Incorporation of 1-^{14}C-sodium acetate into different organic acids accumulated in the culture filtrates of *A. niger* 6N3 grown under light, dark and alternate light and dark conditions

Environmental conditions	Acids (cpm/ml)								
	Oxalic	Oxalacetic	Isocitric	Citric	Glycolic	Malic	Fumaric	Succinic	α-ketoglutaric
Control	628	–	216	2400	–	–	–	–	–
Light	–	–	2681	286	2767	500	785	2755	2297
Dark	–	–	–	2903	–	629	704	947	–
Light–dark	–	783	–	1281	–	–	–	614	–
Dark–light	–	612	–	683	–	–	–	1008	–

L.S.100 liquid scintillation counter using toluene fluor[8] as the counting liquid.

Results and discussion. Cultures grown under light conditions showed the presence of α-ketoglutaric, isocitric, glycolic, succinic, fumaric and malic acids. Incorporation of radioactivity was found to be more in isocitric, succinic, α-ketoglutaric and glycolic acids. It was noticed that succinic acid and citric acid accumulated in cultures grown under all the conditions. Accumulation of citric acid was less when succinic acid accumulated in large quantities. Cultures grown in the dark showed a greater accumulation of ^{14}C-labelled carbon atoms in citric acid, while it was found to be the lowest in cultures grown in the light. When dark treatment was followed by alternate light treatment, the labelled carbon atoms showed a lesser accumulation of citric acid compared to the cultures grown in light followed by dark (Table).

Accumulation of ^{14}C in succinic, α-ketoglutaric, isocitric and glycolic acids in large quantities compared to other acids accumulated in cultures grown in the light suggests the functioning of a 'shunt-metabolism'. From the metabolites accumulated, this might correspond to a short cycle within the TCA cycle, viz. the SKI-cycle (Figure) proposed by CANTINO and HORENSTEIN[3] as functioning in light-grown *Blastocladiella emersoni*.

The low radioactive counts given by fumaric acid and malic acid indicate that the normal conversion of intermediates to the next step does not occur properly after the formation of succinic acid, instead the succinic acid shows a tendency for reversible reaction and in turn gets converted to α-ketoglutaric acid or to glyoxalic acid (in turn to glycolic acid) to enter the SKI-cycle. The low accumulation of citric acid in cultures grown under light condition also shows that depletion of intermediates takes place to facilitate the functioning of a short SKI-cycle within the TCA-cycle. Results of the experiment clearly show that light of the visible range to a great extent inhibits or decreases the biosynthesis and accumulation of citric acid in *Aspergillus niger*.

[8] C. H. WANG and D. L. WILLIAMS, *Radiation Methodology in Biological Sciences* (Prentice-Hall, Inc., Englewood Cliffs., New Jersey 1965), p. 167.

Heritability of Adult Weight in the Tsetse Fly *Glossina morsitans morsitans* Westw. (Diptera: Glossinidae)

R. H. GOODING[1] and J. E. HOLLEBONE

Department of Entomology, University of Alberta, Edmonton (Alberta, Canada T6G 2E3), 8 June 1976.

Summary. By assortative mating the heritability of teneral adult weight in a laboratory colony of *Glossina morsitans morsitans* Westw. was estimated to be 0.09 to 0.16. This finding is discussed briefly in relation to published reports on selection against certain size classes in natural populations of tsetses and on the importance of maternal nutrition in determining larval and pupal size.

GLASGOW[2] discussed the variations of tsetses in nature and reviewed evidence that small male *Glossina morsitans morsitans* are removed from natural populations and that there is selection against both large and small female *Glossina swynnertoni* in nature. More recently, PHELPS and CLARKE[3] have shown that there is selection against the smallest males in a natural population of *G. m. morsitans*. This selection eliminates up to 35% of the smallest quartile during the cool months and up to 75% in the hot months.

In view of this selection against certain sizes of tsetses in nature and because of the present search for new methods of control of the tsetse, we undertook this preliminary study to determine the heritability of adult weight in our colony of *G. morsitans morsitans* Westw.

Heritability (h^2) of a trait is defined as the ratio of additive genetic variance (V_A) to phenotypic variance (V_P) (i.e. $h^2 = V_A/V_P$). Theoretical considerations and methods for estimating variance are discussed by FALCONER[4].

[1] We thank Dr. B. S. HEMING for his comments on the manuscript. This work was financed (in part) by a grant from the World Health Organization.

[2] J. P. GLASGOW, *The Distribution and Abundance of Tsetse* (The Macmillan Company, N.Y. 1963) vol. 11, p. 160.

[3] R. J. PHELPS and G. P. Y. CLARKE, Bull. ent. Res. *64*, 313 (1974).

[4] D. S. FALCONER, *Introduction to Quantitative Genetics* (The Ronald Press Company, N.Y. 1960).

Table I. Productivity of the 3 test populations and the sex ratio of their offspring

	Total number pupae produced	Emergence		Sex ratio ♂:♀
		Number	%	
Control	403	339	84	0.87
Small	325	255	78	0.85
Large	403	292	72	0.91

One method of estimating heritability of weight[5] is based upon inbreeding a sample whose mean weight differs from that of the population, inbreeding a randomly selected sample from the population, and then determining the mean weights of the F_1 generation. Heritability of weight is then estimated by calculating the ratio of weight gain (G) to the selection differential (S) (i.e. $h^2 = G/S$). This assortative mating allows one to maintain the experimental animals in groups rather than as individuals, and the offspring, if obtained in numbers, may be used to establish selected lines.

Materials and methods. Our colony of *G. m. morsitans* originated from approximately 1,000 pupae obtained from the Tsetse Research Laboratory, University of Bristol, England in mid-1973. The colony is maintained at 21–24 °C and uncontrolled (but moderate) relative humidity, and produces about 500 pupae/week. The flies are fed 6 days/week on the ears and backs of cross-bred Flemish giant × French lop-eared rabbits. Flies which emerged on one day were chilled the following day, weighed, and then maintained as indicated above. The control males were weighed first and then, on the basis of their weight distribution, males were selected from the colony to make up the sample selected as small (i.e. weighing 14.36 mg or less) and the sample selected as large (i.e. weighing 19.12 mg or more). When the males were about 1-week-old selection of the females was begun in the same manner (small females weighed 16.40 mg or less, large females 19.86 mg or more). Matings were carried out by placing a 7–10-day old male with a 3-day-old female in a corkstoppered shell vial (22 × 95 mm) having a disc of filter paper covering its floor. If mating did not occur immediately the male was replaced with another from the same sample; the pairs were left together about 17 h. Each male that lived 10 days was used once and a few were used twice; on the second occasion as replacements for males that had died or refused to mate. Most small size males were used twice, due to difficulty in obtaining sufficient numbers of them. Pupae were collected once a week and stored in sand in emergence cages. F_1 flies were weighed the day after they emerged.

Results and discussion. Pupae were collected for 15 weeks during which time large flies produced about the same number of pupae as did controls; but small flies produced fewer pupae (Table I). Emergence of adults and sex ratio were approximately the same in the 3 groups (Table I). Using the mean selection differential (i.e. average for the ♂ and ♀ parent), the heritability of weight of adults was found to have a low value, ranging from 0.095 to 0.163 (Table II).

These results indicate that for our *G. m. morsitans* colony, from 9 to 16% of the variation in weight of unfed teneral adults is due to additive genetic factors. Most of the remaining variation is probably due to environmental factors, the most important probably being maternal nutrition. In this respect our results are in agreement with those of previous studies which established that maternal nutrition is an important factor influencing larval weight of *G. palpalis*[6] and pupal weight of *G. m. morsitans*[7], and *G. austeni*[8,9]. Although none of these studies was concerned specifically with determining the heritability of weight, NASH et al.[8] noted that 'certain individuals tended to produce heavy pupae [while others] produced smaller pupae'. They concluded that production of heavy pupae was associated with heavy maternal weight and consumption of large amounts of blood. Using a multiple regression analysis of pupal weight against the mother's weight at emergence and her blood consumption, BOYLE[9] obtained a very high correlation coefficient ($r = 0.896$) and found that blood consumption accounted for 80% of the variance. Presumably, some of the remaining variance would have been accounted for by the heritability of weight.

In commenting upon the significance of heritability, Falconer[4] (on p. 167) wrote 'On the whole, the characters with the lowest heritabilities are those most closely con-

[5] P. SAINT LAWRENCE, J. W. FRISTROM and W. H. PETRI, *Laboratory Studies in Genetics* (W. H. Freeman and Company, San Francisco 1974), p. 118.
[6] H. MELLANBY, Parasitology *29*, 131 (1937).
[7] P. A. LANGLEY, J. Insect Physiol. *14*, 121 (1968).
[8] T. A. M. NASH, A. M. JORDAN and J. A. BOYLE, Bull. ent. Res. *57*, 327 (1967).
[9] J. A. BOYLE, Bull. ent. Res. *61*, 1 (1971).

Table II. Weights of test populations and calculation of heritability of adult weight

		Parental generation			F_1 Generation[b]			
		N	Weight (mg) Mean ± SD	Selection differential[a]	*N*	Weight (mg) Mean ± SD	Gain[c]	Heritability of weight
Control	♂	90	16.74 ± 2.88		91	17.15 ± 2.61		
	♀	90	18.14 ± 2.72		88	18.38 ± 2.61		
Small	♂	90	12.67 ± 1.33	−4.07 } −4.115	77	16.76 ± 2.47	−0.39	0.095
	♀	90	13.98 ± 1.82	−4.16 }	85	17.70 ± 2.45	−0.68	0.163
Large	♂	91	20.48 ± 2.39	+3.74 } +3.535	97	17.75 ± 2.32	+0.60	0.160
	♀	92	21.47 ± 1.20	+3.33 }	109	18.78 ± 3.10	+0.40	0.120

[a] Selection Differential = mean weight of selected group (S or L) − mean weight of the control group (c). [b] Data are for all flies weighed in the F_1 generation. [c] Gain = Mean weight of F_1 of selected group (S or L) − mean weight of F_1 from the control groups (c).

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*

E. NEVO and GIORA HETH[1]

Department of Biology, University of Haifa, Mount Carmel, Haifa 31 999 (Israel), 18 May 1976.

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;

[1] Acknowledgments. We thank M. Avrahami and Y. Sivan for field assistance; H. Bar-El for karyotyping the animals; Z. Gilula for statistical assistance and I. Kornfield and S. Mendlinger for critically commenting on the manuscript. This study was partly supported by a Volkswagenwerk, grant No. A 3: 11: 1434, and an Israel National Academy of Sciences, grant No. 184 to E. Nevo.

[2] E. MAYR, in *Populations, Species and Evolution* (Belknap Press, Harvard University, Cambridge, Mass. 1970).

[3] E. NEVO, Nato Adv. Study Inst. Vertebrate Evolution, Istanbul (1969).

[4] E. NEVO, Israel J. Zool. *22*, 207 (1973).

[5] E. NEVO and H. BAR-EL, Evolution, in press (1976).

[6] E. NEVO, Science *163*, 484 (1969).

[7] J. WAHRMAN, R. GOITEIN and E. NEVO, Science *164*, 82 (1969).