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Testosterone increase in free-living young Blackcaps *Sylvia atricapilla* and Wood Warblers *Phylloscopus sibilatrix* during post-juvenile molt with possible implications for juvenile dispersal

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Abstract. Testosterone may affect many aspects of passerine maturation including the expression of plumage coloration and spring migration in adult birds. However, how the testosterone level changes during bird development is not well known. We compared the testosterone profile during juvenile development of a sexually dimorphic species, the Blackcap *Sylvia atricapilla*, and a monomorphic species, the Wood Warbler *Phylloscopus sibilatrix*. We first tested whether testosterone influences plumage coloration and increases at the onset of molt in males of the dimorphic but not the monomorphic species. We found that a testosterone level increase occurred in both sexes and species during later stages of molt, and thus was not related to male plumage coloration. We also investigated whether the increase in testosterone level coincides with juvenile dispersal. If testosterone affects dispersal behavior in these species, both sexes should show an elevated testosterone level during dispersal, but this increase should occur earlier in the Wood Warbler, which disperses earlier than the Blackcap. In juvenile Blackcaps, the increase in testosterone level occurred on the 43–68th day after hatching, while in Wood Warblers it occurred on the 32–36th day (i.e. 11–32 days earlier). The increase in testosterone level coincided with the onset of the post-juvenile dispersal in both species. This study provides the first direct evidence of a testosterone level increase during juvenile development in two free-living migratory birds. While not correlated with species plumage coloration, this increase might affect other aspects of behavior, e.g. juvenile dispersal.

Key words: juvenile birds, post-juvenile molt, juvenile dispersal, testosterone, *Sylvia atricapilla*, *Phylloscopus sibilatrix*

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INTRODUCTION

The gonadal steroid hormone testosterone (hereafter T) affects many aspects of reproductive physiology, morphology and behavior in vertebrates (Garamszegi et al. 2005, Hau 2007, Fusani 2008). In male birds, the seasonal peak in T level coincides with peaks in mating behavior and territorial aggression, including singing (Wingfield et al. 1990, 2000, DeVoogd 1991, Cynx & Nottebohm 1992, Wingfield 1994, Wingfield & Hahn 1994). Being a key component of the hypothalamic–pituitary–gonadal axis, T is involved in the process of juvenile maturation (Ball & Wade 2013). However, information about the testosterone role in bird development is scarce and rather fragmented (Ball & Wade 2013).

In adult birds, T is involved in spring premigratory hyperphagia (increased feeding) and fat deposition (Deviche 1995, Tonra et al. 2011), while its role during autumn migration has been studied only in a few species. Especially little is known about T concentration in young birds of migratory species before their first migration.

Silverin et al. (1989) found that about 30% of juvenile Willow Tits *Parus montanus* of both sexes had an elevated plasma level of T, which was not correlated with migration behaviour. Based on this finding, along with the study on the Blackbird *Turdus merula* (Schwabl et al. 1984), the authors concluded that T does not influence autumn migration (Berthold 1996). However, recently it has been shown that T-treated young males of the Blue Tit *Cyanistes caeruleus* exhibited greater locomotor activity than control males (Kurvers et al. 2008). In addition, T may be involved in regulation of post-juvenile dispersal, however, this question is also poorly studied (Berthold 1996).

The possible biological significance of juvenile dispersal is to settle in new areas, to avoid competition and inbreeding, to regulate population density, and to establish links with the future breeding sites, seeking new food sources and molting sites (Sokolov 1997). Consequently, juvenile dispersal potentially requires additional energetic cost, which may be realized by elevating the T level, as T is known to stimulate bird metabolism (Wikelski et al. 1999, Buchanan et al. 2001).

Additionally, it has been suggested that T is involved in the expression of plumage coloration in male birds (Folstad & Karter 1992, Jawor & Breitwisch 2003, Bókony et al. 2008), despite the fact that the role of T in plumage coloration is not obvious (Owens & Short 1995, Cantarero et al. 2019), and T is even known to suppress prenuptial molt (Hahn et al. 1992, Kimball & Ligon 1999, Stoehr & Hill 2001). A positive relation between color brightness and T level has been shown for some dimorphic species (Saino & Møller 1994, Gonzalez et al. 2001, Duckworth et al. 2004, Peters et al. 2006). However, these studies were conducted during the breeding period and not the prenuptial (prealternate) molt when breeding plumage is acquired. Recent studies during the prenuptial molt demonstrate that T does influence male plumage coloration in dimorphic species (Peters et al. 2000, Geslin et al. 2004, Lindsay et al. 2009) and may even have activational effects in the subsequent breeding season (Roberts et al. 2009). Moreover, in species which undergo only one molt before the breeding season, T level in males molting to the adult color is significantly higher than in those molting to subadult plumage (Schwarzová et. al. 2010). Unfortunately, to our knowledge no information is available about differences in T effect on male and female coloration in monomorphic species.

In females, circulating T level is generally lower than in males, but they also vary over the course of the annual cycle, with higher concentrations observed just before or during the breeding season (Elekonich & Wingfeld 2000, Ketterson et al. 2005). In tropical birds T level is associated with female ornamentation (Enbody et al. 2018). However, little is known about T concentration in juvenile females.

In this paper, we investigate two aspects of possible T impact on young birds: 1) Plumage/molt. Many avian species are strongly sexually dimorphic and in the majority of them, male features appear for the first time in autumn during the post-juvenile molt (Vinogradova et al. 1976, Svensson 1992). If T level influences plumage coloration in juvenile males, we hypothesize that young males of dimorphic species should have a T level increase at the onset and propagation of post-juvenile molt in comparison to monomorphic species. Additionally, females of dimorphic species should have a lower T level than males; 2) Dispersal. We also investigate whether the T level increase coincides with juvenile dispersal. If T affects this behaviour, the species that migrate early should have an increase in T level earlier in the life cycle than species migrating later. Also, the T level of females may be similar to that of males in both species.

To test these hypotheses we chose two species abundant in the middle Russia — the dimorphic European migrant Blackcap *Sylvia atricapilla* and the monomorphic long distance migrant Wood Warbler *Phylloscopus sibilatrix* (Ptushenko & Inozemtsev 1968, Svensson 1992). Blackcaps undergo a partial post-juvenile molt in the breeding area and only sometimes have a partial pre-breeding molt in the winter area, while Wood Warblers undergo a partial post-juvenile molt in the breeding area and have a complete pre-breeding molt in the wintering area (Demongin 2016). In male Blackcaps the juvenile brown feathers on top of the head change into adult-like black ones during the post-juvenile molt, while Wood Warbler males remain similar to females after the post-juvenile molt (Vinogradova et al. 1976, Svensson 1992). Both species appear to have only one brood per summer (Ptushenko & Inozemtsev 1968). Wood Warblers, being long-distance migrants, start to disperse earlier than Blackcaps (Ptushenko & Inozemtsev 1968). While, Blackcaps have an extended breeding period in comparison to Wood Warblers (Ptushenko & Inozemtsev 1968).

MATERIAL AND METHODS

Study system

Our research was carried out from June to October of 2004–2013 at Zvenigorod Biological Station of Moscow State University (Moscow Region, Russia, 55°44′N, 36°51′E). Birds were caught using stationary mist-nets (5–15 m long and 2–2.5 m high, with standard mesh size of 14 or 16 mm), which were placed between trees and bushes in the Moscow river flood plain and on the border between the Moscow river flood plain and the forest, in a study plot with an area of 2.75 ha. Every year, 30–50 mist-nets (about 450 m in total length) were used. Mist-nets were opened almost every day during the summer and autumn.

We banded all captured birds with metal rings and determined sex and age for each bird, whenever possible, as well as molt stage (Vinogradova et al. 1976, Svensson 1992). Since juvenile Blackcaps and Wood Warblers do not molt primaries, the molt stage was scored by visual assessment of the progress of body feather molt on a scale from 1 to 3: with 1 (the onset of molt) assigned for less than 1/3 of the body feathers molted, 2 (medium molt) assigned for 1/3 to 2/3 of the body feathers molted, and 3 (the end of molt) assigned for more than 2/3 of the body feathers molted (Bairlein 1995). In total, we defined five stages of juvenile development: pre-molt, the onset of molt, the middle stage, the end of molt and completely molted birds.

To determine the age of juvenile birds at different stages of molt and to estimate how long Blackcaps and Wood Warbles stay in the breeding area before the onset of dispersal, we analysed recapture data of young birds, using our ringing data from 2001–2013. In total, we found 69 recaptures of young Blackcaps and 35 recaptures of young Wood Warblers. In addition, we separately analyzed 8 Blackcaps and 8 Wood Warblers ringed at the nests and later recaptured by mist-nets.

Blood sampling and hormone assay

Blood samples from 90 young Blackcaps and 31 Wood Warblers were collected for measurements of circulating T concentrations (Table 1). The majority of the samples were collected in the morning, but some samples were collected during the day and evening as well (Table 1). Since T levels may vary throughout the day, we divided the day into four periods (Table 1). The delay between capture and taking blood samples was recorded for 51 out of 90 juvenile Blackcaps and for 22 out

of 31 juvenile Wood Warblers (varied from 15 up to 120 min, on average 56 min). The samples were obtained by puncturing the brachial vein using a 26-gauge needle and collecting the sample into 75 μL heparinized capillary tubes. The blood was immediately centrifuged after sampling (3000 r/h for 15 min), and the plasma was separated and frozen at -20 °C until the analysis could be performed.

The T level of the blood plasma was measured by enzyme linked immunosorbent assay (ELISA) using IEA TS (testosterone) commercial test systems (NPO Immunotech, Russia) according to the manufacturer's instructions. 20 μL of plasma was used for one sample assay. The cross-reactivity of antibodies to testosterone was 9% to 5-dihydrotestosterone, 1% to 11-hydroxytestosterone, 1% to 5-androstene-3.17-diol, 0.66% to progesterone, and less than 0.1% for all other tested steroids. The coefficient of inter assay variation across nine assays was 7.9% (data from one year was analysed in one assay), and the intra assay variation was 4.5% (for samples measured in duplicates). The sensitivity was 0.06 ng/ml.

Assay validation

We validated this assay by demonstrating parallelism of a serially diluted plasma sample (2 × to 32 dilutions) with the standard curve. We tested the

Table 1. Testosterone (T) samples per years, time of the day, and sex.

	Number of T samples	
	Blackcap	Wood Warbler
Years		
2004	2	
2005	4	
2006	8	1
2007	36	5
2008	20	
2009	7	
2010	13	5
2011		14
2013		6
Time of the day		
5.00–10.00	46	15
10.00–13.00	22	11
13.00–17.00	9	2
17.00–23.00	13	3
Sex		
Males	53	14
Females	25	8
Sex unknown	12	9
Total	90	31

commercial kit on three passerine species: the Blackcap, the Wood Warbler and the Canary *Serinus canaria* var *domestica*. Serial dilutions of blood plasma were parallel to the steroid standard with no significant difference in slopes (p > 0.05).

Sex of juvenile birds

It was not possible to determine the sex of juvenile Blackcaps before post-juvenile molt in the field, but during and after the molt, their sex was easily distinguished. Since the T level was very low in all Blackcap juveniles before the onset of molt (mean ± SD: 0.38 ± 0.27, see also Table 3 in the Results section), and since after the onset of molt the sex was clearly visible, we did not deem it necessary to determine their sex by genetic analysis. The sex of Wood Warblers was determined by genetic analysis. DNA was extracted using a Diatom DNA Prep 100 kit (Isogene Lab. ltd, Russia). Nestlings were sexed using the primers P2 (5′-TCTGCATCGCTAAATCCTTT-3′) and P8 (5′-CTCCCAAGGATGAGRAAYTG-3′) from the CHD gene (Griffiths et al. 1998). PCR amplification was performed using GenePak® PCR Core kits (0.2 ml). The cycling conditions included an initial denaturing step at 94 °C for 3 min, then 35 cycles at 94 °C for 30s, 50 °C for 30s and 72 °C for 45s, then elongation at 72 °C for 3 min. Males amplified one and females amplified two fragments. PCR products were separated in 2% agarose gels and detected by ethidium bromide.

Statistical analysis

We analyzed the data with linear mixed models (LMM) with the function lme() in the nlme package (Pinheiro et al. 2018) in R 3.4.3 (R Development Core Team 2017). We log-transformed T concentration measurements, to normalize residual distribution. At each step, we started with a full model to see possible effects of interaction between predictors. Year and Time of Day were taken as random factors, while the Stage of Molt, the Delay between capture and blood sampling time (since it was possibly influencing the T level) or Sex were taken as fixed predictors. Variables were excluded from the full model following a backward model simplification protocol (Zuur et al. 2009), and the normality of residuals was checked at each step. The significance of factors was estimated by likelihood ratio tests. Since we missed some data on the Delay between capture and blood sampling time for both species in the first step we checked if it had a significant effect on T concentration. Consequently, for each species the first model included the Stage of Molt and the Delay between capture and blood sampling time as fixed predictors. We first simplified the random part of the model (using restricted maximum likelihood, REML), by the exclusion of the non-significant term Time of Day (see Table 2). Then the fixed part of the model was simplified using the method of maximum likelihood (ML). Since no interactions between the variables had significant effects, they were excluded from the model.

In the second step, we pooled all of the blood samples (with and without data on the delay between capture and blood sampling) into the analysis. At this step, only data from birds of known sex were taken into account. Consequently, for Blackcaps we used a subset of data that excluded the pre-molting stage. The full model for each species at this step included the Stage of Molt and Sex as fixed predictors. Since no interactions between the variables had significant effects, they were excluded from the model. Since sex did not have a statistically significant effect in either species (Table 2), we could pool all the blood

Table 2. Significance of factors by likelihood ratio test (LRT) comparisons of linear mixed models affecting juvenile Blackcaps and Wood Warblers testosterone level. Df — number of degrees of freedom in the model. N — number of birds. * — From the model including the delay between capture and blood sampling time, which was dropped from the final model, ** — From the model including sex, which was dropped from the final model.

Fixed factors	Random variable	Blackcaps				Wood Warblers				Juvenile of both species			
		N	LRT	Df	p	N	LRT	Df	p	N	LRT	Df	p
The delay between capture and blood sampling time*		57	0.79	5	0.37	22	1.8	5	0.18				
	Year	90	7.55	5	**0.006**	31	19.0	5	**< 0.0001**	68	0.13	6	0.72
	Time of the day	90	< 0.0005	6	0.99	31	< 0.0004	6	0.99				
Sex**		78	0.87	4	0.92	22	2.6	4	0.11	99	0.5	6	0.50
Stage of molt		90	8.19	3	**0.004**	31	4.5	3	**0.03**	99	4.8	4	**0.03**
Species										99	8.8	4	**0.003**

samples (with and without known sex) into the analysis and refitted the model without the sex effect using the full dataset. In this case, the model for each species included the Stage of Molt as fixed predictors. A Tukey post-hoc test was performed in function glht in the multcomp package in R to estimate the effects of the stage of molt on T level (Hothorn et al. 2008).

To compare two species we used a model including T level data on both species and Year as random factors, and the Stage of Molt, Sex and Species as fixed predictors. We took into account only data on birds with known sex. A Tukey post-hoc test was performed to estimate the effects of species on T level.

Differences between species in the time spent at the breeding area (time between first capture and last recapture) were analysed non-parametrically using Mann–Whitney U-tests in Statistica version 8.0 (StatSoft Inc. 2007).

RESULTS

Year accounted for variation in testosterone levels between individuals in both Blackcaps and Wood Warblers (Table 2). In the model including two species, Year did not have a significant effect on T level (Table 2). However, this model included three years only, where both species were captured.

Sex and species-specific variation of testosterone level

Sex did not affect T level in either Blackcaps or Wood Warblers and was dropped from the final models (Table 2). In the model including two species, sex did not affect T level either. Species was a significant predictor of T level (Table 2). Wood Warblers had a significantly higher T level than Blackcaps (Table 2, 3, Tukey post-hoc test $p < 0.001$).

Testosterone level and stage of molt

The stage of molt had a statistically significant effect on T level in Blackcaps and Wood Warblers (Table 2). In Blackcap juveniles, T level was highest at the end of the molt and remained considerable higher in completely molted birds (these latter stages did not differ significantly from the other stages of molt, $p < 0.09$, Tukey post-hoc test; Table 3). In juvenile Wood Warblers, T level was highest in the middle of the molt (this stage significantly differed from the others, $p < 0.001$, Tukey post-hoc test), and slightly decreased at the end of it, and in completely molted birds (Table 3).

Molt duration and testosterone increase

In Blackcaps the molt started 18–25 days after leaving the nest, lasted on average 30 days, and finished 48–56 days after the juveniles had left their nests (Table 4). In Wood Warblers the molt started 11–17 days after leaving the nest, lasted on average 16 days, and finished 27–33 days after the juveniles had left their nests (Table 4).

Blackcap juveniles had a T level increase that coincided with the end of molt, i.e. on the 48–56th day after nest departure. Wood Warbler juveniles had a T level increase that coincided with the middle of molt, i.e. on the 20–24th day after nest departure. Consequently, taking into account 12 days in the nest, in Blackcaps the T level apparently increased on the 43–68th day after hatching, and in Wood Warbles the T level increased on the 32–36th day after hatching (about 11–32 days earlier than in Blackcaps).

Days spent in the breeding area

Young Blackcaps stayed in the breeding area for a significantly longer period then Wood Warblers (Fig. 1) ($Z = 2.6$, $p = 0.01$, Mann-Whitney U test, on average 10 days between first and last recapture for Blackcaps and 7 for Wood Warblers). The first juvenile Blackcap and Wood Warbler was

Table 3. The testosterone level (ng/ml) at different molt stages in Blackcaps and Wood Warblers, SD — standard deviation, different letters show differences between stages within species. † — $p < 0.09$, * — $p < 0.05$; Tukey post-hoc test.

Molt stages	Blackcaps				Wood Warblers			
	Number of birds	Mean + SD/ differences between stages	Min	Max	Number of birds	Mean + SD/ differences between stages	Min	Max
Pre-molt	10	0.38 ± 0.27/d	0.06	0.8	0			
Onset of molt	16	0.45 ± 0.61/dc	0.03	2.48	12	3.5 ± 2.2/ab	0.9	8.5
The middle of molt	32	1.67 ± 3.4/dc	0.01	15.7	10	**11 ± 6.11*/a**	0.57	20.7
The end of molt	12	4.33 ± 6.9†/c	0.07	22.8	8	5.04 ± 3.8/b	0.58	10.6
Completely molted birds	20	2.75 ± 4.4†/c	0.02	14.9	1	6.34/ab	6.34	6.34

Table 4. Mean (± SD) duration of some life history stages in Blackcaps and Wood Warblers. * — Only recaptures separated from a capture by more than five days were included.

Life history stage	Blackcap		Wood Warbler	
	N	Days	N	Days
From nest leaving to onset of molt	3	18 ± 2	0	
From nest leaving to the middle of molt	3	37 ± 4	8	24 ± 4
From fledgling to onset of molt	8	14 ± 5	0	
From onset of molt to the middle of molt	9	12 ± 4	5	7 ± 2
From fledgling to the middle of molt	6	20 ± 4	0	
From the middle to the end of molt	5	11 ± 2	6	9 ± 2
From nest leaving to the end of molt	1	56	1	30
Number of recaptures*	35		20	

caught in the middle of June (between 19–26 June in different years). In general, Wood Warblers left the breeding area in August, and the last Wood Warbler was caught on 5 September. The Blackcaps left the breeding area in September, with the last Blackcap being caught on 5 October. Blackcaps (N = 8) left the breeding area at the age of 48 days (31–69, min–max), while Wood Warblers (N = 8) did so at the age of 32 days (27–40, min–max) (from data on birds banded in the nest and later recaptured). The age of juvenile dispersal coincided with a T level increase in both species.

DISCUSSION

Our study is the first to demonstrate an increase in T levels in juvenile Blackcaps and Wood Warblers after nest departure. We hypothesized that if T level did influence plumage coloration in juvenile males, then young males of dimorphic species should have a T level increase at the onset and propagation of post-juvenile molt in comparison to monomorphic species. Additionally, females of dimorphic species should have a lower T level than males. Contrary to our hypothesis, we found that both sexes of both species showed an increase in T levels during the post-juvenile molt. Moreover, the T level was higher and occurred at the early stage of molt in the sexually monomorphic Wood Warbler, compared with the dimorphic Blackcap. Consequently, our data did not support a link between T level and dimorphic and monomorphic species plumage coloration during prenuptial molt.

The duration of the post-juvenile molt of late-hatched birds in general is considerably shorter
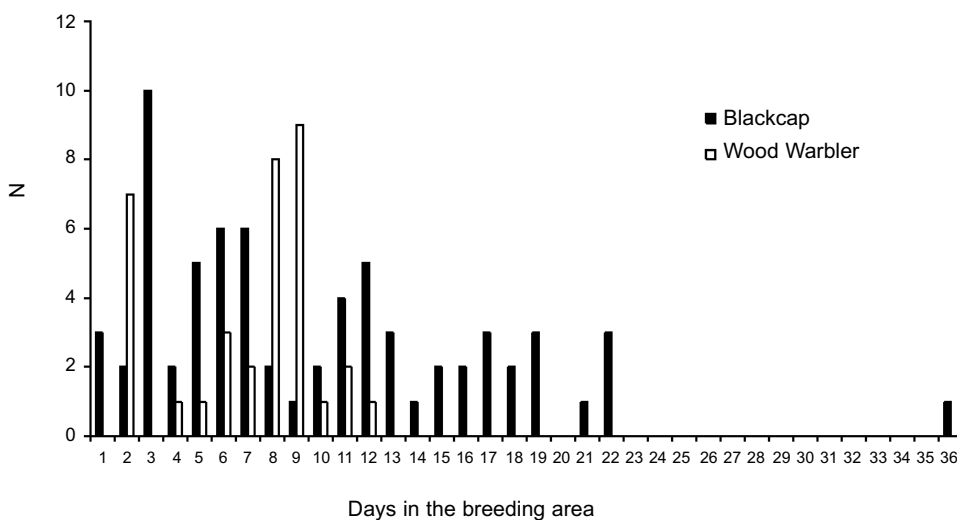

Fig. 1. Days spent in the breeding area (days between the first capture and the last recapture) in young Blackcaps (69) and Wood Warblers (35). On the base of data on birds captured only by mist-nets.

than in early-hatched birds (Jenni & Winkler 2011). Variation in T level at different molt stages (Table 2) may be related to timing of hatching; the T level increase at the later stages coincides with the maturation of the juveniles from early clutches. Consequently, birds from late clutches showed an increase in T levels at an earlier stage of molting. This explains the weak differences in T levels between molt stages in the Blackcaps, which have an extended breeding period in comparison to the Wood Warblers.

An increase in T levels in both species appears to coincide with the onset of post-juvenile dispersal (Newton 2008). In the Wood Warbler, which disperses earlier than the Blackcap, the increase occurred at earlier stages of the life cycle compared to Blackcaps. Consequently, the differences between species in the timing of the T level increase may be related to the period of time they spend in the breeding area. Because the onset of juvenile dispersal varies greatly in Blackcaps (Fig. 1), the T level increase takes place at different stages of molt. In contrast, Wood Warblers' departure from the breeding area is synchronized (Fig. 1) and they have a clearly pronounced peak of T. The increase in T levels in both sexes also supports the idea that there is a relation between T and post-juvenile dispersal. Although the T level increase coincides with the molt stage, we believe that it is related to the onset of post-juvenile dispersal, rather than the molt itself.

The reasons for an increase in T level at the onset of bird dispersal are not very clear. Testosterone, is erythropoietic, stimulating red blood cell synthesis (Domm & Taber 1946, Kern et al. 1972, Nirmalan & Robinson 1972). Researchers have repeatedly found that changes in haematocrit, the ratio of packed red blood cells to total blood volume, are a physiological component of the migratory state, often independent of energetic condition (Wingfield & Farner 1980, Bairlein & Totzke 1992, Morton 1994). The dispersal from the breeding area to new habitats potentially requires energetic and other physiological expenses (costs) which may be achieved by elevating T level. Furthermore, T is involved in pre-migratory hyperphagia (increased feeding) and fat deposition (Deviche 1995, Kurvers et al. 2008, Tonra et al. 2011) and the T level increase may be a preparation for future migration.

None of the species we studied is known to be territorial in autumn, so the T level increase in our study is not related to the territorial interactions observed in some species ("Challenge hypothesis"; Wingfield et al. 1990). On the other hand, unlike Blackcaps, Wood Warblers disperse in flocks, where aggressive interactions have been observed (Goretskaia, unpubl. data), and this may explain the higher T level in Wood Warblers. Indirectly, the relationship between T level and juvenile dispersal is supported by statistically significant T level variation between years, treated as a random effect. Unfortunately, the absence of data on the average annual flock sizes and/or breeding success does not allow us to test this possibility.

In addition T level increase in young birds may be related with other — not studied factors, like song acquisition. In passerine species, song learning and song production as well as the seasonal plasticity of the adult song system are under testosterone regulation (Nottebohm et al. 1987, Ball et al. 2002, Soma et al. 2004). The majority of studies are focused on adult birds, while T function in juvenile birds remains unclear. An increase in T level has been documented in juvenile Canaries of both sexes before the onset and during the beginning of post-juvenile molt, coinciding with subsong production (Weichel et al. 1986, 1989). The juvenile Great Tit *Parus major* undergoes a T level increase, which also seem to coincide with the onset of singing behaviour (Rost 1990). The treatment of juveniles with T elicits premature song production in male Zebra Finches *Taeniopygia guttata* (Leitao & Gahr 2012) and leads to rapid development of song structure in juvenile Song Sparrows *Melospiza melodia* (Templeton et al. 2012). Consequently, the increase of young birds' T levels might also be related to song production (Rost 1990, DeVoogd & Szekely 1998, Templeton et al. 2012). The increase of T level in both sexes may be explained by the necessity for females to recognize conspecific songs, which could be related to the period of formation of song nuclei in the female's brain (Catchpole & Slater 2008).

The lower magnitude of T level increase and its occurrence in the late stage of molt in sexually dimorphic Blackcaps, undergoing partial post-juvenile molt in the breeding area and only sometimes having partial pre-breeding molt in the winter area (Demongin 2016), do not support the impact of T on species plumage coloration during prenuptial molt. However, our data support relationship between T and juvenile dispersal in two species.

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STRESZCZENIE

[Wzrost poziomu testosteronu u młodocianych kapturek i świstunek podczas pierzenia postjuwenilnego jako potencjalny czynnik wpływający na ich dyspersję]

Testosteron może wpływać na wiele aspektów biologii ptaków. U samców sezonowy szczyt poziomu testosteronu zbiega się w czasie z zachowaniami związanymi z kojarzeniem się w pary oraz zachowaniami terytorialnymi. Ma on także związek z ubarwieniem upierzenia samców u gatunków dymorficznych, a także z przygotowaniem do wędrówki wiosennej u osobników dorosłych. Ponadto, ze względu na swoją kluczową rolę w osi podwzgórze–przysadka mózgowa–gonady, testosteron wpływa na proces dojrzewania młodocianych osobników. Niewiele jest jednak wiadomo na temat tego, jak poziom testosteronu zmienia się w trakcie rozwoju ptaków. W pracy badano dwa aspekty potencjalnego wpływu testosteronu na młodociane ptaki: jego związek z pierzeniem i wykształcaniem szaty postjuwenilnej oraz związek z dyspersją. W badaniach porównano zmiany poziomu testosteronu u młodocianych osobników dwóch gatunków — dymorficznej kapturki oraz monomorficznej świstunki. Zakładano, że jeśli testosteron wpływa na ubarwienie upierzenia młodocianych samców,

to w odróżnieniu od gatunku monomorficznego (świstunka), u samców gatunku dymorficznego (kapturka) na początku oraz w trakcie pierzenia powinien być obserwowany wzrost poziomu tego hormonu, zaś samice gatunku dymorficznego powinny mieć poziom testosteronu niższy niż samce tego gatunku. W przypadku dyspersji, przewidywano, że jeśli testosteron wpływa na to zachowanie, to zarówno u samców, jak i samic obu gatunków poziom testosteronu powinien być podwyższony podczas dyspersji, przy czym wzrost poziomu testosteronu powinien być obserwowany wcześniej u gatunku przystępującego do wędrówki wcześniej, czyli świstunki.

Badania prowadzono w latach 2004–2013 na terenie stacji terenowej w Zwienigorodzie (obwód moskiewski, Rosja). Ptaki chwytano w sieci ornitologiczne, obrączkowano, określano płeć (w przypadku świstunek przy pomocy metod molekularnych), wiek a także stopień zaawansowania pierzenia (na podstawie pierzenia piór okrywowych). Wyróżniono 5 etapów pierzenia: przed pierzeniem, początek pierzenia, środek pierzenia, koniec pierzenia, osobnik całkowicie przepierzony. W celu określenia wieku osobników młodocianych w różnych stadiach pierzenia oraz tego, kiedy rozpoczynają dyspersję, wykorzystano dane obrączkarskie z lat 2001–2013. W sumie, w analizach wykorzystano dane o ponownych schwytaniach 69 młodocianych kapturek i 25 młodocianych świstunek. Dodatkowo, wykorzystano dane od osobników (8 kapturek oraz 8 świstunek) schwytanych jako młodociane, a które zostały zaobrączkowane jako pisklęta. Krew z żyły skrzydłowej pobrano od 90 kapturek i 31 świstunek, a następnie w osoczu określono poziom testosteronu. Ze względu na fakt, że nie u wszystkich ptaków określono płeć oraz ile czasu upłynęło pomiędzy schwytaniem ptaka a pobraniem od niego krwi, początkowo analizy ograniczono do tych osobników, dla których zebrano pełną pulę danych. W analizach zostały uwzględnione: płeć i wiek ptaków, rok i godzina pobrania próbek (Tab. 1) oraz ile czasu upłynęło pomiędzy schwytaniem ptaka a pobraniem od niego krwi. Ze względu na to, że zarówno płeć, jak i to, ile czasu upłynęło pomiędzy schwytaniem ptaka a pobraniem od niego krwi, nie wpływały na wyniki analiz (Tab. 2), parametry te nie były

następnie brane pod uwagę, a kolejne analizy przeprowadzono z uwzględnieniem wszystkich schwytanych ptaków.

Świstunki miały znacząco wyższy poziom testosteronu niż kapturki (Tab. 2, 3). Stwierdzono, że zaawansowanie pierzenia a nie płeć u młodocianych ptaków wpływa na poziom testosteronu (Tab. 2). U kapturek najwyższy poziom testosteronu występował pod koniec pierzenia i był stosunkowo wysoki u całkowicie przepierzonych ptaków, chociaż na tych dwóch etapach nie różnił się znacząco od poziomu testosteronu na wcześniejszych etapach pierzenia. Z kolei u świstunki najwyższy poziom testosteronu występował na środkowym etapie pierzenia i był on 2–3-krotnie wyższy niż na pozostałych etapach pierzenia (Tab. 3). Biorąc pod uwagę początek i długość trwania pierzenia (kapturki: początek 18–25 dni po opuszczeniu gniazda, długość trwania — 30 dni; świstunki: początek 11–17 dni po opuszczeniu gniazda, długość trwania — 16 dni, Tab. 4) oraz to na jakim etapie pierzenia stwierdzono najwyższy poziom testosteronu, można przyjąć, że u kapturek wzrost poziomu testosteronu nastąpił w 43–68 dniu po wykluciu, a u świstunek — w 32–36 dniu po wykluciu. Ze względu na fakt, że poziom testosteronu wzrósł u obu gatunków na późniejszych etapach pierzenia oraz że nie było różnic w poziomie testosteronu między samcami i samicami kapturki, można wnioskować, że hormon ten nie jest związany z wykształcaniem ubarwienia upierzenia samców u gatunku dymorficznego. Młodociane kapturki pozostawały na terenie badań znacząco dłużej niż świstunki (Fig. 1). Ogólnie, świstunki opuszczały teren badań w sierpniu, średnio w wieku 32 dni (zakres: 27–40), podczas gdy kapturki — we wrześniu, średnio w wieku 48 dni (zakres: 31–69). U obu gatunków dyspersja młodocianych osobników zbiegała się w czasie ze wzrostem poziomu testosteronu.

Niniejsze badania dostarczają pierwszych bezpośrednich dowodów na wzrost poziomu testosteronu w okresie rozwoju młodocianych osobników u dwóch wolno żyjących gatunków ptaków wędrownych. Wzrost ten, choć nie jest skorelowany z ubarwieniem upierzenia, może mieć wpływ na inne aspekty biologii młodocianych ptaków, np. na ich dyspersję.