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MORPHOLOGICAL PATTERNS OF A NIGHTINGALE POPULATION IN A CONTACT ZONE OF *LUSCINIA MEGARHYNCHOS* AND *L. LUSCINIA*

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We investigated morphological characteristics of a population in a contact zone of two sister species of nightingales in North-East Hungary. The aim of our study was to analyse and interpret the current morphological patterns of a nightingale population in three populations, called Bódva, Tiszalúc–Bárányszeg and Tiszabercel–Tiszatelek. The research was carried out between 2006 and 2010 at the northern sites of rivers Tisza, and Bódva, where 63 nightingales were examined altogether. The three populations were compared with each other for several morphological characteristics. The populations were significantly different in wing-pointedness, wing-symmetry and body mass and, although marginally, in tail length. Based on the combination of two morphological traits (the relative length of the short first primary and the proportion of length of the second and fourth primary), seven (11.1%) of all investigated birds were identified as interspecific hybrids. In conclusion, we suggest a possible interspecific hybridization between the two nightingale species in the studied area. We assume that the modification in the structure of riverine vegetation might be one of the reasons of the relatively high proportion of hybrid individuals.

Key words: contact zones, nightingale, morphology, morphometry, population, hybridization

INTRODUCTION

Hybrid zones are commonly found in regions where the environmental conditions of native habitat of both sister species meet (Barton 1979, Barton & Charlesworth 1984, Barton & Hewitt 1985). These areas often overlap with the spatial structure of vicariant phylogeographical patterns (Taberlet *et al.* 1998, Hewitt 2001, 2004, Schmitt 2007). Although hybridization of closely related bird species generally implies morphological shifts (Mayr 1970, Moore 1977, Grant & Grant 1992), considerable differences can be manifested within species complexes in the case of crossbreeding in subspecies (Leisler *et al.* 1997, Ottosson *et al.* 2005). Carpathian Basin shows a high level of biodiversity (Williams *et al.* 1999, 2000) mostly due to the overlapping of different climatic and vegetation provinces. The varied relief as well as edaphic and hydrogeographic conditions have resulted in suitable conditions for a large number of species belonging to various faunal types to survive (Varga 1995). Thus, mostly or partly allopatric sister species can overlap (e.g. *Parus caeruleus–P. cyaneus, P. palustris–P. montanus* or *Ficedula albicollis–F. hypoleuca*) (Martin 1990, Newton 2003). In case of the species listed above several factors may affect the frequency of hybridization, such as the relative frequency of two closely related species at a given time and location. It is also possible that such differences on a local scale could mask actual differences in species recognition (e.g. Saetre *et al*. 1999).

Some sympatric populations of such partially overlapping species do indeed show little or no consistent differentiation in particular segments of their morphological traits. Hybridization also produces novel combinations of morphological variables that can be used as main identification keys such as wing length, wing shape, relative length of the first short primary and other plumage characteristics. For example, the morphologically similar Old World tit species, marsh tit (*Parus palustris*) and willow tit (*P. montanus*) clearly differ in habitat selection and geographical distribution. The sequence divergence between cytochrome *b* lineages of these species covers a range of 0.4–1.8% per million years. Most recent splits between east and west Palearctic taxa of these tits were dated to the Pleistocene/Pliocene boundary (e.g. Päckert *et al*. 2007). The variation of some habitat characteristics can also be found in other sibling *Parus* species. The blue tit (*Parus caeruleus*) is a forest-dwelling bird over most of its range, whereas the azure tit (*P. cyaneus*) is more restricted to riverine broad leaved scrubs and scrubby forests of the southern taiga and northern steppe zones or to the edges of montane coniferous forests (Voous 1970). The two species overlap in the western part of the Ural Mountains. This overlap has changed owing to hybridization as a consequence of the westward expansion of the azure tit (Pleske 1912, Frank & Voous 1969).

In Europe, two sister species of nightingales interbreed continuously in a narrow contact zone (Sorjonen 1986, Reifová *et al.* 2011a). The common nightingale *Luscinia megarhynchos* Brehm, 1931 which is widely distributed in the western Palearctic, is a frequent breeding and migratory species (Cramp 1988). According to Svensson (1992) it was subdivided to three subspecies (*L. m. megarhynchos, L. m. hafizi* and *L. m. africana*). However, only *L. m. megarhynchos* breeds in the Carpathian Basin (Cramp 1988). The thrush nightingale *Luscinia luscinia* Linnaeus, 1758 is monotypic, widespread in temperate Asia (mostly in Western Siberia) and northeastern Europe, and a rarely nesting but regularly migrating passerine in autumn in the Carpathian region (Cramp 1988). Hungary is situated on the south-western edge of its distribution area (Moreau 1972).

When two populations of distinct but closely related bird species come into secondary contact, individuals of the population can mate with each other (Hewitt 2001). In case of nightingales hybridization occurs in some areas where populations of the two species meet, whereas in other areas of overlap it is not at all observed (Becker 2007). Supposedly, these two sister species have diverged by geographical isolation during the Pleistocene and came into secondary contact in a narrow hybrid zone from north Germany via Poland

and Hungary to the Black Sea (Sorjonen 1986). Recent studies have demonstrated the existence of mixed pairs and interspecific hybrids, especially in some sympatric localities in the northern and western part of Europe (Becker 1995, 2007, Kverek *et al*. 2008, Storchová *et al*. 2010). However, no study on the morphometric differentiation and hybridization of the two nightingale species has been performed in the Carpathian Basin. Although the relative abundance of the two species is well documented in northern countries (Becker 1995, 2007, Kverek *et al*. 2008, Sorjonen 1986), the relative abundance and morphological relationships of their populations are virtually unknown in Hungary.

The two nightingale species have different song patterns in general, although thrush nightingale males often sing like the common nightingale, where their habitats overlap (Sorjonen 1986, Lille 1988). According to former studies, the two nightingale species occurred in an apparently stabilized overlapping zone in the northeastern part of Hungary, especially in the Upper-Tisza valley (Farkas 1954*a,b*, Schmidt 1986). According to Schmidt (1973), territories of the two nightingale species were not clearly separated from each other along the river Tisza and the males of common nightingale and thrush nightingale were frequently heard singing close to each other. In 1979, Schmidt found that overall plumage coloration of the mixed singing birds showed interim patterns of the two species (Schmidt 1986). Thus, Schmidt (1986) hypothesized a gradual hybridization between the thrush nightingale and common nightingale populations occurs in the Upper-Tisza region in North-East Hungary and attributed habitat loss as one of the most important reason to this phenomenon. It means that the total area of ancient soft-wood riparian (gallery) forest has decreased; therefore, lower quality of fragmented habitats became more suitable as a breeding territory for the common nightingale. According to Schmidt's observations, the number of singing thrush nightingales along the Upper-Tisza region was 20–30 between 1968–1975, 8–12 between 1976-1979, and 12–20 between 1980–1982. Later, there were only seven individuals in 1983, six in 1984, and four individuals in 1986 (Schmidt 1986). In 1993, five thrush nightingales were also found by Schmidt, but he could not find pure singers among these birds in 1993 (Haraszthy 1998). Based on those findings, a gradual hybridization between of the thrush nightingale population can be hypothesized. Although the overall morphology is relatively similar, the two nightingale species can be clearly distinguished by the relative length of the first primary, the maximal wing length and the presence of emargination on the outer vane of the third or fourth primary and plumage coloration (Cramp 1988, Svensson 1992). However, these morphological characteristics are not always clear especially in areas where both species occur (Kverek 1998, 2002, Kverek *et al*. 2008). For example, few nightingales with very short first outermost primaries which are an inherent trait for thrush nightingales were regularly observed in the northeastern part of Hungary (Kováts *et al.* 2009). Based on Becker (2007) and Kverek *et al.* (2008), if the second primary (P_2) is shorter than the fourth primary (P_4), the bird should be identified as *L. megarhynchos*, while as *L. luscinia* with opposing characteristics. Taking into consideration those observations, we wanted to investigate the morphometrical patterns of nightingales in the contact zone of North-East Hungary.

In this study we aim to conduct population-level description and classification of the nightingales, using morphological characteristics. Since the former surveys (Haraszthy 1998, Schmidt 1986) have suggested the existence of hybridization between *L. luscinia* and *L. megarhynchos* in our studied area, we suppose that surveyed individuals might form three morphological groups (*L. luscinia, L. megarhynchos* and their interspecific hybrids). Finally, we tested whether for a possible hybridization between the two nightingale species in a contact zone in North-East Hungary.

MATERIAL AND METHODS

Study area

The study areas were situated on the riversides of the Upper-Tisza region between Tiszabercel and Tiszatelek Flood Nature Protection Areas (48°10′25.4″ N, 21°42′16.7″ E), in the Kesznyéten Landscape Protection Area (48°01′10.5″ N, 21°06′52.4″ E), and along the ancient river flood plain areas of River Bódva, in the Aggtelek National Park (48°27′16.9″ N, 20°43′34.5″ E) (Fig. 1). The climate is dominantly continental with a mean annual temperature of 9.5–10 °C, and with a mean annual precipitation of 550–700 mm. In general, habitat and vegetation types are closely related to those of typical riparian areas. Its vegetation is primarily dominated by soft-wood riparian forests (*Salicetum albae-fragilis*) consisting of *Salix alba, S. fragilis, Populus alba* and *P. nigra* which play an important role in the general ecological functioning of those wetlands. The habitat structure of the study area consists of mixed old hard-wood riparian (gallery) forests (*Querco-Ulmetum*) dominated by *Quercus robur*, *Ulmus laevis* and *U. minor*, *Fraxinus excelsior* or *F. angustifolia* ssp. *pannonica* (*Ulmenion-minoris*), *P. alba*, *P. nigra* and different sections of associated small oxbow lakes and backwaters with *Alnus glutinosa, S. alba*, *P. alba* and *Sambucus nigra* at the Bódva study site.

Biometric measurements

Birds were captured during the peak of breeding season in May and June between 2006 and 2010 using 2.5 m high and 7 or 12 m length Ecotone® mist-nets [mesh: 16x16 sq. mm] by tape luring. Mist netting started at 4.00 o'clock and lasted to 8.00.

All birds were measured and ringed with individually numbered aluminium rings by DK. Essential ringing data (e.g. date, ring No., age, sex) of each bird are available through the Hungarian Bird Ringing Centre (BirdLife Hungary, Budapest). Each captured bird was measured following the recommendations of the Protocol of Actio Hungarica (Szentendrey *et al*. 1979) (based on Protocol of Actio Baltica (Busse & Kania 1970, Busse 1974) in the following succession:

Wing characteristics – Wing characteristics were measured with a plastic ruler to the closest 1 mm according to maximum chord for live birds, with wing flattened and straightened sideways. Specifically, the following variables were recorded for each individual:

- maximal wing length (MWL) [mm],
- wing index (difference in length between the first primary and wing tip) (W^{ix}) [mm],
- distance between the wing point and the first secondary (I/II) [mm],
- relative length of the first primary (outermost) (rLP_1) (difference in length between the first primary and the longest primary covert) [mm],
- length of the third primary (P_3) [mm],

Additional characteristics:

- tail length (TL) [mm],
- body mass (Bm) [g].

Wing formula measurements

Wing formula was recorded in all cases with the primaries measured from tip of wing to tip of each primary on the absolutely closed wing in millimetre and numbered in an ascending order. The wing formula was used to calculate two indices: (1) an index of wing pointedness (W^p) as the sum of the distances divided by the wing length, (2) an index of wing-symmetry (W^{sym}), as a ratio of sums of distances of primaries ascendently and descendently from the tip calculated by the following relationships:

$$W^p = 100 * (dPF\text{-}dDF)/MWL,$$

$$W^{sym} = dPF/dDF,$$

where dPF: the sum of distances from wing-tip to tips of proximal primaries and dDF: as above the added distance from wing–point of distal feathers in millimetre (HOŁYNSKI 1965).

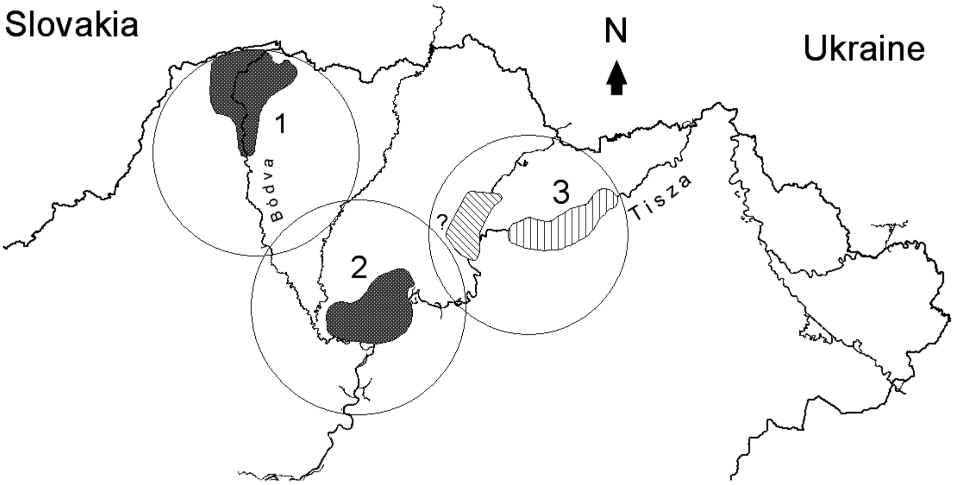

Fig. 1. Location of the study areas based on the studied populations (1: Bódva, 2: Tiszalúc–Bárányszeg, 3: Tiszabercel–Tiszatelek). Empty circles show the distribution of *L. megarhynchos,* vertical striping indicate *L. luscinia,* while slantwise striped area (Bodrogköz) is not studied.

This is a sensitive approach to separate populations based on wing shape characteristics (Csörgő & Lövei 1990). The relative length of the second primary (P_2) and of the fourth primary (P_4) was also calculated from the wing formula. Body mass was recorded to the closest 0.1 g using a 60-g Pesola spring scale. In total, we used 11 different variables in our analyses. After capture and measurements each bird was individually identified by applying numbered aluminium rings for further control. Birds were released in the same sites where they were caught.

Statistical analyses

To determine differences in independent variations between the three populations we used ANOVA (analyses of variance). Statistical analyses were carried out using the statistical software SPSS 16.0. First, we classified three populations and determined to which extent do these differ from each other. Further, we identified individuals with interspecific sizes, using the combination of two morphological characteristics based on identification described by Kverek *et al.* (2008): the rLP_1 and the combination of relative length of P_2 and P_4 were used: if $rLP_1 > 1$ mm and $P_2 < P_4$ birds were specified as *L. megarhynchos*, when $rLP_1 <$ 1 mm and $P_2 > P_4$ as *L. luscinia* and if $rLP_1 < 1$ mm like in the *L. luscinia*, but $P_2 < P_4$ birds were specified as interspecific hybrids (*L. megarhynchos* × *L. luscinia*). Birds with $rLP_1 = 0$ were not considered as potential hybrids. All morphometrical data are given in the Supplementary Table.

RESULTS

In total, 63 birds were captured, ringed and measured between 2006 and 2010 in the breeding season. The summaries of all measurements of each population with descriptive data for morphological characteristics are presented in Table 1.

Comparison of the studied populations

According to the statistical results of ANOVA, high significant differences between the population were found for W^p, W^{sym} and Bm, while TL was only marginally significant between the studied populations (ANOVA: W^p: $F = 29.351$, df = 2, $p < 0.001$; W^{sym}: $F = 13.924$, df = 2, $p < 0.001$; Bm: $F = 5.766$, df = 2, $p = 0.005$, TL: $F = 3.455$, df = 2, $p = 0.05$). The Tukey post hoc tests indicated the following differences: Bódva/Tiszalúc–Bárányszeg: W^p: $p < 0.001$, W^{sym}: $p = 0.005$; Bm: $p < 0.03$, TL: $p = 0.05$; Bódva/Tiszabercel–Tiszatelek: W^p: $p < 0.001$, W^{sym}: $p = 0.005$, Bm: $p < 0.03$, TL: $p > 0.3$. Tiszalúc–Bárányszeg/Tiszabercel–Tiszatelek: W^p: $p < 0.001$, W^{sym}: $p = 0.005$; Bm: $p < 0.03$, TL: $p > 0.5$.

Based on the results of ANOVA, individuals of Bódva population have significantly more pointed and symmetrical wings than population of Tiszalúc–Bárányszeg and Tiszabercel–Tiszatelek. Wings of nightingales of Tisza-

Table 1. Individual measurements of the three studied populations.

Studied population		Variable	Mean	Range	sd	N
Bódva		MWL	87.0	83.0–92.0	2.58	20
		W^{ix}	49.2	43.0–59.0	3.60	20
		W^{p}	61.0	55.68–71.59	4.10	15
		W^{sym}	42.7	37.0–56.0	5.12	15
		rLP_1	2.7	1/–4	0.97	20
		P_2	61.3	59.0–65.0	1.98	15
		P_3	66.9	64.0–70.0	1.75	15
		P_4	65.6	62.0–69.0	1.92	15
		I/II	26.7	23.5–28.5	1.25	20
		TL	73.2	67.0–80.0	3.32	20
		Bm	24.2	22.0–28.5	1.59	20
Tisza	Tiszabercel–Tiszatelek	MWL	88.2	86.0–92.0	1.81	10
		W^{ix}	48.8	47.0–54.0	2.30	10
		W^{p}	48.8	43.18–61.49	5.50	10
		W^{sym}	37.9	31.50–72.00	12.16	10
		rLP_1	2.8	0/–4	1.04	10
		P_2	62.2	59.0–65.0	1.90	10
		P_3	67.6	65.0–72.0	1.84	10
		P_4	66.3	64.0–71.0	2.00	10
		I/II	26.40	24.0–28.0	1.35	10
		TL	75.4	73.0–78.0	1.34	10
		Bm	24.0	21.5–26.0	1.35	10
Tisza	Tiszalúc–Bárányszeg	MWL	87.5	82.0–91.0	2.22	33
		W^{ix}	49.5	44.0–54.0	2.35	33
		W^{p}	49.5	31.71–64.44	7.58	32
		W^{sym}	35.1	22.0–48.0	5.38	32
		rLP_1	2.1	–3/5	1.76	33
		P_2	61.9	57.0–65.0	2.15	32
		P_3	67.6	63.0–72.0	1.97	33
		P_4	66.5	62.0–71.0	1.93	32
		I/II	26.3	23.0–29.0	1.33	32
		TL	75.9	68.0–80.0	2.84	33
		Bm	22.7	19.0–24.0	1.46	33

Table 2. Morphological characteristics of seven individuals described as interspecific hybrids. Additionally, the most important variables and their mean, range and standard deviation (SD) are given.

Date	Ring no.	MWL	rLP$_1$	Bm	Em	P$_2$	P$_4$	P$_2$/P$_4$
9.06.2006	AE36781	89.0	–2	23.8	3	64.0	66.0	<
9.05.2007	AE36873	89.0	–4	24.2	3	65.0	67.0	<
23.05.2007	AE36892	88.0	–1	23.5	3	62.0	68.0	<
24.05.2007	AE36896	85.0	–3	24.1	3	61.0	66.0	<
25.05.2007	AE36899	89.0	–1	22.4	3	65.0	68.0	<
31.05.2007	AE44809	86.0	–1	23.5	3	60.0	66.0	<
2.06.2010	N115812	86.0	–1	24.0	3	60.0	67.0	<
	mean	87.42	–1.85	23.64		62.4	66.9	
	range	85.0–89.0	–1_–4	22.4–24.2		60.0–65.0	66.0–68.0	
	sd	1.71	1.21	0.61		2.23	0.90	

lúc–Bárányszeg population are significantly more pointed, but less symmetrical than population of Tiszabercel–Tiszatelek. Individuals of Bódva and that of Tiszabercel–Tiszatelek have significantly greater body mass than birds of Tiszalúc–Bárányszeg. In relation to the TL, birds of Tiszalúc–Bárányszeg population have slightly longer tails than that of Bódva, while individuals of Tiszabercel–Tiszatelek population did not differ from the others in this trait.

Presence of interspecific hybrids

Out of 63 birds, seven nightingales (two birds of Bódva population and five individuals of Tiszalúc–Bárányszeg population) were specified as potentially interspecific hybrids in total, based on the criteria given by Kverek *et al.* (2008). These individuals had emargination on the outer vane of the third primary only, the relative length of the first (rLP$_1$) primary was shorter than 1 mm compared to the longest greater wing covert and the relative length of the second primary (P$_2$) was shorter than of the fourth primary (P$_4$). Furthermore, undertail coverts of our birds identified as hybrids were only poorly-streaked as it described in Fig. 1 (B) in Kverek *et al.* (2008). Morphological data of each interspecific hybrid are given in Table 2.

DISCUSSION

Morphological conclusions of the three studied populations

The results confirmed that the three studied populations could be separated from each other by wing-shape (included wing-pointedness and wing-symmetry) and body mass, while other morphological traits showed greater similarities between the populations. It suggests that all available morphometrical characteristics, in particular to wing formula should be measured to classify individuals into the correct population of the study area.

According to our findings, 11.1% of the studied birds could be specified as interspecific hybrids. Thus, we assume that our studied area might be a spatial continuation of the sympatric zone of Poland (via Slovakia) of the two nightingale species.

The exact width of the sympatric belt of North-East Hungary is still unknown. There could be several possible reason for the hybridization. Based on our experience, the quality of undergrowth is not suitable, where the forest environment has already been considerably altered, thus, the composition of the nightingale population probably changed accordingly during the last decades. Due to modified habitat conditions, interspecific competition could have started between peripheral populations of thrush nightingale and common nightingale, resulting in a hybridization process between the two closely related species.

Other plausible reasons for the greater proportion of interspecific hybrids occur in North-East Hungary remain untested. One possible explanation which has often been mentioned in connection with fluctuations in the distribution of the two nightingale species relate to changes in habitat structure and climatic patterns as well (Voous 1960, Sorjonen 1983). However, we believe that climatic and habitat conditions might be more stable in Poland, where breeding habitats are relatively undisturbed and thus, it is possible that the proportion of hybrid individuals is smaller than in North-East Hungary. In addition, Czech Republic is allopatric region for *L. megarhynchos* and north-eastern Poland is allopatric region for *L. luscinia,* and so, hybridization can occur relatively rarely between the two species, while Central Poland and North East Hungary are situated in their sympatric zone (Reifová *et al.* 2011*b*). Based on repeated field experiences, woodlands have been intensively exploited by humans along the river Bódva and Upper-Tisza during the last decades (Varga, pers. comm.). Because of the regulation of the river course carried out in the 19th century, habitats have been decreased significantly during the last hundred years (Dobrosi & Szabó 2002) in the studied area. Owing to exploitation, populations have had to move to other territories, which thus became fragmented, excluding the possibility of meeting other populations.

Consequences of isolation vs hybridisation in other European passerine birds

The significance of intraspecific and interspecific variation in morphology of three sympatric *Phylloscopus* warblers, the willow warbler (*Ph. trochilus*), the chiffchaff (*Ph. collybita*) and the subspecies of the chiffchaff (*Ph. c. tristis, Ph. c. abietinus*) have been extensively studied in Europe (Lövei 1983, Tiainen 1982, Tiainen & Hanski 1985). The chiffchaff and the willow warbler are morphologically similar to each other and can often be found in contact zones. For example, the chiffchaffs arrive approximately one week before the willow warblers, which results in interspecific competition in Finland (Tiainen 1982). This difference is a result of the wing structure of the chiffchaff which is more symmetrical and less pointed than that of the willow warblers. Therefore, chiffchaffs can occupy breeding habitats earlier than willow warblers. In consequence, chiffchaff populations can be separated into different geographical areas. Based on the wing-pointedness indices, chiffchaffs have been separated into two morphologically different populations. *Ph. c. collybita* is a short distance migrant, while *Ph. c. abietinus* is a long-distance migrant subspecies. In general, long-term migrant subspecies have more pointed and less rounded wings so they usually breed in more northerly areas, than subspecies performing short-distance migration (Gaston 1974).

The two sister species of nightingales are not known to hybridize with other similar genera or species (see: www.bird-hybrids.com). Based on the investigation of speciation of the Z-chromosomes, the two closely related nightingale species have diverged approximately 1.8 Mya. According to their nucleotide variation, it was found that rates of introgression more intensively occurred from *L. megarhynchos* to *L. luscinia* than vice versa (Storchová *et al.* 2010). Taking this into consideration, this process may also be present in North-East Hungary. In conclusion, morphological traits may constrain, the range of foraging sites for each population to some degree, evolutionary changes between populations are still possible, as suggested by the above splits (Suhonen *et al.* 1994).

Recent declining population trends of thrush nightingales might be owing to long-lasting floods which became increasingly frequent in the past decades. Additionally, annually repeated floods (a similar process during ice-ages) might force nightingales to occupy a variety of habitats, which may accelerate speciation. In hybrid nightingales, F_1 females are sterile and F_1 males fertile in accordance with Haldane's rule (Stadie 1991) implying that heterogametic sex is more frequently sterile than homogametic sex (Haldane 1992). In Czech Republic (near Mladá Boleslav in central Bohemia) a female hybrid confirmed by genetic analyses was found, without any reproductive activity suggesting sterility (Reifová *et al.* 2011*a*). As sterility can be inheritable in females only, and thus, male thrush nightingales can only mate with fertile (non-hybrid) female common nightingales in such a situation of a hybrid population in the studied area. Due to the increasing hybridization process, fertile females of thrush nightingale will eventually disappear sooner or later from the studied area. Hence, dispersion of the *megarhynchos*-shape may expand successfully. This could be the reason of the relatively large percentage of hybrid individuals in the questionable area. On the other hand, thrush nightingales prefer larger expanded gallery forests along riversides, while common nightingales often breed further away from rivers and exhibit a decreased sensitivity in habitat use, which increases its dispersion abilities.

To obtain a better overview of the hybrid zone we need to carry out further examinations (e.g. in the Bodrogköz Landscape Protection Area).

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