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CASES OF MIXOPLOIDY IN BROWN FROGS OF UKRAINE

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INTRODUCTION

Polyploid cells were detected for the first time in testicle preparations of *Rana arvalis* from surroundings of Zhitomir city (Ukraine) in 1998 (Manilo, 2000). This observation encouraged the further, more detailed cytogenetic investigation of the genus *Rana* in Zhitomir Oblast', as well as in other regions of Ukraine. This paper is focused on the description of karyotypes in brown frogs, but now we have also similar results of the cytogenetic investigation in other species.

MATERIAL AND METHODS

The material investigated is shown in Table 1. Chromosome preparations were obtained by the dripping (pipetting) method from blood cells, marrow and testicles of the colchycinized animals according to the routine methods (Ford and Hamerton, 1956; MacGregor and Warley, 1986). In order to increase mitotic activity, most of animals were treated with phytogemagglutinine M (Difco Laboratories) according to the method described earlier (Manilo, 1986).

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RESULTS

Principal morphological features of chromosomes in karyotypes of *Rana arvalis arvalis* and *R. temporaria* are presented in Table 1. Below we describe those characters, which are not shown in Table 1, but are considered important.

***Rana arvalis arvalis*.** Metaphase plates in blood and marrow cells of specimens from all studied populations had the standard karyotype and were identical in their ex-

TABLE 1. Karyotypes of the Brown Frogs

No.	Locality, year	Number of studied specimens (males, females)	Chromosome numbers		Rate of polyploid sets and their chromosome numbers	Karyotype	*NF*	Presence and location of secondary constrictions
			n	2*n*				
					Rana arvalis arvalis			
1	Bogunia, to north near Zhitomir (1998)	4♂ 2♀	12	24	Up to 15% 4*n* = 48 5*n* = 60 6*n* = 72	12V + 4sV + 8sT 24V + 8sV + 16sT 30V + 10sV + 20sT 36V + 12sV + 24sT	48 96 120 144	2nd pair, short arm 5th pair, long arm
2	Zhitomir Oblast', Korosten District, near Ushomyr (2000)	2♂ 1 juv	12	24	10 – 20% 4*n* = 48 6*n* = 72	12*V* + 4*sV* + 8*sT* 24*V* + 8*sV* + 16*sT* 36*V* + 12*sV* + 24*sT*	48 96 144	—
3	Zhitomir Oblast', Chernyakhovsky District, near Andreevka (2001)	2♀	12	24	10 – 45% 4*n* = 48 6*n* = 72	12*V* + 4*sV* + 8*sT* 24*V* + 8*sV* + 16*sT* 36*V* + 12*sV* + 24*sT*	48 96 144	—
					Rana temporaria			
4	Zakarpatskaya Oblast', Rakhovsky District, near Yasenya (2000)	1♀	13	26	Up to 30% 4*n* = 52 6*n* = 78	6*V* + 18*sV* + 2*sT* 12*V* + 36*sV* + 4*sT* 18*V* + 54*sV* + 6*sT*	52 104 156	10th pair, long arm
5	Bogunia, to north near Zhitomir (2000, 2001)	3♂ 6♀	13	26	Up to 20% 4*n* = 52 6*n* = 78	6*V* + 18*sV* + 2*sT* 12*V* + 36*sV* + 4*sT* 18*V* + 54*sV* + 6*sT*	52 104 156	10th pair, long arm
6	Zhitomir Oblast', Korosten District, near Ushomyr (2000)	1♂ 2♀	13	26	Up to 20% 4*n* = 52 6*n* = 78	6*V* + 18*sV* + 2*sT* 12*V* + 36*sV* + 4*sT* 18*V* + 54*sV* + 6*sT*	52 104 156	10th pair, long arm

Abbreviations. *n*, haploid chromosome set; 2*n*, diploid set; *NF*, basic chromosome number; 4*n*, tetraploid set; 5*n*, pentaploid set; 6*n*, hexaploid set; *V*, metacentric; *sV*, submetacentric; *sT*, subtelocentric.

ternal chromosome morphology ($2n = 24$, $NF = 48$). At preparations from tikles two types of dividing cells were present: cells of spermatogonial division (metaphase of mitosis II) and gametes (meiotic metaphase I – diakinesis and metaphase II). Metaphase plates of speramatogonial division included 24, 36, 48, 60, and 72 chromosomes. All of them represented multiplied haploid set ($n = 12$) and did not differ in chromosome morphology from the standard karyotype (Fig. 1*a – c*; Table 1). Furthermore, the part of dividing cells had the incomplete (aneuploid) chromosome set with several chromosomes more or less than in the standard karyotype. The total rate of polyploid or aneuploid cells was 15 and 5% of the investigated cells of spermatogonial division, respectively. Meiotic metaphases I and II in gametes also showed unusual chromosome sets: the number of diakinetic bivalents varied from $n = 12$ to $4n = 48$ (Fig. 1*d – h*). Secondary constrictions were observed in most of the metaphase plates from Bogunia on the long arm of the 5th chromosome pair, and on the short arm of the second pair. Sex chromosomes were not identified.

***Rana temporaria*.** Individuals of this species have been examined from three localities (Bogunia and Ushomyr villages, Zhitomir Oblast') and vicinities of Yasenya village [Urdu-Flavanchuk ridge (alt. 800 m) in Zakarpatskaya Oblast' (Table 1)]. All studied cells of blood and marrow had the standard karyotype ($2n = 26$, see Table 1). Karyologycal features of testicle cells were similar to those in *R. arvalis*: mixoploidy was recorded in gametes ($n = 13$, $2n = 26$, $3n = 39$) as well as by spermatogonial division ($2n = 26$, $3n = 39$, $4n = 52$, etc., see Fig. 2). Numbers of dividing cells in some microscopic slides exceeded 100, making their counting difficult. The total rate of polyploid cells (in relation to all studied cells) varied between 10 and 30%, aneuploid cells were markedly less frequent — about 5%. As in the first species, no sex chromosomes were identified.

DISCUSSION

The results of our cytogenetic investigations of Zhitomir and Zakarpatskaya Oblast' populations of *R. arvalis* and *R. temporaria* are unexpected and unusual for Ranidae. Since this group is rather ancient and conservative, any hypotheses presuming active evolutionary processes or speciation seem improbable. More likely, the observed multiplied chromosome sets were caused by aberrations of meiosis, while polyploid cells appeared, because the chromosomes did not separate properly during cell division. Furthermore, we suggest that the described aberrations in

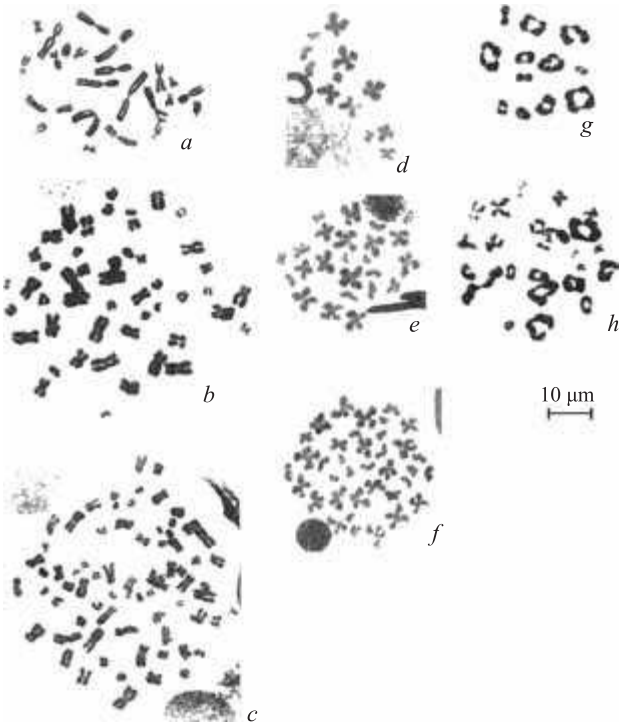

Fig. 1. Metaphase plates of *Rana arvalis*: *a*, metaphase plate of the normal karyotype ($2n = 24$), spermatogonial cell; *b*, metaphase plate of a tetraploid ($4n = 48$), spermatogonial cell from Bogunia near Zhitomir; *c*, metaphase plate of a hexaploid ($6n = 72$), spermatogonial cell from Ushomir; *d*, metaphase of meiosis II ($n = 12$), haploid gamete of a male from Andreevka; *e*, metaphase of meiosis II ($2n = 24$), diploid gamete of a male from Andreevka; *f*, metaphase of meiosis II ($3n = 36$), triploid gamete of a male from Andreevka; *g*, metaphase of meiosis I, diakinesis ($n = 12$), haploid gamete of a male from Bogunia near Zhitomir; *h*, metaphase of meiosis I, diakinesis ($2n = 24$), diploid gamete of a male from Bogunia near Zhitomir.

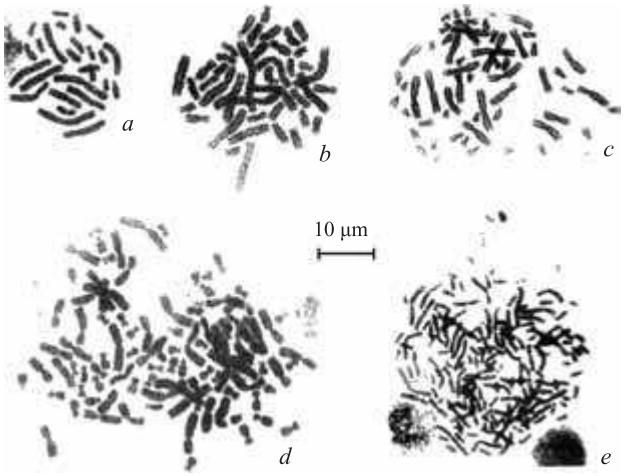

Fig. 2. Metaphase plates of *Rana temporaria*: *a*, diploid metaphase plate $2n = 26$, spermatogonial cell from Yasenya, Zakarpatskaya Oblast'; *b*, metaphase plate of a tetraploid ($4n = 52$), spermatogonial cell from Bogunia near Zhitomir; *c*, *d*, ihcomplete (aneuploid) metaphase plate $Xn = 60$; $Xn = 101$ (?) from Yasenya, Zakarpatskaya Oblast'; *e*, hexaploid metaphase plate $6n = 78$ from Ushomir, Zhitomir Oblast'.

testicles where determined by ecological factors (chemical and radio nuclide pollution of the environment). Indeed, such factors affect mainly germ cells (Mitrochenko et al., 1999). It is known, that amphibians belong to animal groups, which are sensitive to environmental factors, in particular to geochemical and radioactive influences, and may serve as very good markers of such influences (Petrov and Sharygyn, 1981; Israel, 1984; Ilyenko and Krapivko, 1989). In addition, biological test systems are often more sensitive than chemical, physical and radiometrical methods (Brusick, 1987). Being a consequence of the negative influence of different mutation agents on somatic and germ cells, development of aneuploid and polyploid cells severely disturbs genetic balance and suppresses viability and reproductive ability of animals. Thus, the obtained results may be used in future for ecological and genetic monitoring in the studied regions.

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