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On the hybrid zone between *Bombina bombina* and *Bombina variegata* in Livada Forest, north-western Romania

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Abstract. In the forest from Livada there is a mosaic hybrid zone found at an altitude of 145-155 m a.s.l. The aspect of the hybrid populations is determined by the morphology of the habitats; thus between the populations found at very close altitudes there is a high degree of differences. All the studied populations, including the ones closer to one of the species, present characteristics of the other species. The hybridizing area from Livada is situated at similar altitudes with other segments of the hybridizing area from the north-western Romania.

Key words *Bombina bombina*, *Bombina variegata*, hybrid zone, altitude, habitat.

Introduction

The hybrid zone between *Bombina bombina* and *Bombina variegata* is one of the best documented examples of genetic interaction from the borders of the species (Gollmann et al. 1993). The analysis of the hybridizing areas offers the possibility of investigating problems tied to the evolution (Gollmann 1991) and to the genetics of species (Hewitt 1988). The hybridizing area between the two European species of the *Bombina* genus has a great length across Europe (Szymura 1993). Thus, this hybrid zone between these two species has been intensely studied (Szymura 1976, Szymura et al. 2000, Gollmann 1984, 1987, Yanchukov et al. 2006, Hofman & Szymura 2007). Even in Romania there have been many recent studies on this aspect (Ghira & Mara 2000, Ghira et al. 2003, Covaciu-Marcov et al. 2003a, 2004, 2005, 2006, Sas et al. 2005, Ghiurcă & Gherghel 2007). However, most of the research has analyzed the hybridizing area between the *B. variegata* populations from the Apuseni Mountains and the *B. bombina* group from the Pannonian Plain. There is only one study focused on the hybrid zone between *B. variegata* from the Eastern Carpathians and *B. bombina* from the Pannonian Plain.

The aim of this study is to establish the location, the type and the particularities of the hybrid zone from the Livada forest.

Materials and methods

Our study took place between April and May 2007. The toads were captured by hand or using different round nets. We analyzed 6 populations, investigating 214 toads. After concluding our research, the toads were released in their habitat of origin. The forest from Livada is situated in the north-western part of Romania, near the border with Ukraine and Hungary (Fig. 1). The investigated region is in direct contact with the Pannonian Plain and the Oas Mountains, which represent a subunit of the Eastern Carpathians.

The analyzed populations inhabited different biotopes, situated at various altitudes (Table 2). Habitat 1. is a temporary puddle with a surface of about 3 m² and with a depth of about 20-30 cm, situated on a path linking the forest to the railroad. There is no aquatic vegetation here, but there is plenty of peat. Habitat 2. is a marsh of over 30 m², situated between the woods and a road. The depth of the water reaches over 1 m and the biotope presents ample aquatic vegetation. Habitat 3. is a system of temporary puddles located on a path inside the forest. These puddles have a surface of only few tens cm² and a depth of about 20 cm, being connected to each other but also to a larger pond near the path. Habitat 4. is represented by a long canal of about 100 m, situated inside the forest, in the higher sector and lacking aquatic vegetation. The water has a width of 50 cm the most and a depth of about 20-30 cm, drying out completely in the summer time. Habitat 5. is made of a series of temporary puddles, with no vegetation, formed in an abandoned quarry. Habitat 6. is similar to the precedent one, but occupies a larger surface.

Aiming to establish the origin of each population, we examined the main morphologic and chromatic characteristics

of the two species, grouped in two grids that represent the standard models in this domain. The method consists of analyzing some morphologic and chromatic characteristics of the two species. The used features are the most important diagnose characteristic of the two species and several authors have used them (Stugren 1980, Ghira & Mara 2000, Ghira et al. 2003, Gollmann et al. 1993, Szymura & Barton 1991). We analyzed 20 features, using two grids, each grouping 10 characteristic. The first grid analyzed the morphology, the dimension and the ratios of light ventral spots, colored in red at *B. bombina* and in yellow at *B. variegata*. The degree of confluence or separation of different ventral spots is scored for 10 chromatic groups (Table 1). If the light spots are separated among them by black pigment the character belongs to the *B. bombina*. When the spots are united among them and the light pigment on the body appears uniform the character belongs to *B. variegata*. The second grid analysis 10 features as well and was used by Stugren (1980) and modified by Ghira & Mara (2000) (Table 1).

Both of the grids use a binary system (0, 1). For both grids each feature receives a mark: 1 if it is expressed like *B. variegata* and 0 if it is expressed like *B. bombina*. Summing the marks obtained for each characteristic, a certain individual can receive on each grid a score ranging from 0 to 10; the score equal to 0 means a pure *B. bombina*, the score equal to 10 means a pure *B. variegata*. After assessing the score for each individual, we calculated the average score of all individuals of each population, for each grid and then the average of the two grids. This method allows the transfer of the feature into percentages and their statistical interpretation. The final mark indicates the amount of *B. variegata* species features.

Results

In the forest from Livada, we identified populations very close to *Bombina bombina*, others close to *Bombina variegata*, but also hybrid populations (Table 2.). Generally, the rule is this: the higher the altitude, the higher the amount of *Bombina variegata* characteristics.

However, this growth isn't uniform, some great variations occurring among the populations found at smaller differences of altitude. All the populations present some characteristics of the other species and therefore we didn't find any pure population.

By analyzing the 20 characteristics we found that there are features that manifest in the case of the populations closer to *Bombina bombina* like in the case of *Bombina variegata*. Equally, there are features that manifest in the case of most of the individuals from the populations closer to *Bombina variegata* just like they would do in the case of *Bombina bombina* (Table 3). The situation is general, signaled at other populations from western Romania (Covaciu-Marcov et al. 2002, 2003b, 2007).

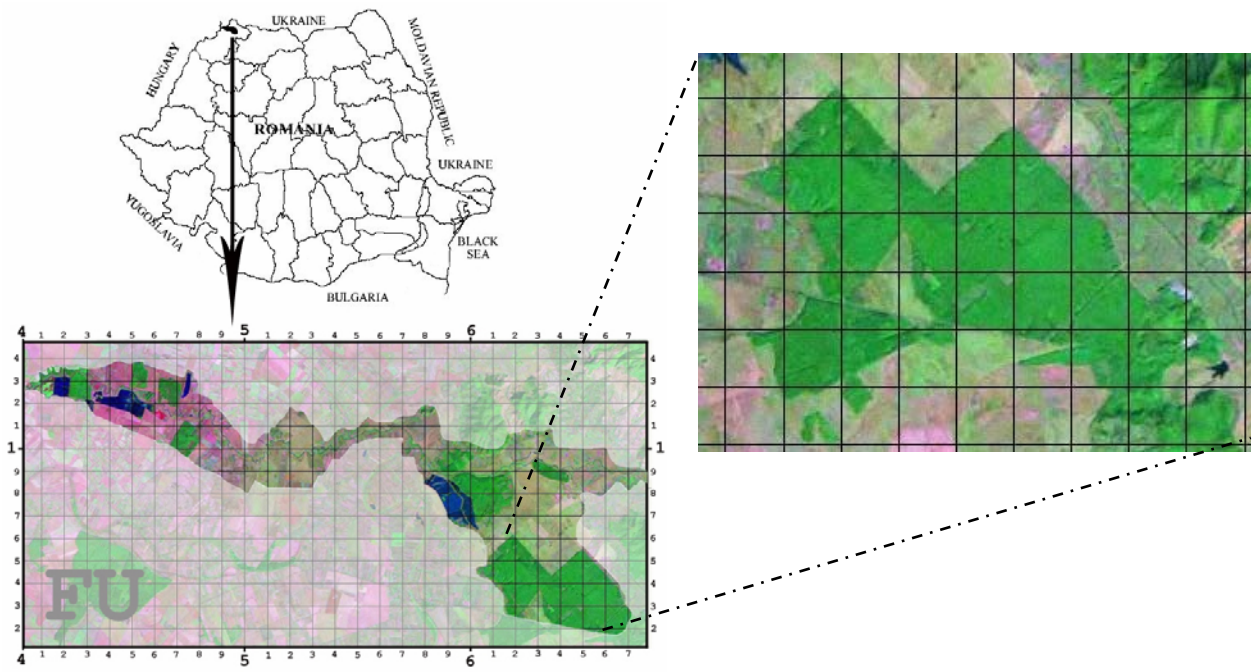

Figure 1. The geographical position of the Livada Forest
in the Natural Reservation of the Inferior Course of the River Tur, North-western Romania

Table 1. The two Grids of differentiation of the European species of *Bombina*

Grid 1			
Character		***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

Grid 2			
Character		***Bombina bombina***	***Bombina variegata***
1	Color of light ventral spots	Red, orange, yellowish	Yellow
2	Color of the top of fingers	Black	Yellow
3	Dorsal color	Black	Pale grey
4	Relation between tarsal and plantar light spots	Separated	United
5	Ventral color	Orange spots on black background	Black spots on yellow background
6	Relation between the head length and width	Length > width	Length < width
7	The drawing of the lateral and ventral parts	White spots around the verrucae	Without white spots around the verrucae
8	Aspect of the dorsal black tubercles	Regulated	Scattered or absent
9	The aspect of the dorsal verrucae	Lens-shaped, squatted	Sharp, rough
10	Ratio of tibia-tarsian joints when the legs segments are parallel	Not touching	Touching

Discussions

The altitude at which this hybridizing area from the Livada forest is located is extremely low (Fig. 2), even in comparison to other regions from western Romania and more, in comparison to other regions of the country. Thus, the hybrid populations are present here at 145 m a.s.l., while in other regions of Romania they reach altitudes of about 400 m a.s.l. (Fuhn 1960, Ghira et al. 2003, Vines et al. 2003, Strugariu et al. 2006, Gherghel et al. 2007).

However, this altitude here is similar with that of the hybrid populations from the Tur River basin, which are situated at altitudes between 130 and 160m (Covaciu-Marcov et al. 2006). This is how it seems that the hybridizing area that flanks to the west the Oas Mountains functions after the same rules across its entire length. The low altitude at which the hybrid zone is found is consequences of the strong descend of the mountain relief. The Oas Mountains don't present hills (Mihăilescu 1969) and therefore they come in direct contact with the plain. Thus, the Mujdeeni Hummock has a rocky substratum and presents suitable habitat for *Bombina variegata* even at very low altitudes. This fact pushes a lot towards the plain *Bombina variegata*'s area and implicitly the hybridizing area.

All of the 6 studied populations are found at the limit of the hybridizing area, being each more or less impure. Thus, the purest *B. bombina* population presents 18.09% characteristics from *B. variegata* (Fig. 2, 3, 4). This percentage is very high compared to other populations from the northern part of the Western Plain, where the amount of *B. variegata* characters is usually of 6-7% (Covaciu-Marcov et al. 2002, 2003a, 2006). However, these amounts are higher than those recorded at 238 m a.s.l. in Dobrudja (Groza et al. 2007). This is a consequence of the fact that Dobrudja represents a glacial refuge of this species (Szymura et al. 1993), where as the north of the Pannonian Plain was only recently populated by *B. bombina*, entering the

Transylvanian Plateau about 10.000 years ago (Vines et al 2003). In the Livada area, it probably reached a bit sooner than that, possibly with a few hundred years.

In the Livada forest, *B. variegata* comes down to lower altitudes than in other regions from Romania, in Transylvania it can be found only from 300 m up (Ghira et al. 2002). Further more, the *B. variegata* populations here are purer than those from the Apuseni Mountains situated at much higher altitudes (Covaciu-Marcov et al. 2003b), but are similar to the ones previously indicated in the Tur River basin (Covaciu-Marcov et al. 2006). As a conclusion, we can say that the populations from the western Oas Mountains are overall purer than those from the same altitudes but in the Apuseni Mountains. With all that, the amount of *B. bombina* characters is higher than in the case of the *B. variegata* populations from the Danube's Gorge, situated at even lower altitudes (Covaciu-Marcov et al. 2007). Thus, there appear to be differences between the *B. variegata* populations from different mountain chains in Romania, just as how more distinct groups have been described in general for this species (Vukov et al. 2006).

Table 2. The affiliation of the studied populations

Habitat	*Habitat 1*	*Habitat 2*	*Habitat 3*	*Habitat 4*	*Habitat 5*	*Habitat 6*
No. of studied individuals	25	42	25	50	37	35
Altitude (m.)	144	137	145	155	171	190
Grid 1 average	35.6	22.38	53.4	56.9	67.43	74.71
Grid 2 average	25.8	13.8	45.2	52.7	65.13	72
Both grid average	30.7	18.09	49.3	54.8	66.28	73.35
The affiliation of the populations	***Bb. - like***	***Bombina bombina***	***Hibrid population***	***Hibrid population***	***Bv. - like***	***Bombina variegata***

Table 3. The features' ratio in the two grids of the studied populations

Habitat	*Habitat 1*	*Habitat 2*	*Habitat 3*	*Habitat 4*	*Habitat 5*	*Habitat 6*
Character	***Grid 1***					
1	76.00	76.19	96.00	80.00	91.89	100.00
2	6.00	2.38	10.00	17.00	21.62	25.71
3	12.00	4.76	12.00	26.00	35.13	45.71
4	24.00	2.38	48.00	47.00	67.56	74.28
5	96.00	90.47	100.00	98.00	98.64	100.00
6	16.00	0.00	24.00	31.00	27.02	32.85
7	44.00	14.28	66.00	66.00	82.43	94.28
8	26.00	11.9	58.00	62.00	79.72	92.85
9	20.00	9.52	56.00	70.00	81.08	88.57
10	36.00	11.9	64.00	72.00	89.18	92.85
Character	***Grid 2***					
1	40.00	14.28	60.00	74.00	89.18	91.42
2	44.00	21.42	66.00	74.00	85.13	95.71
3	34.00	9.52	48.00	60.00	75.67	94.28
4	36.00	16.66	54.00	59.00	66.21	78.57
5	32.00	19.04	72.00	70.00	83.78	97.14
6	40.00	23.8	44.00	56.00	21.62	28.57
7	4.00	0.00	0.00	0.00	10.81	5.71
8	4.00	0.00	20.00	34.00	67.56	68.57
9	8.00	0.00	36.00	50.00	78.37	91.42
10	16.00	33.33	52.00	50.00	72.97	68.57

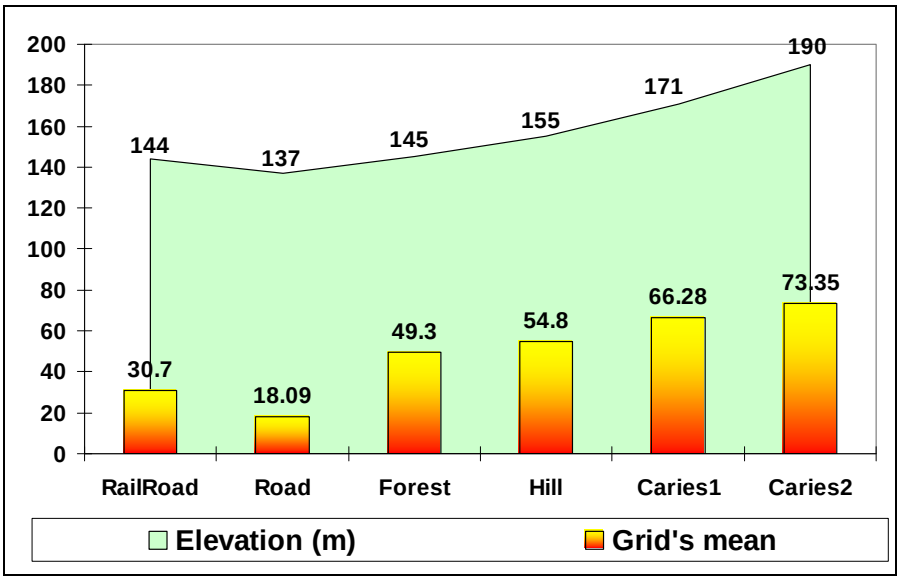

Figure 2. The correspondence between the elevation and the grid`s mean

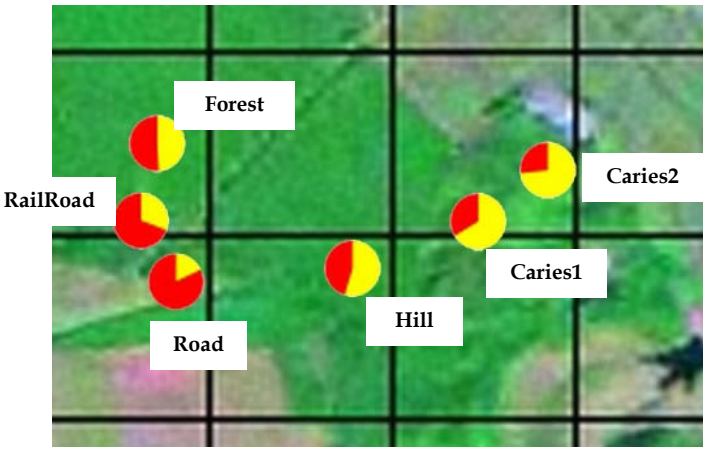

Figure 3. The distribution model of the studied hybrid zone

Figure 4. *Bombina* individuals from the studied region: *B. bombina,* hybrid and *B. variegata.*

In the investigated region, the morphology of the habitats has a crucial role in selecting the hybrid phenotypes and implicitly in the aspect of the entire population, a fact signaled in other regions, too (MacCallum et al. 1998). Thus, even though between habitats 1 and 3 there is an altitude difference of only 1 m, the difference between the affiliations of the two populations is 18%. Therefore this distinction can only be explained through the dissimilarities of the habitats. Hence, habitat 3 – a system of temporary puddles from a path inside the forest - is more favorable for *B. variegata* than habitat 1 – a larger puddle situated on a gravel road linking the forest to a railroad - *B. bombina* avoiding the afforested habitats in general (Yanchukov et al. 2006). Also, habitat 2 is at only 7 m altitude difference and a few meters away from habitat 1, but the differences here are again due to the aspect of the habitat 2, a large permanent pond, corresponding to the needs of *B. bombina* (Gollmann et al. 1988).

In the Livada forest we don't have a slow and uniform transition from populations resembling *B. bombina* to populations close to *B. variegata*, together with the increase of the altitude. This transition is cut and modified at a certain level by the morphology of the habitats. Therefore, in the Livada forest, the hybridizing area is more of a mosaic one. But right near the Livada forest, in the Tur River basin, a smooth cline hybrid zone has been documented (Covaciu-Marcov et al. 2006). These differences are given by types of the habitats overlapping the area of the two species, also a fact stated before (Vines et al. 2003). Hence, if the habitats change slowly, from bigger ponds in the plain areas to smaller puddles in the hills, we have a smooth cline hybrid zone. On the other hand, should the habitats be mixed and very diverse on small sectors, this fact modifies the slow transition and determines a mosaic type hybridizing area. In such cases, the difference among the populations can be amplified by the migration of some individuals towards habitats that correspond more to their ecological needs (Vines et al. 2003). The stability of the hybridizing areas is therefore partially based on the adaptation of the two species to different habitats (Vorndran et al. 2002).

The differences obtained from the two grids are greater for the hybrid populations and smaller for the populations better resembling one of the species. This fact suggests that the hybrid populations aren't stable and depend on the intake of parental genotypes from the neighboring habitats. This speculation is sustained by the fact that in the case of stable, isolated hybrid populations, the two grids have very similar values (Sas et al 2005). So, this fact is probably a consequence of the constant change of individuals among neighboring populations, which in turn modifies the balance that is established in time in the case of hybrid populations. Such a migration among habitats has been previously documented, too (Schmidtler & Schmidtler 2001, Yanchukov et al. 2006, Hartel 2008).

The above mentioned statements are underlined by the great differences between the most distinct individuals of each population. Thus, for the populations from habitat 1, the difference between the closest individual resembling *B. bombina* and the one closest to *B. variegata* is 87.5. The smallest difference (45) is recorded for the puddle from near the forest. Great differences between the most distinct individuals were also documented for other hybrid populations, situated in regions with favorable habitats for both species, where migration among habitats is as well possible (Vesea et al 2004). Even for the populations from the second quarry – habitat 6 – the one closest to a *B. variegata* biotope, we found two specimens that, after inspecting both grids, present more *B. bombina* characteristics. The existence in this habitat of some individuals with a majority of *B. bombina* characteristics is a consequence of the fact that the low area from the Tarna River meadow is only at 100 m away. Thus, *B. bombina* can move forward using the system of puddles and streams that in the rainy season flow into the Tarna River.

The width of the hybridizing area corresponds to that of the literature (Szymura & Barton 1986) and is of about 4 km between the farthest populations. However, because for all the populations the amount of the characteristics from the other species is still relatively high, the hybridizing area stretches more than we investigated, reaching about 6 km. Possibly the greatest difference between this and the one to the west of the Apuseni Mountains is the fact that the *B. variegata* populations are purer at similar altitudes. This detail can be explained by *B. variegata*'s different history for colonizing various regions of the Carpathian Mountains.

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