THE PRELIMINARY CYTOGENETIC INVESTIGATIONS IN *RANA RIDIBUNDA* PALL. AND *RANA ESCULENTA* L. FROM THE NORTH OF MOLDAVIA

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Abstract: The cytogenetic investigations in *Rana ridibunda* (Pall.) and *R. esculenta* (L.) species pointed out that the two species have the karyotype formed of 13 pairs of chromosomes (2n = 26). The chromosomes of these species can be divided into two groups: the first group comprises the pairs of large chromosomes 1–5, and the second group is made of the pairs 6–13 of smaller chromosomes.

INTRODUCTION

Some research made on green frogs of the *Rana ridibunda* Pall. and *Rana esculenta* L. species pointed out some remarkable differences between the number of chromosomes and the value of C index. Thus, while in *Rana ridibunda* Pall. a number of 13 chromosomes were found in the genome, and a value of C index (measured by Feulgen densitometry) between 5.51 and 8.10 pg (9, 10, 19, 23), C index in *Rana esculenta* L. (with the same chromosome number in the haploid set) ranged between 5.6 and 11.5 pg (3, 16, 21 – 23).

The aim of this study is to establish the karyotype of *Rana ridibunda* Pall. and *Rana esculenta* L.), to point out the possible differences which exist between the two species of green frogs and compare the results we obtained with specific literature data (1, 2, 6, 8).

MATERIAL AND METHODS

Our cytogenetic investigations were achieved on males of *Rana ridibunda* Pall. and *Rana esculenta* L. species. These males were collected from a pond which is situated in the neighborhood of Dorohoi (Botoşani County).

The study of mitotic chromosomes can be achieved either directly – with the help of cytogenetic analyses on some tissues with intense mitotic activity (hepatic, splenic, gonadal tissue, corneal epithelium) or indirectly – in cell cultures.

Thus, we tested many techniques in order to point out the mitotic chromosomes of the two species and we finally chose the method suggested by Spurway and Callan (1960), this method being quoted in literature (7, 17). In this view, we injected 0.1 ml colchicine solution (0.2% for a 10 g weight) in the lymphatic dorsal sacs of the frog. A period of time of 2 hour treatment is recommended in the above-mentioned technique, but we obtained better results in case of an 8 hour treatment. When this period of time was over, the animals were sacrificed and various organs were drawn (kidneys, gonads, spleen) to be used as a biological material appropriate for this type of investigation. We obtained good results using the testicles, which were minced in small fragments. Contrary to the original method, we did not introduce these fragments in colchicine solution, but we immersed them for 2 hours in a hypotonic solution (NaCl 0.075M) at room temperature. The fixing of the biological material was achieved including these fragments of tissue in a fixing solution ethanol/glacial acetic acid (3/1), 2 or 3 hours (the time of fixing can be extended up to 24 hours, the results being as good as before). The biological material was kept in a refrigerator during the fixing time. The biological material was coloured with an acetic – carmine solution (20 minutes), and for the accomplishment of the microscope preparations the “squash” technique was used. The preparations were examined with a binocular microscope (type IOR Bucureşti, SR. 3886 – 70), in view of identification of some metaphases with well displayed and non-superposed chromosomes. The metaphases chosen for the setting of the karyotype were photographed with a digital camera, Sony DSC – W30 (with a 6 Mp resolution) which was attached to the ocular (objective 40X and ocular 10X).

The chromatides of each chromosome were measured (on photographs made on paper support), and depending on their dimensions and position of the centromer, we established the pairs of homologues and subsequently the chromosomes in the karyotype were displayed. In the case of the chromosomes which are partially superposed we used photographical copies of the same metaphases. The results are presented in figures 1 – 4.

RESULTS AND DISCUSSIONS

Our study pointed out that the diploid number of chromosomes in the *Rana ridibunda* Pall. and *Rana esculenta* L. species is 26, confirming the results obtained by other authors, (MORESCALCHI, 1967; quoted by AL-SHEHRI and AL-SALEH, 2005), (5, 8, 11, 12, 18, 20). The information displayed in literature points out that the most frogs of the *Rana* genus have the karyotype formed of 26 chromosomes (metacentric and submetacentric).

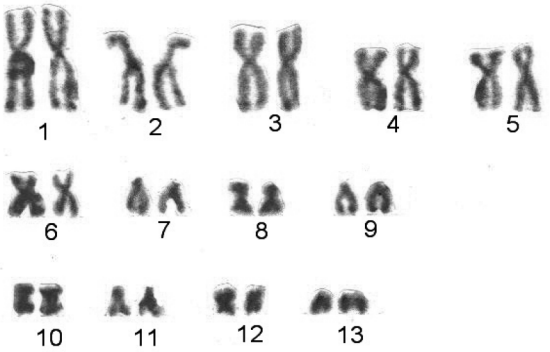

Fig. 1- The karyotype of *Rana ridibunda* (Pall.) (♂)

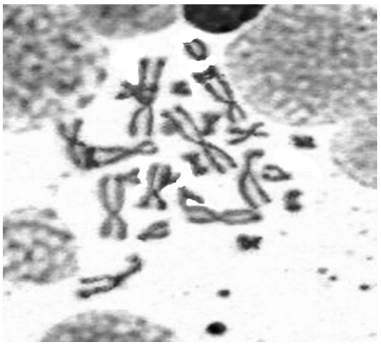

Fig. 2 - The metaphase used for the achievement of the karyotype presented in *Rana ridibunda* (Pall.)

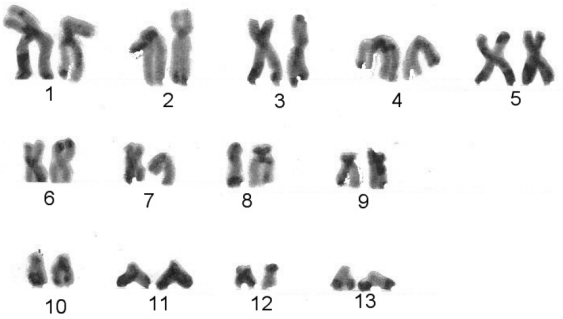

Fig. 3 - The karyotype of *Rana esculenta* (L.) (♂)

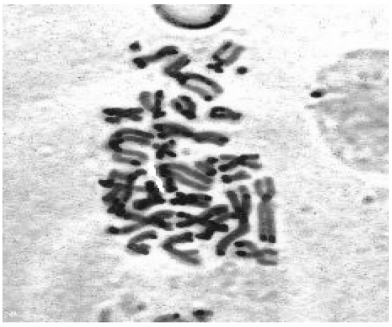

Fig. 4 - The metaphase used for the achievement of the karyotype presented in *Rana esculenta* (L.)

The chromosomes specific for the green frogs were classified, according to their size (8, 11, 18), into two groups: the first group comprises the larger chromosomes belonging to the pairs 1 – 5, and the second group is formed of the other 8 pairs of chromosomes, pairs 6 – 13.

We cannot make any certain statement concerning the centromer position of the thirteen chromosome pairs. Nevertheless, our study points out that the chromosomes belonging to the 7^{th}, 9^{th} and 13^{th} pairs of *Rana ridibunda* Pall. and 11^{th} and 13^{th} pairs in *Rana esculenta* L. are more likely subtelocentric than meta- and submetacentric.

According to the study achieved by KOREF-SANTIBANEZ and GÜNTHER (1984), the dimensions of the chromosomes in *Rana esculenta* (L.) are intermediate between *Rana ridibunda* Pall. and *Rana lessonae* Cam. This fact would represent an argument for the hybrid nature of the *Rana esculenta* L. However, we noticed that there are no notable differences regarding the chromosome size for both investigated species.

Some data show significant differences regarding the centromeric region of mitotic chromosomes that belong to the European species of green frogs. Using the C banding technique or the fluorescence in UV, BUCCI et al. (2005) pointed out that the centromers of the *Rana*

ridibunda Pall. (as well as the chromosomes of the *ridibunda* type from the hybrid karyotype of *esculenta* type) are extremely visible, shaped as black granules or as evident fluorescent spots. The centromers of the *Rana lessonae* Cam. (like those of the *lessonae* type from the *esculenta* hybrid karyotype) are not visible. The authors pointed out significant differences at the chromosomes of “lampbrush” type too, from the parental species of green frogs (*Rana ridibunda* Pall. and *Rana esculenta* L.), which consist in the shape, number and the distribution of the gigantic loops within chromosomes. These chromosomes can be easily evinced when they are present in the hybrid complement of the *Rana esculenta* L. Using G banding technique, RAICU and GEORMĂNEANU (1977) pointed out a great variability of the colouring of the centromeric regions in *Rana ridibunda* Pall. Thus, the second pair of the large chromosomes stands out because they have a greater amount of pericentromeric heterochromatine than the other chromosomes in the same group or even in the entire karyotype. The 7th, 9th, 11th and 13th pairs of chromosomes are poorly coloured, though they are uniformly coloured along the arms, the fact that points out a more uniform distribution of the heterochromatine.

A controversial aspect of these species is the presence (or absence) of the sex chromosomes in the genome. Although some papers (BĂRA and CÎMPEANU, 2003) point out that the sex of Amphibians is determined by genes having male effect (their manifestation being influenced by the conditions of the environment, especially by temperature), more recent data stated that in Anura Order, and especially within the *Rana* gender, the sex chromosomes would be involved in the determining of the sex (1, 12 – 15). As an example, we provide below the male and the female karyotype in the *Rana ridibunda* Pall. (fig. 5), achieved by AL-SHEHRI and AL-SALEH (2005) on individuals caught in Saudi Arabia (oases Al-Hassa and Al-Qatif), in which the second pair of chromosomes was considered as representing the two heterosomes (XX type for female, and XY type for male).

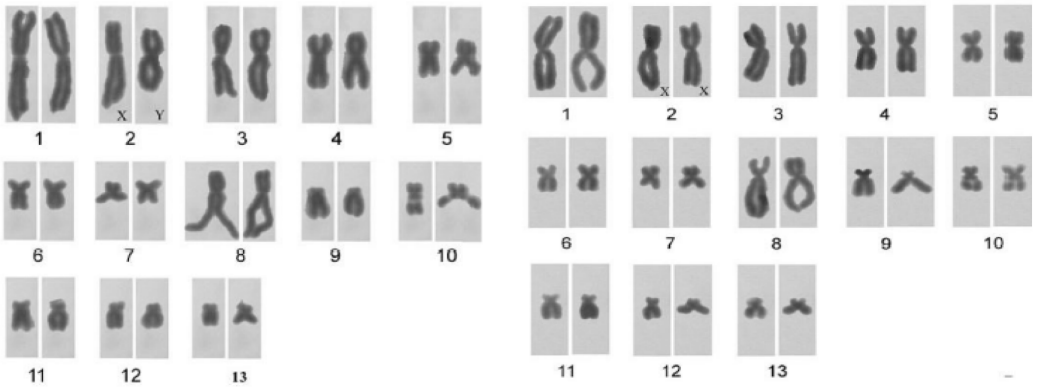

Fig. 5 - The karyotype of *Rana ridibunda* (Pall.) (left – male, right – female)
(after AL-SHEHRI and AL-SALEH, 2005)

Our results do not point out differences between the pairs of homologues established before and implicitly they do not confirm the existence of sexual chromosomes at the two investigated species.

The last aspect regarding the green frogs karyotype implies the finding out of a pair of chromosomes which is specific to *Rana ridibunda* Pall. According to some research (1, 5, 8, 18, 20), the tenth pair of chromosomes has secondary constrictions at the level of long arms, which

can be considered a characteristic element for this species. In their study of the constitutive heterochromatine of this pair of chromosomes, RAICU and GEORMĂNEANU (1977) pointed out the presence of a heterochromatic block at the level of the centromers, which has approximately equal dimensions to that from the second pair of chromosomes, and the secondary constriction region is not marked. Our karyotype study did not evince the presence of the secondary constriction at the level of the 10th pair of chromosomes in *Rana ridibunda* Pall.

CONCLUSIONS

Our cytogenetic investigations in *Rana ridibunda* (Pall.) and *Rana esculenta* (L.) species pointed out the fact that:

The two species have the karyotype formed of 13 pairs of chromosomes (2n = 26);

The chromosomes of these species can be divided into two groups: the first group comprises the pairs of large chromosomes 1 – 5, and the second group is made of the pairs 6 – 13 of smaller chromosomes;

The cytogenetic studies accomplished in the frog populations that we investigated do not confirm either the existence of the sex chromosomes in the two species or the presence of secondary constriction in the chromosomes of the tenth pair.

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