

Kapitola I.

Schwarzová, L., Štros, P., Frynta, D., Fuchs, R.:

**Arrival timing in subadult and adult Black
Redstart males: competition-dependent
behaviour?**

Rukopis v recenzním řízení v časopise
Ecology Ethology & Evolution

Kapitola II.

Schwarzová, L. & Exnerová, A.:

**Territory size and habitat selection in
subadult and adult males of Black Redstart
(*Phoenicurus ochruros*) in an urban
environment**

Kapitola III.

Schwarzová, L.:

Aggressive behaviour in subadult and adult Black Redstarts: status – signalling strategy?

Rukopis v recenzním řízení v **Journal of Ethology**
(toho času již upravený na základě požadavků dvou anonymních
recenzentů, očekává se finální rozhodnutí editora)

Kapitola IV.

Schwarzová, L., Fuchs, R., Frynta, D:

**Delayed plumage maturation correlates with
testosterone levels in Black Redstart males**

Rukopis v recenzním řízení v časopise
Wilson Journal of Ornithology

Kapitola V.

**Schwarzová, L., Šimek, J., Coppack, T.,
Tryjanowski, P:**

**Male-biased sex of extra pair young in the
socially monogamous Red-backed Shrike,
*Lanius collurio***

Rukopis v recenzním řízení v časopise
BMC Ecology

Arrival timing in subadult and adult Black Redstart males: competition-dependent behaviour?

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Abstract

Different arrival time of one-year old and older males is widely recognised phenomenon in most migrating passerines. ~~C. Exceptionally there is being found~~ ~~converse pattern, i.e. the yearlings arriving to the breeding grounds at the same time as adults, has been reported only exceptionally.~~ Two hypotheses had been formulated to explain delayed arrival of yearling males: reduction investment, and energetic constraint hypotheses, respectively. However, ~~it is often unclear~~ ~~which one of them is the right~~ ~~more appropriate~~ ~~appropriate~~ way to explain the phenomenon ~~in each case is often~~ ~~remains unclear.~~

We studied arrival timing in two distinct urban populations of Black Redstart (*Phoenicurus ochruros*), a small passerine exhibiting delayed plumage maturation, whereupon dull coloured yearlings are easily distinguishable from black adults. The birds inhabiting South Moravian villages did not show differential timing of arrivals between yearlings and older birds in the ~~high dense~~ ~~population~~ ~~resource-rich~~ ~~environment.~~ On the other hand, ~~under suboptimal~~ ~~conditions in Prague~~ ~~lower dense~~ ~~population~~ the yearlings were delayed of three days on average compared to the adult males. We discuss the possibility for the young birds of this species to sample the ~~situation~~ ~~on a~~ ~~potential~~ ~~potential~~

breeding site/constant locality during autumnal singing period and flexibly fit its migrating behaviour according to the circumstances. In such a case the reduction investment hypothesis is the best explanation for different arrival timing of yearling and adult males in Black Redstart.

Key words: arrival timing, *Phoenicurus ochruros*, DPM, intraspecific competition

Running title: Arrival timing in Black Redstart males

Introduction

In migratory passerines, usually two-2years old or older males are-arriving to their breeding sites earlier in spring than yearling males (Stewart et al. 2002). Earlier arrivals of older males were reported in 19 out of 20 examined American Passerines (STEWART Stewart et al. 2002). FRANCIS Francis and COOKE Cooke (1986) noted indirect evidence that yearling birds arrived later than the older ones in eight warbler species. Similar pattern was reported in several single species studies (YUNICK unick 1988, JOHNSON Johnson 1965, CATCHPOLE catchpole 1972, WALKINSHAW walkinshaw 1968, Hopp HOPP et al. 1999). Converse results are less numerous. In Savannah Sparrows (*Passerculus sandvicensis*) BÉDARD Édard and LAPOINTE aPointe (1984) found earlier arrival in older males during one year only, and not in other studied seasons. No differences were found between arrival times of one-1 year old and older males in White-crowned Sparrow (*Zonotrichia leucophrys*) (MORTON orton 1992).

Bird species with delayed plumage maturation (DPM) represent a group where the arrival-date/arrival dates of yearling (dull coloured) and older (bright coloured) males-ismales are easily detectable. Strong evidence of difference in arrival time according to age of male is available for American Redstart (*Setophaga ruticilla*),

the species exhibiting duller plumage in the first year (FRANCIS & COOKE Francis and Cooke 1986). The adult Rose-breasted Grosbeaks (*Pheucticus ludovicianus*) arrived prior to the second-year males, and among them the most adult-like individuals tended to arrive earlier than the others (FRANCIS Francis and COOKE Cooke 1990), and this pattern was found also in Indigo Bunting (*Passerina cyanea*) (QUAY Quay 1987) and Purple Martin (*Progne subis*) males (MORTON Morton and DERRICKSON Erickson 1990) as well. Among European Passerines exhibiting delayed plumage maturation (DPM), older males of Pied Flycatcher (*Ficedula hypoleuca*) arrived to breeding sites earlier to breeding sites than younger males (BREIHAGEN Reichagen et al. & SÆTRE Aetne 1992, POTTI otti 1998).

Two hypotheses have been proposed to explain the delay in arrival time of yearling birds.

At first, energetic constraints may lead up to later migration time in yearlings because of its poor competitive ability on winter grounds (ROHWER Rohwer et al. 1983, ROHWER Rohwer 1986, ROHWER Rohwer et al. & BUTCHER Butcher 1988). Yearling males of lower rank in dominance hierarchy have the only access to inferior feeding resources (SMITH Smith et al. & METCALFE Metcalfe 1997, EKMAN Ekman 1990) and they are not able to cope with harsh conditions of earlier arriving (STEWART Stewart et al. 2002, Marra ARRA et al. 1993, 1998).

The second hypothesis propose investment reduction in yearling males via avoiding competition for the best breeding sites if there is only little probability for yearlings to defence better territory against the adults in the case of earlier arrival (PROCTER-roeter-GRAY ray et al. & HOLMES Holmes 1981, FRANCIS Francis et al. & Cooke Ooke 1986, MORTON Morton et al. & DERRICKSON Erickson 1990). Although there is good evidence for delayed arrival in Passerine yearlings, it is often hard to provide exclusive test of the hypotheses noted above due to widespread overlap of its predictions (SMITH Smith et al. & MOORE Moore 2005, QUAY Quay 1987, FRANCIS Francis et al. & COOKE Cooke 1990).

In the case of energetic constraints hypothesis the adult males are bearers of the main effort to arrive as ~~earliest~~ as possible to the breeding grounds, and so all subadults wintering on given area are delayed in their arrival timing as they are limited by its poor physical condition. On the other hand, the difference in arrival date should also depends on the yearling males' autonomous decision to avoid unsuitable conflicts with the higher quality adult competitors, according to -by reduced investment hypothesis. We can expect the actual situation on the breeding grounds would affect such decision. Yearling males can benefit from delayed arrival primarily under strong competition for limited number of the best territories, whereas in areas of low ~~competitive pressure~~ there is no reason for such a delay in spring arrival. On that ground, reduction investment hypothesis can well explain the contradictory information ~~comes coming~~ from previous studies of Black Redstart (*Phoenicurus ochruros*) males. The adult ones arrived about 2 weeks earlier than subadults in mountain villages (LANDMANN ~~and~~ Landman et al. & KOLLINSKY ~~and~~ Kollinsky 1995). However, WEGGLER ~~and~~ Reggler (2001) found only slightly earlier arrivals of adult males in similar urban habitat.

—To test this interpretation, we focused on arrival time in subadult and adult males of Black Redstart inhabiting two different urban habitats. This small passerine wintering in Mediterranean arrives to the Central European breeding grounds in during late March and April (ŠTASTNÝ ~~Štastný~~ et al. 2006). The nest site is usually situated on the buildings in urbanized areas. South Moravian villages offer the best conditions for Black Redstart breeding and breeding density varies from 0,38 to 6,7 ex. - /-ha around this area (ŠTASTNÝ ~~Štastný~~ et al. 2006). Prague city environment is less attractive for Black Redstart and breeding density varies from 0,09 - 0,15 pairs /—ha in this area (SCHWARZOVÁ ~~Šchwarzová~~ and & EXNEROVÁ ~~Exnerová~~ 2004), or 0,3 pairs / ha, respectively, in another part of Prague city (ŠTASTNÝ ~~Štastný~~ et al. 2006). We ~~hypothesises~~ suppose that the occurrence of ~~apparent~~ strong competition for attractive territories in Prague ~~should induce~~ connected with delayed arrival of subadult males trying to reduce their investment to intraspecific conflicts. In contrast, for South Moravian villages with higher offer of suitable habitats and thus lower competition, we expect ~~lower competition owing to higher offer of~~

~~suitable habitats and no considerable overlap difference~~ in arrival time ~~of between~~ adult and subadult Black redstart males.

Methods

The study was ~~carried out~~~~provided in localities in~~ of two different urban habitats ~~exemplified by~~~~presented in two locations~~: group of villages in South Moravian countryside of ~~South Moravia~~ (48°49'N, 16°47'E, 200 m asl), and ~~in~~ two built up areas of Prague (50°06' N, 14°30' E, 280 - 360 m asl). One of Prague localities was ~~studied~~~~worked out~~ in 1997, 1999 and 2002 seasons, ~~and, while~~~~while~~ the second one ~~and the South Moravian countryside~~ in 2001 ~~only season as well as countryside locality~~.

The countryside South Moravian study area consisted of 6 villages (total area of 219ha). Low buildings ~~there~~ are concentrated to the centre of each village, with extensive area of utility gardens around. ~~We can expect high offer of insect food due to presence of dunghills connected with high abundance of insect, abundant in sheds and hutches as well.~~

~~In Prague, I~~ locality of garden city in Prague (total area 125ha) consisted of solitary villas surrounded by gardens or rows of buildings up to 3 floors with smaller gardens. As the second locality of Prague we choose housing estate (total area of 90 ha) consisted of high prefab houses (up to 9 floors) surrounded by areas of artificial ground and parks with young low trees, grass and flower beds.

Longer distances are

~~In Prague, I~~ there are longer distances among buildings in Prague and typically that the houses are not so ragged as the village ~~houses ones and (especially the animal houses sheds and barns in South Moravian villages).~~ For that we presume more opportunities for birds to find a place appropriate for nest building as well as higher amount of food resources in villages than in Prague city. ~~We also presuppose higher amount of food resources in villages due to presence of dunghills connected with high abundance of insect, abundant in animal houses as well.~~

In each locality the arrivals of birds were observed every day from the day of the first bird presence through the season to reveal if the territory was established for each observed bird. For analyses were used only arrival dates of territorial males. Because we did not band all the birds in studied localities, we regarded as the territory owner the male of particular age category defending given territory throughout the breeding season. However, any case of known change of one bird by another was excluded from further analyses. During controls the presence of birds, their activities, and age and plumage characteristics was noted.

The mean date of arrival was subsequently specified for each locality and/or year, and the difference from this means was assigned for arrival date of every each individual male. Arrival date was further analysed by GLM procedure in STATISTICA 6.0 software package. The effects of male age, locality and its interaction were introduced as fixed factors, and year as a random factor. The final model explained 33.8% of variation in arrival date, and were tested by visual inspection revealed that distribution of residuals closely resemble normal distribution, although deviations from normality was detected by the strongest diagnostic method (Kolmogorov-Smirnov and Lilliefors ns. at $P=0.05$; Shapiro-Wilk's $w = 0.97$, $p = 0.0084$). GLM procedure in STATISTICA 6.0 software package.

Results

Breeding densities

In Prague the breeding densities varied from 0,09 pairs / ha to 0,32 pairs / ha in particular seasons, the mean value of breeding density was $0,16 \pm 0,11$ (SD) there. In South Moravia we found breeding densities between 0,24 to 0,69 pairs / ha, the mean value was $0,48 \pm 0,18$ (SD).

Arrival time

The first bird appeared in particular seasons ~~March 27th-3-~~ 1997, ~~March 18th-3-~~ 1999, ~~March 25-3th-2001th~~ 2001, ~~March 29th-3-~~ 2002 in Prague locality, and ~~March 25th 2001~~ ~~25-3-2001~~ in South Moravia, respectively. So, in the same year the first male appeared at March 25th, i.e. exactly the same term in both localities.

[Totally, we recorded arrival dates of 60 males in Prague localities and 80 males in South Moravia.](#)

___The mean date of arrival in Prague varied from ~~April 3rd-4-~~ (seasons 1997, 2001) to ~~April 6th-4-~~ (seasons 1999, 2002), in South Moravia the mean date was ~~March 30th-3-~~ 2001. The birds arrived ~~on~~ average four days later in Prague within the same breeding season. [There was found no effect of year in the overall model.](#)

___The arrival date was affected by age of male and locality and between effects of these variables was also significant in the model (GLM: age of male: $F = 19.5$, $p < 0.0001$; locality: $F = 19.5$, $p < 0.0001$; age of male * locality: $F = 11.8$, $p < 0.001$; [Fig. 1](#)). There was no difference in arrival dates of subadult and adult males in South Moravia (mean difference from average arrival date was 0.29 days for adults, and 1.1 day for subadults, respectively). On the contrary, the adult Black Redstart males arrived earlier than the subadults in Prague (mean difference from average arrival date was -1.6 days for adults, and 1.5 day for subadults, respectively). The mean arrival date in South Moravia was five days earlier than in Prague.

Discussion

Breeding density of Black Redstart was found generally higher in South Moravian villages than in Prague, what is in accord with previous literary data ([ŠŤASTNÝ](#) ~~řastný~~ et al. 2006). Despite the exact cause of these findings is not a matter to resolve in this study, we may speculate about two main reasons for higher carrying capacity of village environment for Black Redstart population. At first, we presume more abundant potential nest sites to be found in clumped multifunctional [\(associated residential and utility rooms](#) ~~spojující obytné a hospodářské objekty)~~ village houses ~~being build up continuously~~. Such ~~an~~ [environmental](#) heterogeneity was previously found to be a criterion of territory

choice (ANDERSSON~~andersson~~ 1995, LANDMANN & KOLLINSKY~~landmann et Kollinsky~~ 1995, WEGGLER~~eggler~~ 2001, SCHWARZOVÁ~~chwarzová & et et~~ EXNEROVÁ~~xnerová~~ 2004). Secondly, higher amount of available food concentrated in particular patches such as dunghills, ~~and animal houses sheds and hutches~~ or utility gardens suitable above that owing to high amount of perches used for lookout the insect food.

___The delay in arrival of subadult black redstart males as compared with adult ones is in accord with a common pattern previously found in many ~~p~~Passerines (STEWART~~tewart~~ et al. 2002). However, this pattern of arrival was found only in one of studied localities, i.e. in Prague, where the breeding density is lower. Such a delayed arrival of subadults appears there despite the fact that the birds arrives generally later than in South Moravian locality. We consider higher competition in Prague because of small offer of potential high quality territories. South Moravia is rich of habitats suitable for Black Redstart breeding and all the birds have a good chance to find appropriate territory throughout this area. In such a situation subadults do not benefit so much from avoiding competition of adults, and they arrive as early as possible together with the older birds. Similar situation seems to be in mountain habitats studied previously. In a locality with lower competition (the empty territories after owner's death did not attract new inhabitants) the males arrived at the same time (WEGGLER~~eggler~~ 2000, 2001). On the other hand, in the locality with higher competition among males, where is not enough territories and the dominant adult males are frequently able to chase out previously settled subadults from the preferred areas, the adults arrived generally 14 days earlier (LANDMANN~~andmann et&~~ KOLLINSKY~~ollinsky~~ 1995~~b~~). It seems, the two factors stated by ROHWER~~ehwer~~ et al (1980) as working together in evolution of DPM, i.e. high variance in habitat quality and later arrival ~~time,time,; these~~ continue to be functional only in some urban localities.

To adjust the date of arrival with a view to presupposed competitive situation on breeding ground, subadult males would have some knowledge about the conditions on the locality. This is true in the case of Black Redstart, which is the

species exhibiting autumn territoriality and namely the adults defend the territories during autumn (WEGGLER 2000). The subadults can get to know if there are some empty territories to acquire in the locality and they can improve their decision-making when it is better to arrive during the spring.

Although delayed arrival of subadult birds to breeding grounds is well documented in Passerines (JOHNSON 1965, BÉDARD & LAPOINTE 1984, FLOOD 1984, QUAY 1987, HILL & HILL 1989, FRANCIS & COOKE 1990, MORTON & DERRICKSON 1990, POTTI 1998, LOZANO & LEMON 1999, STEWART et al. 2002), the entire cause of this phenomenon is still unknown. In some cases the role of reduction investment hypothesis is emphasised, but it is usually not possible to rule out one of these exclusively (Francis et Cooke 1990, Smith et Moore 2005). However, there are some examples giving evidence for one or another hypothesis. In Purple Martin (*Progne subis*) the first year breeding males reduce their overall reproductive investment due to high competition for the best nesting-cavities and delayed arrival is part of the strategy how to avoid hard nest-site competition with older males (MORTON & DERRICKSON 1990). Also in Black-headed Grosbeaks (*Pheucticus melanocephalus*) study, the reduction investment hypothesis was reported as an explanation of delayed arrival and dull subadult plumage presence in yearling males (HILL & HILL 1989). On the other hand, evidence for constraint hypothesis comes from study of Yellow Warbler (*Dendroica petechia*). In this species, within-individual size increased with age and individual males arrived earlier in each subsequent years, and within-individual size increased as well (LOZANO & LEMON 1999). However, in other cases authors are not able to rule out one of these hypotheses exclusively (FRANCIS & COOKE 1990, SMITH & MOORE 2005). Our results are unambiguously in concordance with reduction investment hypothesis. The yearling males delay its arrival only under the condition of higher intraspecific competition.

According to ROHWER Rohwer et al. (1980), DPM should be favoured in habitats of high variance in quality, subadult plumage makes yearling males safer against strong conflicts with the adults and the birds exhibiting DPM should not show ~~within~~between-age category overlap in spring arrival timing. Therefore, delayed arrival of yearling yearling males is a part of their complex adaptation to cope with high intraspecific competition. Moreover, ~~DPM should be favoured in habitats of high quality variance, and subadult plumage makes yearling males safer against strong conflicts with the adults (Rohwer et al 1980).~~ Although Even though ~~However~~ such a strong rule was not confirmed in all studies (QUAY Quay 1987, FRANCIS Francis et & COOKE Cooke 1990), the arrival of yearlings and older birds is generally more separated in species with apparent subadult plumage (HILL Hill 1989). ~~We can propose positive selection favoured more delayed arrival in yearling males exhibiting subadult plumage owing to their effort to reduce overall investment during the first breeding season under constant environmental conditions with strong competition for higher quality territories. Three examples noted above also point to dichotomy in explanation of delayed arrivals, i.e. higher probability to find reduced investment in species exhibiting DPM and constraints in the species not selected for such a complex defence against competition with more experienced adults.~~ However, this pattern may not be irreversible in a species able to respond flexibly to environmental variability ~~such Black Redstart is~~. Our results, as well as the previously published studies of black redstart (~~Landmann~~ LANDMANN et & KOLLINSKI Kollinsky 1995b, WEGGLER Weggler 2001), well document the case of once evolved behavioural trait subsequently maintained or disappeared dependently on actual conditions on breeding grounds. DPM had evolved under extreme conditions –of primarily inhabited craggy habitats (cliffs, boulder-strewn detritus fields right up to snowline) in Black redstart (Harrison CRAMP 1988)-. Habitats as human settlement areas can offer so optimal foraging and breeding conditions that the intraspecific competition is widely diminished and the adaptation previously evolved to suppress the competition could get inappropriate for this purpose.

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WEGGLER M. 2001. Age-related reproductive success in dichromatic male *Phoenicurus ochruros*: Why are yearlings handicapped? *Ibis* 143: 264 – 272.

se u BRG vyvinulo v extrémních podmínkách původně obývaných stanovišť (suťová pole nad horní hranicí lesa). náhradní stanoviště (lidská sídla) mu však mohou poskytnout natolik optimální hnízdní i potravní podmínky, že zde je vnitrodruhová kompetice oslabena a adaptace směřující k jejímu potlačení se pak stávají neúčelnými.

1. Většina prací o nich nespekuluje

2. Ty co si něco myslěj a proč si to myslěj (nevím, co to obnáší)

3. RI však předpokládá Rohwer u všech DPM ptáků. Podle něj je pozdní přilet stejně jako DPM opatřením jak se vyhouť střetům s adulty, ke kterým dochází protože....

4. V takovém případě lze předpokládat pozitivní selekci později přilétajících subadultů, ta by měla vyústit ve zvětšování rozdílů. Hill skutečně ukázal, že jsou u DPM větší, což hypotézu o obecném výskytu RI u DPM podporuje.

5. Naše i Wegglerovy výsledky však ukazují, že tento posun nemusí být nezvratitelný.

some evidence points to energetic constraints hypothesis. (Potti 1998, Lozano et Lemon 1999, Bédard et LaPointe 1984, Johnson 1965), — nedoloženo, nechat jen ty, co to opravdu tvrděj

mainly the evidence in the species exhibiting DPM shows reduction investment hypothesis as probably explanation of delayed arrival of subadults (Francis et Cooke 1990, Morton et Derrickson 1990, Quay 1987, Hill 1989). — nedoloženo, nechat jen ty, co to opravdu tvrděj

The species with DPM also show higher difference between subadult and adult males' arrival times (Hill 1989). — toto nejspíš součást tvé argumentace pro DPM a investments,

← Naformátováno: Odrážky a číslování

Okomentoval(a): [RF1]: Toto do několika vět, proč si to myslěj

To bych vyhodil, respective zařadil do tvých úvah v bodě 3

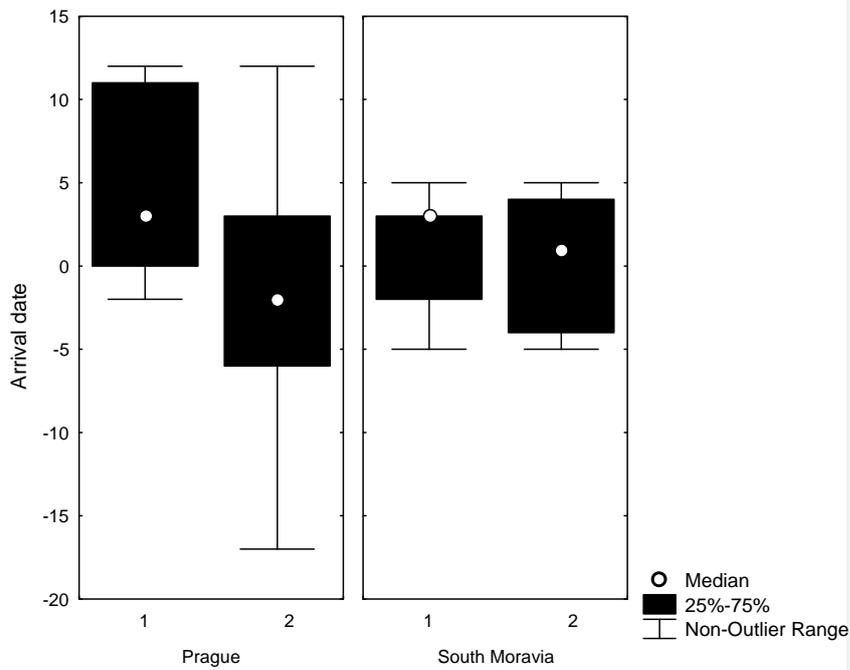


Figure- 1. Arrival date (days) expressed as difference from locality means in two studied populations of Black Redstart (*Phoenicurus ochruros*). The results are categorized according to male age (feather colour): 1 denotes yearling (dull coloured) males while 2 denotes adult (full coloured) males.

Naformatovano: Odrážky a číslování

Naformatovano

Territory size and habitat selection in subadult and adult males of Black Redstart (*Phoenicurus ochruros*) in an urban environment

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Territory size, distribution of territories and habitat selection were studied in Black Redstart (*Phoenicurus ochruros*) populations in three urban habitats (garden city, old and new housing estates) of Prague (Czech Republic). We tested if the territory size and quality of territories occupied by adult and subadult males differed from each other. Average territory size of the adult males was 1.21 ± 0.80 (SD) ha and of the subadult males 1.08 ± 0.58 (SD) ha. This difference was not statistically significant. Moreover, males of particular age-class were not spatially clustered. Microhabitat analysis shows that the habitat selection depends on the presence of buildings. Apparently buildings offer a good nesting, singing and foraging places for the Black Redstart. The territory size of Black Redstarts differed between habitats, being largest in the garden city (1.98 ± 1.06 (SD) ha), intermediate in the old housing estate (1.48 ± 0.45 (SD) ha) and smallest in the new housing estate (0.85 ± 0.33 (SD) ha). Our results indicate that in urban environment the relationships between adult and subadult territory owners differ from those referred to in mountain villages.

1. Introduction

The Black Redstart, *Phoenicurus ochruros* (S. G. Gmelin, 1774), a small European passerine exhibiting delayed plumage maturation, is a suitable model for studies of variation in territory quality and spatial relationships between the two age categories of males. Subadult males have female-like, grey-brown plumage; second-year and older males (adults) are characterized by black face,

throat and breast, grey-black forehead and crown, and grey mantle-back area. The Black Redstart occupies various habitats: dry, rocky zones, mountain habitats in higher altitudes and anthropogenous environments, i. e. villages, towns and industry agglomerations (Cramp 1988, Zamora 1991). In Europe the highest densities of this species are reported from the mountain and urban habitats (Hagemeijer & Blair 1997). In mountain villages the subadult males are displaced by earlier

arriving adults to suboptimal and/or peripheral village zones with few, usually also young neighbours (Landmann & Kollinsky 1995). The males compete for the territories in the centre of human settlement, and the differences between territories of subadult and adult males may ensue from sharp gradient of resources. The situation may be different in large agglomerations owing to even distribution of human settlement and less harsh environmental conditions than in mountain villages.

We studied the Black Redstart territory distribution in three representative city habitats (i. e. garden city, new and old housing estate) to test whether there is any difference in the quality of territories occupied by subadult and adult males. We used the territory size, and proportion of microhabitats in the territory as a measure of the territory quality. Following the concept of economic defendability (Krebs & Davies 1993), we assumed smaller territories to be of higher quality (Davies 1992, Searcy & Yasukawa 1995).

We compared the distances between territories of subadult and adult males within each locality to find whether there are clusters of males of the same age. Another aim of this study was to test if the presence of vertical surfaces is the major criterion of territory choice.

2. Material and methods

2.1. Study sites

The study was carried out in three types of urban habitats situated in Prague (50°06'N 14°30'E, 280–380 m a. s. l.) in 1998 and 1999 viz. garden city, old (from 60's) and new (built in last 5 years) housing estate. The garden city adjoins the old housing estate, both are situated in NW side of the town, the border was made by the highway. The new housing estate is situated on the other side (NE) of Prague agglomeration. Size of the localities was 24.9–124.9 ha; they differed mainly in a vegetation structure (Kruskal-Wallis test for high dense trees with undergrowth: $H_{2,30} = 22.7$, $P < 0.05$) and types and density of buildings (Table 1). In the garden city, there were no houses higher than 3 floors. In contrast, buildings up to 5 floors occurred in the old housing estate. In the new housing estate there were even 6 to 12 floor high

buildings standing close to each other. The borders of investigated areas were changed from the first season to the second one relative to human influence on localities. The influence of the year was tested in the GLM: year, habitat type and age of male were taken as dependent variables in the model, territory size as the independent one; there were found the significant effect of the year ($F_{1,54} = 16.87$, $P < 0.05$) and between effect of habitat and year ($F_{2,54} = 4.21$, $P < 0.05$, $n = 66$; whole model $R^2 = 39.93\%$). So, the combination of the habitat and the year was given as a single variable "locality" to simplify the model. For that reason, the term "locality" means "one habitat in one season" for all the following analysis.

2.2. Bird surveys

Localities were visited during the breeding season weekly (over 10 visits per locality, i. e. 150 to 300 min of observations per bird, from 3:00 to 7:00 CET) and all the activities of birds were recorded and mapped. Birds were observed with a 8 x 50 telescope. Only the data from the first breeding were taken into account. All the locations transferred to one map for each locality enabled us to estimate territory boundaries. The territory size was assessed as the area delimited by lines drawn closely outside (5 m) of the lines connecting marginal points where the territory owner was observed (minimal convex polygon), with the exception of evidently remote observations. Colour rings for exact individual identification marked the birds settled in overlapping territories mainly. We have mapped from 8 to 19 territories per locality.

2.3. Microhabitat measurements

Microhabitats were classified into five categories on the basis of vegetation structure, ground type, and presence of buildings (Table 1). Proportions of microhabitats in territories were taken from the maps. The same procedure was done for the control plots, areas of the approximately circular shape and the same average size as territories, randomly placed (by uninitiated person) into maps of studied areas (ten plots per locality).

Table 1. Proportion (in %) of different microhabitats in studied localities (i.e. garden city, old housing estate and new housing estate), and their overall areas.

Micro habitat	Garden city		Old housing estate		New housing estate	
	1998	1999	1998	1999	1998	1999
Overall area (ha)	109.3	124.9	54.8	57.9	24.9	62.8
Built-up area	14.22	14.18	15.50	15.93	14.67	15.96
Bare grounds	26.76	25.26	33.81	33.56	28.49	24.06
Herbaceous vegetation	3.02	3.03	12.74	12.46	10.86	18.42
Low rare trees with undergrowth	34.30	31.83	12.56	13.03	44.05	40.81
High dense trees with undergrowth	23.04	24.69	25.42	25.02	1.88	0.74

2.4. Statistical testing

Mann-Whitney U-test was applied to compare the proportion of each microhabitat between territories and the control plots. To test the dependence of territory size on microhabitat composition of territory we performed multiple linear regression analysis (forward stepwise variable selection, critical value for variable removal $P = 0.05$). To measure potential habitat-dependent and/or age-dependent differences in territory size we compared the territory sizes using GLM procedure (locality and male age were taken as the independent variables, territory size as the dependent one, between effect of these variables was also computed in the model). The proportions of one chosen microhabitat (differing in territories and control plots) in territories of subadult and adult males were compared by ANCOVA when territory size variance was taken as a covariate. We used log-transformed values of territory size and areas covered by specific microhabitats in these analyses. All the analyses were computed using STATISTICA 6.0 software.

Another studied feature was the mutual position of Black Redstart territories. The distance of each territory from the nearest subadult territory and from the nearest adult territory was defined as the shortest distance between the territory borders. These distances were compared by Wilcoxon matched-pair rank test (using S-Plus 4.0 software). Year-to-year territory overlap on each locality was defined as the proportion of territories situated at

the same position in both years of the study. The position of two territories was considered as the same if at least 25% of territory area occupied in the second year was placed over the first year territory area.

The significance level is $P < 0.05$ for territory sizes, and distances between neighbouring territories. To keep the overall error of the five tests set below 5% we used Bonferroni correction (Sokal & Rohlf 1995) in the analyses of microhabitat composition of territories and randomly generated areas; in these analyses the significance level is $P < 0.01$.

3. Results

The parameters of 37 territories occupied by adult males and 29 occupied by the subadults were recorded. Average territory size was 1.15 ± 0.51 (SD) ha; range 1.08–4.05 ha. Territory sizes of subadult (1.08 ± 0.58 (SD) ha) and adult males (1.21 ± 0.80 (SD) ha) did not differ (GLM: $F_{1,54} = 1.62$, ns, $n = 66$, Table 2). The locality had considerable effect on territory size (GLM: $F_{5,54} = 6.20$, $P < 0.001$, whole model $R^2 = 39.93\%$, $n = 66$, Table 2). The interaction between variables had no considerable effect on the model. Garden city in 1998 differed from all other localities except from the old housing estate; the largest territories were found there, the breeding density was 0.09 pairs/ha only (Table 2). In contrast, the smallest territories

Table 2. Adult and subadult males' average territory sizes (\pm SD), and breeding densities (pairs/ha) found in three urban habitats of Prague.

	Garden city		Old housing estate		New housing estate	
	1998	1999	1998	1999	1998	1999
Adult males	2.46 (\pm 1.38)	0.95 (\pm 0.64)	1.65 (\pm 0.42)	0.72 (\pm 0.26)	0.96 (\pm 0.23)	0.98 (\pm 0.50)
Subadult males	1.66 (\pm 0.76)	0.73 (\pm 0.36)	1.21 (\pm 0.42)	1.14 (\pm 0.74)	0.85 (\pm 0.28)	0.75 (\pm 0.08)
Breeding density	0.09	0.15	0.18	0.13	0.40	0.14

were found in the garden city in the season 1999. However, the breeding density in the garden city was 0.15 pairs/ha in this season (Table 2).

In 1999 the territories there were concentrated to the same regions as in the previous season (68.4% of territories were placed over the territories from the last season). The same situation was found in the old housing estate, where 62.5% of the territories overlapped between the years. In the new housing estate, only 22.2% of them were situated likewise. No cases of presence of the same ringed male (about 20% of males) in his last year territory were recorded in any of the localities. Presumably, there is a possibility that some of the non-marked adult males occupied the same territory during both years.

Comparison of the microhabitat proportions between the territories and control plots shows significant difference for built-up areas only (Mann-Whitney U-test: $Z = 2.44$, $P < 0.05$, $n = 132$). The higher proportion of buildings was found in territories (Fig. 1). Regression analysis shows a significant negative dependence of the territory size on the proportion of the territory area covered by buildings ($r = -0.37$, $F_{1,64} = 9.88$, $P < 0.05$, $R^2 = 13.37\%$). In smaller territories the proportion of built-up area was higher. The distribution of territories in ordination space based on the proportion of microhabitats was not affected by the owner's age (Fig. 2). The territories occupied by adult and subadult males did not differ in the representation of built-up areas (ANCOVA: $F_{1,62} = 0.38$, ns, $n = 66$).

The breeding sites were occupied randomly relative to owner's and neighbour's age (Wilcoxon rank test: $Z = 0.10$, ns, $n = 61$), there were no

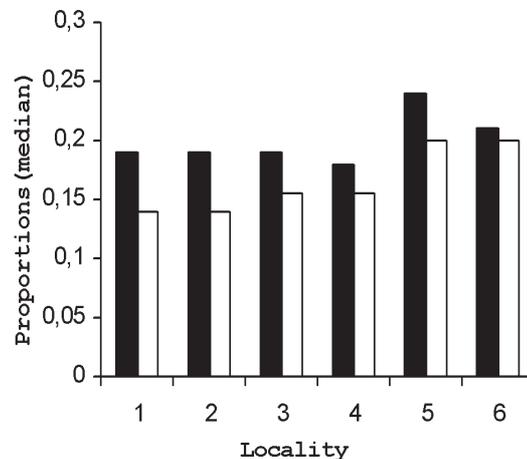


Fig. 1. Median values of proportions of built-up area in occupied territories (black columns) and control plots (white columns). Localities: (1) garden city in 1999; (2) garden city in 1998; (3) old housing estate in 1999; (4) old housing estate in 1998; (5) new housing estate in 1999; (6) new housing estate in 1998.

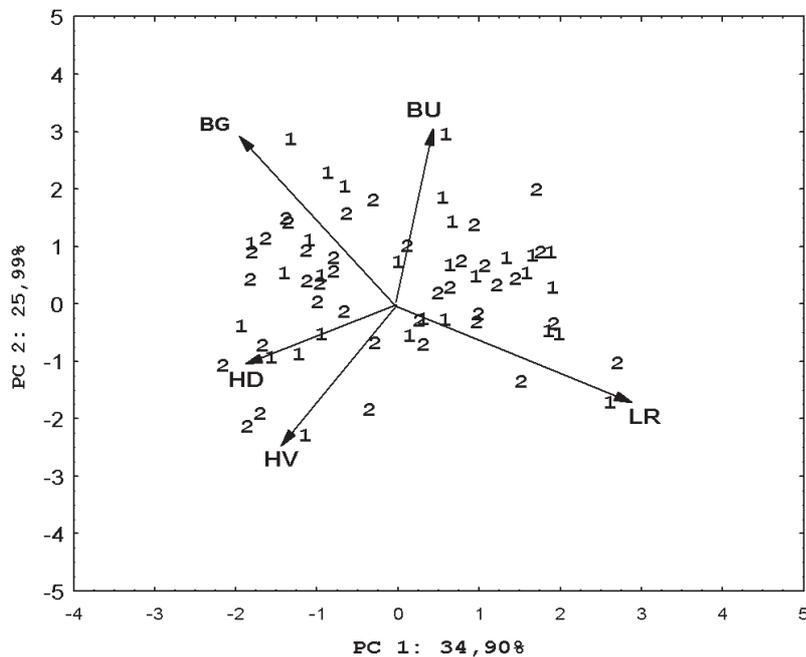
significant differences in distances between adult and subadult nearest neighbour relative to owner's age. Thus, the birds did not form any clusters composed exclusively by the same age category.

4. Discussion

4.1. Territory quality

Previous studies suggest overall structural diversity (Andersson 1995, Landmann & Kollinsky 1995) and particularly structural diversity of buildings (Weggler 2001) to be a criterion of habi-

Fig. 2. Distribution of the territories occupied by subadult (1) and adult (2) males in the ordination space determined by the first two principal components based on the proportion of different microhabitats in the territories. BU = built-up area; BG = bare grounds; SG = surfaced grounds without vegetation; HV = herbaceous vegetation; LR = low (less than 2 m) rare trees with undergrowth; HD = high and dense trees (over 2 m) with dense undergrowth.



tat selection of Black Redstart. Our analysis of microhabitat composition of Black Redstart territories suggested presence of vertical surfaces as a possible criterion of habitat selection. The vertical walls seem to be the only common feature of different habitats inhabited by Black Redstarts (rocky, bleak land, and modern cities or industrial agglomerations; Cramp 1988). The walls in human settlements, and the rocks in montane areas represent the potential nest sites (Weggler 2001) and the food source (the insects resting frequently on the surfaces exposed to sun, or hilltopping around their tops) for the Black Redstart. The top position of the singing male should be important for the control of all the territory area and the possible intruders. The visibility and audibility of the male sitting on the top position is much better for the other individuals. So, the presence of suitable vertical surfaces should determine the size of the area occupied by male. Contrastingly, in some other urban breeders the negative relationships between bird abundance and built structure proportion were found (Jokimäki 1999).

The proportion of built-up area differs significantly between the real territories and control plots (Fig. 1). In the garden city, the larger territories were situated in the areas of villas surrounded by

large gardens, while numerous but smaller territories were found in areas where family houses stand close to each other, i.e. forming long rows. The houses in the new housing estate are very similar and spaced in regular distances, what may explain the absence of preferences for particular regions. Accordingly to this observation, the regression analysis shows the significant dependence of the territory size on the proportion of the area covered by buildings in the territory. The larger territories included the higher proportion of non-preferred microhabitats (i. e. those, which proportion in territories was not significantly higher than in control plots). The territories in new housing estate were the smallest during both seasons. Larger territory area may be needed mainly in the garden city for the acquisition of a sufficient number of walls in the situation, when the buildings are far from each other in the areas covered by large gardens. However, the cause of such a pattern would arise from the food competition in garden-covered areas (Solonen 2001). The concentration of the other breeding bird species is higher than in more urbanized areas of garden city. These are less attractive for most species. If the food and nest sites availability are equal, as the urbanization increases, the density of breeding pairs increases (Kosiński

2001). Such a bird-human relationship (safe zones hypothesis) may explain the observed distribution and density pattern of Black Redstart's territories as well.

4.2. Territory size

The territory size of Black Redstart varies according to different habitats occupied by the species in Europe. We have found a wide variation in territory size (range 0.22–4.05 ha) in the urban habitats we studied. The Black Redstarts inhabiting montane areas occupy fairly smaller territories of the average size 0.28 ha for subadult and 0.57 ha for adult males (Landmann & Kollinsky 1995). The density of breeding pairs and the number of singing males per kilometer of transect in Alpine valleys suggest the smaller territory size as well: 4.5–9 pairs per 10 ha, 4.3–6.9 males per 1 km of transect respectively (Landmann 1987, Kollinsky & Landmann 1996). The territory size varies between 0.35–7.4 ha (Nesenhöner 1956, Menzel 1983, Cramp 1988) in urban landscape. These examples illustrate the plasticity of the territory size, demonstrated by the comparison between different habitat types, and seen within one habitat type as well.

4.3. Comparison of subadult and adult males

Territory parameters showed no differences between the territories occupied by the two age categories of males, contrary to findings of Weggler (2001) in mountain villages, where young males occupied territories less structurally diversified than adults. Breeding sites in our localities were occupied randomly relative to the owner's and the neighbour's age, and the birds did not form clusters of the same age category. Subadults were not displaced to peripheral areas. The density of breeding territories varied from 0.09 to 0.4 pairs per 1 ha in our localities. These values are low in comparison with Black Redstart population in mountain villages. The territories of subadults are situated rather marginally and solitarily in the mountain village zones. Such a pattern, when subadults are separated from the adult males in suboptimal habitats, should facilitate avoidance of

competition of the young males with the adult, more experienced ones (Procter-Gray & Holmes 1981). The distance of subadult's territory from the adult's one is expected to be larger in situations of high competition than in the environment with evenly distributed resources and lesser competition in which the territories are randomly distributed (as in urban habitats studied, where the birds choose from a sufficient amount of preferred habitats).

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Selostus: Aikuisten ja esiaikuisten mustaleppälintukoiraiden reviirikoosta ja elinympäristönvalinnasta kaupunkiympäristössä

Artikkelin kirjoittajat tutkivat eri-ikäisten mustaleppälintukoiraiden reviirikokoa, reviirien sijoitumista ja elinympäristönvalintaa Prahassa vuosina 1998–1999. Tutkimusalueet sijaitsivat puistomaisessa kaupunginosassa, vanhalla asuntoalueella (ikä noin 60 vuotta) sekä uudella asuntoalueella (ikä noin 5 vuotta). Puistomaisessa kaupunginosassa ei ollut yli 3-kerroksisia kerrostaloja, vanhalla asutusalueella oli 5-kerroksisia taloja ja uuden asutusalueen talot olivat lähellä toisiaan sijaitsevia, 6–12 kerroksisia rakennuksia. Mustaleppälintu on sopiva mallilaji eri-ikäisten koiraiden elinympäristönvalinnan tutkimiseen, koska eri-ikäisten koiraiden höyhenpuvut ovat selvästi erilaisia. Kirjoittajat olettivat, että pienemmät reviirit olivat laadukkaampia kuin suuret reviirit. Vanhojen koiraiden keskimääräinen reviirikoko (1,21 ha, n = 37) ei eronnut esiaikuisten koiraiden reviirikoosta (1,08 ha, n = 29). Mikrohabitaattitasoanalyysi osoitti, että rakennusten esiintyminen vaikutti mustaleppälinnun habitaatinvalintaan.

Pienikokoisilla reviiireillä rakennetun alan osuus oli suurempi kuin suuremmilla reviiireillä. Ilmeisesti rakennukset ovat hyviä pesimä-, laulu- ja ruokailupaikkoja mustaleppälinnuille. Lajin reviiirikoossa esiintyi vaihtelua habitaattien välillä. Reviiirin keskikoko Keskimääräinen oli suurin puistomaisessa kaupunginosassa (1,98 ha) ja pienin uudella asuntoalueella (0,85 ha). Vanhalla asuntoalueella reviiirin keskikoko oli 1,48 ha. Toisin kuin aikaisemmin on havaittu vuoristokylissä, eri-ikäisten mustaleppälintukoiraisten reviiirin laatu ei eronnut kaupunkialueella toisistaan. Mustaleppälintujen reviiirit eivät olleet kaupungissa myöskään ryhmittyneet ikäluokittain. Oletettavasti nämä erot johtuivat siitä, että resurssit ovat jakautuneet kaupunkialueella tasaisemmin ja kilpailu reviiireistä on kaupungissa vähäisempää kuin vuoristokylissä.

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Aggressive behaviour in subadult and adult Black Redstarts: status - signalling strategy?

Naformátováno: Vlevo: 4,13 cm, Dole: 2,85 cm

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Abstract

In some birds, yearling males (subadults) are less brightly coloured than the adult ones. To explain this phenomenon known as delayed plumage maturation (DPM), several hypotheses have recently been proposed. The aim of this paper was to test predictions of status signal hypothesis (SSH). This hypothesis claims that the adults less frequently attack dull coloured subadult males, and consequently DPM facilitates survival of subadults during the first breeding season.

The experiments were performed in two habitats in the Prague suburbs: a housing estate and garden city. Subadult and adult males of Black Redstart (*Phoenicurus ochruros*) were exposed to stuffed dummies of both age categories. Contrary to predictions of SSH, brown (subadult like) coloration of the dummy had no suppressive effect on defensive behaviour of the resident males. On the other hand, subadult males were more active in vocal territorial defence such as singing and producing snarl and whistle sounds during the experiments.

The defenders of smaller territories generally behaved more aggressively than defenders of large territories in habitat of garden city, and especially adult males responded more aggressively against dummies in this habitat. In housing estate, the habitat of evenly distributed resources (i. e. vertical surfaces), the birds tended to observe the dummy prior to attack it directly.

Key words: *Phoenicurus ochruros*, aggressive behaviour, DPM, status-signalling

Naformátováno: Doprava, Vpravo: 0,63 cm

Introduction

The onset of the breeding season is associated with elevated aggression in male birds. Fighting for territories or females is usually strong until the pairs and territory boundaries are already established, and females start to incubate the eggs (Hill 1989; Searcy and Yasukawa 1995). There are, however, several mechanisms that may reduce the rivalry hardness. These are for example ritualisation of fights (Lumsden and Hölldobler 1983), establishment of dominance hierarchy within a social group (D'Eath and Keeling 2003; Seibert and Crowell-Davis 2001), and signalisation of individual social status (Rohwer 1975). Young but sexually mature males (subadults) are sometimes less brightly coloured and/or their plumage resembles that of juveniles or females. This phenomenon usually referred as delayed plumage maturation (DPM) may be viewed as mechanism of status signalling facilitating survival of yearling males (Rohwer and Butcher 1988). Immature plumage does not considerably handicap these males in reproduction, and thus, such males may breed successfully (Nicolai 1994; Greene et al. 2000). Nevertheless, social status hypothesis is not the only one aimed to explain presence of such a controversial phenomenon as DPM is. In all, 15 hypotheses have been formulated in this context (Cucco and Malacarne 2000).

Selander (1965) has interpreted the yearling's dull plumage as decreased conspicuousness for predators. Under high competition between males, the young inexperienced male would not be as successful as its more mature competitors. The inconspicuous plumage facilitates survival of the yearling male. Consequently, possessing fully developed reproductive organs inconspicuous male may breed successfully in a situation of reduced competition between males. Although, some experimental data correspond with this hypothesis (Procter-Gray and Holmes 1981; Flood 1984; Hill 1988; Enstrom 1993; Prum and Razafindratsita 1997), we cannot ignore the potential significance of bright plumage as a warning signal to predators (Guilford 1988; Götmark 1994,

Götmark and Unger 1994; Slagsvold et al. 1995).

Rohwer et al. (1980) noted the frequent female plumage resemblance in the first-year males. The female mimicry hypothesis (FMH) perceives this fact as crucial in explaining DPM adaptively. If the major factor of female mate choice is based on some other character, the dull-coloured males succeed in attracting females to their territories as well as the bright plumaged ones. Older males should attack the first year males less frequently if faced with imitated female plumage. Therefore, both territorial defence and survival are facilitated throughout the first breeding season.

By the status signal hypothesis (SSH; Lyon and Montgomerie 1986) dull plumage represents an honest signal of the subordinate status of first-year males against the adults. Thus, the honest signalisation results in reduced aggression of adult males towards subadults, and similarly as in the case of FMH it makes territorial defence easier and helps subadults to survive the first breeding season. Although evidence of reduced aggression against less colourful competitors has been described in yellow warblers (*Dendroica petechia*; Studd and Robertson 1985), northern orioles (*Icterus galbula*; Flood 1984), lazuli buntings (*Passerina amoena*; Muehter et al. 1997) and red-flanked bushrobins (*Tarsiger cyanurus*; Morimoto et al. 2005) this is not the case in other bird species (Procter-Gray 1991; Breiehagen and Saetre 1992).

Juvenile mimicry hypothesis was designed for the specific case of manakins of the genus *Chiroxiphia* mating system. Subadult plumage may reduce aggressive behaviour of adult males on leks (Foster 1987). This explanation, however, cannot be used for the monogamous species passing the part of year in the breeding territory area. Males cannot meet juveniles during the arrival time: therefore, the mimetic function of dull coloration is out of the question until the second breeding.

Several hypotheses explain DPM as a non-adaptive trait in terms of breeding. These are the winter crypsis hypothesis (Rohwer and Butcher 1988), winter female mimicry hypothesis (Brown and Brown 1988), and winter status-signalling hypothesis (Rohwer 1975). Persistence of dull plumage in the breeding season is explained through the absence of pre-breeding moult in discussed species - moult constraint (Rohwer 1986; Rohwer et al. 1983). Some of the other

hypotheses related to DPM are modifications of those as noted above or associated with a special case of DPM – carotene based feather coloration (Alatalo et al. 1990; Björklund 1991; Collis and Borgia 1993; Hill 1996).

The black redstart, *Phoenicurus ochruros* (S. G. Gmelin 1774), a small European passerine exhibiting DPM, is a suitable model for studies of DPM and relationships between the two age categories of males. The melanin-based colouration does not seem to be condition-dependent trait in this species, and it is still unknown if there is developmental constraint, or the sharp difference in colouration between subadult and adult males evolved and/or persists due to selection pressure acting on subadults via mate choice (Greene et al. 2000) or signalling function (Lyon and Montgomerie 1986). However, high variability in frequency of adult-like subadults (between 4.8 – 20 %) in particular populations (Nicolai 1992a) suggests possible environmental effect incorporated in a problem of DPM in black redstart. The studies performed in urban populations of black redstart focused on the breeding ecology of the species, but only marginally on DPM (Nicolai 1992a, b; Nicolai 1994). There is, however, some information concerning DPM in a mountain population (Landmann and Kollinsky 1995a).

The aims of the study are: (1) to compare aggressive behaviour of subadult and adult resident males of black redstart to brown (subadult) and black (adult) coloured dummies (simulating intruders). (2) Variation in quantity of structured vertical walls that represents a basic criterion of territory choice due to offer of nest sites and singing posts (Schwarzová and Exnerová 2004) allows me also to compare level of aggression in two contrasting urban habitats. (3) Moreover, I assessed aggression exhibited by owners of large (i.e., less profitable) and small (more profitable) territories to test prediction of economic defendability hypothesis (Krebs and Davies 1993).

Study area

I studied two habitats of Prague city (Czech Republic; 50°06'N, 14°30'E, 280-380 m asl) defined according to the type of buildings, i.e. villas and high prefab houses.

In the garden city there are two main types of buildings, i. e. solitary villas surrounded by large gardens, and rows of low family houses in close proximity to each other. The height of the buildings there is up to four floors. The vegetation comprises grass, flowerbeds, and dense coniferous or fruit-trees.

The habitat of housing estate comprises buildings of five to nine floors standing in large blocks. Surroundings of these prefab houses are artificial surfaces mainly, or grass with much lower proportion of full-grown trees than in garden city. For detailed descriptions of localities see Schwarzová and Exnerová (2004).

Materials and methods

Studied species

Subadult males of black redstart (*P. o. gibraltariensis* J. F. Gmelin, 1789) have female-like, grey-brown plumage typically without any black feathers or bright white wing panels in their plumage. Although, 4.8 to 20 % of subadult individuals wear intermediate plumage, *i.e.* black parts developed with only one or no wing panel (Nicolai 1992a); no such a bird bred on localities in the time of study. Second-year and older males (adults) are characterized by dark black face, throat and breast, grey-black forehead and crown, and grey mantle-back area; panels on both wings shine brightly white. The black redstart occupies various habitats: dry, rocky zones, mountain habitats in higher altitudes and villages, towns and industrial agglomerations (Cramp 1988; Zamora 1991). In Europe, the highest densities of the species are reported in mountainous and urban habitats (Hagemeijer and Blair 1997). Earlier-arriving adults in mountain villages usually displace subadult males to suboptimal peripheral zones (Landmann and Kollinsky 1995b). Such distributional pattern was not observed in the urban population of Prague (Schwarzová and Exnerová 2004).

Experimental design

In all, 68 resident males were studied, 38 of them were subadults and 30 were adults. The number of focal birds was balanced between the two years of study, and 19 subadults plus 15 adults were involved each year in the study. Each experimental male was mated to a single female through ongoing breeding. The territories were visited weekly through the breeding season and no socially polygamous male was detected among experimental males. Each male was tested twice during the first seasonal breeding (30 March to 15 May). 136 experiments were performed during the 1998 and 1999 seasons. Finally, six dummies (stuffed birds wearing distinctive female-like subadult and/or adult plumage) of each class of age were used for the experiments. I have chosen the dummy-based experimental design for the difficulties to observe real interaction between the birds in highly heterogeneous and inaccessible urban habitat. This method was formerly used for DPM studies (Studd and Robertson 1985, Flood 1984) and for the study of agonistic interactions between two species of redstarts (Sedláček et al. 2004). Such a design uncovers the entire motivation of the focal bird, although the information about potential male-male encounter result is lost. The subadult dummy alternates the adult one or vice versa for each focal male. I placed the dummy high off the ground (on a palisade, garage roof, and so on) near the assumed centre of the territory (the most inner part of area defended by each male), and the recorder was placed close beside. Black redstart song was played on an RQ-L309 (Panasonic) mini tape recorder. I used a song of an old male for all experiments with the exception of song differences between subadult and adult males (Cucco and Malacarne 1999). After starting the playback, I recorded all activities of the focal bird by compact VHS camcorder (Panasonic NV-RX5EG). Duration of experiments was 10 minutes. I performed the experiments at the time of peak singing activity, i.e. early in the morning (0300 – 0700 hours CET) and closely before dusk (1800 – 2100 hours CET). Only if the male was singing at the moment of my approach to the territory I did proceed with the experiment. The data of dummy location, and singing activity of the bird immediately prior to and closely after the experiment were noted. I counted the motifs sang by the male during one minute. However, as a result of disturbance by local people, complete data covering both pre- and post-experimental term are

not available for all the experiments. The interval of two experiments for one male was about one week. The experiments were assembled for the period of peak aggression during acquisition of territories (Flood 1984; Hill 1989; Searcy and Yasukawa 1995) prior to arrival of females, during ~~their arrival~~their arrival and laying period, respectively. Both territory defence and mate guarding motivations of males together are evaluated in experiments provided during these breeding stages. Territory size was measured by minimal convex polygon method modified for the specifics of urban habitats (Schwarzová and Exnerová 2004).

Statistical analyses

For collecting behavioural data of Black Redstart males from the tapes, I used The Noldus Observer version 4.0 software. I noted the behavioural elements belong to five categories as follows: singing (number of motifs sang during the experiment), two other vocalisations such as snarl and whistling, latency of the first attack, and number of all aggressive displays recorded during the experiment (i.e. all cases of air attacks on the dummy plus pecking into it). To determine all the effects of dummy colour (black or brown, simulating adult or subadult intruder respectively), age of male, year, and habitat on male response, the GLMM with repeated measurements was performed. I used log link function for the data of Poisson distribution. All possible interactions between the independent variables were also included in the whole model. Because of the censored observations of the latency data, I have used the frailty model with gamma distribution for this variable. A frailty model is a random effects model for survival data or other time-to-event data. It is similar to the mixed models, so that conditional on some random variable (which in survival data is the term denoted frailty), the observations are independent. The frailty is not observable, and therefore has to be integrated out. Unconditionally, the observations are dependent (Armitage and Colton 1998). Frailty is specific for bird; it individualizes level of unobservable heterogeneity. In this model I have tested the effects of dummy colour, age of male and habitat. In addition, latency of the first direct air attack to the dummy was compared to latency of presence nearby the dummy without attack using Wilcoxon matched pairs test. As the repeated use of a statistical test increases the probability of committing type I error, I adjusted the

level of significance to 0.0125 using Bonferroni correction (Sokal and Rohlf 1995).

Relationship between the individual aggression output (expressed as the sum of all the aggressive air attacks to the dummy recorded during experiment) and territory size defended by the male was assessed by Spearman rank order correlations procedure. In addition, I calculated rank correlation between territory size and the number of motifs sang by the male (either prior to or after the experiment). The data from both studied habitats were taken separately for the mentioned analyses.

The analyses were performed with STATA (GLMM, frailty model) and STATISTICA 6.0 (nonparametric tests) software packages.

Results

Vocalisation

In the habitat of housing estate, post-experimental singing activity was not significantly higher than singing activity before the experiments in both adult and subadult males (Wilcoxon matched pairs test for pooled data: $T = 905$, $p = 0.77$, $N = 80$; adults: $T = 157.5$, $p = 0.89$, $N = 36$; subadults: $T = 323$, $p = 0.87$, $N = 44$). In contrast to this, males in garden city showed a tendency to lower intensity of singing after the experiments (Wilcoxon matched pairs test for pooled data: $T = 293$, $p = 0.02$, $N = 56$). This tendency was even more apparent in adult males (adults: $T = 47.5$, $p = 0.05$, $N = 26$; subadults: $T = 101.5$, $p = 0.16$, $N = 30$; Figure 1). Thus, males were not incited to sing more after the incident in any habitat.

The results revealed the effect of the year on singing activity during the experimental procedure (Table 1), so the results for each year were computed in separate analyses. These unveiled the effect of age of male in both study years ($z = 2.93$, $p = 0.003$ for 1998 and $z = -5.26$, $p = 0.000$ for 1999), and the interaction of age with habitat in the year 1998 ($z = -2.72$, $p = 0.007$), respectively. Subadult males tended to sing more during the experiments, and generally they sing more

in the housing estate habitat, however no significant effect of this interaction was found in the year 1999.

Moreover, the models did show marginally significant effect of the age of male on snarl counts (subadult males produced more these sounds), and of the age of male, colour of the dummy and its interactions with habitat and year on the whistle counts, respectively (Table 1). However there was found statistically significant effect of age of male ($z = 302.52$, $p < 0.0001$), colour of the dummy ($z = -135.82$, $p < 0.0001$) and habitat ($z = 319.4$, $p < 0.0001$) in 1998 season, no such effects affected number of whistle vocalisations in year 1999. More counts of the whistle vocalisations were recorded in housing estate from subadult males, and during the experiments arranged with brown (subadult) dummies.

Aggressive behaviour

Dummy colour, age of male and habitat did not predict significantly the latency time of direct attack on the dummy in frailty model (dummy colour: $z = 1.70$, $p = 0.08$; age of male: $z = -0.49$, $p = 0.62$; habitat: $z = 1.51$, $p = 0.13$). Although this difference is not statistically significant, resident males approached the black (“adult”) dummies somewhat later when compared to the brown (“subadult”) dummies (Figure 2).

The latency of presence nearby and latency of the first direct air attack on the dummy were not significantly different in the garden city (Wilcoxon matched pairs test: $T = 217$, $N = 56$, $p = 0.38$; Figure 3), but in the housing estate the former trait was significantly shorter (Wilcoxon matched pairs test: $T = 203$, $N = 80$, $p = 0.01$; Figure 3) due to frequent presence and skipping of the territory owner nearby the dummy preceding the direct flight-attack.

The total number of aggressive elements recorded in a particular experiment was significantly predicted by age of male, habitat and year, and the interactions between these variables, respectively (Table 1). Age of male and habitat predicted significantly aggressive behaviour of males in 1998 season (age: $z = 4.9$, $p < 0.0001$; habitat: $z = -4.01$, $p < 0.0001$); more aggressive behaviour was recorded in adult males in garden city habitat. However, no effect of any variable was revealed in the year 1999.

Aggression relative to territory size

Responses to intruders varied greatly among tested males. In 46% of cases, I recorded no response in any experiment involving a particular male. Moreover, males in the garden city showed a negative correlation between aggressive behaviour and territory size: the smaller the territory, the higher the number of aggressive elements (Spearman rank correlation: $r_s = -0.42$, $p = 0.02$, $N = 28$). No such correlation was found on the housing estate ($r_s = -0.05$, $p = 0.76$, $N = 40$). As regards to singing activity, such a relationship was not detected either before the experiment (garden city: $r_s = 0.14$, $p = 0.45$, $N = 28$; housing estate: $r_s = 0.18$, $p = 0.26$, $N = 40$) or during the period immediately following the experiment (garden city: $r_s = 0.15$, $p = 0.43$, $N = 28$; housing estate: $r_s = 0.14$, $p = 0.37$, $N = 40$).

Discussion

Only about one half of tested males apparently responded to dummies. Most of them engaged in patrolling prior to direct aggression, although the subadult males tended to intensify their singing activity. Especially in the habitat of housing estate, territorial males spent some time by presence nearby the intruder prior the attack, producing snarl sounds during this time. This time is thus available for decision to attack the intruder or not, and/or more importantly to care for female guarding, as was seen in several cases during experiments (unpublished data). According to these findings, more aggressive effort was observed in garden city habitat, and especially adult males behaved more aggressively toward dummies, regardless of the colour of the dummy. On the other hand, subadult males invest more energy to singing rather than to direct attacking the dummy. Also snarling (the vocalisation expressing aggressive mood of the bird) and whistling (more frightened sound) was produced by the subadult males mainly. Higher number of these sounds was produced in the presence of brown dummies in the housing estate habitat. So, it means that the birds prefer less aggressive approach in a situation of lower competition, especially if the model of the same quality intruder was presented. Even if the owner of the territory had lost it, he can find another territory of the same quality nearby.

Brown coloration of the dummy had no suppressive effect on expression of direct aggression recorded in resident males. Moreover, there was a slight tendency to attack brown coloured dummies earlier. The apparent contradiction to finding noted above may arise from high individuality in approach of the black redstart males to the dummies. If the bird decides to fight, it does not matter what the colour of intruder is, even if younger males generally prefer more peaceful approach. Even so, these findings are in contradiction with both hypotheses predicting considerable reduction of aggressive behaviour towards dull coloured subadult intruders, i.e., SSH (Lyon and Montgomerie 1986) and FMH (Rohwer et al. 1980). Thus, we may further reject these hypotheses as an explanation of DPM in the studied species. Nevertheless, there are the species as Lazuli Bunting (*Passerina amoena*), Northern Oriole (*Icterus galbula*), Yellow Warbler (*Dendroica petechia*) or red-flanked bushrobin (*Tarsiger cyanurus*) in which it was unequivocally demonstrated that adult males showed less aggression towards dull yearlings (Flood 1984, Studd and Robertson 1985, Muehler et al. 1997, Morimoto et al. 2005). Specifically in Lazuli Buntings such a strong competition for the best territories resulting in disruptive sexual selection may reasonably explain DPM (Greene et al. 2000).

Surprisingly, adult and subadult Black Redstart males exhibited comparable responses to the dummies in housing estate, and not in garden city habitat. Therefore, intruding males can utilise feather colour for prediction of rival investment into territorial defence under certain circumstances. Adult males show higher effort to chase out the intruder under conditions of high competition for the best territories, whatever the quality (colour) of the intruder is. This is not in accord with previous findings of Landmann and Kollinsky (1995a) who reported no obvious relationship between male age and occurrence of agonistic behaviour in the Black Redstart population of mountain villages. In conclusion, experimental data conform fairly those hypotheses not suggesting that DPM in Black Redstart is a product of signal evolution. If DPM is not a tactic of brown coloured subadult males how to exploit intraspecific signals, such hypotheses as, e.g., reduced investment hypothesis (Studd and Robertson 1985) should be further evaluated. The more placid reactions found in subadult males support this explanation as well.

We may speculate that the behavioural plasticity and high inter-individual variance found in Prague populations would be interpreted as a good precondition for success in acquiring a wide scale of habitats inhabited by the species, e.g. rocky zones of mountains or urban areas (Cramp 1988). The correlation of the number of aggressive air attacks and territory size was found in the garden city habitat, but not in housing estate. This may arise from territorial distribution pattern in the localities concerned. While usually smaller territories are evenly distributed on a housing estate, the attractive territories are situated to the zone of specific building type in garden city (Schwarzová and Exnerová 2004). Maintaining better territory is more important for males in a mosaic habitat where the number of the best territories is limiting factor. Strong competition for these high quality territories results in higher aggression towards all intruders. In this case, aggressive motivation is predetermined by the territorial quality instead of intruder feather quality. In territories of equivalent parameters the criterion of decision-making is the quality of the intruder directly, and the male takes some time to evaluate if it should start the fight or not. Probably age of male, *i.e.* plumage characteristic, also represents the major factor in female choice. Namely older females prefer the adult males in Black Redstart and these experienced females are carriers of higher annual reproductive success (Weggler 2001). These results also contradict to FMH prediction of dull plumage convenience in mosaic, sparse habitats (Rohwer and Niles 1979; Rohwer et al. 1980). In populations of Ipswich sparrows (*Passerculus sandvicensis*) a similar phenomenon was reported only when male territories were of nearly the same size. Females preferred males according to song quality (Reid and Weatherhead 1990). Similarly, in least auklet (*Aethia pusilla*), female choice depends on bill colour pattern only under poor breeding conditions (Jones and Montgomerie 1992).

In Black Redstart population in Prague, the adult males settled in habitat of evenly distributed resources showed more tolerance to settlement of subadult neighbours (expressed as longer latency of direct attack in experiments) what may result to some genetic benefits from extra pair copulations with mates of these subadult neighbours similarly as in Lazuli bunting (Greene et al. 2000). We may speculate that certain benefits resulting from keeping exclusive high quality

territory outweighed the potential genetic gain of extra-pair copulations in Black Redstart males settled in the garden city habitat of Prague. To prove this hypothesis, detailed genetic study of this Black Redstart population is needed.

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Table 1: Results of GLMMs for Black Redstart males' vocalisations and aggressive behaviour during experiments performed with subadult and adult like coloured dummies. The models were performed for four dependent variables according to dummy colour, age of male, habitat and year, and all between-interactions (marked *) of these independent variables ($N_{\text{subadults}} = 38$, $N_{\text{adults}} = 30$).

Dependent variable	Singing		Snarling		Whistling		Total aggression	
	z	p	z	p	z	p	z	p
Independent variable								
Age of male	2.06	0.04	-2.67	0.008	-2.4	0.016	-5.44	< 0.001
Dummy colour	0.22	0.823	1.57	0.115	2.34	0.019	-0.74	0.457
Habitat	0.91	0.36	0.35	0.724	1.94	0.053	-6.72	< 0.001
Year	5.36	< 0.001	0.83	0.405	0.52	0.603	-6.46	< 0.001
Colour*year	-1.27	0.206	-1.87	0.061	-2.72	0.007	0.85	0.396
Colour*habitat	1.06	0.29	-1.02	0.307	-4.14	< 0.001	0.55	0.585
Colour*age	0.4	0.686	0.51	0.608	0.05	0.964	-0.17	0.866
Age*year	-8.41	< 0.001	2.31	0.021	1.4	0.162	9.85	< 0.001
Age*habitat	-3.98	< 0.001	1.51	0.131	2.44	0.015	7.22	< 0.001
Habitat*year	-1.52	0.13	-2.21	0.027	-2.78	0.005	4.62	< 0.001

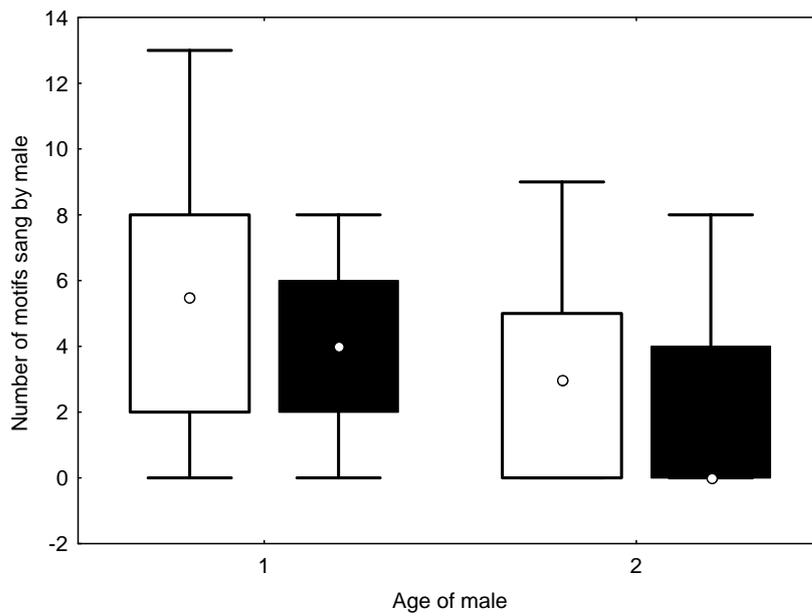


Figure 1: Number of motifs sang by subadult (1) and adult (2) Black Redstart males in habitat of garden city before (white boxes) and immediately after (black boxes) the dummy experiments. The motifs were counted during one minute and compared by Wilcoxon matched pairs test ($T = 293$; $P = 0.02$; $N = 56$). Males of both age categories showed tendency to sing less after the experiment, however this difference was statistically significant in adult males only (adults: $T = 47.5$, $p = 0.05$, $N = 26$; subadults: $T = 101.5$, $p = 0.16$, $N = 30$).

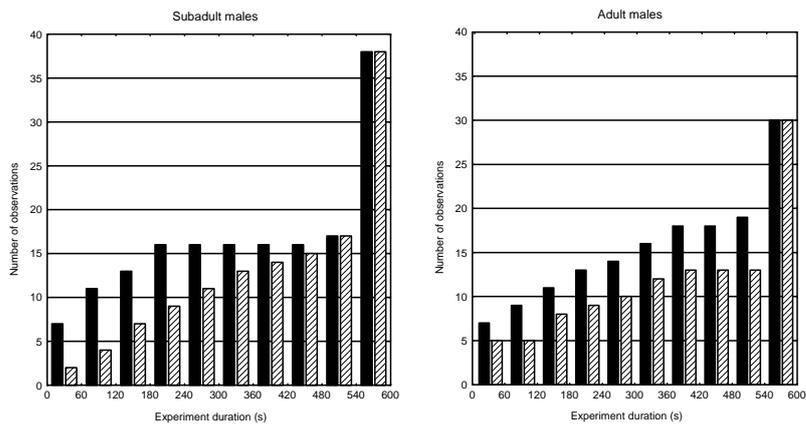


Figure 2: Cumulative frequency of latencies of direct attack during experiments. Data were pooled for both studied habitats, *i.e.* garden city and housing estate. Black Redstart males tended to approach the adult-coloured (black) dummies (shaded columns) later than the subadult-coloured (brown) dummies (black columns) during the experiments. However, the overall model did not show significant differences in these variables (Frailty model: $z = 1.70$; $P = 0.08$; $N=136$).

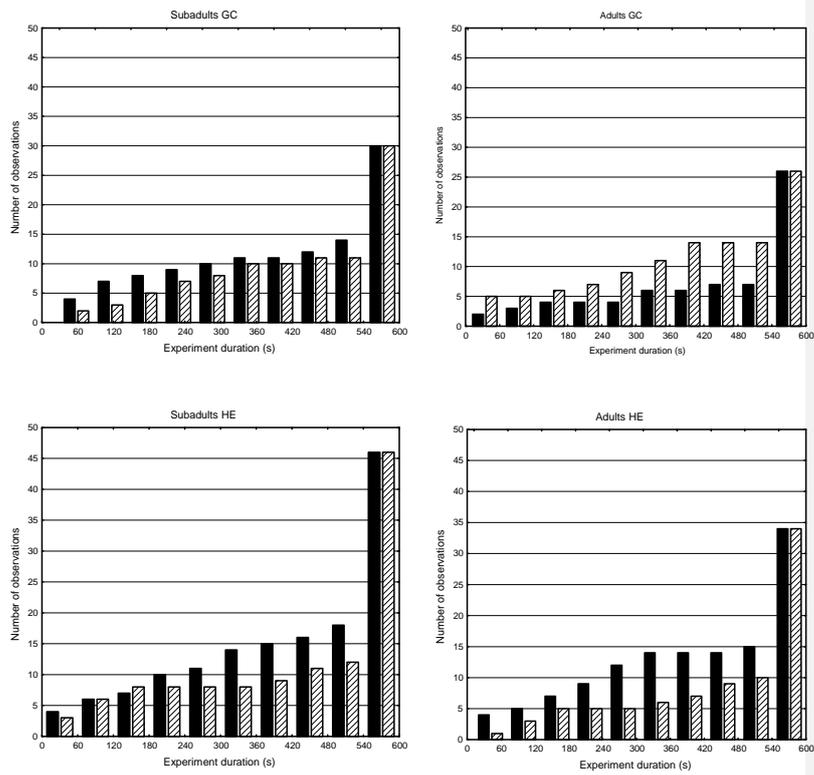


Figure 3: Black Redstart males' behaviour in the habitats of garden city (GC) and housing estate (HE). Latency of presence nearby the dummy without attack (black columns) and latency of the very first direct attack (shaded columns) did not differ significantly in garden city. In housing estate there the males observed the dummies skipping nearby prior to direct attack on it (Wilcoxon matched pairs test: $T = 203$; $P = 0.01$; $N = 80$). The figure shows cumulative frequency of these latencies categorized according to age and habitat.

Delayed plumage maturation correlates with testosterone levels in Black Redstart males

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Abstract

Black Redstart males are usually female-like (olive-brown) feathered during ~~their~~ first breeding season. This subadult colouration sharply contrasts with the grey and black feathers of the adults. To examine proximate mechanisms of this phenomenon known as delayed plumage maturation (DPM), we assessed levels of testosterone (T) in circulating blood of subadult and adult males. The results confirmed seasonal T pattern typical for most temperate passerines, however, there were no marked differences between males of different colouration and/or age neither during the breeding season nor in the autumn and winter months. Nevertheless, analysis of blood samples collected during the moult, i.e., at the time of plumage development, revealed significantly higher T levels in males moulting to adult colour than in those moulting to subadult one. This statistical association may suggest a certain role of T in regulation of DPM. This view may be indirectly supported by the fact that body weight as a fundamental covariate of physiological and life history

variable is not correlated with T levels and do not differ between males moulting to subadult and adult feather coat.

Key words: testosterone, endocrine status, delayed plumage maturation, passerines,

Phoenicurus ochruros

Running head: Testosterone in Black Redstart

Introduction

The plumage coloration plays a significant role in female mate choice (e.g., Sundberg and Dixon 1996, Hill et al. 1999, Greene et al. 2000). As a rule, brightly coloured males are the preferred ones. Nevertheless, coloration of sexually mature yearling males is regularly dull or even female-like in numerous bird species [including some turdids \(Escalona-Segura and Pederson 1997\)](#). This phenomenon known as delayed plumage maturation (DPM) may cause an obvious paradox concerning sexual selection. Regardless to their dull plumage, yearlings are comparably attractive for females and successful in breeding and acquiring the territories, as are the adult males with full coloured feathers (Flood 1984; Nicolai 1994; Schwarzová ~~&~~ Exnerová 2004).

It was hypothesised that dull subadult coloration is favoured by natural selection as its bearers are less likely and/or less severely attacked by their adult rivals (Selander 1965, Rohwer 1975, Rohwer et al. 1980, Rohwer et al. 1983, Rohwer 1986, Lyon ~~et-and~~ Montgomerie 1986, Foster 1987, Brown ~~et-and~~ Brown 1988). A contrasting explanation of DPM is proposed by the reduced investment hypothesis suggesting that subadults reduce their overall investment to the first breeding event and therefore they lower energetic investment to the building bright feathers during their preceding moult (Studd ~~et-and~~ Robertson 1985, Procter-Gray ~~et-and~~ Holmes 1981, Procter-Gray 1991; [for comparative evidence suggesting costs of ornamental feathers see Cuervo and Møller 2000](#)).

Testosterone (further referred as T) is an important steroid hormone affecting primarily timing and magnitude of the reproductive functions and associated morphological and behavioural traits (including [singing, mating](#) and parental behaviour; e.g., Gill 1990, [Schwable and Kriner 1991, Absil et al. 2003](#)). Moreover, high T level may indicate better quality of bird males (Ligon et al. 1990). Only high quality males are able to cope with elevated T level known to suppress immune response according to immunocompetence handicap hypothesis ([Folstad and Karter 1992, Saino and Møller 1994, Merila and Sheldon 1998, for a review see Roberts et al. 2004, Saino et Møller 1994](#)). [Consequently, T related traits are frequent subjects of mate choice \(Ligon et al. 1990, Enstrom et al. 1997\) and related reproductive decisions \(e.g., maternal investment; Foerster and Kempenaers 2005\). It is of interest,](#)

[that yearling males exhibit lower T levels in some passerines \(e.g., *Zonotrichia leucophrys*; Morton et al. 1990\)](#)

In temperate passerines, T level exhibits considerable seasonal variation. Typically, the level of circulating T is elevated at the onset of the breeding season and gradually falls during the nestling stage (Morton et al. 1990, Raouf et al. 1997, Romero et al. 1997, Romero et al. 1998). Although actual T levels during the moulting period are rather low, [and artificially elevated levels may even preclude the moult initiation \(Schleussner et al. 1985, Nolan et al. 1992, Dawson 1994\)](#), there are studies suggesting correlation between T level and/or volume of the testes (i.e., the trait functionally correlated with T_2 —Gwinner ~~in Farner a King~~ 1975) with feather coloration (e.g. in *Carduelis chloris*: Merila et Sheldon 1998; *Pheucticus melanocephalus*: Hill 1994; *Ptilonorhynchus violaceus*; Collis ~~and~~ Borgia 1992; but see Romero et al. 1997 in *Carduelis flammea*). Nevertheless, the evidence confirming the role of testosterone in development of the plumage coloration is somewhat ambiguous and even its putative mechanisms differ between melanin, carotene and structural colours ([Hill ~~et and~~ Brawner 1998, Møller ~~et and~~ Eritzoe 1988, Bennett ~~et and~~ Owens 2002](#)).

As concerned melanin ornaments, enlargement of black bib appeared under elevated testosterone level during moult in House Sparrows (*Passer domesticus*; Gonzales et al. 1999, Evans et al. 2000, Gonzales et al. 2001). Hormonal control of melanogenesis could be affected by food availability, as well as by deficit of specific amino acids in diet receiving in energetically sufficient amount. Such a deficient food during the moult stage may widely affect the quality of melanin ornaments (Jawor ~~et and~~ Breitwisch 2003).

Black Redstart (*Phoenicurus ochruros* S. G. Gmelin, 1774), a small European passerine bird, exhibits usually two plumage colourations during its life. Mostly black and grey feathers with the bright white wing panels characterize the adult males. The most of breeding yearlings carry grey-brown feathers with no white wing panels, similar to female of the species. Small proportion of them appears adult-like black feathers with the absence of at least one white wing panel (Nicolai 1992, Cramp 1988). Referring to physiological constrains possible influence during the first year moulting in Black redstart (Landman ~~et and~~ Kollinski 1995) we should pay attention to the importance of testosterone role in the process of feather colouration development (Gill 1990, Bezzel ~~et and~~ Prinzing 1990). The period of the first post-

juvenile moulting ([for difficulties associated with moult terminology see Howell et al. 2003](#)) is crucial to solve this question. In the Black Redstart this moult takes place typically before migration lasting from August to September in Central Europe (Cramp 1988).

The aims of this study were: (1) to compare testosterone levels in Black Redstart males exhibiting adult and subadult feather colouration; (2) in particular, we paid attention on the period of moult when plumage is developed. (3) We described also seasonal pattern of testosterone levels, and (4) we examined relationship between the T level, feather colour and the body weight. (5) Finally, stability of T levels was challenged by exposure to conspecific song.

Material and Methods

Subjects

Studied males of *Phoenicurus ochruros* were captured on their breeding grounds in Prague city (Czech Republic; 50°06'N, 14°30'E, 280-380 m asl) in housing estate or garden city habitats supporting high abundance of the species. They were caught in Japanese mist nets or flap traps using the specific song playback. Such a short simulated territorial intrusions and attacks do not increase plasma testosterone levels in experimental birds (Silverin 1993, Meddle et al. 2002). This assumption was also tested as a part of this study in a separate experiment (see below). Birds were banded for subsequent identification with a unique combination of colour rings and in addition with aluminium rings of National Museum in Prague. Some of the juvenile birds were captured in the nests, and hand raised subsequently up to independency. These were maintained in captivity up to the time of moulting to subadult plumage. These birds were fed *ad libitum* by mealworms supplemented with the vitamin mixture (Roboran H, UNIVIT). They were kept in aviary room under natural daylight cycle. The Committee for Animal Care and Use at Charles University approved these experimental procedures.

A 100- μ l blood sample was collected from each male immediately after capture and/or the time of testing (see below). The blood was collected from *vena ulnaris* into a heparinized tip. Blood samples were kept on ice in microtubes until they were centrifuged (within 1 hour), and obtained sera were stored frozen at -20 °C until

analyzed. Analyses of samples were provided in Czech Academy of Sciences, Institute of Endocrinology, T was determined by radioimmunoassay (RIA). After extraction to diethylether, T was measured by using rabbit antisera against testosterone-3-CMO:BSA conjugate and radioiodinated tyrosine methyl ester testosterone as tracer (Hampl 1994). Interassay variability was estimated for 10,7 %, intraassay variability for 8.2%, respectively. STATISTICA 6.0 software package was used for computing all the statistical analyses.

Experimental design

Testosterone blood levels in males actually moulting to different feather colour. The birds were captured during August and September 2001, 2002 and 2003 to provide this part of study. The males were examined for the proportion of growing feathers on their body; We sampled exclusively those males exhibiting the proportion of new feathers above 25 %. The colour of new feathers was recorded. Juveniles were sexed according to a universal PCR method based on the amplification of CHD genes located on the avian sex chromosomes (Griffiths et al. 1998). To evaluate the possible confusing effect of captivity, this factor was included in the overall model; however, no significant effect of human care was revealed by the (ANOVA, $F = 0.13$, $p = 0.71$, $R^2 = 18.8\%$, $n = 25$, $R^2 = 18.8\%$).

Testosterone levels in Black Redstart males during the different stages of annual cycle.

Altogether, fifteen males were sampled during territory acquisition in breeding seasons 2001 and 2002. The samples of four migrating birds were taken at the end of October; all of them were juveniles of the current year. Three males from the group of hand-raised juveniles were maintained throughout the winter in captivity, and they were sampled in the mid of December.

Interrelations of bird weight and the testosterone blood level. During the 2002 and 2003 seasons, respectively, we collected the data of body weight in the birds after the breeding season. The number of actually non-moulting birds (collected during late July and August) was 24 males, and the group of moulting birds consisted of 16 individuals. These data were analysed for each moulting and non-moulting group separately. The test of weight differences in the males of different feather colouration was performed for a group of moulting males.

Hormonal response to ~~singing~~ conspecific song. We carried out this experiment to verify the expectation of playback song used in catching the birds has no influence to plasma testosterone level in Black Redstart males. 11 experimental males were caught during the September 2003 and blood was sampled after one-week acclimatisation in the aviaries. The experimental exposure to song of territorial conspecific started in the morning and continued until darkness. Each male has received several repeated bouts of playback. Each bout was 45 min long and was followed by a 60 min pause. The following day the males were exposed to two bouts, and the blood samples were taken immediately after the last song.

Results

Testosterone blood levels in males actually moulting to different feather colour.

In adult Black Redstart males during their moulting time in September, the mean testosterone level was 1.07 ± 0.43 (n = 3). In juvenile males, the corresponding values were 1.21 ± 0.36 (n = 10) and 0.82 ± 0.34 (n = 12) for those moulting to black feathers and olive-brown feathers, respectively (Fig. 14).

As adults and subadults moulting to black had similar testosterone levels (ANOVA: $F = 0.49$, $p = 0.50$, $n_1 = 3$, $n_2 = 10$), these groups were pooled. Comparison of the males moulting to black feather and those moulting to olive-brown (subadult) feather has revealed that the former males had significantly higher testosterone levels than the latter ones (ANOVA: $F = 4.75$, $p = 0.04$, $R^2 = 18.8\%$, $n = 25$).

Testosterone blood levels in breeding subadult and adult males.

At the beginning of the breeding season, i.e., during the period of territory acquisition, the mean value (\pm SD) of testosterone level in adults was 2.33 ± 0.89 nmol/l (range: 1.46-3.41; $n = 4$). Nearly the same value (2.37 nmol/l) we recorded in the only black-feathered yearling male. Corresponding values in female-like yearling males were also fairly similar 2.81 ± 2.61 nmol/l (0.45-7.71; $n = 10$) and thus the comparison of testosterone levels in subadults and adults (Fig. 12) has revealed no statistically significant difference ($F = 0.12$, $p = 0.72$, $n = 14$).

Testosterone blood levels in males during autumn and winter months. Testosterone levels assessed in juvenile males during the autumnal months (prior their first breeding season) were markedly lower than in breeding males. The mean values were 0.78 ± 0.09 nmol/l (n = 4) at migration time, and even 0.38 ± 0.12 nmol/l (n = 3)

during the winter period (mid December). In spite of small sample sizes, the difference between these two means appeared significant ($t = 5.03$, $p = 0.003$; see Fig. 3).

The mean values of T blood levels in still non-moulting males collected after the breeding season of the current year were as follows: 1.22 ± 0.27 nmol/l for adult birds ($n = 4$); 1.71 ± 0.99 for subadult birds (i.e. moulting to their first black (adult) plumage ($n = 5$); 1.33 ± 1.10 for juvenile birds subsequently moulting to black plumage ($n = 2$); 1.03 ± 0.28 for juvenile birds subsequently moulting to female-like plumage ($n = 13$).

Interrelations of bird weight, testosterone blood level and feather colouration. We found no correlation between the testosterone level and the body weight, neither in non-moulting males out of the breeding season (Spearman $R = -0.32$, $p = 0.127$, $n = 24$), nor in the males examined during moulting period (Spearman $R = 0.19$, $p = 0.49$, $n = 16$). Moreover, males moulting to adult black feather were not significantly heavier than those moulting to subadult olive-brown feathers ($F = 0.01$, $p = 0.89$, $n = 16$).

Hormonal response to conspecific song.

No difference in the plasma testosterone level before and after the experimental exposure of the territorial male to the playback of conspecific song was detected ($Z = 0.53$, $p = 0.59$, $n = 11$; Fig 24).

Testosterone levels in juvenile males during autumn and winter months.

Testosterone levels assessed in juvenile males during the autumnal months (prior their first breeding season) were markedly lower than in breeding males. The mean values were 0.78 ± 0.09 nmol/l ($n=4$) at migration time, and even 0.38 ± 0.12 nmol/l ($n=3$) during the winter period (mid December). In spite of small sample sizes, the difference between these two means appeared significant ($t = 5.03$, $p = 0.003$; see Fig. 3).

Testosterone levels in moulting males of different feather colour.

In adult Black redstart males during their moulting time in September, the mean testosterone level was 1.07 ± 0.43 (n=3). In juvenile males, the corresponding values were 1.21 ± 0.36 (n=10) and 0.82 ± 0.34 (n=12) for those moulting to black feathers and grey-brown feathers, respectively (Fig. 4).

As adults and subadults moulting to black had similar testosterone levels (ANOVA: $F = 0.49$, $p = 0.50$, $n_1 = 3$, $n_2 = 10$), these groups were pooled. Comparison of the males moulting to black feather and those moulting to grey-brown (subadult) feather has revealed that the former males had significantly higher testosterone levels than the latter ones ($F = 4.75$, $p = 0.04$, $R^2 = 18.8\%$, $n = 25$).

Relationship between body weight, testosterone and feather colouration

We found no correlation between the testosterone level and the body weight, neither in non-moulting males out of the breeding season (Spearman $R = -0.32$, $p = 0.127$, $n = 24$), nor in the males examined during moulting period (Spearman $R = 0.19$, $p = 0.49$, $n = 16$). Moreover, males moulting to adult black feather were not significantly heavier than those moulting to subadult olive-brown feathers ($F = 0.01$, $p = 0.89$, $n = 16$).

Discussion

Unlike most temperate passerines, Black Redstart males regularly exhibit territorial behaviour during the autumn (Wegglér 2004). In spite of this, playback experiment successfully verified our expectation that the T level assessed in circulating blood is not affected by exposure of experimental males to song of a conspecific male. Although previous studies carried out in some other passerine species led to the same conclusion (Silverin 1993, Meddle et al. 2002), this was an obligatory step allowing us to confirm reliability of adopted catching method of sampled males in the Black Redstart. It should be emphasised here that the experimental design involved latent presence of some visual stimuli (birds were allowed to observe conspecific males kept in the same room) in addition to the auditory ones. As demonstrated in other species during breeding season, simultaneous presence of both these stimuli may be essential for the elevation of testosterone levels (Wingfield et al. 1987).

Blood samples collected during the period of territory acquisition in the spring showed fairly similar T levels in males of different feather colour and/or age. Although sample sizes are insufficient, it is remarkable that subadult males (i.e., those dull feathered) even tended to show slightly higher testosterone levels than the adults. Thus, we cannot support the expectation about inferior endocrine phenotype of these males sampled during their first breeding season.

It was not much surprising that T levels recorded outside the breeding season were markedly lower (with minimum in winter months) and males of different feather colour and/or age exhibited comparable T levels. There is, however, moulting period in which we recorded higher T values in males moulting to adult (black) feather colour than in those moulting to subadult (olive-brown) one. This finding suggests that testosterone may play signal and/or regulatory role affecting colour of actually growing feathers. It is in accord with the fact that testosterone (besides luteinizing hormone and non-hormonal factors) was repeatedly reported as a proximate cause of plumage dichromatism in many phylogenetically derived bird taxa including passerines (Kimball and Ligon 1999). It may be argued, however, that our results ~~this reflect~~ is just a functional link caused by partial correlation of T levels with another traits causally affecting feather colour. The absence of significant relationship between the body mass and T-levels assessed in this period might be interpreted as independence of T level on the trait reflecting body condition. There is, however, some evidence supporting relationship between body mass and testosterone in some other bird species as, e.g., Redheaded Bunting (*Emberiza bruniceps*; Kumar ~~et~~and Kumar 1990) or Spotted Munia (*Lonchura punctulata*; Gupta ~~et~~and Thapliyal 1984) However, the evidence of relationship between body mass and testosterone was found in some birds, e.g. in Redheaded Bunting (*Emberiza bruniceps*; Kumar ~~et~~ and Kumar 1990) or Spotted Munia (*Lonchura punctulata*; Gupta ~~et~~ Thapliyal 1984).

In view of the fact that testosterone production requires high energetic investment, it could seem somewhat surprising that testosterone level did not decrease to its basal minimum during migration (but Schwabl et al. 1984 reported similar results in European Blackbird *Turdus merula*), and was significantly higher than the winter level. Moreover, due to high metabolic rate of testosterone (Graner 2003- ~~in *Passer* *illustrated bison*~~) one should expect higher than basal testosterone production persistently during migration. This may follow many reasons, from by-product stimulation by longer day in lower latitudes or need of a higher aggressive level for

strong resource competition on the way, stimulation of singing activity that appears during autumn in Black Redstart, or simply slowly working regulation mechanism decreasing blood testosterone production to its basal level. However, there is some evidence that aggression and singing activity performed during non-breeding season does not depend on testosterone blood level in some species ([Logan and Wingfield 1990](#); ~~Canoine et and~~ Gwinner 2002, Pinxten et al. 2003).

)- On the other hand, testosterone is a factor stimulating locomotion activity (Deviche 1992) and so it may play a role in regulation of migrating behaviour. The effect of testosterone is also resistance to the anorectic effect of endogenous opioids, what was found in Dark-eyed Juncos (*Junco hyemalis*; Deviche 1992). The hormone may be a part of highly complex regulation of stress-induced anorexia during migration, when the sufficient amount of food intake is one of the crucial points for bird survival. However, account that the data set concerning the problem of basal and migration testosterone level is rather small in the case of Black Redstart, it remains the anecdotic inspiration for a view to the problematic of physiological changes during migration.

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As we have seen, most of the studies of testosterone in birds focus to the problems closely related to breeding and aggression. However, somewhat few studies have

focused on the problem of testosterone relation to feather colour or blood circulation levels of testosterone out of the breeding season in temperate migrating birds (e.g. Schlessner et al. 1985, Schwabl et Kriner 1991, Kriner et Schwabl 1991, Dawson 1994).

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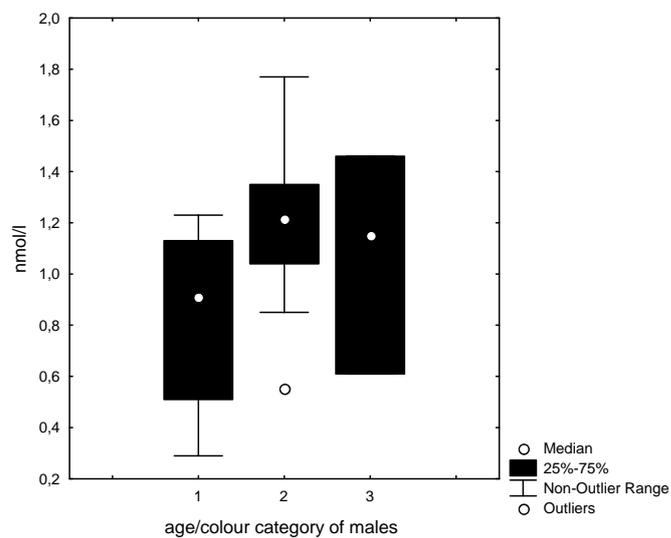


Figure 1: Testosterone blood levels in Black Redstart males during moulting. The figure shows a difference of T levels in juvenile individuals moulting to brown feather (denoted as 1; n = 10), juvenile individuals moulting to black feather (denoted as 2; n = 12), and the adult individuals (denoted as 3; n = 3). The statistical significant difference was found between the T levels in birds moulting to black plumage and the birds moulting to olive-brown plumage (($F = 4.75$, $p = 0.04$, $R^2 = 18.8\%$, $n = 25$).

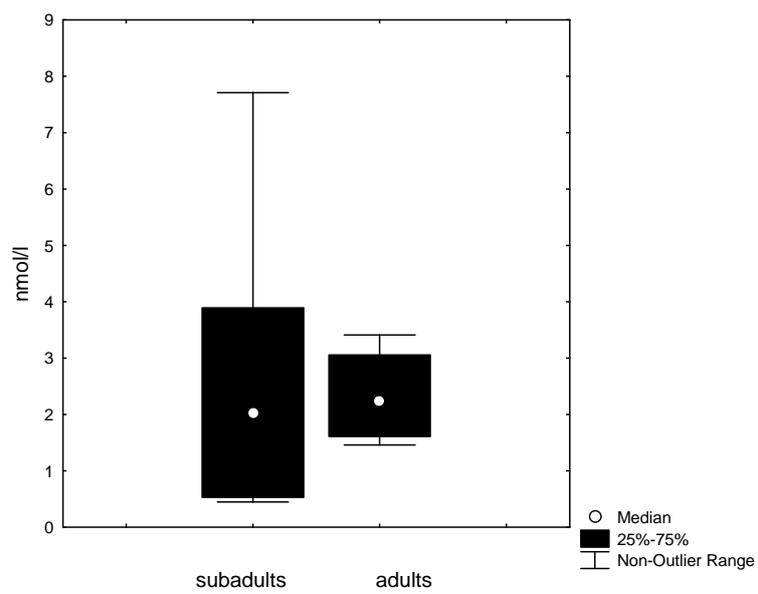


Figure 2. Testosterone blood levels in subadult and adult males of Black redstart during breeding season. The testosterone levels of subadults did not differ significantly from these of adult birds ($F = 0.12$, $p = 0.72$, $n_{\text{subadults}} = 10$, $n_{\text{adults}} = 4$).

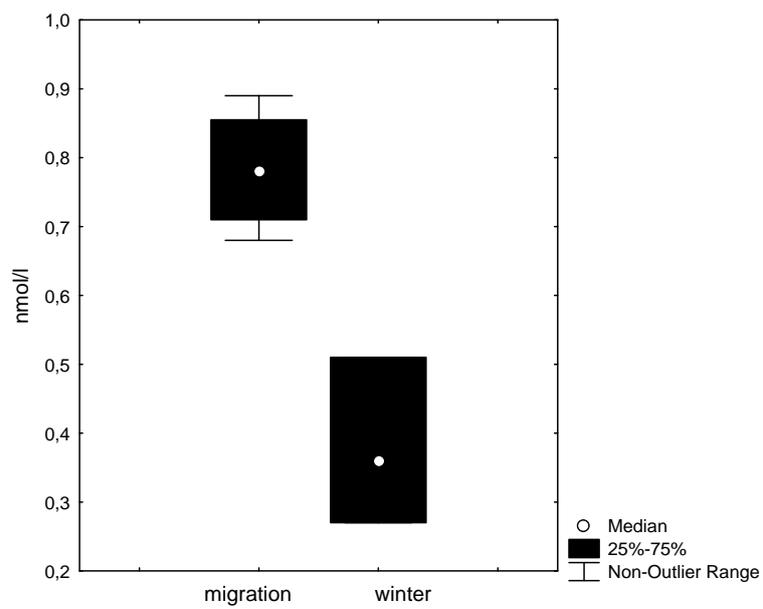


Figure 3: Testosterone blood levels in Black Restart males during migration (late October) compared to data of base level in the species ($t = 5.03$, $p = 0.003$, $n_1 = 4$, $n_2 = 3$). During migration, the blood T level did not still decrease to base levels. Base levels of testosterone were measured at mid December in caging birds.

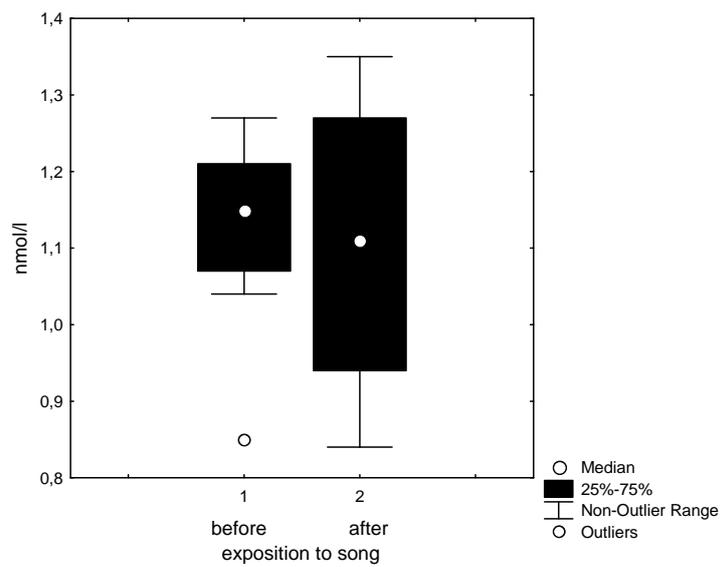


Figure 4. Testosterone blood levels found in experimental Black Redstart males before and after the exposition to specific song repeated regularly during one day. Total number of the birds included in this experiment is eleven, and no significant

difference in the testosterone levels was revealed before and after the exposition to song bouts ($Z = 0.53$, $p = 0.59$, $n = 11$).

Male-biased sex of extra pair young in the socially monogamous red-backed shrike, *Lanius collurio*

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Abstract

Females of many socially monogamous bird species engage in - or even actively seek - copulations outside their social pair bond. However, in socially monogamous birds with low breeding abundance density, such as the red-backed shrike *Lanius collurio*, extra-pair paternity (EPP) was thought to be an exceptional and random incident. Here we provide evidence that EPP in the red-backed shrike increases with breeding density and results in male-biased brood sex ratio. Drawing on samples collected in an unusually dense red-backed shrike population in the Czech Republic, we show through DNA microsatellite typing that among 65 chicks from 15 nests, 10 individuals (26.15.54%) had been sired by males other than the nest-attending social mate. All 10 extra pair young were of male sex. In all cases, genetic fathers of extra pair young stemmed from neighbouring territories. Extra pair fathers had significantly longer tarsi than social mates, indicating that female choice was a function of age-dependent body size. Our findings support sex allocation theory, which suggests that promiscuous females mating with higher quality males should produce mostly sons.

Naformátováno: Řádkování: jednoduché

Key words: extra pair paternity, sex ratio, secondary sexual character,

Naformátováno: Přístupy klávesou tabulátor: 13,15 cm, (Zarovnání vlevo)

Naformátováno: Přístupy klávesou tabulátor: 13,15 cm, (Zarovnání vlevo)

Introduction

The existence of extra pair paternity (EPP) arising from extra pair copulations (EPCs) has been demonstrated in numerous socially monogamous songbird species (for review see Griffith et al. 2002). While the evolutionary advantage of promiscuity can be easily understood from the male perspective, the benefits to females seem less clear. It is generally assumed that females may gain indirect genetic benefits from EPP through increased offspring heterozygosity or through the potential to produce sons of higher genetic quality (Petrie and Kempenaers 1998; Griffith et al. 2002). If the latter were the case, females engaging in EPCs with high quality mates should invest more into the production of sons, since male offspring would receive a relative advantage from inheriting paternal traits (Leech et al. 2001).

Despite the sheer quantity of studies devoted to avian mating systems, the causes of variation in the frequency of EPP between species and populations remain elusive (Petrie and Kempenaers 1998). In some socially monogamous bird species, such as shrikes (Laniidae), EPCs occur regularly but do not necessarily lead to EPP. For instance, in the lesser grey shrike *Lanius minor*, females frequently engage in EPCs after courtship feeding (Carlson 1989; Valera et al. 2003; Tryjanowski and Hromada 2005), but remain genetically faithful to their social mate, as has been revealed by genetic fingerprinting of offspring (Valera et al. 2003). In the closely related red-backed shrike *Lanius collurio*, there is to date only one genetic estimate of the level of EPP, which is based on a very small sample size (EPP among nestlings, 5.26 %, n=19; Fornasari et al. 1994).

The level of EPP in a given species is likely to depend on demographic as well as life-history attributes, including breeding [abundance density](#), length of the reproductive season and number of broods per season. Any biological inference drawn from estimates from single study sites and years (or averaged EPP estimates based on small sample sizes) must be handled with caution, since the frequency of EPP may vary in a density-dependent manner rather than reflect a predisposed

species-specific feature of the mating system (for the pied flycatcher *Ficedula hypoleuca*, see Lubjuhn et al. 2000). Because red-backed shrikes occur in small (and continually declining) numbers, the low frequency of EPP reported so far may simply be due to reduced encounter probability rather than reflect a low intensity of sexual selection in this species.

There is growing awareness that individual behavioural decisions may impact the genetic structure and demography of populations and are thus relevant to conservation and management issues (Quader 2005). Threatened species apparently experience more intense post-mating sexual selection than less threatened species (Morrow and Pitcher 2003). At the population level, habitat heterogeneity is expected to promote polygyny, since females may directly benefit from producing offspring with males occupying territories of higher quality (polygyny threshold hypothesis, Verner & Wilson 1966; see Quader 2005). However, if populations reach low densities, the encounter probability of males and females decreases, which reduces mating opportunities and restricts females from selecting among a wide variety of males and upgrading mating decision (Quader 2005). As a consequence, females may reduce their investment into offspring or may even decide not to breed at all, given the limited possibilities to increase their fitness. Thus, ongoing population declines in small and fragmented populations may be augmented through the intensity of sexual selection (Quader 2005).

The aim of the present study was to test whether the probability of EPP in the red-backed shrike increases with higher breeding density and whether female choice and offspring sex ratio vary in a predictable way. We show that the frequency of EPP in red-backed shrike has so far been underestimated. Furthermore, we provide evidence that promiscuous females choose older, higher quality males and skew offspring sex ratio strongly towards the male sex.

Material and methods

Study area and birds

The study area (15 km²) was situated in South Bohemia, north-east of the town of Písek, Czech Republic (49°19'N, 14°15'E). Population density of red-backed shrikes

in this area averaged 3.5 pairs / km², which is among the highest breeding densities documented in this species (see Kuźniak and Tryjanowski 2003). Brood size ranged from two to five nestlings (mean ± SD = 4.0 ± 1.0, n = 15 nests), which lies within the range of values published elsewhere (review in: Kuźniak and Tryjanowski 2003). Adult birds were trapped in bowl-traps baited with mealworms, or with mist-nets (Šimek 2001) and individually marked with a combination of colour rings and one aluminium ring. Adult birds were sexed and aged according to plumage characters described by Svensson (1992). In addition, we took basic morphometrical measurements, including the length of the right wing (maximum chord, to the nearest 0.5 mm, using a metric ruler), the length of the right tarsus (to the nearest 0.05 mm, using callipers) and body mass (to the nearest 0.1 g, using a spring balance). All morphometrical measures were conducted by one and the same person (JŠ).

We took 5-50µl blood from the brachial vein in adult birds and nestlings. Nestlings were sampled and ringed from day 7 onwards (day 0 = hatching day). Blood samples were collected in 70 µl capillary tubes, cooled on ice in the field and stored in a freezer at -18oC on the same day. Social parentage of each brood was determined by observing nest attendance of individually colour ringed adults during chick rearing.

Molecular genetic techniques

DNA was extracted from blood samples using a phenol/chloroform protocol (Milligan 1998). Individual birds were genotyped using four polymorphic microsatellite loci (*LS1*, *LS2*, *LS3*, *LS4*), which were originally developed for the loggerhead shrike *Lanius ludovicianus* (Mundy and Woodruff 1996), but amplify in the red-backed shrike (Pustjens et al. 2004). For each PCR step, a total volume of 20 µl contained 0.5 units Taq DNA Polymerase (Invitrogen), 1 x PCR buffer (20mM Tris-HCl (pH = 8.4), 50 mM KCl), 0.2 mM dNTPs each, 1.5 mM MgCl₂, 0.5µM primers each and 2 µl of template DNA. PCRs were run on GeneAmp 9700 Thermocycler (Applied Biosystems). The PCR profile consisted of an initial denaturing step at 94 °C for 3 min, followed by 35 cycles of 94°C for 30s, X°C for 1min (X = 47°C for LS1, 50°C for LS2, 53°C for LS3 and 55 °C for LS4 loci), and 72°C for 1min. The cycle was terminated with a step of 72°C for 7 min followed by 4°C. The bands were visualized in 6 % polyacrylamide gels stained by SYBR® Green (Molecular Probes).

Nestlings were sexed according to a universal PCR method based on the amplification of CHD genes located on the avian sex chromosomes (Griffiths et al. 1998). Adult birds could be sexed by their plumage characters, yet information was confirmed by PCR based methods. PCR amplifications were run in a total volume of 20µl. Each reaction contained 0.5 units Taq DNA Polymerase (Invitrogen), 1x PCR buffer (20mM Tris-HCl (pH = 8.4), 50mM KCl), 0.2 mM dNTPs each, 1mM MgCl₂, 0.5µM primers each and 2 µl of template DNA. The PCR profile consisted of an initial denaturing step at 94 °C for 1 min, followed by 30 cycles of 94°C for 30s, 48°C for 45s, 72°C for 45s, and terminated by 94°C for 30s, 48°C for 1 min and 72°C for 5 min. Bands were visualized in 2 % agarose gels stained with SYBR® Green (Molecular probes). Figure 1 shows the resulting sex-specific bands in a gel. Because the number of sampled broods (n = 15) and applied microsatellite loci (n=4) was relatively small, we conducted paternity assignments only for those broods where both social parents had been genotyped (Kasumovic et al. 2003, Kalinowski et al. 2007). Offspring were defined as extra-pair young (EPY) when two or more loci deviated from the social male. The likelihood of genetic paternity was established using the software package CERVUS 3.0 (Kalinowski et al. 2007).

Statistical analyses

Statistical analyses were performed using SPSS 12.0 for Windows. All tests were two-tailed with a significance level of $P < 0.05$. Data are presented as means \pm SD.

Results

Frequency and distribution of extra-pair paternity

Extra pair young (EPY) were detected in 6 (40.0%) of the 15 analysed broods, each containing one to three (2.0 ± 1.1 , $n = 6$) EPY. Among the 65 sampled nestlings, 55 (84.6%) had been sired by social mates, whereas 10 (15.4%) resulted from EPCs of 3 males. In all cases, genetic fathers of EPY stemmed from neighbouring territories. All individuals observed outside their own territories (intruders) were males. This pattern differed significantly from random ($n = 7$, binominal sign test, $P = 0.016$).

Offspring sex ratio

The sex ratio of offspring shared among social pairs was 1.39 (32 males versus 23 females). All 10 nestlings sired by extra pair males were of male sex. Thus, the net sex ratio of all sampled nestlings accumulated to 1.83. The contrast in sex ratio between offspring among social partners and those sired by extra pair males was statistically significant (χ^2 with Yates correction = 4.792, $P = 0.029$).

Estimates of male quality

Extra pair fathers had significantly longer tarsi than faithful males (24.30 ± 0.10 versus 23.22 ± 0.40 mm, sample size for extra pair males and faithful males was 3 and 14, $t_{1,14} = -8.67$, $P < 0.0001$). Interestingly, in three cases, extra pair young were sired by genetic fathers of the females' social partner. All males identified as extra pair mates had territories with established social bonds in the same local population, as documented through genetic paternity analyse and observations of colour marked individuals.

Discussion

Extra-pair paternity is widespread among birds, and illegitimate offspring have been found in approximately 90% of the examined species (reviewed by Griffith et al. 2002). Our study adds to the plethora of studies reporting significant genetic promiscuity in a socially monogamous songbird. In contrast to the low level of EPP found in a red-backed shrike population in the Italian Alps (Fornasari et al. 1994), we show that EPP can reach higher levels in this species. Furthermore, compared with other passerines with a similar mating system, i.e. social monogamy (16% EPP averaged across 61 species; value derived from Appendix 1 in Griffith et al. 2002), our EPP estimate for the red-backed shrike lies exactly in the range of EPP estimates in this group of birds. Our estimate of the frequency of EPP in the red backed shrike lies above the estimates found in all other species of Laniidae studies so far. Using multi-locus DNA fingerprinting, Yamagishi et al. (1992) found that 10 of 99 (10%) nestlings of the bull-headed shrike (*Lanius bucephalus*) had been sired by extra pair

males. In the loggerhead shrike (*Lanius ludovicianus*) eight offspring from five families (4% of offspring) were sired by extra pair males (Etterson 2004). In the lesser-grey shrike, although females frequently engaged in EPC, EPP has so far not been genetically detected (Valera et al. 2003).

Our result questions the previous view that the red-backed shrike is strictly monogamous, which was based on observational data alone, with only one case of social polygyny found in this species (Olsson 2001). This had led to the assumption that either females are unwilling to accept EPCs, or that males cannot monopolize the territorial resources necessary to attract more than one female. Our study shows that female red-backed shrikes do accept EPCs from owners of neighbouring territories. Moreover, there was a tendency towards longer tarsi in males chosen by females outside their social pair bond. The association between tarsus length and EPP may be linked to age-dependent male quality, since older males of the red-backed shrike generally have longer tarsi than younger males (Jakober and Stauber 1989; Tryjanowski and Yosef 2002; Tryjanowski and Šimek 2005). Such age-dependent female choice is further supported through cases where females mated with fathers of their social mates. Together, this matches the general pattern seen in songbirds where older males are favoured by female choice (e.g. Lubjuhn et al. 2000; Griffith et al. 2002; Kleven et al. 2006).

Age-dependent quality of extra pair males may also explain why the sex-ratio of their offspring was male-biased. Theory predicts that cryptic, post-copulatory female choice should favour the production of 'sexy sons' after mating with males of higher quality, through which they may gain an indirect fitness advantage (Wedell and Tregenza 1999).

Previous behavioural observations such as strong defence responses at territory borders by males (Durango 1956; Fornasari et al. 1992; 1994), as well as intensive mate guarding of receptive females (Jakober and Stauber 1994), support the high probability of EPP resulting from EPCs in the red-backed shrike. Our study underpins these suggestive observations and calls for further detailed studies on mate choice, sexual selection and genetic variation in red-backed shrike populations (*cf.* Pustjens et al. 2004)

In conclusion, our study shows that the level of detected EPP in the red-backed shrike is higher than previously reported. Since the red-backed shrike is critically endangered in many parts of Europe, and has gone extinct on the British Isles, its

prospects for recovery are not the best. With increasing habitat fragmentation and locally decreasing habitat quality, the relatively high level of sexual selection through female choice found in this species may limit overall fecundity. Female choice could potentially drive populations into evolutionary traps, since the cues upon which females base reproductive decisions may fall below threshold, leading to maladaptive mating decisions or even complete reproductive failure (*cf.* Quader 2005).

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Figure legend

Figure 1. PCR products after gel electrophoresis of amplified CDH genes located on the avian sex chromosomes (W, Z). The size of the large W band (estimated according to 100bp DNA ladder – Invitrogen) is approximately 390bp, the shorter Z consists of about 325bp. The image shows the genotypes of two males and one female (symbols).

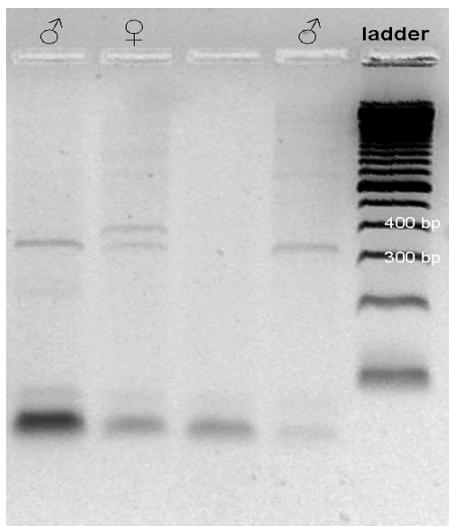


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