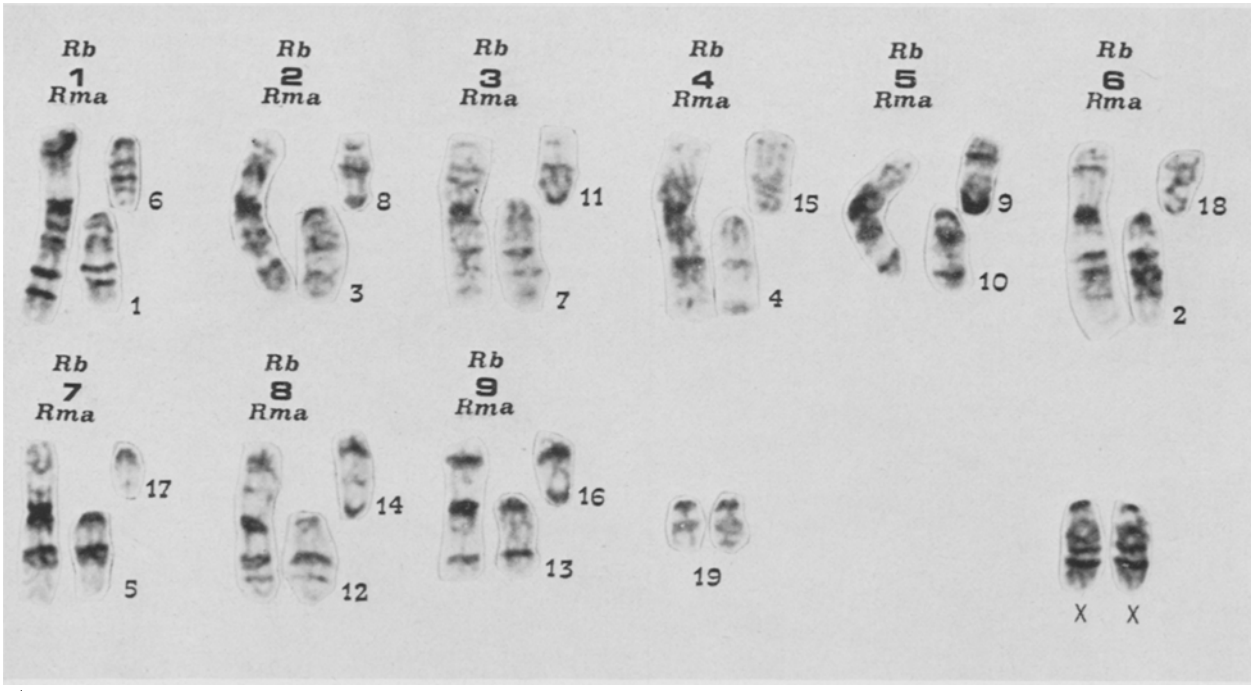


Fig. 2. The Trypsin-Giemsa-banding pattern of the karyotype of a hybrid mouse in which the haploid sets of both parental strains – i.e. 22- and 40-chromosomes – are shown.

drift allows the homozygous condition for chromosomes having undergone Robertsonian translocations to be set up in small isolated populations. The opposite is true in a densely anthropized cultivated plainland environment, or in towns, or in any case, where human activities favour large-scale exohybridization phenomena. Here any Robertsonian translocation mutants are at an immediate disadvantage as regards their reproductive rate because of their gametic aneuploidy[14], and are thus quickly eliminated from the natural populations. Therefore, in order to exist in a natural population, a centric fusion must succeed in reaching a homozygous situation, a condition which is offered by isolation at the end of a valley in a mountainous district.

*Riassunto.* Le nove traslocazioni Robertsoniane, responsabili della trasformazione del cariotipo standard del topo nel cariotipo a 22 cromosomi caratteristico dei *Mus musculus* dell'Appennino Centrale, sono state caratterizzate individuando, attraverso la tecnica del T-G-banding, gli elementi acrocentrici coinvolti nel processo di fusione centrica. Le fusioni Robertsoniane presenti nel popolamento appenninico sono differenti da quelle realizzate sia in *Mus poschiavinus* sia in *Mus musculus* di altre popolazioni Alpine.

E. CAPANNA, M. CRISTALDI, P. PERTICONE and M. RIZZONI[15]

*Istituto di Anatomia Comparata dell'Università di Roma, Via Borelli 50, I–00161 Roma (Italy); and Centro di Studi di Genetica Evolutiva, Istituto di Genetica, Università di Roma, Roma (Italy), 7 November 1974.*

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[15] Authors' address: E. CAPANNA and M. CRISTALDI, Istituto di Anatomia Comparata, dell'Università di Roma; P. PERTICONE and M. RIZZONI, Centro di Studi di Genetica Evoluzionistica del C.N.R. presso l'Istituto di Genetica dell' Università di Roma. Reprint requests should be addressed to: Prof. E. CAPANNA, Istituto di Anatomia Comparata, Via Borelli 50, I–00161 Roma (Italy).

## Lactate Dehydrogenase Isozymes in Xiphophorin Fish Melanoma Conditioned by the Locus *Sd*.*

In the Xiphophorin fish the formation of melanomas occurs by hybridization- or mutation-conditioned derepression of specific loci[1,2]. Biochemical investigations on these genetically defined neoplasms should be able to add information on mechanisms of tumor formation. For the present study lactate dehydrogenase (LDH, EC 1.1.1.27) was chosen as reference enzyme. The LDH-isozyme pattern of various types of tumors is known to be different from that of normal tissues, as has been reviewed by CRISS[3]. In human malignant melanoma, however, no appreciable differences were detected in the LDH-isozymes between the malignant and the normal tissue[4]. This investigation was undertaken to find out whether any changes in the LDH-isozyme pattern occur during the

* Supported by Deutsche Forschungsgemeinschaft through Sonderforschungsbereich 103, Zellenergetik und Zelldifferenzierung (Marburg).

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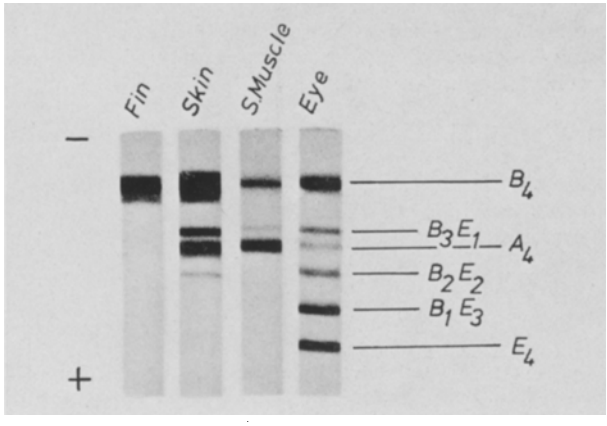


Fig. 1. Lactate dehydrogenase isozymes in *P. maculatus*.

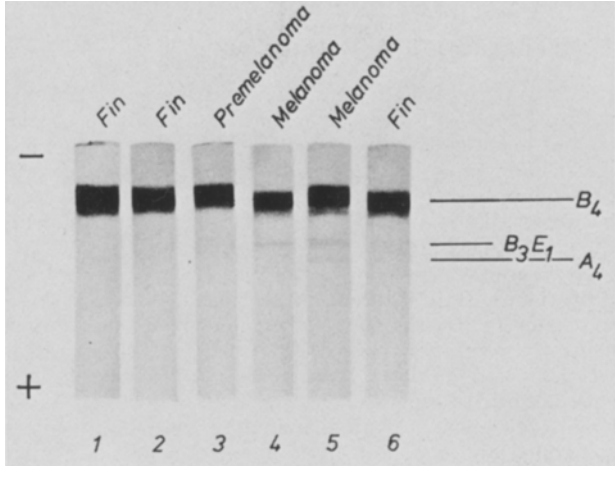


Fig. 2. Lactate dehydrogenase isozymes of 1, normal fin from *P. maculatus*; 2, normal fin from *X. helleri* (wildtype); 3, fin-premelanoma from their $F_1$; 4, fin-melanoma, fast-growing, and 5, fin-melanoma, slow-growing, both from the backcross pregonies; 6, normal fin from the non-tumor segregant.

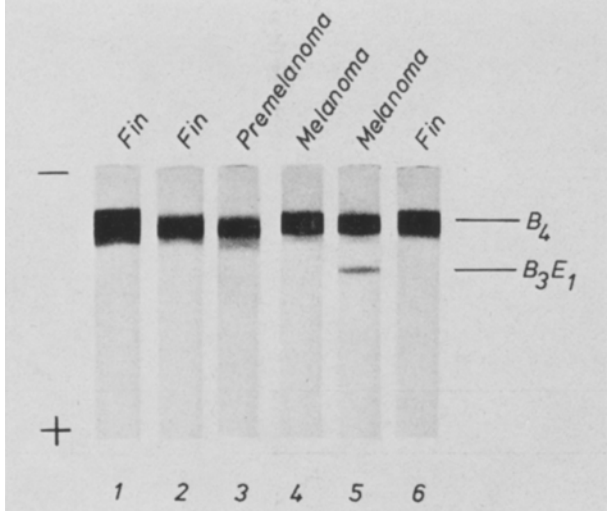


Fig. 3. Lactate dehydrogenase isozymes of 1, normal fin from *P. maculatus*; 2, normal fin from *X. helleri* (albino); 3, fin-premelanoma from their $F_1$; 4, fin-melanoma, fast-growing, and 5, fin-melanoma, slow-growing, both from the backcross progenies; 6, normal fin from the non-tumor segregant.

process of melanomatogenesis in Xiphophorin fish and whether these changes can be correlated with the development of the disease.

*Materials and methods.* For the present study only those premelanomas and melanomas were used which develop on the dorsal fin controlled by the locus *Sd*[1,2]. Normal fins were taken from both parental species, the platyfish (*Platypoecilus maculatus* from Rio Jamapa) carrying *Sd*, which in the normal strains is repressed, and from the Swordtail (*Xiphophorus helleri* from Rio Papaloapan), which does not carry *Sd*. Fin-premelanomas were obtained from the $F_1$-hybrids. Fin-melanomas, both fast-growing and slow-growing, were taken from the sibs of backcrosses to *X. helleri*; also the non-tumor segregants were included as controls. For comparison, normal fins, premelanomas and melanomas from the same genotypes as above were employed involving the albino *X. helleri*[5] instead of the wild type. The tissues were homogenized in 3 parts of 0.1 *M Tris*-HCl, pH 8.0 (w/v), and the homogenates were centrifuged at $20,000 \times g$ for 15 min at 4 °C. The resultant supernatant was subjected to disc electrophoresis in 5% gels according to the method of Davis[6], but omitting both spacer and sample gels. Before application of the samples, the gels were 'prerun' to wash out the ammonium persulphate. Electrophoresis was carried out for 30 min 4 °C with a current of 3.5 mA per gel. The gels were stained according to Shaw and Prasad[7]. Control staining was done using the staining solution without the substrate. The staining was stopped by immersing the gels in 7% acetic acid.

*Results and discussion.* The LDH isozyme pattern of normal fin, and for comparison, skin, skeletal muscle and eye is shown in Figure 1. It is evident that in the fin only the heart-type LDH ($B_4$-isozyme) is present (for designation of isozyme-subunit composition see Whitt and Booth[8], and Scholl[9]). The isozyme patterns of normal fins from the other genotypes involved in this study, as well as from the premelanomas and the melanomas, are presented in Figures 2 and 3. In all tissues the heart-type LDH is the prominent isozyme. In some tumor tissues, additionally 2 minor isozymes, the $B_3E_1$ and $A_4$ are detectable. In the fast and slow-growing melanotic melanomas the patterns were somewhat variable, showing the presence of the minor isozymes $B_3E_1$ and/or $A_4$. However, in the albinotic melanomas a consistent difference could be found between the fast-growing type showing only $B_4$ activity and the slow-growing type, exhibiting in addition to $B_4$ also $B_3E_1$ activity (Figure 3).

From these results, it appears that in LDH-isozymes within the group of the normal and premelanoma fins there is only little or no variation. However, within the fin melanomas some LDH variation can be seen, which may be dependent on the age and growth rate, but not on the genotype.

In many types of malignant neoplasms there occurs a shift in LDH isozymes towards the muscle-type LDH[10-12],

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indicating that these tissues switch to higher activity in anaerobic metabolism. In the *Sd* conditioned melanomas of Xiphophorin fish, no such shift towards the muscle type LDH ($A_4$) could be detected. These results, which are in agreement with those recently reported by PRASAD et al.[4] on human malignant melanoma, suggest that melanoma type neoplasms may not be as dependent on anaerobic metabolism for their energy as many others.

*Zusammenfassung.* Elektrophoretische Untersuchungen an benignen und malignen Rückenflossenmelanomen sowie an normalen Rückenflossen lebendgebärender Zahnkarpfen zeigen, dass in den Geweben in erster Linie die für gut mit Sauerstoff versorgtes Gewebe typische Herz-LDH ($B_4$-Isoenzym) vorkommt. Die für schlecht mit Sauerstoff versorgtes Gewebe typische Muskel-LDH ($A_4$-Isoenzym) wird nur in manchen der untersuchten Gewebe gefunden, und ist auch dort nur in sehr geringer Menge vorhanden. Die Melanome scheinen also im Gegensatz zu vielen anderen Neoplasmen anderer Systeme aeroben Stoffwechsel durchzuführen.

M. R. AHUJA[13], M. SCHWAB and F. ANDERS

*Genetisches Institut der Justus Liebig-Universität Giessen, Leihgesterner Weg 112–114, D-6300 Giessen (German Federal Republic, BRD), 28 October 1974.*

[13] On leave from Pant University of Agriculture and Technology, Pantnagar, India. Richard Merton – Guest Professor through Deutsche Forschungsgemeinschaft.

## Selection in Parthenogenetic Lines of *Asplanchna sieboldi* (Leydig) 1854 (Rotatoria)

Most authors who were concerned with heterogonic Rotifers assumed that amictic females reproduce by ameiotic parthenogenesis, and this view was supported by extensive research of TAUSON[1], Whitney[2], SHULL[3] and others. Therefore the progeny of a founder female was considered a clone, that is a genetically homogeneous group, where all the variability, appearance of mictic females and cyclomorphosis included, is determined by the action of environmental conditions on a single genotype. Such purely phenotypical interpretation has been applied until recently not only to Rotifers, but to widely different groups, such as Aphids and Daphnids.

A few authors, on the other hand, have supported an interpretation of heterogony on the basis of natural selection, beginning with WEISMANN[4–6] and LAUTERBORN[7], until BACCI[8,9] approached the problem in terms of population genetics. This approach allowed COGNETTI[10,11] to demonstrate the existence of different genetic pools in population of Aphids living under different climatic conditions.

Following these results, we wanted to verify whether in parthenogenetic lines of Rotifers variability is due to environmental factors only, or is due to the different reaction norms which are shown by different genotypes within progenies, which until now are generally considered as genetically homogeneous.

In order to discriminate environmental from genetic components of variability, selection experiments have been carried out within parthenogenetic lines, following the classic scheme for the study of hereditability.

*Asplanchna sieboldi* (Leydig), was collected in Lago Sirio (Ivrea, Italy) in September 1972 and mass cultures were kept, following the method described by BIRKY[12], using *Paramecium aurelia* and green unicellular algae (*Oöcystis* sp.) as food. Different parthenogenetic lines were obtained from single resting eggs, which were taken from such mass cultures and they were kept under controlled and constant environmental conditions, until they showed a certain degree of variability. The experiments started with 5th generation, when selected parents were isolated and the analysis of their offspring carried on.

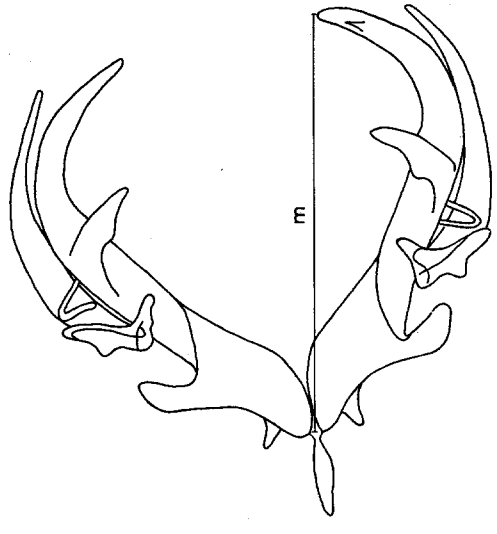


Fig. 1. Length of mastax (m).

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Results of selection experiments in parthenogenetic lines of *Asplanchna sieboldi* (Leydig)

| Line | $h^2$ | *n* | *t* | *P* |
|---|---|---|---|---|
| 1a | 0.97 | 79 | 3.60 | $< 0.01$ |
| 2a | 0.86 | 38 | 2.23 | $< 0.05$ |
| 2b | 1 | 38 | 2.37 | $< 0.05$ |
| 3a | 0.81 | 81 | 3.10 | $< 0.01$ |
| 3b | 0.83 | 79 | 2.12 | $< 0.05$ |