

large chromosomes (18 pairs in *Xenopus* and 9 in *Hymenochirus*): if it is true, as regards the Amphibia, that evolution is generally accompanied by a reduction of the chromosome material<sup>6,15</sup>, this fact may constitute a characteristic of karyological primitiveness with respect to the Microhylidae, the Pelobatidae and the higher Anura, which have, at the most, 7 pairs of large homologues in the more primitive forms and a smaller number in the higher forms.

(3) As previously stated, the Pelobatidae show various affinities with the Leptodactylidae; compared with the latter, they do not appear more primitive. What seems more interesting is that certain Leptodactylidae, in their turn, show karyological relationships, on the one hand with the Hylidae and on the other hand (certain Australian Leptodactylidae) perhaps with the Bufonidae and the Atelopodidae<sup>16,17</sup>. All these families therefore constitute a group of karyologically associated forms, perhaps converging in a leptodactiloid (or pelobatoid) stock; they form a natural group, also from the anatomo-comparative point of view, because they comprise the higher Anura having an arciferal shoulder girdle<sup>1</sup>.

(4) The Microhylidae, karyologically heterogeneous but not obviously primitive, may be related to the Ranidae, which are related to the Hyperolidae<sup>13</sup>. This vast group of forms, comprising the firmisternal Anura<sup>1</sup>, is contrasted, from its extent and the number of species, with the group discussed in section (3), together with which it comprises all the higher Anura.

The fact that the Bufonidae, considered 'a pedomorphic offshoot' of the primitive Australian Leptodactyli-

dae<sup>1</sup>, approach the karyotype of the families of group 4, may signify that (perhaps among the Leptodactylidae) there may exist forms of which the karyotype has characteristics intermediate between these of the 2 large groups of families (cf. <sup>19</sup>).

*Riassunto.* Lo studio kariologico di specie di Anuri appartenenti alle famiglie più primitive dell'ordine conduce a formulare le seguenti ipotesi: (1) Ascaphidae e Discoglossidae sono strettamente collegati fra loro, e si differenziano da quasi tutti gli altri Anuri; (2) i Pipidae africani e quelli americani sono molto diversi fra loro; (3) i Pelobatidae hanno varie affinità kariologiche con i Leptodactylidae, e questi con gli Anuri superiori prevalentemente arciferi; (4) i Microhylidae hanno rapporti kariologici con i Ranidae.

A. MORESCALCHI

*Istituto di Istologia ed Embriologia dell'Università di Napoli (Italy), 26 March 1968.*

<sup>15</sup> A. E. MIRSKY and H. RIS, *J. gen. Physiol.* **34**, 451 (1951).

<sup>16</sup> A. MORESCALCHI and G. GARGIULO, *Rc. Accad. Sci. fis. mat.*, Napoli, in press.

<sup>17</sup> F. H. ULLERICH, *Chromosoma* **21**, 345 (1967).

<sup>18</sup> R. LAURENT, *Bull. Mus. r. Hist. nat. Belg.* **18**, 1 (1942). — O. A. REIG, *Act. Trab. prim. Congr. sudam. Zool.* **1**, 271 (1961).

<sup>19</sup> Research carried out through a contribution from the C.N.R. (Genetics Enterprise).

## The Mitotic Chromosomes of the Lamprey *Mordacia mordax* (Agnatha: Petromyzonidae)

As representatives of the most primitive group of living vertebrates, the lampreys are of considerable phylogenetic interest. However, little is known about the cytogenetics of this group because the very small size and high number of chromosomes has made the analysis of karyotypes extremely difficult. It would appear that centromere positions and length variation of chromosomes within complements have not been clearly established for any lampreys. ZANANDREA and CAPANNA<sup>1</sup>, in the only study which deals in some detail with lamprey chromosomes, have given counts for 3 species within the genus *Lampetra*. Other references to lamprey diploid numbers<sup>2-4</sup> also refer to northern-hemisphere species.

The exclusively southern-hemisphere genus *Mordacia* has been regarded by most authors as sufficiently different from northern genera to merit being placed in at least a separate sub-family<sup>5</sup>. The current study was carried out on *M. mordax* (Richardson), to provide morphological details of the chromosomes of a member of the Petromyzonidae and to determine the diploid number of 1 of the 4 species of southern-hemisphere lampreys for comparison with those already recorded for northern species.

*Material and methods.* *M. mordax* occurs in rivers in south-eastern Australia. The difficulty of obtaining adults precluded the use of gonadal material for the study of mitotic and meiotic configurations. Ammocoete larvae were therefore employed for counts, the source of material being the epithelial cells lining the gut. Tissue was re-

moved from animals, after they had been placed in 0.01% colchicine solution for 5 h, and macerated in saline. The resulting cell suspension was treated hypotonically and fixed in 3:1 methanol-glacial acetic acid. Counts were made from photographs of air dried preparations stained with either Giemsa or 2% aceto-orcein. Bright field and phase microscopy helped to distinguish individual chromosomes which could not be resolved from photographic prints.

*Results and discussion.* Many preparations, made from the guts of numerous ammocoetes, were examined in order to find spreads clear enough for accurate counting. The distribution of counts obtained from the 23 satisfactory spreads is shown in the Table.

Despite the small size, large number and occasional overlapping of the chromosomes, a range in counts of 75-79, and the occurrence of 76 chromosomes in more than half the total number of counts, indicated that

<sup>1</sup> G. ZANANDREA and E. CAPANNA, *Boll. Zool.* **31**, 669 (1964).

<sup>2</sup> L. A. CHUBAREVA, *Vest. leningr. gos. Univ.* **9**, 83 (1957).

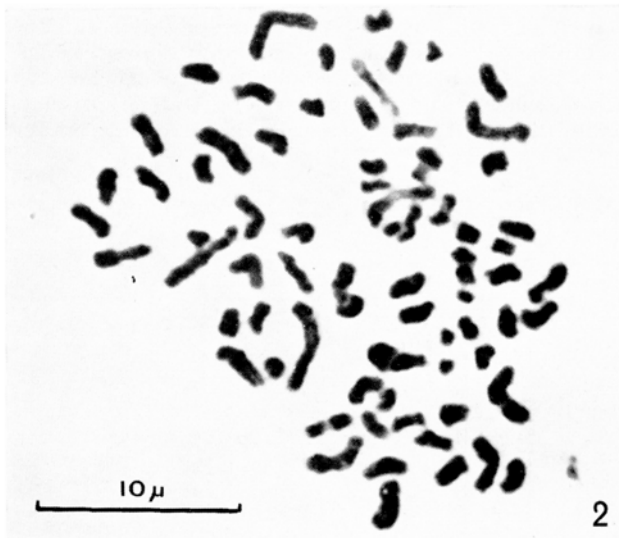
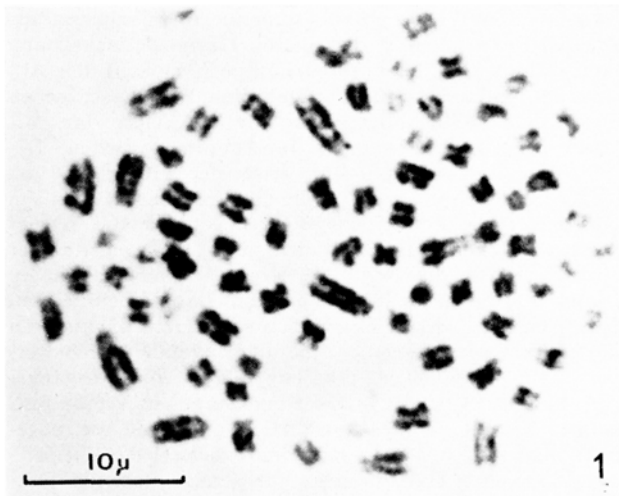
<sup>3</sup> W. J. R. LANZING, *Studies on the River Lamprey, Lampetra fluvialis, during its Anadromous Migration* (Uitgeversmaatschappij Neerlandia, Utrecht 1959).

<sup>4</sup> S. NOGUSA, *Mem. Hyogo Univ. Agric.* **3**, 1 (1960).

<sup>5</sup> I. C. POTTER and R. STRAHAN, *Proc. Linn. Soc. Lond.* **179**, in press.

Distribution of somatic chromosome counts for 23 spreads

Diploid numbers					
No. of counts	75	76	77	78	79
	5	12	3	2	1



Figs. 1 and 2. Mitotic metaphases in gut cells of ammocoetes of *M. mordax*. Figure 1. Chromosomes showing distinct chromatids and many showing the median position of the centromere. Figure 2. Spread showing variation in length of chromosomes within a single complement.

preparative techniques had not greatly disrupted the spreads. It should be mentioned, however, that 2 spreads were rejected because they had widely scattered chromosomes and drastically lowered chromosome numbers, a result that could be clearly attributed to rupturing of nuclei during spread preparation. The fact that the distribution of chromosome numbers is not negatively dis-

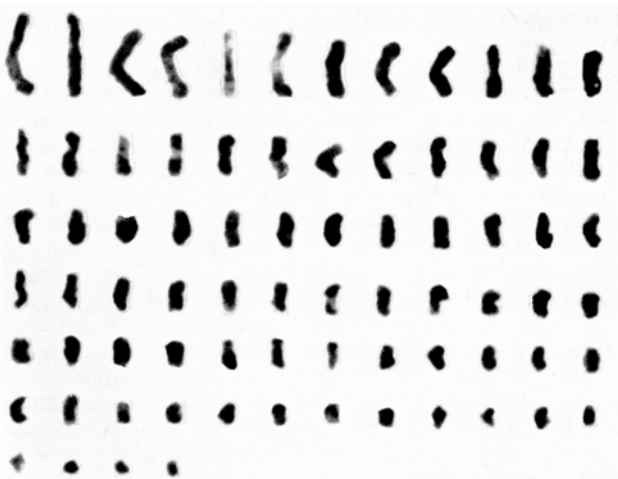


Fig. 3. Serial alignment of somatic metaphase chromosomes made from the spread shown in Figure 2.

torted is probably due to difficulties in the accurate counting of large numbers of very small chromosomes.

The location of the centromeres which is easily seen in many of the chromosomes in Figure 1, indicates that the majority of chromosomes are metacentric or sub-metacentric. Figures 2 and 3 show the considerable variation in the length of the chromosomes in a single complement. Furthermore, the longest homologous pair is clearly distinguished in spreads containing chromosomes that are not highly contracted.

The high diploid number in *M. mordax* and *Entosphenus reissneri*<sup>4</sup> and the extremely high numbers recorded for 3 *Lampetra* species, warrant the generalization that the lampreys as a group are characterized by high chromosome numbers. Diploid numbers as high as 76 for *M. mordax* have been recorded from fishes, amphibians, birds and mammals but a diploid complement of 156 in *L. fluviatilis*<sup>1</sup>, appears to be among the highest, if not the highest, recorded for a member of the Phylum Chordata. However, it should be noted that there is considerable discrepancy in other figures given for the latter species<sup>2,3</sup>.

Most of the chromosomes of *M. mordax* are metacentric or submetacentric, while NOGUSA has reported that those of *E. reissneri*<sup>4</sup> 'seem to be of telomitic nature'. If the centromere position in the chromosomes of these 2 species does, in fact, differ so markedly, a detailed study of chromosome morphology in other northern- and southern-hemisphere lampreys is desirable, since it may present important information regarding phylogenetic relationships within the group.

**Résumé.** L'étude des chromosomes somatiques de la lamproie de l'hémisphère sud, *Mordacia mordax* (Richardson), montre que l'équipement chromosomique diploïde de cette espèce est composé de 76 chromosomes très petits. La plupart des chromosomes sont métacentrique ou bien submétacentrique. Ces chromosomes sont différents en nombre de ceux décrits pour les lamproies de l'hémisphère nord.

I. C. POTTER, E. S. ROBINSON  
and SHIRLEY M. WALTON

School of Zoology, University of New South Wales  
(N.S.W., Australia), 10 May 1968.