


Short Communication

Explorative behaviour is not associated with metabolism in the European Pied Flycatcher *Ficedula hypoleuca*

 OONA PORANEN  &

 SUVI RUUSKANEN* 

Department of Biology, University of Turku, Turku, 20014, Finland

The pace-of-life syndrome hypothesis (POLS) represents an attractive theoretical framework suggesting that physiological and behavioural traits have evolved together with environmental conditions and life-history strategies. POLS predicts that metabolic differences covary with behavioural variation such that high metabolic rate is associated with risk-prone behaviour and a faster pace-of-life, whereas a low metabolic rate is associated with risk-averse behaviour and a slower pace-of-life. We tested the POLS hypothesis in captive European Pied Flycatchers during their first year by examining the relationship between explorative behaviour and basal metabolic rate. Our results are inconsistent with POLS. The positive association of explorative behaviour with basal metabolic rate was not recovered for either sex, possibly due to foraging conditions in the aviaries where control and trial groups were fed twice a day, the birds' young age, developmental plasticity, or a non-existent syndrome.

Keywords: basal metabolic rate, birds, novel environment test, novel object test, pace-of-life syndrome, personality.

INTRODUCTION

The pace-of-life syndrome hypothesis (hereafter POLS) expands classic life-history theory by including behaviour (i.e. personality) and physiology into an evolutionary framework. This is based on the observation that environmental conditions favour not only certain life-

history traits but also physiological and behavioural traits, and therefore shape adaptive responses among species, among populations and even among individuals (Ricklefs & Wikelski 2002, Wolf *et al.* 2007, Réale *et al.* 2010, Dammhahn *et al.* 2018).

According to POLS, species, populations and individuals are positioned along a slow to fast pace-of-life continuum that reflects conflicts between maximizing survival and fecundity. Individuals that adopt a fast pace-of-life are more active and bold, grow faster, have a shorter lifespan, reproduce earlier and have more offspring, whereas other individuals within the same population with a slower pace-of-life are less active, are shy, grow slower, have a longer lifespan, reproduce later and have fewer offspring. Individuals with a fast pace-of-life prioritize current reproduction over future breeding and their own survival, whereas individuals with a slower pace-of-life prioritize future breeding and their own survival over current reproduction (Réale *et al.* 2010, Dammhahn *et al.* 2018). The functional integrations among multiple traits can be maintained by correlational selection favouring the evolution of genetic covariance between only certain traits and removing individuals with different trait combinations (Bouwhuis *et al.* 2014).

POLS predicts that metabolic differences covary with behavioural variation, with a high metabolic rate associated with faster pace-of-life (White *et al.* 2016). Greater capacity to produce energy leads to a higher daily energy expenditure and allows individuals to mobilize energy for energetically costly behaviours such as aggression (Careau *et al.* 2008, Réale *et al.* 2010). Aggressive individuals are usually also bold and explorative, whereas docile individuals tend to be shy and avoid novel situations (Sih *et al.* 2004).

The correlation between personality and metabolism has not been proved unambiguously within species or populations. Although Møller (2009) discovered a positive correlation between riskier flight behaviour and higher metabolic rates across 76 bird species, a study of Great Tit *Parus major* populations in the wild by Bouwhuis *et al.* (2014) found no significant correlation between explorative behaviour and metabolism in males and a surprisingly negative correlation in females. White *et al.* (2016) found that although personality differences are clearly present in a population of Trinidadian Guppy *Poecilia reticulata*, they do not covary with metabolism. Thus, even though POLS is relatively well supported by studies comparing suites of traits at species or population levels (e.g. Hall *et al.* 2015, Byer *et al.* 2019), empirical support has been ambiguous within populations (Bouwhuis *et al.* 2014, White *et al.* 2016, Dubuc-Messier *et al.* 2017, Royauté *et al.* 2018).

In this study we examined whether there is a relationship between personality and metabolism in captive European Pied Flycatchers *Ficedula hypoleuca* (hereafter Pied Flycatchers) during their first year. Explorative

*Corresponding author.

Email: skruus@utu.fi

Twitter id: @RuuskanenSuvi

behaviour was used as an indicator of personality and basal metabolic rate represented metabolism. Both traits were measured at two time points (between 8 and 12 weeks and at 28 weeks). Following POLS, we predicted a positive correlation between explorative behaviour and metabolic rate, and investigate whether the association is sex-dependent (following Bouwhuis *et al.* 2014).

METHODS

Study species

The Pied Flycatcher is a small (10–17 g), migratory, insectivorous and hole-nesting passerine bird. It is an abundant species over a large geographical range in Europe and winters in tropical Africa. Pied Flycatchers are single-brooded and their typical clutch size is five to six eggs (Lundberg & Alatalo 1992). The Pied Flycatcher is a common model species in the experimental studies of ecology, as it easily accepts artificial nestboxes.

Field protocols

The study was conducted with birds from a nestbox breeding population in Turku (Ruissalo), in southwestern Finland (60°26.055'N, 22°10.391'E). Data were originally collected to study the effects of egg androgens on several behavioural (Ruuskanen & Laaksonen 2010) and physiological traits (Ruuskanen *et al.* 2013). Therefore, a full description of the housing conditions and details of the measurements are found in Ruuskanen and Laaksonen (2010) and Ruuskanen *et al.* (2013). All experimental tests were conducted under licence from the Animal Experiment Committee of the State Provincial Office of Southern Finland (ESLH-2008-03693) and the Environmental Centre of Southwestern Finland (LOS-2007-L-264-254).

The laying dates of the nestbox breeding population were determined from the beginning of May. After the estimated day of clutch completion each clutch was randomly assigned to androgen treatment (i.e. egg androgens were elevated via injection prior to incubation) or control treatment (4 µL of sesame oil). The hormone treatment has been taken into account in the statistical analyses (see below). On the fifth day after hatching, nestlings were ringed and a small blood sample was collected for molecular sexing.

Housing

Twelve days after hatching one male and one female from each of 30 broods were transferred to the aviaries (15 androgen-treated and 15 controls). These 60 nestlings were formed into 10 new foster broods with a

brood size of six nestlings. Because of the accidental death of three chicks, two replacement pairs were transferred to the aviaries a few days later. The final sample size was 61 individuals, 31 females and 30 males (from 32 nests).

Each brood was kept in an aviary 2.5 × 3 × 2.5 m in size and fed by two foster parents until it was certain that the youngsters could forage independently (at an age of about 40 days) after which the foster parents were released into the wild and the two aviaries connected. Each smaller aviary contained a nestbox, two artificial trees and a feeding table. The birds were fed twice a day until they had collected fat stores for migration and after that once a day. The photoperiod of the aviaries corresponded to the Finnish summer and the temperature was set to a minimum of 15 °C during the day and 10 °C at night. From the beginning of September to the beginning of December the photoperiod and aviary temperature were gradually altered to mimic the changes encountered during migration and arrival at wintering areas.

Basal metabolic rate

Basal metabolic rate (BMR) is the minimum maintenance metabolism by endothermic animals at rest. It requires a thermoneutral environment and the absence of thermoregulatory, digestive, circadian and other increments in metabolic heat production (McNab 1997). As a proxy for metabolic rate, the oxygen consumption of the birds was determined individually. Oxygen consumption values (O₂ µmol/min) were calculated from the reduced oxygen partial pressure recorded from the measurement (animal) chamber outflow, compared with ambient oxygen partial pressure.

BMR measurements were performed twice: at the age of 8 weeks in August (during simulated autumn migration, i.e. trial 1, *n* = 58) and at the age of 28 weeks in January 2009 (during simulated wintering period, i.e. trial 2, *n* = 50). At each measurement round, six birds were caught from one aviary and weighed (17.15 ± 1.84 g). Roughly the same number of birds from both treatments and sexes were included in each round. BMR was measured overnight, during 10 h in the first trial and 13 h in the second trial. The average BMR was 41.52 (± 9.26) O₂ µmol/min.

Exploration behaviour

Exploration behaviour was measured using the novel environment and novel object protocols as described in Ruuskanen and Laaksonen (2010) modified from Verbeek *et al.* (1994). One day before the experiments the birds in aviaries were captured and placed in individual cages (60 × 35 × 55 cm) in which the birds had no

visual contact with each other. Each bird was tested when they were approximately 12 weeks old (trial 1; $n = 61$ (novel environment activity), $n = 60$ (novel object activity)) and this test was repeated when they were about 29 weeks old (trial 2; $n = 58$).

The novel environment experiment was conducted in an observation room $4 \times 2 \times 2.5$ m in size. The room contained five artificial wooden trees with four cylindrical branches placed on opposite sides of the trunk. The doorway of the experimental room was covered with a curtain and a video camera was placed behind it. The birds' behaviour was observed for 15 min and the time and duration of every flight or hop were recorded. All the flights and hops were summed as a measure with which to express the bird's novel environment activity.

The novel object experiment measured the boldness of individuals regarding novel objects and their activity in the presence of a novel object. The experiment was conducted 1 day after the novel environment experiment. The novel object (a pink and gold toy rubber duck) was placed on a perch in the home cage. The behaviour of the bird was recorded with a video camera for 3 min after the object was introduced. The total number of hops and flights indicated an individual's novel object activity.

Statistical analyses

Statistical analyses were conducted with the SAS Enterprise Guide (version 7.1). To find out how personality links to basal metabolic rate, a linear mixed model (procedure GLIMMIX) was built with BMR as a response variable and explorative behaviour (i.e. novel environment and novel object activities), sex, trial (1 or 2) and hormone treatment as explanatory variables. The body mass of the bird was added to a model as a covariate because it is known to affect BMR (Careau *et al.* 2008). The nestbox of origin was included as a random effect to control for non-independence of the siblings and, because of the two trials, the identity of the individual was included as a repeated factor. Furthermore, six interactions were added to this model separately. As Bouwhuis *et al.* (2014) found that the relationship between explorative behaviour and BMR differed between males and females, the interaction terms of novel environment activity \times sex and novel object activity \times sex were tested. We also tested the interactions of novel environment and novel object activities with hormone treatment and trial to determine whether the association between activity and BMR differs between the two different treatments and trials. There was no strong correlation between novel environment activity and novel object activity ($r_s = 0.17773$, $P = 0.524$), and thus the multicollinearity of these two variables was not a problem. The Kenward–Roger method as implemented

in the SAS Enterprise Guide (version 7.1) was used to calculate the degrees of freedom of the fixed effects and normality was checked from the residuals.

RESULTS

The birds' activity in the novel environment was 6.48 ± 10.45 hops and flights in 15 min and their activity in the presence of a novel object was 50.89 ± 33.97 hops and flights in 3 min. Results from the linear mixed model testing the association between explorative behaviour and BMR are presented in Table 1. Predictably, hormone treatment increased BMR ($F = 4.37_{1,27.11}$, $P = 0.046$), as reported in Ruuskanen *et al.* (2013). None of the other main factors or interactions was associated with BMR (all P -values > 0.05). Thus, we found no overall association between activity in the novel environment or activity in the presence of a novel object and BMR. The association was not dependent on sex (Fig. 1, Table 1), trial or hormone treatment (Table 1). There was a moderate correlation between the first and second trial in novel environment activity ($r_s = 0.312$, $P = 0.017$) and novel object activity ($r_s = 0.458$, $P < 0.001$; Fig. 2).

DISCUSSION

We found no support for the correlation between BMR and explorative behaviour when the sexes were combined or analysed separately; our results are not

Table 1. Results from a general linear mixed model (GLMM) testing the effect of explorative behaviour (novel environment activity (NE) and novel object activity (NO)) on basal metabolic rate ($n = 107$ observations, 52 individuals) in the Pied Flycatcher. Interaction terms (below the solid horizontal line) were added individually to the base model.

Variable	Estimate \pm se	$F_{ndf,ddf}$	P
NE	0.038 \pm 0.084	0.21 _{1,54.45}	0.652
NO	0.038 \pm 0.028	1.92 _{1,52.43}	0.171
Mass	0.107 \pm 0.641	0.03 _{1,72.57}	0.868
Sex (female)	2.199 \pm 1.435	2.35 _{1,27.1}	0.137
Treat (control)	-3.785 \pm 1.811	4.37 _{1,27.11}	0.046
Trial (1)	1.097 \pm 2.634	0.17 _{1,89.24}	0.678
+ NE \times sex (female)	0.109 \pm 0.183	0.35 _{1,60.24}	0.555
+ NO \times sex (female)	0.026 \pm 0.053	0.24 _{1,75.26}	0.623
+ NE \times treat (control)	0.0183 \pm 0.174	0.01 _{1,47.54}	0.917
+ NO \times treat (control)	0.017 \pm 0.054	0.10 _{1,60.57}	0.749
+ NE \times trial (1)	-0.169 \pm 0.192	0.77 _{1,89.31}	0.383
+ NO \times trial (1)	0.062 \pm 0.061	1.06 _{1,86.5}	0.307

Nest of origin was included as a random effect and individual ID as a repeated factor.

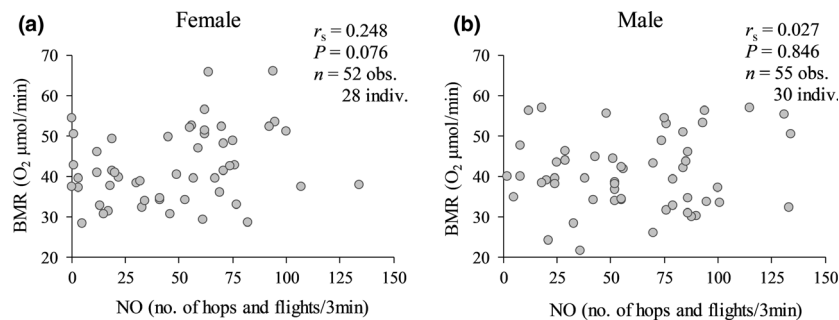


Figure 1. Correlation between basal metabolic rate (BMR) and novel object activity (NO) in female (a) and male (b) Pied Flycatchers with Spearman's correlation coefficient.

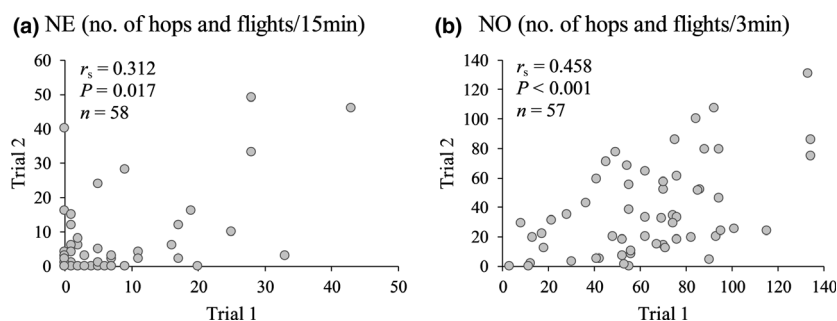


Figure 2. Correlation between the first and second trial in (a) novel environment activity (NE) and (b) novel object activity (NO) in the Pied Flycatchers with Spearman's correlation coefficient.

consistent with POLS theory and there are several possible explanations for this.

First, activity and exploration behaviour are suggested to covary with POLS if foraging requires continuous effort and food availability is unpredictable (Montiglio *et al.* 2018). For example, it has been shown that in poorer environmental conditions (less food available) populations express a slower pace-of-life, whereas in richer environmental conditions, a faster pace-of-life is more common (Dubuc-Messier *et al.* 2017, Dammhahn *et al.* 2018). In this study, all birds were fed similarly and the conditions were not resource-limited, which could have masked the natural foraging behaviour of the birds and minimized variation in metabolic rate. Our rather limited sample size may also have led to limited variation in the measured traits (but see Fig. 1 for two- to three-fold differences among individuals in BMR and up to 100-fold differences in activity). On the other hand, changes in environmental conditions can weaken or even reverse the association between behaviour and other traits (Réale *et al.* 2010, Dubuc-Messier *et al.* 2017) and thus, in our controlled environment, such environmental influences should have been minimized.

Second, POLS is critically associated with investment in reproduction: birds that have a lower future

reproductive value should take more risks and invest more in current reproduction and offspring defence. In contrast, individuals that have a higher residual reproductive value should display risk-averse behaviour and invest in future reproduction (Wolf *et al.* 2007, Dubuc-Messier *et al.* 2017). Birds in our experiments were all of the same age (29 weeks old at the most) and not reproductive, and hence we lack variation in the age and reproductive status of individuals, which may explain the lack of association between the measured POLS traits.

Third, a potential weakness with POLS is that short-term reversible plastic changes are not incorporated into the theory (Dingemanse *et al.* 2012) and can obscure the relationships among traits. For example, Dubuc-Messier *et al.* (2017) found that differences in behaviour and life history among three populations of Corsican Blue Tits *Cyanistes caeruleus* were consistent with POLS in some years but not in others. Within a population, individuals may differ in their pace-of-life as a result of plastic changes and traits that independently affect reproduction and survival (Montiglio *et al.* 2018).

Fourth, when comparing explorative behaviour between two different trials we found a moderate correlation in both cases. This suggests that birds do have individual temperaments even though this is not linked to

differences in metabolism. Montiglio *et al.* (2018) stress that the personality trait in question should be associated with resource acquisition in order to be linked with POLS. We may speculate that our measurement of personality, i.e. activity in a novel environment, is not tightly linked to resource acquisition in this species, as its prey may not be clumped or may be ephemeral, but rather quite abundant in the environment. The Pied Flycatcher is known as an opportunistic forager that uses very variable diets in different habitats (Lundberg & Alatalo 1992, Eeva *et al.* 2005) and hence it may be less vulnerable to changes in food acquisition than Great Tits for which the novel environment test was originally developed. Furthermore, the choice of the type of metabolic measurements may also explain contrasting outcomes of POLS in the literature, given that the commonly used resting metabolic rate can vary with daily energy expenditure in response to environmental changes; for that reason, Bouwhuis *et al.* (2014) suggest that studies should focus on more complete metabolic profiles over longer time periods instead of using just one measurement.

Therefore, to validate or reject fully the assumptions underlying the POLS hypothesis, the correlation between life history, physiology and behaviour should be studied across wide-ranging natural populations over several years, over different gradients of resource abundance and predation risk, and with the help of the determination of underlying genetic structures. More work is required to appropriately evaluate the general explanatory power of the POLS hypothesis. As Montiglio *et al.* (2018) underline, it is also important to keep in mind the possibility that life history, physiology and behaviour do not form a syndrome in all systems.

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AUTHOR CONTRIBUTION

Oona Poranen: Conceptualization (supporting); Data curation (lead); Formal analysis (lead); Validation (equal); Visualization (lead); Writing-original draft (lead); Writing-review & editing (lead). **Suvi Ruuskanen:** Conceptualization (lead); Funding acquisition (lead); Investigation (lead); Methodology (lead); Project administration (lead); Supervision (lead); Validation (equal); Writing-original draft (supporting); Writing-review & editing (supporting).

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are openly available Data S1 to this article (IBIS-2020-SC-012_Data.xls).

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Data S1. Original data used in Poranen and Ruuskanen: 'Explorative behaviour is not associated with metabolism in the European Pied Flycatcher *Ficedula hypoleuca*'.