Position of Y-chromosome at somatic metaphase

Parameters	Location of the Y-chromosome*										
	1	2	3	4	5	6	7	8	9	10	Total
CML:											
Frequency	1	33	44	70	78	90	75	66	43	37	537
Percent	0.186	6.145	8.190	13.035	14.520	16.759	13.966	12.290	8.009	6.890	100
Control:											
Percent	0.452	3.060	8.590	12.050	14.040	16.740	15.670	13.650	7.960	7.790	100
Expected:											
Percent	0.319	4.602	8.390	12.542	14.280	16.749	14.818	12.970	7.984	7.340	100

^{*}for detailed description of these locations (1–10), see Verma et al.⁴.

Y-chromosome from metaphase be due to its position in hematopoietic cells? Therefore, we examined the position of the Ychromosome at metaphase in bone marrow cells from patients with CML and compared it with normal controls.

Materials and methods. 50 male patients whose clinical diagnosis was CML were studied. All had the Ph'-chromosome with the 9q; 22q translocation as determined by QFQ and RFA techniques^{2,3}. The chromosome preparations were made from bone marrow aspirates and QFQ cells were photographed on tri-X film (Kodak) using a Zeiss photomicroscope II. At least 20 cells from each individual were initially photographed. Recording of the location of the Y-chromosome was performed directly by enlarging the metaphase onto a circle or square as previously described⁴. The square and circle were divided into 10 equal parts (1–10) at a distance of 1 cm.

The following criteria were introduced to identify the location of the Y as either peripheral or non-peripheral. If the Y-chromosome fell in area No. 10 and there was no other chromosome located further from it, then Y was described as being peripheral. The location of the Y-chromosome was also recorded when it was not on the periphery. Only complete metaphases with 46 chromosomes and those which fitted into the square or circle were included. The logic behind this approach has been described elsewhere⁴. A total of 537 metaphases were included. The control data were taken from an earlier report⁴.

Results and discussion. There is a subgroup of patients with Ph'-positive CML (8–10% of male cases) who have been described as having a chromosome constitution of 45 chromosomes⁵. The missing chromosome was the Y, unequivocally identified by fluorescence banding techniques⁶. The mechanism underlying this selective loss, however, remains a mystery. The loss of the Y-chromosome is a phenomenon that appears to be secondary to the induction of the Ph' but may be closely related to it. Nevertheless, the missing Y-chromosome in marrow cells has been postulated to have significance in the prognosis of CML⁷. Does the position of the Y-chromosome and metaphase

play any role in loss from somatic cells? The distribution of Y-chromosome at metaphase is recorded (table). The normal distribution of the Y-chromosome was noted when compared to the control value. There was no significant difference from the expected value. Therefore, it is concluded that the position of the Y-chromosome in hematopoietic cells in patients with CML at somatic metaphase is random. The missing Y-chromosome in bone marrow cells may be a normal aging phenomenon. Nevertheless, there is a strong suggestion that a missing Y is a reflection of a basic defect occurring in elderly males in whom the marrow is affected by a hematologic disorder. Thus, the position of the Y-chromosome does not appear to influence loss from bone marrow cells.

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- 1 Comings, D.E., Hum. Genet. 53 (1980) 131.
- Verma, R. S., and Lubs, H. A., Am. J. hum. Genet. 27 (1975) 110.
- 3 Verma, R.S., and Dosik, H., Microscopy 108 (1976) 339.
- 4 Verma, R.S., and Rodriquez, J., Shah, J.V., and Dosik, H., Heredity 52 (1984) 227.
- 5 Atkin, N. B., and Taylor, M. C., Cytogenetics 1 (1962) 97.
- 6 Hossfeld, D.K., and Sandberg, A.A., Klin. Wschr. 48 (1970) 1431.
- 7 Sandberg, A. A., The Chromosomes in Human Cancer and Leukemia. Elsevier, New York 1980.

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Hematocrit and hemoglobin concentration in four chromosomal species and some isolated populations of actively speciating subterranean mole rats in Israel

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Summary. Hematocrit (HCT) and hemoglobin (Hb) concentration were measured in four chromosomal species and some peripherally semi-isolated and isolated populations of the mole rat superspecies Spalax ehrenbergi in Israel. HCT was 52.0, 51.4, 50.9, and 47.8%, and Hb was 16.0, 16.6, 16.3, and 14.7 g/100 ml for 2n = 52, 58, 54, and 60, respectively. The species 2n = 60, which lives in arid habitats, had lower HCT and Hb than the other three species. HCT decreased as aridity increased between the species and within the species 2n = 60. Changes in HCT probably reflect clinal changes in both soil permeability to gases and ambient temperature. Key words. Blood; aridity; habitat selection; fossoriality.

The fossorial mole rat superspecies *Spalax ehrenbergi* is in the final stages of an active process of speciation and has produced four chromosomal subspecies as it invaded more arid habitats,

in the order of 2n = 52, 58, 54, and $60^{1,2}$. The species 2n = 60 extends furthest into arid regions, where it forms peripherally semi-isolated and isolated border populations³ and, because of

The chromosomal species tested and their ecogeographical background

Chromosomal species (2n)	Weight (g) mean \pm SD	Population site	Population type*	Sample size (N)	Mean annual precipitation	Temperature mean (°C)	
					(mm)	January	August
52	122 ± 33	Kerem Ben-Zimra	С	13	625	7	24
		Maalot	H	8	783	8	23
		Qiryat Shemona	M	6	653	9	26
54	144 ± 28	Quneitra	C	8	820	6	22
		El-al	H	8	464	10	26
		Hermon	M	6	1400	3	21
58	123 ± 12	Zippori	С	5	507	10	25
		Carmel	C	1	686	11	24
		Kabri	H	7	620	12	26
		Afik	H, M	6	450	11	27
60	119 ± 30	Lahav	C	16	303	12	26
		Anza	Н	20	451	11	26
		Jerusalem	M	13	550	9	24
		Wadi Farah	S	6	300	13	29
		Jiftlik	S	1	250	14	31
		Dimona	I	1	91	9	26
		Sde Boker	I	11	91	9	26

^{*}C, central population; H, near hybrid zone; M, ecologically marginal; S, semi-isolate; I, isolate.

the limited mobility and increased genetic polymorphism of $2n = 60^4$, isolation, natural selection, and genetic drift may form different genetic populations in the border zones. The mole rat is well adapted to living in an underground atmosphere where hypoxia and hypercapnia prevail⁵, and its gas-exchange physiology is very different from that of nonfossorial mammals⁶⁻⁹. Because the soil texture and humidity affect the burrow's atmosphere⁵, different selective pressures should operate in the different microhabitats of the mole rat. In this study we examined blood hematocrit (HCT) and hemoglobin (Hb) concentrations

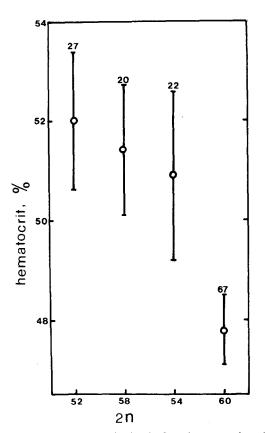


Figure 1. Hematocrit; mean \pm SE for the four chromosomal species of mole rat, in order of increasing aridity. Sample size appears at upper margin.

of different populations of the four chromosomal species of mole rats in Israel. The Hb of the mole rat is in the upper range for terrestrial mammals⁶; this high O₂-carrying capacity is advantageous in the hypoxic burrow atmosphere. Therefore, one would expect that the 2n = 52 species, which inhabits a more humid region, would be likely to have a higher Hb concentration than the species which inhabits an arid region (2n = 60), where the soil is more aerated and the burrow atmosphere less hypoxic. Materials and methods. Blood was taken via heparinized syringes from the hearts of mole rats anesthetized with sodium barbital (40 mg/kg b.wt), which were then sacrificed for the study of other organs. The blood was either analyzed immediately or refrigerated for later analysis. Hb concentration was measured using the cyanmethemoglobin method in duplicate determinations, and HCT by centrifugation in triplicate determinations.

The animals had been captured in the field and kept in captivity between 4 days and 2 years or more. Since HCT as well as other blood parameters are not affected by length of captivity 10, we could combine the results for the same population, regardless of captivity duration. A total number of 136 mole rats were examined. The table shows the population characteristics, weights, places of capture, and climate.

We used analysis of variance to compare the four chromosomal species, and we also checked the correlation of either HCT or Hb to the order of aridity using the Spearman rank correlation

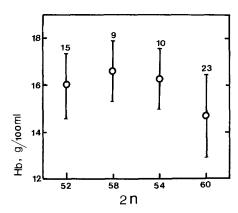


Figure 2. Hemoglobin concentration of the four chromosomal species of mole rat. Symbols as in figure 1.

coefficient. The five populations from different habitats belonging to 2n = 60 were compared by linear relations between HCT and annual precipitation as an approximate measure of soil permeability to gases.

Results. The HCT of the four chromosomal species is shown in figure 1. There was no difference in HCT between 2n = 52, 54, and 58; only the species 2n = 60 had low HCT compared to the other species (p < 0.01). The correlation coefficient of HCT to the order of habitat aridity was significant (r = 0.308, p < 0.01). A similar pattern was seen in the Hb concentration (fig. 2), where Hb was lower in 2n = 60 than in the other three groups (p < 0.01), and the correlation of Hb to the order of arid to humid habitats was significant (r = 0.411, p < 0.01). Comparison of HCT of 2n = 60 as a function of increased aridity showed that HCT decreased from the semihumid habitat of Jerusalem to the arid habitats (fig. 3). The slope of regression of HCT against annual precipitation was different from zero (p < 0.01).

Discussion. The two species of mole rat which inhabit a humid climate (2n = 52 and 58) exhibited high HCT values as compared to some other terrestrial mammals, but were comparable to some burrow-dwelling rodents¹¹ and to the ground squirrel¹², which lives in burrows and is well adapted to hypoxia. The species 2n = 54, which is distributed in a continental dry climate, had lower HCT than the 'humid' species, and the species 2n = 60, which inhabits a more arid region, had lower HCT and Hb than the other species; however, it seems that HCT is related more to the humidity (fig. 3), and therefore to the permeability of the soil to gases⁵, than to the chromosomal species. It is obvious that precipitation is not the only factor which determines the permeability of the soil to gases. Other factors, such as soil structure, evaporation, and drainage, would also affect permeability. Thus, high precipitation in a mountainous area where drainage is good and evaporation is high would not caused reduced permeability; for example, no difference in HCT could be found between the Hermon population with high precipitation and the El-al population with much lower precipitation (table). Also, when the soil is fully saturated with water, addi-

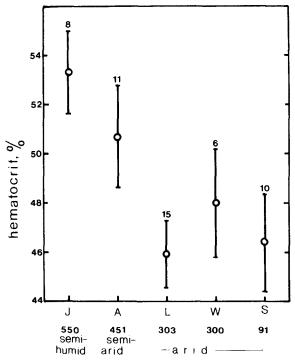


Figure 3. Hematocrit of five populations of mole rats (2n = 60) in sequence of the mean annual precipitation (numbers at abscissa in mm). Locations of origin are: J, Jerusalem; A, Anza; L, Lahav; W, Wadi Farah; S, Sde Boker. Other symbols as in figure 1.

tional precipitation would not have any effect; therefore, the effect of rainfall is clearly seen in the dry section of the distribution of the mole rat.

The reduction of HCT as aridity increases is also correlated with a reduced metabolic rate^{13,14}. In the arid region, the oxygen consumption of the mole rat is low^{13,14}, and therefore the blood can have a reduced O2-carrying capacity. However, the low Hb concentration of 2n = 60 is in the normal range for other mammals¹². This reduction of carrying capacity of the blood is concomitant with a decreased viscosity. Populations of 2n = 60live in a hot climate, and for thermoregulation in situations of heat load when blood is directed to arteriovenous anastomoses, where no gas exchange occurs, a reduced blood viscosity is advantageous. This can be compared to the increased plasma volume in man during acclimation to heat¹⁵ or to the reduction of HCT in various rodents in the summer in comparison to the winter11.

Differences related to the chromosomal species can be tested using neighboring populations sharing similar climate and soil. Such a comparison between the El-al population (2n = 54) and the Afik population (2n = 58) revealed a nonsignificant difference: HCT was 52.8 (N = 8) and 49.8 (N = 6) for the El-al and Afik populations, respectively. The question as to whether these differences are due to the chromosomal species awaits further experimentation on bigger samples.

Although the amino acid sequence of the Hb of 2n = 52 and 60 is identical (Kleinschmidt, Nevo, Goodman, and Braunitzer, personal communication), a genetically based physiological difference is evident, which supports the suggestion of only small genetic differences that adapt the different populations to their habitats1.

Adaptation of the mole rats HCT and Hb to different habitats, in addition to other clinal changes of genetically based physiological properties of the mole rat such as heart rate and breathing frequently¹⁶, tissue gas tensions¹⁷, oxygen consumption¹³, and nonshivering thermogenesis¹⁴, adds to our understanding of the active evolution of genetic types which radiate into more arid habitats.

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- Nevo, E., in: Mechanisms of Speciation, p. 191. Ed. C. Barigozzi. A. Liss, New York 1982.
- Nevo, E., in: Animal Speciation and Contact Zones. Ed. P. Omodeo. Bull. zool. 52, in press.
- Nevo, E., Heth, G., and Beiles, A., Evolution 36 (1982) 1283.
- Nevo, E., and Shaw, C. R., Biochem. Genet. 7 (1972) 235.
- Arieli, R., Comp. Biochem. Physiol. 63A (1979) 569.
- Ar, A., Arieli, R., and Shkolnik, A., Resp. Physiol. 30 (1977) 201.
- Arieli, R., and Ar, A., J. appl. Physiol. 47 (1979) 1011.
- Arieli, R., and Ar, A., Physiol. Zool. 54 (1981) 14. Arieli, R., and Ar, A., Physiol. Zool. 54 (1981) 22.
- Arieli, R., The Mole Rat (Spalax ehrenbergi): Adaptations to Fossorial Life. Ph.D. Thesis. Tel Aviv University, Tel Aviv, 1978.
- 11 Lee, A. K., and Brown, J. H., Mammalogy 51 (1970) 669.
- Altman, P. L., and Dittmer, D. S., Respiration and Circulation. Federation of the American Societies for Experimental Biology, Be-
- Nevo, E., and Shkolnik, A., Experientia 30 (1974) 724.
- Haim, A., Heth, G., Avnon, Z., and Nevo, E., J. comp. Physiol. [B] 154 (1984) 145.
- 15 Ross, D.E., Kleenman, C.R., Quinn, M., Henschel, A., and Heghnuer, A. H., Medicine 34 (1955) 323
- 16 Arieli, R., Heth, G., Nevo, E., Zamir, Y., and Neutra, O., Expe-
- 17 Arieli, R., Arieli, M., Heth, G., and Nevo, G., Experientia 40 (1984)

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