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On the karyology of trematodes of the genus *Microphallus* and their intermediate gastropod host, *Littorina saxatilis* II. Karyological study of *Littorina saxatilis* (Gastropoda: Prosobranchia)

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Abstract

The karyotypes of *Littorina saxatilis* Olivi specimens from exposed areas of the Yarnyshnaya inlet littoral (the Kola Peninsular, USSR) consist of 34 chromosomes, i.e. 10 pairs of meta- and submetacentrics and 7 pairs of subtelo- and telocentrics. Two pairs of bi-armed homologues are much bigger than the others. On the whole these karyotypes are similar to those of the individuals from the Swedish and English populations.

Introduction

Littorina saxatilis Olivi is a standard object for studies in morphological, physiological and genetical polymorphism (Snyder, Gooch, 1973; Smith, 1981; Janson, 1983a; Janson & Ward, 1984, etc.). To date, specimens of only two *L. saxatilis* populations, from the Swedish coast and Cornwall (England), were investigated karyologically (Janson, 1983b). It was shown that there is no difference between two morphs, E and S in chromosome number, 2n = 34, or in chromosome morphology. The S-morph inhabits sheltered boulder inlets, and the E-morph inhabits exposed rocky shores of the Swedish coast.

The purpose of this work is to make a karyotypic analysis of *L. saxatilis* individuals from the population inhabiting the south-western coast of the Barentz Sea. Its population substructure depends mainly on trematode invasion (Sergievsky, 1985; Granovitch, 1986; Mikhailova *et al.*, 1988). Usually *L. saxatilis* is infected by the microphallids of the '*pygmaeus*' group (Galaktionov, Dobrovolsky, 1984). The karyotypes of the microphallids were described in the preceding paper (Birstein & Mikhailova, 1989, 1990).

Material and methods

The *L. saxatilis* specimens were taken from exposed areas of the Yarnyshnaya inlet littoral near the Murmansk Institute of Marine Biology (USSR). Only the uninfected molluscs were used for the karyological investigation. After breaking the shells of 5–10 molluscs, these were placed in a 3% colchicin solution in sea water for 6 h. In the same manner, 10 embryos taken from the dissected brood pouches of females were incubated. Then, the prepared ctenidia, pericard, kidneys and fragments of the generative system of adults, or whole embryos were placed in distilled water or in a 0.5% KCl solution for 40 min (with the hypotonic treatment, the results were better). The

tissues or embryos were fixed in a cold ethanol-glacial acetic acid mixture, 3 : 1. After that they were suspended in 50% acetic acid, the suspension was dropped on glass slides and air-dried. The process of staining and analyzing of the preparations obtained was described in the previous papers (Birstein & Mikhailova, 1989, 1990).

Results

Figure 1 shows the chromosome set of *L. saxatilis*. The karyotype of all individuals investigated consists of 34 chromosomes, 10 pairs of bi-armed meta- and submetacentrics and 7 pairs of subtelo- and telocentrics. The homologues of two large pairs, the metacentrics No. 1 and submetacentrics No. 2, are considerably greater than the other chromosomes. The homologues Nos. 11, 12, 15, 16 seem to have very short arms, while the other small chromosomes are apparently telocentrics. The karyotypes of adults and embryos are the same. In some cases sets consisting of 35 chromosomes were found in the embryos. The latter result was, probably, an artifact of slide preparation.

Discussion

The chromosome number, 2n = 34, in *L. saxatilis* specimens investigated by us does not differ from that in individuals from the Swedish and English coasts (Janson, 1983b). Janson (1983b) did not find any karyological difference between periwinkles of these two distant west European populations, nor between two (E and S) morphs of the molluscs from the Swedish coast.

On the whole the karyotypes of the individuals from the Barentz Sea coast are the same. But a small difference between the karyotypes described by us and by Janson seems to exist. Thus, telocentrics are absent in the karyotypes of molluscs from the Swedish and English populations, while in the karyotypes of the Barentz Sea periwinkles three small pairs, most likely, consist of telocentrics.

As for the diploid number in other littorinid species, in most of them it equals 34 (Table 1). The karyotypic structure changes little in the *Littorina* species, as in the Mediterranean *L. neritoides*, as well as in *L. saxatilis*, two pairs of metacentrics are greater than the other chromosomes of the set and the karyotype of *L. neritoides* includes 5 pairs of subtelo- and one pair of telocentrics (Thiriot-Quiévreux & Ayraud, 1982). But as the genome size differs appreciably between the two species studied, *L. littorea* and *L. irrorata* (C = 1.0 and 0.82 pg respectively, Hinegardner, 1974), one may conclude that considerable changes in the amount of different genome fractions took place in this genus during speciation events, and these processes were accompanied by chromosome rearrangements.

It seems possible that more intensive karyological changes occurred in other littorinid genera since in two *Nodilittorina* species chromosome number differs significantly, 2n = 30 and 36 (Nishikawa, 1962) (Table 1). *Rissoa ventricosa*, representing Hydrobiidae, related to the littorinids, has a karyotype consisting of 32 chromosomes, including the heteromorphic pair of XY-chromosomes (Thiriot-Quiévreux & Ayraud, 1982). Similar diploid numbers are also characteristic of other Mesogastropoda genera. For instance, the Naticidae species have 32–34 chromosomes (Patterson, 1969; Vitturi *et al.*, 1982). At the same time there are tetraploid species within the Mesogastropods, such as *Capulus hungaricus* (Capulidae), 2n = 62 (Vitturi *et al.*, 1982).

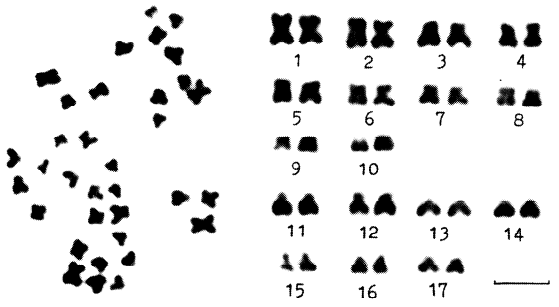

Fig. 1. Littorina saxatilis metaphase plate (a) and karyotype (b). Bar represents 10 μm.

Table 1. Chromosome numbers of the Littorinidae species.

	2n		
Littorina strigata	34	Japan	Nishikawa, 1962
Littorina brevicola	34	Japan	Nishikawa, 1962
L. neritoides	34	France (the Mediterranian Sea)	Thirioit = Quiévreux, Ayrand, 1982
L. neritoides	♀♀34(XX) ♂♂33(XO)	Italy (the Mediterranean Sea)	Vitturi *et al.*, 1988
L. obtusata	33–34	Sweden	Janson, 1983
L. saxatilis	34	Sweden	Janson, 1983
	34	England	Janson, 1983
	34	Kola Peninsular, USSR (the Barentz Sea)	This paper
L. punctata	32	Italy (the Mediterranean Sea)	Vitturi *et al.*, 1986
Nodilittorina granularis	36	Japan	Nishikawa, 1962
N. picta	30	The same	The same

Interspecies karyological polymorphism is very rare in the molluscs. In marine *Nucella lapillus* (Prosobranchia: Muricidae) there are populations of individuals having sets of 26 or 36 chromosomes (Staiger, 1957; Hoxmark, 1970; Bantock & Cockayne, 1975). Centric fusions do occur in this species with only chromosomes of some pairs being fused. The morphology of some chromosomes in the karyotypes of *L. saxatilis* from distant populations seems to be a little different. Such difference can be a result of small inversions, which may be caused by the influence of trematode infection. Thus, the trematodes can exert a mutagenic effect on their host (Shubber & Salih, 1987). But the action of microphallids on *L. saxatilis* can be more complicated. The trematode infection changes the behavior of *L. saxatilis*: the infected molluscs do not move as fast as the uninfected ones and become preys of birds (Mikhailova *et al.*, 1988). Therefore, trematodes and birds seem to be selective factors, which gradually change the genetical structure of *L. saxatilis* populations. As a result of this selection pressure a karyological differentiation can emerge in remote populations. But for the final conclusions it is necessary to do detailed morphometric examination of karyotypes of specimens from different populations of *L. saxatilis*.

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