

Short Note

Karyological Analysis of Two Allopatric Populations of Planarian *Polycelis felina* (Daly.) in Croatia

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Accepted January 27, 2004

KALAFATIĆ M., KOPJAR N., ZRNA G., ZUPAN I., KOVAČEVIĆ G., FRANJEVIĆ D. 2004. Karyological analysis of two allopatric populations of planarian *Polycelis felina* (Daly.) in Croatia. Folia biol. (Kraków) 52: 131-133.

The results of preliminary karyological investigations of two geographically distant and morphologically slightly different *Polycelis felina* (Daly.) populations from central Croatia are reported. The results have shown that individuals of both populations are diploids, with the same chromosome number in their neoblasts ($2n=18$). Their karyotypes were composed of nine chromosome pairs, three of them are metacentric and the other six are submetacentric. Statistical evaluation of data indicated that both populations of *Polycelis felina* (Daly.), despite minor differences observed between them, belong to the same karyological biotype.

Key words: Planarian (*Polycelis felina* Daly.), karyological analysis, neoblasts.

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Planarians are an important component in the aquatic ecology of unpolluted streams. The most commonly studied are the freshwater triclads (class Turbellaria, phylum Platyhelminthes). They are well adapted to strong water flow and usually inhabit clean water rich in oxygen, with a temperature range between 6-15°C (ROCA *et al.* 1992). Planarians have long been known to possess remarkable regenerative abilities. Besides differentiated cells, adult planarians possess totipotent stem cells (neoblasts) that remain mitotically active during their whole life span. They are scattered throughout the parenchyma and their function is to replace cells lost during the course of cell turnover in the intact animal (BAGUNA 1981). In planarians, variation in chromosome number is related to the reproductive strategy, while the degree of polyploidy changes relative to climatic conditions. Ecological factors such as temperature, light and food have a strong influence on the type of reproductive mode in planarians. Some planarian species reproduce only sexually and some asexually, with architomy (BENZAZZI 1973). The species *Polycelis felina* (Daly.) has been reported to repro-

duce both sexually and asexually. The existence of diploid, triploid and some aneuploid populations of *Polycelis felina* (Daly.) was also reported previously. In this study two geographically distant populations of *Polycelis felina* (Daly.) were analyzed karyologically by assessing the morphology and number of chromosomes in neoblasts. Since the planarians studied showed slight morphological differences (in color and body shape), the degree of polyploidy and the possible existence of different karyological biotypes among them was determined.

Material and Methods

Polycelis felina (Daly.) individuals that were surveyed in this work were collected at two geographically distant sites in central Croatia: The Gračanski ribnjak pond near Zagreb and spring of Lonja River, situated approximately 70 km north-east of Zagreb. After field collection, the animals were kept and fed in the laboratory in glass dishes filled with aquarium water, in order to maintain

their normal behavior and morphological appearance. They were 10-12 mm long. Each animal was transversally cut into four pieces with a surgical scalpel. The planarian pieces were kept for three days in clean water to regenerate. For the karyological analysis, three-day-old planarian regenerates were treated with a 0.002 g/l Colcemide solution (Ciba) and placed on clean glasses. Every regenerate was treated with a 2% acetic acid solution, for the fixation and disintegration of the tissue. Staining with lacto-orcein was carried out for 45-60 min at room temperature (BENZAZZI *et al.* 1972). Slides were prepared by using the "squash" technique. The preparations were frozen in liquid carbon dioxide, dried in room temperature and embedded in euparal. Chromosome arm lengths were measured and their mean values were calculated. Statistical significance of the obtained results was evaluated by using analysis of variance (Statgraphics package).

Results and Discussion

Depending on ecological factors, freshwater planarians may reproduce sexually or asexually. For example: temperature, food availability, seasonal changes or light intensity may influence the reproductive mode in *Dugesia* (PALA *et al.* 1987). Planarians that reproduce asexually often show variation in chromosome number. Frequent variation in chromosome number and the appearance of more than one karyological biotype are characteristic of organisms that reproduce both sexually and asexually. Allopatric populations that inhabit geographically distant habitats, after a long period of time, may also show differences in chromosome number. Since *Polycelis felina* (Daly.) reproduces both sexually and asexually, and since the studied individuals were collected in geographically distant and ecologically isolated habitats, variation in chromosome number can be expected.

According to data obtained in the present investigation, regeneration processes in the individuals of both populations proceeded in a similar way. A few hours after the dissection, regeneration blastemas, rich in neoblasts, were formed in the injured areas. Cytological analyses showed that the neoblasts of the individuals collected from both locations contained 18 chromosomes (Figs 1 & 2). No variation in chromosome number was observed. All individuals studied were stable diploids $2n=18$. The neoblast karyotype in both populations consisted of nine chromosome pairs. Three pairs (e.g. 1st, 2nd and 8th) are metacentric and the other six submetacentric (Fig. 3). The mean values of total length of single chromosomes for both populations are listed in Table 1. Small differences

in chromosome length observed between the two populations were not statistically significant and could be accounted for by cytostatic use during chromosome preparation. The results of the statistical analysis confirmed that these two populations of the same species belong to the same karyological biotype. The F-ratio value estimated by analy-

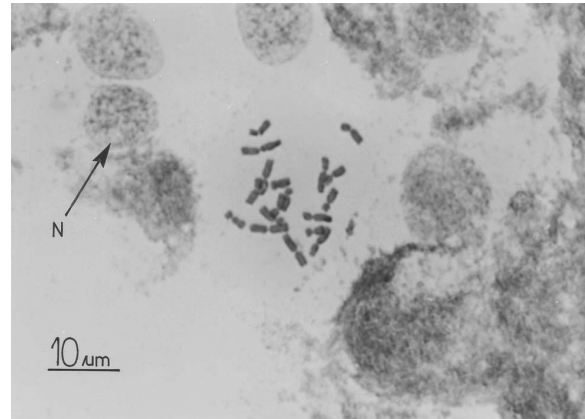


Fig. 1. Metaphase chromosomes isolated from the neoblasts of a *Polycelis felina* (Daly.) population collected at the Lonja River spring. The arrow indicates an interphase neoblast. Preparation was stained with lacto-orcein.

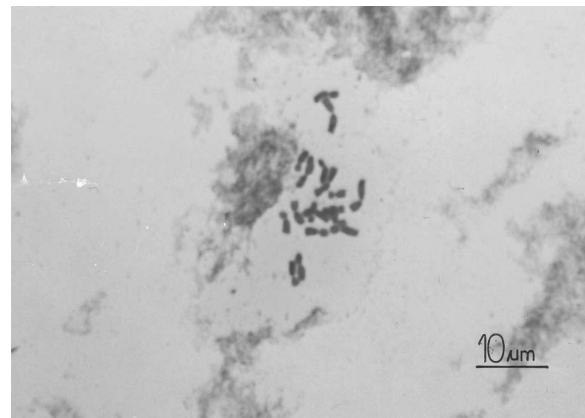


Fig. 2. Metaphase chromosomes isolated from the neoblasts of a *Polycelis felina* (Daly.) population collected at the Gračanski ribnjak pond. Preparation was stained with lacto-orcein.

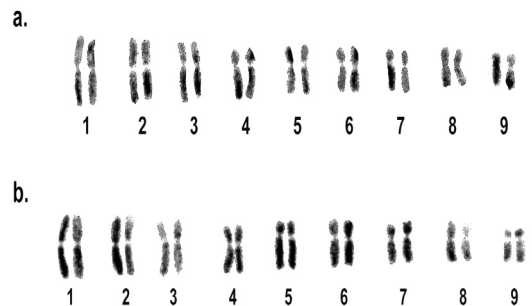


Fig. 3. Karyotype of *Polycelis felina* (Daly.) species: a) population collected at Gračanski ribnjak pond; b) population collected at the spring of Lonja River. Bar = 10 μ m.

Table 1

Mean length and shape of chromosomes in neoblasts of the planarian *Polycelis felina* (Daly.) belonging to two allopatric populations

Chromosome pair	Location	Mean chromosome length (μm)			(\pm) SD	Shape
		longer arm	shorter arm	total length		
1.	GR	2.81	2.54	5.35	0.131946959	metacentric
	L	2.90	2.44	5.35	0.073673454	
2.	GR	2.64	2.35	4.98	0.085479042	metacentric
	L	2.58	2.34	4.92	0.067667488	
3.	GR	2.65	1.72	4.37	0.072793467	submetacentric
	L	2.67	1.72	4.39	0.058887841	
4.	GR	2.61	1.51	4.12	0.132010101	submetacentric
	L	2.62	1.57	4.20	0.064601342	
5.	GR	2.53	1.34	3.87	0.202495542	submetacentric
	L	2.50	1.35	3.85	0.025033311	
6.	GR	2.21	1.28	3.49	0.104008547	submetacentric
	L	2.25	1.29	3.55	0.122479023	
7.	GR	2.02	1.14	3.16	0.095852897	submetacentric
	L	2.05	1.14	3.19	0.035901099	
8.	GR	1.58	1.44	3.02	0.090584522	metacentric
	L	1.54	1.50	3.04	0.025033311	
9.	GR	1.76	0.81	2.57	0.078095383	submetacentric
	L	1.80	0.83	2.64	0.020682789	

GR – Gračanski ribnjak pond, L – spring of Lonja River, SD – standard deviation.

sis of variance at 0.028 was lower than the theoretical value of the F-ratio proposed for the given degrees of freedom (3.84).

Based on data obtained, it can be concluded that the two allopatric populations of *Polycelis felina* (Daly.) belong to the same karyological biotype, despite minor morphological differences and the geographical distance separating their habitats. The fact that both populations are diploid instead of polyploid also indicates that the ecological factors in their habitats are fairly stable. Despite their limitations, the results also point to the possible prevalence of sexual over archytomic reproduction among both planarian populations studied. However, in order to obtain more precise data on planarian karyology and morphological changes, other sensitive techniques such as chromosome banding, fluorescent staining or biochemical analysis of proteins should be employed. Moreover, a molecular approach using techniques such as repetitive DNA analysis, Southern blotting and RAPD-PCR should give more insight into the genetic characteristics of these planarian populations (BAGUNA *et al.* 2000; BATISTONI *et al.* 1998; BATISTONI *et al.* 2001). Possible differences in reproductive modes should also be investigated.

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