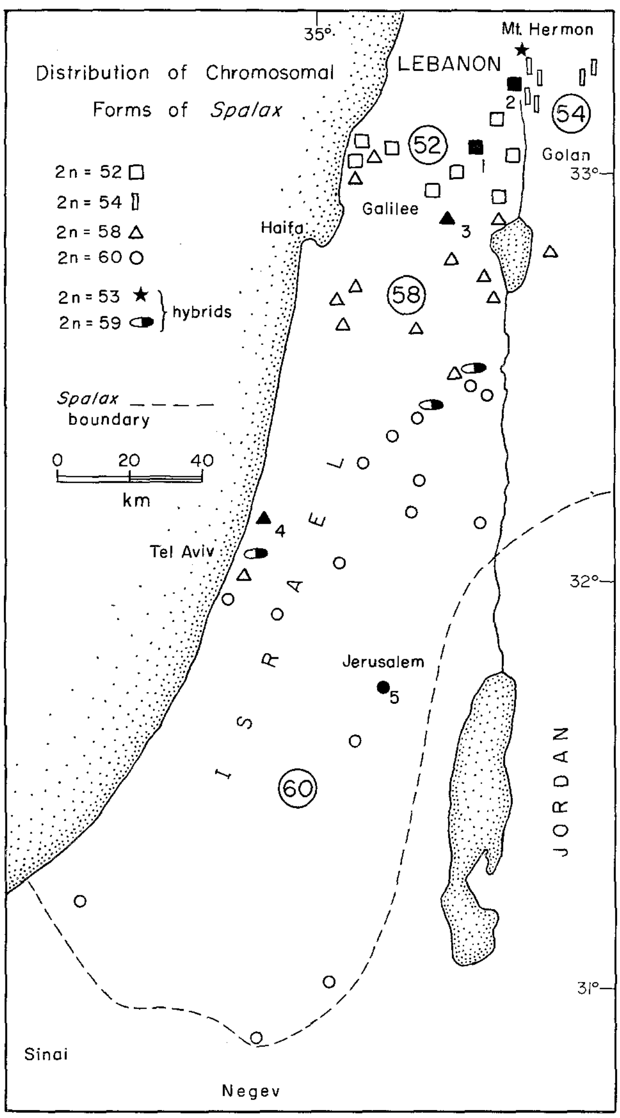
[21] Woods Hole Oceanographic Institution Contribution Number 3147.

Adaptive Metabolic Variation of Chromosome Forms in Mole Rats, *Spalax*

Speciation through chromosomal rearrangement is widespread in animals[1], yet the significance of chromosome variation is still largely speculative and needs further elucidation. MAYR[2] suggested that it may act both as an isolating mechanism and a protection for favourable supergenes, as initially pointed out by WALLACE[3]. Evidence suggesting association between karyotypic variation and ecophysiological adaptation(s) may at least partly provide the selective basis for chromosomal differentiation, and indirectly support the supergene hypothesis.

Chromosome variation in the East-Mediterranean fossorial rodent *Spalax* is remarkable in both morphology and diploid numbers which range from 48 to 62[4]. Four main chromosome forms occur in *Spalax ehrenbergi* from Israel and vicinity (Figure). These involve diploid numbers (*2n*) 52, 54, 58 and 60, which are distributed clinally and parapatrically from north to south Israel along a steep and short (160 km) ecological gradient of increasing aridity[5]. Their pattern of distribution, karyotypic homozygosity, relative rarity of natural hybrids and probable selective matings[6] suggest that these forms are closely related biological species. The present study was designed to test the hypothesis that the different karyotypes of *Spalax ehrenbergi* are distinct adaptive ecophysiological systems.

Materials and methods. Oxygen consumption was measured and compared in 5 populations belonging to 3 chromosome forms of *Spalax ehrenbergi* (Figure and Table). Two geographically distant populations were studied in the karyotypes 52 and 58 in order to compare the basal metabolic rate (BMR) and its variance within and between chromosome forms.



Distribution of chromosome forms of *Spalax ehrenbergi* in Israel. Open symbols indicate all karyotyped localities. Black symbols, numbered 1 to 5, indicate populations studied for BMR.

Prior to measurements, all animals were kept in the laboratory under similar conditions (20–23°C) and diet for at least 2 weeks. Food was denied to experimental animals 24 h prior to testing. The measurements were taken in an open flow system. Oxygen coneentration in the air leaving the metabolic cell was recordcd in a Beckman paramagnetic oxygen analyzer after passing a column of water and CO_2 absorbent. Mole-rats are active and restless animals, and only at thermoneutrality they become calm for prolonged periods to enable reliable measurements to be made. When ambient temperature exceeded body temperature (which is normally between 35.5°C and 36°C in the 3 chromosome forms under study), the animal struggles vigorously and soon afterwards collapses before proper measurements of oxygen can be taken. At ambient temperatures higher than 36°C (e.g., 37.0–37.5°C) some animals collapsed, having at that time a body temperature of 43°C.

Results and discussion. Measurements of the minimal oxygen consumption in resting mole-rats (BMR), BMR deviation from the expected[7], and lower critical temperatures of the thermoneutral zone are summarized in the Table together with results of Anova tests for statistical significance and climatological data. The results suggest a) an overall low metabolic rate, and b) a clinal decrease in BMRs southwards inversely related to the southward increases in aridity and dipolid number. The NE Jerusalem population (No. 5 on the Table) representing the $2n = 60$ karyotype, which ranges mostly in semi-arid and arid environments, shows a significantly lower metabolism as compared to the mesic populations having $2n = 52$ karyotype (Nos. 1 and 2 on the Table).

BMR values similar to those of the 52 chromosome form of *S. ehrenbergi* were found in *S. leucodon* from SE Europe. SAVIĆ[8] reported an average BMR of 1.0 cm^3 O_2/g h in 26 animals from Yugoslavia. GORECKI and CHRISTOV[9] recorded an average BMR of 0.96 cm^3 O_2/g h in 8 animals from Petrochan, Bulgaria, amounting to 66% of the expected BMR. McNAB[10] recorded in one specimen from Kastamonu, Turkey, a BMR of 0.77 cm^3 O_2/g h, 86% of the expected[7].

Low metabolic rates characterize desert mammals in general and rodents in particular[11–15]. Fossorial rodents

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Comparison of climatic type and basal metabolic rates (BMR) of 3 chromosome forms of *Spalax ehrenbergi* in Israel

Population No.	Chromosome form (2*n*)	Locality	Climatic type and moisture index [a]	Sample size (*N*)	Mean and range wt. (g)	Lower critical temperature (°C)	Basal meatbolic rate (BMR) mean and S.D. ($cm^3 O_2/g/h$)		BMR measured / BMR predicted [b] ×100
1	52	Kerem-Zimra	humid to semihumid (0 to +20)	5	104.0 (92.0–11.5.5)	26.40	1.03	0.16	96.0
2	52	Kiryat-Shemona	semihumid to semiarid (−20 to −40)	6	128.0 (101.0–145.0)	28.31	0.86	0.07	85.0
3	58	Eilabun	humid to semihumid (0 to +20)	4	117.0 (93.0–149.0)	30.40	0.85	0.27	82.0
4	58	NE of Tel-Aviv	semihumid to semiarid (−20 to −40)	4	124.0 (115.0–140.0)	28.40	0.87	0.12	83.0
5	60	NE of Jerusalem	semiarid to arid (−40 to −60)	4	121.0 (102.0–147.0)	30.00	0.62	0.10	60.0

[a] C. W. THORNTHWAITE, Geograph. Rev. *38*, 55 (1948). [b] Predicted after KLEIBER[7], assuming 4.8 Kcal/LO_2.

Results of analysis of variance between populations and chromosome forms:

Population No.	Chromosome form	F	*P*
1–2	52–52	8.028	< 0.01
3–4	58–58	0.119	< 0.50
(1 + 2)–(3 + 4)	52–58	3.074	0.10
(1 + 2)–(5)	52–60	18.960	< 0.001
(3 + 4)–(5)	58–60	9.702	< 0.01

have low BMRs (compared with those expected for their size), high conductances, and high ranges of thermoneutrality[10]. MCNAB suggested that low rates of metabolism may serve to reduce water expenditure and the probability of overheating. This may particularly be true in the subterranean niche where evaporative and convective cooling are greatly reduced.

The BMRs of the 3 chromosome forms of *S. ehrenbergi* decrease progressively and significantly toward the desert, whereas their deviations from the expected values increase correspondingly. This physiological cline along an increasingly arid gradient suggests that the BMR is sensitive to environmental pressures and is adapted to the aridity index. The Kiryat-Shemona population (No. 2, Table) has a lower BMR value, possibly due to its location along the warmer and drier eastern slopes of the Upper Galilee Mountains. This reinforces the suggestion that local ecotypic adaptation within a karyotype probably reflects the sensitivity of BMR to local conditions. It is of special interest to find owser and progressively declining BMR values in animals which approach the desert and are exclusively fossorial.

Association between karyotypes and BMRs may suggest one of the selective factors involved in the successful adaptive radiation of spalacids to aridity via chromosomal mutations. The latter may have resulted in physiologically better coadapted supergenes, reproductively isolated both cytogenetically and ethologically[6,16].

Résumé. Nous avons comparé la consomption minimale d'oxygène (BMR) chez 23 rats taupes au repos, provenant de 5 populations du *Spalax ehrenbergi* en Israel, avec 3 formes chromosomales. Il s'agit de diploides (2*n*) 52, 58, et 60 s'étendant suivant un axe Nord–Sud. Lorsque l'on passe du nord humide au sud aride, leurs BMR diminuent progressivement et d'une façon significative, mais la différence entre la valeur du BMR et son espérance mathématique augmente. Ce gradient physiologique suggère a) que le BMR s'est probablement adapté a l'index d'aridité, sa valeur faible près du désert indiquant une réduction du besoin d'eau et de dépense calorifique; et b), que la spéciation chromosomale en spalacides est due, en partie du moins, au développement de supergènes adaptés à l'aridité de l'environnement.

E. NEVO and A. SHKOLNIK[16]

Department of Biology, Haifa University, Mount Carmel, Haifa (Israel), and Department of Zoology, Tel-Aviv University, Tel-Aviv (Israel), 14 January 1974.

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