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STUDIES ON TWO *Bombina variegata* POPULATIONS FROM TWO VALLEYS IN THE IEZER MOUNTAINS, ROMANIA

Sára FERENȚI*

University of Oradea, Faculty of Sciences, Department of Biology,
Universităţii str. 1. 410087 – Oradea, Romania
E-mail: ferenti_sara@yahoo.com

Abstract. *Our study took place in the August of 2009, when we studied the affiliation of two Bombina variegata populations from two parallel valley form Iezer Mountains (Arges County, Romania). The amount of the B. variegata characters within the populations is influenced by the isolation level of the habitats, their morphology and distribution in space. An accentuated instability of the characters was observed in correlation with the distance between the ponds.*

Key words: *altitudes, habitat morphology, isolation level, yellow bellied toad.*

INTRODUCTION

In contradistinction to other animals, hybridization on amphibians was signaled by many authors in the scientific literature (e.g. Berger 1973, Elinson 1981, Littlejohn & Watson 1985, Kocher & Sage 1986, Parris 2004, Lemmon et al. 2007, Alexandrino et al. 2007, Lengagne et al. 2008). Hybridization can be caused by the reproductive isolation that results from the contact between congeneric species, which permits gene flow in sympatric or parapatric manner, after that the divergence between the species had been beginning (Mallet 2005).

Bombina bombina and *Bombina variegata* are two species with the same origin, which were separated during the Pleistocene glaciation (Szymura 1998). This geographical isolation allowed the adaptation to different environmental conditions, but was not enough for the complete reproductive isolation (Hewitt 2004, Hofman et al. 2007). At the common limit of their distribution area there is a sector where they can reproduce, forming a hybrid zone, this sector covering the zone between 100-400 m altitudes (Szymura 1988, Gollmann 1984). These hybrid zones were token on interest by researchers, the scientific literature containing many studies with this theme (e.g. Maccallum et al. 1995, 1998, Hofman & Szymura 2007, Arntzen 1996, Nürnberger et al. 2005, Gollmann et al. 2002, Groza et al. 2007, Covaciu-Marcov et al. 2009).

The *B. bombina* characters were also observed at *B. variegata* populations situated to large distances from the congeneric species` area (Covaciu-Marcov et al. 2003, Kovacs & Covaciu 2009). Our study took place in an area with high altitudes, our objective being to analyze the causes of the occurrence of *B. bombina* characters in two populations of *B. variegata* from two parallel valleys in Iezer Mountains (Arges County, Romania).

MATERIAL AND METHODS

Our study was made in August 2009 on the valley of Bratia River and Doamnei River in Arges County.

The first habitat is represented by a pound system localized on the large meadow of the river. The depth and surface of these ponds was reduced, but for all that they never dried because of the high humidity offered by the river. The second valley is narrower and is represented by a zone affected in the past by deforestation. The ponds depth and surface is bigger than in the case of the previous habitat.

For establishing the affiliation of the populations of *B. variegata* we used the main morphological and chromatic characters of these two species, grouped in two grids, which represents the standard work models in this domain (Stugren 1980, Ghira & Mara 2000, Ghira et al. 2003, Gollmann et al. 1993, Szymura & Barton 1991).

We analyzed 20 characters, using two grids, each of them grouping 10 features. The first grid contains the features about the morphology, dimension and ratio of the ventral light spots, that are red at *Bombina bombina* and yellow at *Bombina variegata* (Table 1). The second grid also contains 10 features, and was used by Stugren (1980) and modified by Ghira & Mara (2000) (Table 2).

Both of the grids use the binary system (0, 1), where every feature gets a mark: 1, if it shows like at *B. variegata* and 0 if it is like at *B. bombina*. Summarizing the marks for every feature an individual for every grid have a value between 0 and 10, the close value to 10 meaning a pure *B. variegata* individual, but the individual with the value close to 0 indicates *B. bombina*.

After laying of the value for every individual, we calculated the average of the values for all the individuals of each of the population, first separated for every grid and after that together.

Table 1.
Grid 1 of differentiation of the European species of the Bombina gender (the characteristics of the ventral pattern)

Characteristic (light spots on):		*Bombina bombina*	*Bombina variegata*
1.	Chin – chin	Separated	United
2.	Chin – chest	Separated	United
3.	Chest – chest	Separated	United
4.	Chest – shoulder	Separated	United
5	Shoulder – arm	Separated	United
6	Chest – abdomen	Separated	United
7	Abdomen – abdomen	Separated	United
8	Abdomen – basin	Separated	United
9	Basin – basin	Separated	United
10.	Basin – thigh	Separated	United

Table 2.
Grid 2 of differentiation of the European species of the Bombina gender (after Stugren, Ghira & Mara, modified)

Character		*Bombina bombina*	*Bombina variegata*
1.	Colour of open ventral spots	Red, orange, yellowish	Yellow
2.	Colour of upper part of the first finger and the top of fingers	Black	Yellow
3.	Dorsal colouring	Black	Pale grey
4.	The relation tarsian and plantar open spots	Separated	United
5.	Ventral colour	Orange spots on black background	Black spots on yellow background
6.	The relation between the length and width of the head	Length > width	Length < width
7.	The drawing of lateral and ventral parts	White spots around the verrucae	Without white spots around the verrucae
8.	The drawing of the dorsal part	Regulated black tubercles	Black scattered verrucae
9.	Dorsal verrucae	Lens – shaped, squatted	Sharp, rough
10.	The ratio of tibia – tarsian joints when the stylopode and the zeugopode are parallel	Not touching	Touching

This method allows the transformation of some features in percents, and their statistical interpretation. The final value indicates the percentage of the *B. variegata* features.

RESULTS AND DISCUSSION

The general observed phenomenon at many populations of *B. variegata* also seems to be valid in our case too. The populations we analysed being not constituted of pure individuals belonging to one of the species, but appear characters of both of species, such as it was also signalled in the case of other populations (Covaciu-Marcov et al. 2009, Groza et al. 2007).

After analyzing the results at the population at Doamnei River we can observe that this is a *B. variegata* population, though it is not pure, the values being a little bit raised that 75 %, which would indicate a *B. variegata* - like population (Fig. 1). The population from the Doamnei River has *B. variegata* characters in 81.99 % by the first grid and 83.16 % by the second one, the average value being 82.57 % (Fig. 1). In the case of the population from Bratia River the amount of the *B. variegata* characters by the first grid was 75.99 %, but by the second grid 78.83 %, the average being 77.41 %. Thus this is a *B. variegata* - like population, the values being closer to 75 % (Fig. 2).

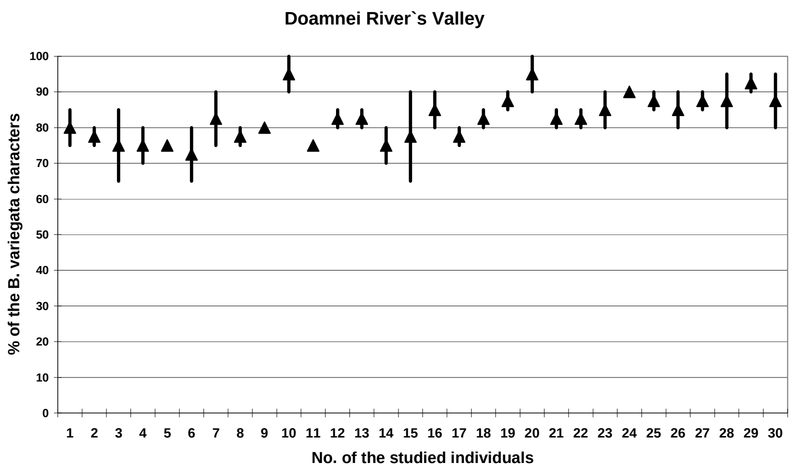

Figure 1. The distribution of the *B. variegata* characters at the individuals from the Doamnei River Valley.

Both of the studied populations are localized at similar altitudes (Doamnei River: 695m asl, Bratia River: 707m asl), the valleys being parallel, despite the amount of the characters are different. This is probably a result of the habitat morphology. At the Doamnei River the habitat is typical for *B. variegata* such as temporary ponds with rich vegetation, with reduced surface and depth.

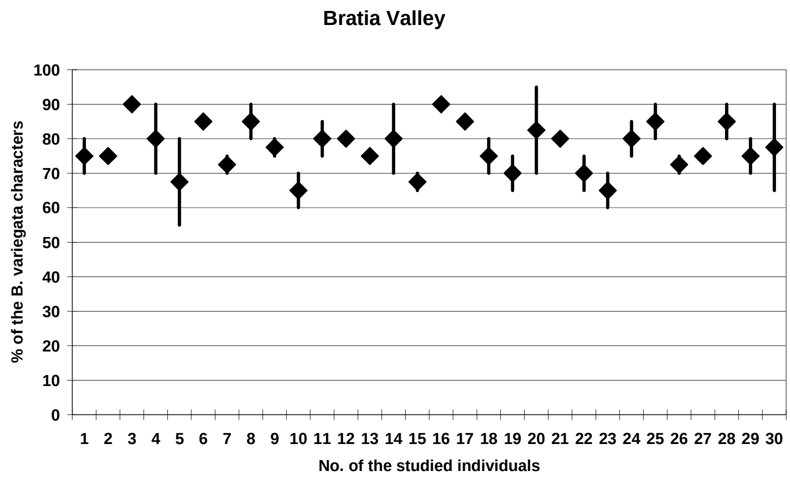

Figure 2. The distribution of the *B. variegata* characters at the individuals from the Bratia Valley

In the case of the Bratia River the studied individuals emanates from a large zone with temporary ponds with rich vegetation, continued by a sector with rocky substratum and large ponds as it is preferred by *B. bombina.* On the other hand the valley of the Doamnei River is narrow, with wider zones in the interior of the valley, being thus closed for the influences from the exterior of the valley. Thus in this valley the toads migration is more difficult. Instead of it the valley of the Bratia River is larger, with a wide meadow with wetlands. In this valley with favorable habitats the migration was facilitated by the environmental conditions. Thus these populations could make contact easily with the populations from the lowest altitude, which along with the descent of the altitude contains more and more characters of *B. bombina.* On the other hand rising of the genetic instability in the case of the hybrid populations was observed concomitant with the rising of the distance between the ponds (Reh & Seitz 1990, Hitchings & Beebee 1997).

In the case of the populations from the Doamnei River we can observe a relative stability of the individuals in point of the characters of *B. variegata*. None of the individuals have the amount of the characters fewer then 70%, but the majority has upwards of 80%. This uniformity is a result of the stability of the environmental conditions of the habitat. Secondly these individuals were collected from a limited space, the narrow valley ensuring the more stable microclimate. Even if the forest was very close, the vicinity of the habitat was affected by the deforestation in the past, and the reforestation is realized by the white alder in an inchoate phase. This aspect is favorable for *B. variegata* that prefers opened areas without forest (Madej 1973). This affirmation is also sustained by the small difference between the individuals with the extreme values.

In the case of the population from the Bratia valley the values of each of the grids are variable, the results by the first grid showing a tendency to lower values, but the second grid having characters with raised value. Sometimes the contact of these species with their congener can result disequilibrium of the genes, which leads to the fact that some genes from the one of the species can occur to the other species (Szymura et al. 2000). This is the cause of the occurrence of some characters of *B. bombina* at *B. variegata*, being a result of this disequilibrium of the genes. For this phenomenon in our case a good example is the 7th character from the second grid. The amount of this character is very low in the case of both populations (Doamnei valley: 0 %, Bratia valley: 10 %). In the case of other populations of *B. variegata* - like was also observed the presence of this *B. bombina* character (Covaciu-Marcov et al. 2003). In contradistinction to the population from Doamnei River valley, at the Bratia valley the distances between the extreme values of the individuals are big. We can observe disequilibrium within the population, the polymorphism being more accentuated. The scientific literature points out as a cause of this polymorphism the diversity of the habitats (Vesea et al 2004).

The occurrence of the instability of the characters at such a high altitude can be explained by the presence of some genes, which persisted since the period when these two species were not yet isolated. The presence of *B. bombina* characters was also signaled at the other yellow bellied toad populations from high altitudes (Ghira & Mara 2000).

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