The Mitotic Chromosomes of the Lamprey, *Petromyzon marinus* L.

There are comparatively few records of the chromosome numbers of lampreys, mainly due to the difficulty of counting the large numbers of very small chromosomes that are generally found in this group[1]. The limited data present a confusing picture. Counts for *Lampetra fluviatilis* range from 60[2,3] to 156[4], and for *Entosphenus reissneri* the range is from 94–96[5] to 165–174[6]. There is also variation in the details of the centromere position, even within a single species. *E. reissneri* is regarded by NOGUSA[5] as having chromosomes of 'telomitic nature' while SASAKI and HITOTSUMACHI[6] recorded the occurrence of 'some mediocentrics'. POTTER, ROBINSON and WALTON[7] showed that the majority of the chromosomes of the Southern Hemisphere species, *Mordacia mordax*, were metacentric or submetacentric. On the other hand the centromere was described as being 'subterminal to terminal' in the chromosomes of the North American lamprey, *Lampetra aepyptera*[8].

In this study, the chromosomes of specimens of *Petromyzon marinus* L. from the rivers Teme and Tawe in England were studied using air-dried preparations of the gill epithelium stained with 2% aceto-orcein[8]. Although over 200 preparations were examined, only 20 sets of chromosomes were satisfactory for counting, one of which is shown in Figure 1. The distribution of these counts from 7 different animals is listed in the Table.

The majority of counts lie between 164 and 168 but there is no sharp mode. However, a diploid number of 168 was obtained in more cases than any other value and probably constitutes the true number for 2 other reasons. Firstly, it was the count recorded for the 3 best spreads and secondly it represents a modal value at the upper end of a distribution with a pronounced negative skew. It seems likely that the problem of discerning a few of the overlapping chromosomes in some of the spreads is the reason for the counts between 163 and 167. Three other spreads giving counts of 140–150 presumably represent ones in which some chromosome loss had occurred. The chromosomes of each individual complement showed considerable variation in length with the largest chromosomes being as long as 2.7 μ while the smallest were only 0.7 μ in length, a range very similar to that found in *L. aepyptera*[8].

The position of the centromere could not be resolved in the vast majority of preparations. However, 2 spreads, in which the chromosomes were visible as chromatids and which were not as contracted as in other preparations, showed that a few of the longer chromosomes were metacentric. Additional evidence for some metacentry amongst the complement was obtained from embryonic tissue, whose rod-like chromosomes occasionally showed a marked central constriction. Although it was not possible to establish definitely whether any of the chromosomes were

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[1] E. S. ROBINSON and I. C. POTTER, Copeia *4*, 824 (1969).

[2] L. A. CHUBAREVA, Vest. leningr. gos. Univ. *9*, 83 (1957a).

[3] L. A. CHUBAREVA, Dok. Akad. Nauk. S.S.R. *112*, 945 (1957b).

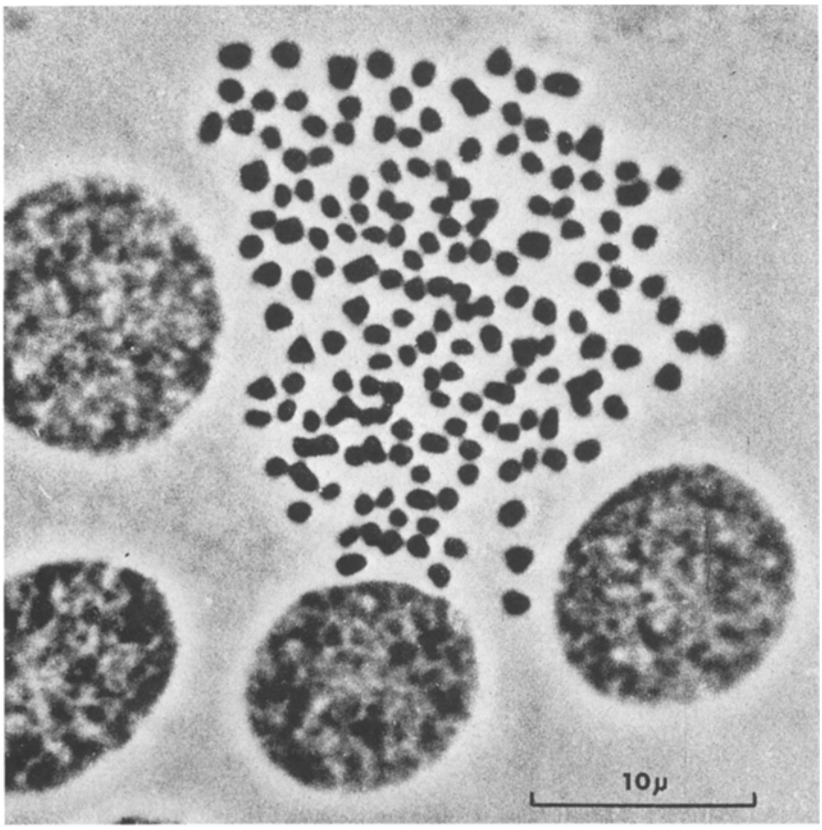
[4] G. ZANANDREA and E. CAPANNA, Boll. Zool. *31*, 669 (1964).

[5] S. NOGUSA, Mem. Hyogo Univ. Agric. *3*, 1 (1960).

[6] M. SASAKI and S. HITOTSUMACHI, Chromosome Information Service *8*, 22 (1967).

[7] I. C. POTTER, E. S. ROBINSON and S. M. WALTON, Experientia *24*, 966 (1968).

[8] W. M. HOWELL and T. E. DENTON, Copeia 1969 (2), 393 (1969).



Chromosome spread from gill epithelium of an ammocoete larva of *Petromyzon marinus* L., $2n = 168$.

acrocentric, some indication of the presence of a terminal or subterminal centromere was obtained in several cases.

The count of 168 for *P. marinus* appears to be the highest diploid number yet recorded for a vertebrate species for which there are at least several counts. This value is close to that obtained for *Lampetra aepyptera*[8] and to the 3 counts recorded for *E. reissneri* by Sasaki and Hitotsumachi[6]. The counts obtained for 3 *Lampetra* species from Italy by Zanandrea and Capanna[4] are also extremely high, ranging from 142 for *Lampetra zanandreai* to 156 for *Lampetra fluviatilis*. While the chromosome numbers of lampreys are generally very high, more work is required to establish the reason for the very considerable intraspecific differences recorded for *L. fluviatilis*[2–4] and *E. reissneri*[5,6]. The fact that far higher diploid numbers, than are apparently present in any other vertebrate group, have now been recorded for species from 3 different genera of lampreys lends support to the view that polyploidy played a role in lamprey evolution, as has already been suggested by Ohno, Wolf and Atkin[9], Howell and Denton[8], and Robinson and Potter[1].

The diploid number of 168, and the presence of at least a few metacentric chromosomes in the karyotype of *P. marinus*, should be considered in the context of polyploidy. Ohno et al.[9] suggested that the ancestral vertebrate genome contained 48 acrocentric chromosomes, the situation found in hagfishes and several teleosts. They thus postulated on the basis of Nogusa's[5] counts, that *E. reissneri* was a tetraploid. *P. marinus* may therefore represent an octoploid condition in which some reduction in the total number of chromosomes has occurred through centric fusions. While the numbers of chromosomes in lampreys is now beginning to form a pattern, it is difficult to make generalizations about the centromere position. This is epitomized by the conflict between the descriptions for *E. reissneri* with respect to this character, and the difference between *P. marinus*, which possesses some metacentric chromosomes, and *L. aepyptera* which apparently does not. It is quite evident that further work is required on the chromosome morphology of lampreys before the significance of centromere position can be confidently discussed in terms of the overall pattern of karyotype evolution within the group.

Distribution of counts for the diploid chromosome number of *Petromyzon marinus* in gill epithelium

Diploid Nos.:	163	164	165	166	167	168	169	170	171
No. of counts:	1	4	3	3	2	5	1		1

Résumé. L'étude des chromosomes somatiques de la lamproie, *Petromyzon marinus* L. a montré que l'équipment chromosomique diploïde de cette espèce est composé de 168 chromosomes très petits, dont queleues-uns des plus grands sont métacentriques. Le chiffre semble représenter le plus grand nombre de chromosomes trouvé dans une espèce de Vertébré. On a comparé les chromosomes de *P. marinus* avec ceux des autres espèces de lamproies.

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[9] S. Ohno, V. Wolf and N. B. Atkin, Heriditas *59*, 169 (1968).

[10] Holder of a Science Research Council Fellowship.

Prediction and Explanation of Chemical Mutation Sites

I wish to report a possible cause for the location of sites on nucleic acid molecules particularly susceptible to chemical mutation. As a model of the approach, I propose a consideration of the formation of mutations in tobacco mosaic virus (TMV) vulgare coat protein by the action of nitrous acid on TMV ribonucleic acid[1].

Since one quickly observes that, for example, of the many arginine residues present in this protein, only 4 were replaced in mutation, it appeared therefore that only the RNA chemical environment about the codon for arginine might disclose a possible explanation for the reaction specificity. This same argument may presumably hold true for all other chemical mutations.

Since TMR-RNA codes directly its coat protein, a knowledge of the protein sequence allows the sequence of the RNA to be written – with, however, several triplets per amino-acid since the genetic code is degenerate. Which of the actual triplets comprise the RNA can only be determined in the laboratory and has not yet been done.

Clearly, neighboring codon patterns which fit the RNA sequence described herein containing all possible codons will obviously fit the single true sequence. Therefore, the RNA sequence about each of the codon corresponding to each mutated amino acid was examined with all of the codons listed. Continuing with the above example, in the case of TMV coat protein, arginine underwent the largest number of mutations (4), all to glycine.

Consideration of the codons of these 2 amino acids immediately indicate the location of the nucleotide which has undergone reaction with nitrous acid, as follows:

Arg	*Gly*
AGA	GGU
AGG	GGC
CGU	GGA
CGA	GGG

The change corresponds to position one of the triplets and involves a base-pair substitution of the transition type.

One next lists all of the RNA regions corresponding to the mutation in question. Thus, in this case, the RNA

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[1] A. Tsugita and H. Fraenkel-Conrat, Proc. Nat. Acad. Sci. *46*, 636 (1960); A. Tsugita, J. Mol. Biol. *5*, 284 (1962); A. Tsugita and H. Fraenkel-Conrat, J. Mol. Biol. *4*, 73 (1962).