

Stability of a behavioural syndrome vs. plasticity in individual behaviours over the breeding cycle: Ultimate and proximate explanations



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ABSTRACT

Animals often show correlated suites of consistent behavioural traits, i.e., personality or behavioural syndromes. Does this conflict with potential phenotypic plasticity which should be adaptive for animals facing various contexts and situations? This fundamental question has been tested predominantly in studies which were done in non-breeding contexts and under laboratory conditions. Therefore, in the present study we examined the temporal stability of behavioural correlations in a breeding context and under natural conditions. We found that in the great reed warbler (*Acrocephalus arundinaceus*) females, the intensity of their nest defence formed a behavioural syndrome with two other traits: their aggression during handling (self-defence) and stress responses during handling (breath rate). This syndrome was stable across the nesting cycle: each of the three behavioural traits was highly statistically repeatable between egg and nestling stages and the traits were strongly correlated with each other during both the egg stage and the nestling stage. Despite this consistency (i.e., rank order between stages) the individual behaviours changed their absolute values significantly during the same period. This shows that stable behavioural syndromes might be based on behaviours that are themselves unstable. Thus, syndromes do not inevitably constrain phenotypic plasticity. We suggest that the observed behavioural syndrome is the product of interactions between behavioural and life history trade-offs and that crucial proximate mechanisms for the plasticity and correlations between individual behaviours are hormonally-regulated.

1. Introduction

Behaviour is considered one of the most flexible phenotypic traits allowing individuals to adapt rapidly to changing conditions. However, animals often exhibit consistent and/or correlated behaviour across different contexts or situations (i.e., personality or behavioural syndromes: Sih et al., 2004a; Réale et al., 2007) that can seriously limit individual behavioural plasticity (Sih et al., 2004b). Moreover, associated behaviours may not be free to evolve independently of one another and, thus, stable correlation structures between different behavioural traits can also have important evolutionary implications (Sih et al., 2004a; Dochtermann and Dingemanse, 2013; Krams et al., 2014a). A fundamental question related to behavioural syndromes is therefore how correlations between behaviours are stable through time.

Nevertheless, despite this research challenge, few studies to date have investigated the temporal stability of individual personality traits and stability of entire behavioural syndromes (e.g., Bell and Stamps, 2004; Lee and Berejikian, 2008; Wuerz and Krüger, 2015). Results of some of these studies suggest that the existence of a stable behavioural

syndrome does not necessarily imply a total absence of behavioural plasticity as single behaviours that constitute such a syndrome, or at least some of them, may not be stable through time. This means, for example, that in the case of a stable bold-aggression syndrome, individuals that are initially shy and non-aggressive may later become bold and aggressive, or *vice versa*. The notion that stable behavioural syndromes can coexist with unstable individual behaviour through ontogeny has been well documented in some fish and invertebrate species (Bell and Stamps, 2004; Lee and Berejikian, 2008; Fisher et al., 2015; Müller and Müller, 2015). These examples involve behavioural correlations across non-breeding contexts examined under laboratory conditions.

However, given that behavioural syndromes can have severe fitness consequences (Sih et al., 2004a; Smith and Blumstein, 2008), investigating the stability of behavioural correlations in a breeding context and under natural conditions can lead to a much better understanding of the adaptive significance of consistent behavioural variation. Recently, behavioural ecologists have also become interested in proximate mechanisms inducing covariation between behaviours as

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such an approach can provide deeper insights into the evolution and stability of behavioural syndromes (Duckworth and Sockman, 2012; Briffa and Sneddon, 2016). At this proximate level, genetic and neuroendocrine mechanisms are proposed to underlie behavioural correlations (Sih and Bell, 2008).

One of the suitable behavioural traits for testing the temporal stability of behavioural syndromes in the breeding context is avian nest defence. Active defence of the nest can markedly increase the chance of offspring survival, but it also entails high costs for defenders in terms of time, energy, injury or even death caused by predators (Montgomerie and Weatherhead, 1988). Parents therefore face the trade-off between current reproductive success and their own survival or, more generally, between current and future reproduction, and this compromise may influence their willingness to defend their nests. In addition, according to parental investment theory, the intensity of nest defence should increase with the age of the offspring as they become relatively more valuable to the parents and greater parental investment is necessary to replace older offspring if they die (Regelmann and Curio, 1983; Redondo, 1989).

Therefore, we can expect parents to change their nest defence behaviour over the nesting cycle and defend their nests more aggressively during the nestling period than in the incubation period. Importantly, the intensity of nest defence has been found to correlate (either positively or negatively) with a wide variety of other behavioural traits, such as exploration (Hollander et al., 2008; Krams et al., 2014b), aggression towards conspecifics (Duckworth, 2006), nestling provisioning (Wetzel and Westneat, 2014), handling aggression (Fresneau et al., 2014; Trnka and Grim, 2014a) and the breath rate (Krams et al., 2014b) suggesting nest defence can be an important component of behavioural syndromes in birds. In summary, if nest defence behaviour is a part of a stable behavioural syndrome, then it should be repeatable and correlated with other repeatable behavioural traits that comprise this syndrome regardless of its changing/increasing intensity through the nesting cycle.

We tested this hypothesis in a wild-living passerine bird, the great reed warbler (*Acrocephalus arundinaceus*), by assessing aggressive and stress responses of females towards a simulated intruder near the nest (nest defence aggression) and towards the observer during handling (handling aggression and breath rate). Great reed warbler females are suitable candidates for testing the stability of correlations between these behavioural traits because they show large variation in both aggression towards nest intruders and when being handled and female nest defence behaviour is highly repeatable and strongly correlates with handling aggression (Trnka et al., 2013; Trnka and Grim, 2014a). Moreover, females usually play a leading role in defending the nest against predators, and this behaviour is independent of male nest defence effort (Trnka and Grim, 2013a). On the other hand, handling aggression and breath rate, together with aggression against nest predators, are widely accepted and commonly used personality traits in studies of behavioural correlations in birds (David et al., 2012; Class et al., 2014; Klueen et al., 2014). In addition, breath rate has also been suggested as a reliable proxy of the physiological response of animals to acute stress (Carere and van Oers, 2004; Fučíková et al., 2009).

We first examined temporal consistency and stability of single behaviours, i.e., nest defence behaviour, handling aggression and breath rate, to determine whether these behaviours truly represent personality traits in great reed warbler females and whether these traits are stable or not over the nesting cycle. We differentiate between consistency and stability of individual behaviours: consistency refers to rank-order differences between individuals and stability refers to the absolute behavioural values. Thus, for example, individuals may become more aggressive during the breeding cycle, i.e., absolute behavioural values may change between stages (the behaviour is therefore unstable) but the rank order of individual aggression scores within each stage may remain the same (thus the trait is consistent). To quantify individual consistency and stability of traits over ecologically relevant time period,

each female was measured for all three behaviours early in the egg incubation stage and again at the end of the nestling stage. We then explored whether these behaviours were also correlated with each other at the both measured time-points and hence whether they formed a stable behavioural syndrome.

In our previous study we found a positive association between nest defence intensity and aggression during handling in great reed warbler females (Trnka and Grim, 2014a). These two behavioural traits are known, on the other hand, to correlate negatively with breath rate stress responses in other passerines (Brommer and Klueen, 2012; Krams et al., 2014b). We therefore predicted that aggressive behaviour of great reed warbler females against a nest intruder would correlate positively with their aggressive behaviour during handling and negatively with breath rate and that these correlations would remain the same across repeated measurements of different individuals. We further predicted that females would be more aggressive in defending their nests and when being handled but less stressed (i.e., lower breath rates) during the nestling stage than during the egg stage whereas rank-order of their response scores within each stage would remain the same. Hence, these individual behaviours would be stable in terms of rank order between stages, but unstable in terms of absolute values between stages.

2. Methods

2.1. Study sites and general field procedures

The study was conducted in 2016 at three fishponds near Štúrovo, SW Slovakia (47°51'N, 18°36'E, 115 m a.s.l.), where the great reed warbler population comprised 50–60 breeding pairs. Birds breed at this site in narrow (approximately 3–10 m wide) reed (*Phragmites australis*) belts fringing the ponds. All tested birds were individually marked. They were either ringed in previous breeding seasons or mist-netted and colour-ringed during the nest defence experiments (see 2.2. Aggression against nest intruder below).

The great reed warbler is an altricial, medium-sized (~30 g) and highly aggressive passerine species that strongly attacks almost all intruders near its nest, including humans (Kleindorfer et al., 2005; Honza et al., 2010; Trnka and Prokop, 2010; Trnka and Grim, 2013b 2014b; but see Trnka and Požgayová, 2017). In spite of this, its nests suffer relatively high rates of predation and common cuckoo (*Cuculus canorus*) parasitism. In the study area, the nest predation rate averaged 20% and the rate of successful parasitism (i.e., the cuckoo chicks hatched and evicted host offspring) reached 16–20% (Trnka and Prokop, 2012). The most frequent predators of great reed warbler nests were the little bittern (*Ixobrychus minutus*) and the marsh harrier (*Circus aeruginosus*). Based on a previous experimental work from the same study area, the little bittern was responsible for about 18% of nest failures (Trnka et al., 2010). There is also known intraspecific nest predation in the great reed warbler where lower-ranking females destroy eggs of higher-ranking females in order to gain a higher proportion of male parental investment (Bensch and Hasselquist, 1994; Trnka et al., 2010). For these reasons, and given that only the female builds the nest, incubates eggs and broods in this species, great reed warblers breed usually once per breeding season in our study area (Trnka, 2011).

To locate great reed warbler nests, we systematically searched the reed beds at 4–5 day intervals from May to late June. Most nests were found during *building* or *egg-laying*. Once a nest was found, we checked it at 2–3 day intervals to determine the day of clutch completion and the final clutch size. The great reed warbler has a facultatively polygynous mating system in which monogamous females receive more male nest defence assistance than females of polygynous males, which might influence their nest defence behaviour (Trnka and Prokop, 2010; Požgayová et al., 2013). Therefore, all experiments were conducted on monogamous nests only. The percentage of monogamous nests was 69.1% (n = 55) in the study area in the year when this study was done.

The social status of each nest was determined on the basis of direct observations of colour-ringed birds defending their nests during the nest defence experiment (see the next section). Each monogamous female was tested twice, first 2–3 days after clutch completion, and then when the nestlings were 8–10 days old. For each female we measured the following behavioural responses.

2.2. Aggression against nest intruder

We tested female responses to the taxidermic dummy of the little bittern. This species was chosen because (i) it is one of the main predators of great reed warbler nests in our study area (see above) and (ii) great reed warblers clearly consider the mount of this species a threat to their nests and attack it aggressively (Trnka and Grim, 2014b). However, given that the aggression of great reed warbler females towards the little bittern dummy could be moulded by their previous experience with this heron (see Trnka and Grim, 2014b), we conducted all nest defence experiments only on nests located more than 10 m from active little bittern nests. This distance was sufficient given the known maximum distance at which great reed warblers defend their own nests in our study population is ~7 m (Trnka and Grim, 2014b). We employed two different dummies of the little bittern to reduce the possibility that potential differences between treatments could be caused by a particular dummy. Although using only one specimen may hold the stimulus constant, employing different dummies ameliorates potential pseudoreplication, and thus, it is more preferable (e.g., Sealy et al., 1998). Both specimens were adult females and were mounted in life-like positions with folded wings and heads pointing forwards. We did not use a control dummy because we were not interested specifically in enemy recognition in the present study (see also Trnka and Prokop, 2010; Trnka and Grim, 2013a).

Parents often cooperate in defending their nests and can adjust their nest defence to the defence intensity of their partners. Although nest defence responses of great reed warbler females are independent of their mate's presence or absence near the nest (Trnka and Grim, 2013a), we removed a male from the focal nest 2 h before beginning the nest defence experiment in order to test female responses without any potential confounding effect of male activity. According to our previous experience (Trnka and Grim, 2013a) this time is adequate for the female to adjust to this novel situation. The male removal was done by catching the male in a 10-m long mist-net stretched in his territory, 5–8 m away from the nest so as to not disturb the female. Each male was ringed (if not already ringed before) and then placed into a cloth bag where it was held until the nest defence experiment was finished.

Following established protocols, in each experiment, a randomly chosen dummy was placed 0.5 m from the focal nest, at the same height above water as the nest rim and with the head facing the nest (Grim, 2005). Observations were consistently made by the first author from a hide placed in reeds 5 m from the nest. This distance was enough to avoid disturbing the nest owner (see Trnka and Prokop, 2012; Trnka et al., 2013; Trnka and Grim, 2014b). The experiment started when the female arrived in the immediate vicinity of the nest and spotted the dummy. Then her behaviour was recorded for 5 min or until the first contact attack on the dummy. When the female physically attacked the dummy, the number of all strikes and pecks delivered to the dummy by the female for 1 min was counted. If the female had not appeared near the focal nest for 15 min since the dummy was exposed, the experiment was terminated and was not included in the analyses. Immediately after completing the nest defence experiment, a female was mist-netted, placed into a cloth bag and then subjected to two additional experiments (see below). At the same time the male from the focal nest was released back into his territory.

Avian nest defence includes various activities, such as alarm calls, distraction displays, dive flights and contact attacks. Therefore, to assess the willingness of great reed warbler females to take risks in defence of their nests, the responses of females were recorded according to

a predetermined scale. This scale was based on our previous experiences with this species (Trnka and Grim, 2013a, 2014b): 0 = the female watched the nest silently from a distance, 1 = the female approached the little bittern dummy at a distance of 0.5–1 m giving short warning calls, 2 = the female jumped closely around the dummy giving alarm calls and attacked it less than 8 times per 1 min, 3 = the female flew around the dummy giving alarm and distress calls and attacked it 8 to 15 times per 1 min, and 4 = the female persistently uttered distress calls and attacked the dummy more than 16 times per 1 min. The maximum response of each female was recorded.

2.3. Handling aggression and breath rate

Immediately after capture, each female was ringed (if previously not ringed). Of all tested females, 17 (71%) were not ringed at the time of their first capture and thus probably inexperienced with mist-netting and handling by humans. Then morphometric measurements (see Confounding factors below) were taken. During this period the female's response to being handled and measured was scored, consistently by the first author, on an interval scale ranging from 0 (lowest handling aggression score when an individual is completely passive and docile during all measurements) to 4 (highest handling aggression score when an individual struggles continuously during the measurements) (for details see Brommer and Klun, 2012). Although it was difficult to standardize the time of handling in the field, the entire procedure took about 3–4 min. After all measurements were taken, the bird was put back into a cloth bag and kept there for 5 min to stabilize its respiratory rate (see also Torné-Noguera et al., 2014). Thereafter, it was carefully removed from the bag, held in hand following the procedure used by Fučíková et al. (2009) and measured for the duration of 30 breaths with a stopwatch for two consecutive times. Breath rate (number of breaths per 1 s) was then calculated based on these two measurements. All measurements were taken by the first author.

No nest was depredated or abandoned and no adult or nestling died within 2 days after the experiments. Thus, mist-netting and handling did not negatively affect nesting behaviour of tested females.

2.4. Confounding factors

There are several factors that could influence the responses of great reed warbler females in nest defence and handling experiments. The intensity of nest defence could be affected, for example, by the weather conditions, timing in the breeding (re-nesting potential), clutch or brood size and offspring age (nest reproductive value), the size and body condition of defenders, mating status, etc. (Montgomerie and Weatherhead, 1988; Fisher et al., 2004; Grim, 2005; Hogstad, 2005; Pavel and Bureš, 2008; Trnka and Prokop, 2010). Similarly, the rate of breathing could be influenced by the ambient temperature, time of day and body size (Fučíková et al., 2009; Torné-Noguera et al., 2014). Some of these potential confounders were avoided by experimental design. All experiments we conducted under appropriate weather conditions (no rain or strong wind) and only at monogamous nests containing either eggs in the early stage of incubation (within 2–3 days following clutch completion) or 8–10 day old nestlings.

Other potentially relevant factors that could not be avoided in this study design, namely clutch and brood size, female body condition, experiment date in the season, time of day and ambient temperature, were statistically controlled for (see section Statistical analyses below). Female body condition was calculated as residuals from linear regression of mass on tarsus length. The body mass was measured with a Pesola spring balance to the nearest 0.1 g and the length of the tarsus with a sliding calliper to the nearest 0.1 mm. Ambient temperature was measured by a digital thermo-hygrometer and was recorded at the same time when breath frequency was measured.

2.5. Statistical analyses

2.5.1. Within-trait repeatability

We first calculated simple within-trait correlations between the egg and nestling stage, using a Pearson correlation for the trait "breath rate" and a Spearman correlation for "nest defence" and "handling aggression" (for details on this approach see Samaš et al., 2011; Grim et al., 2014). Next, we calculated adjusted within-trait repeatability (Nakagawa and Schielzeth, 2010) using univariate models with the package lme4 (Bates et al., 2015) for the trait "breath rate" (linear mixed model) and package ordinal (Christensen, 2015) for "nest defence" and "handling aggression" (ordered logit mixed models) of R v. 3.4.3 (R Core Team, 2017). Full models contained fixed predictors of "nest stage" (binary; egg and nestling), "date" of the experiment (continuous; group centred by "nest stage"), "daytime" (continuous; hour), ambient "temperature" (continuous), "clutch size" (continuous), "body condition" (continuous) and "female ID" as a random intercept effect.

We did not include first egg laying date as another predictor because we already included the date of experiment and the high correlation between these two predictors would invalidate our statistical models due to multicollinearity (see Graham, 2003). Potential collinearity among the covariates was satisfactory: variance inflation factors (VIF) were < 1.6 for all predictors (Zuur et al., 2010).

We used stepwise regression with backward elimination to create the final models (see Appendix for model outputs). Adjusted repeatability was calculated as the between-individual variance divided by the sum of the between-individual plus within-individual variance from the final model (Nakagawa and Schielzeth, 2010). Adjusted repeatability from ordered logit models was calculated by the formula $r = VB/(VB + VE + \pi^2/3)$, where VB is between-group variance, VE is the residual variance fixed to 0, and $\pi^2/3$ is the inherent distribution-specific variance (Nakagawa and Schielzeth, 2010). The asymptotic 95% confidence intervals for the repeatability estimates from the (G) LMMs were calculated using the package psych (v. 1.5.8; Revelle, 2018).

2.5.2. Between-trait correlations

We first calculated simple between-trait Spearman correlations (separately for egg and nestling stage). Next, we employed a Bayesian multi-response mixed model (package MCMCglmm; Hadfield, 2010) to estimate covariance between all three repeatable behavioural traits at the individual level and again separately for egg and nestling stage. The response of "breath rate" was modelled as Gaussian (link identity), "nest defence" and "handling aggression" were ordinal (link probit; family threshold). We included "female ID" as a random intercept effect (other predictors were shown to be non-significant in previous analyses). We used the same fixed and random effect structure and model selection approach as for the calculation of adjusted repeatabilities above. We set an uninformative flat prior and ran 5×10^6 iterations with a burn-in phase of 10,000 and a thinning interval of 10. We fitted an unstructured covariance matrix for the grouping effect "female ID" (calculates both variances in traits and covariances among trait variances). We obtained a posterior distribution of covariances and calculated among trait correlations with 95% credible intervals.

3. Results

Females ($n = 24$) showed very high within-individual repeatability of all three behavioural traits (Table 1, Fig. 1). Thus, behaviour of individual females was highly consistent (rank-order) between the egg and nestling stages. Breath rate showed highest repeatability and, based on non-overlapping 95% CIs, was significantly more repeatable than nest defence (Table 1). Handling aggression repeatability did not statistically differ from either breath rate or nest defence (overlapping 95% CIs, Table 1).

However, despite this consistency, the female behaviour was

Table 1

Repeatability of three behavioural traits of great reed warbler females measured at egg and nestling stages. For these simple within-trait correlations (i.e., egg stage measurement vs. nestling stage measurement for each trait), we used a Pearson's correlation for "breath rate" (during handling) and Spearman's correlations for "nest defence" (against dummy nest predator), and "handling aggression". We calculated adjusted repeatability from variances estimated by linear models (see Methods). We present estimates with 95% confidence intervals (95% CI).

Trait	Simple correlation (95% CI)	Adjusted repeatability (95% CI)
Nest defence	0.78 (0.54, 0.90)	0.79 (0.57, 0.91)
Handling aggression	0.87 (0.71, 0.94)	0.88 (0.74, 0.95)
Breath rate	0.96 (0.91, 0.98)	0.96 (0.91, 0.98)

unstable as evidenced by significant differences in absolute values of their behavioural traits between egg vs. nestling stages: compared to the egg stage, females in the nestling stage showed increased nest defence (median [interquartile range]: egg stage = 2 [0.5–3], nestling stage = 3 [2–4]; $\chi^2 = 24.0$, $p < 0.0001$; Table S1), increased aggressiveness during handling (egg = 1.75 [0.625–2.875], nestling = 2.75 [2–3.5]; $\chi^2 = 25.2$, $p < 0.0001$; Table S2) and decreased breath rate (mean \pm SD: egg = 2.0 ± 0.3 , nestling = 1.8 ± 0.3 ; $F_{1,23} = 40.4$, $p < 0.0001$; Table S3). All other confounding factors were non-significant (Tables S1–S3).

Correlations among these repeatable behavioural traits were high in the egg stage (Table 2) and remained high in the nestling stage (Table 2). Quantitatively, correlations slightly decreased from the egg stage to the nestling stage (Table 2) but statistically remained the same (as seen from the overlapping 95% CIs for correlations at the egg vs. nestling stages: Table 2). Specifically, in both the egg stage and the nestling stage, nest defence correlated positively with handling aggression whereas breath frequency correlated negatively with both nest defence and handling aggression.

4. Discussion

In line with our predictions, great reed warbler females showed high repeatability in all measured behavioural traits between the two stages of the nesting cycle in our study population. More importantly, females that defended their nests more intensively were also more aggressive during handling and also less stressed (estimated from breath rates) when being handled than females that defended their nests less intensively at both egg incubation and nestling stages. These patterns provide clear evidence that the intensity of female nest defence formed a behavioural syndrome with her aggression and stress response during handling. Additionally, this syndrome was stable throughout the nesting cycle as the same behavioural correlations occurred at different stages in time, both early at the start of the egg stage and later at the end of the nestling stage.

However, in spite of the temporal stability of the behavioural syndrome (rank-order), individual behavioural traits changed significantly (absolute values) over the course of the nesting period. Specifically, females were consistently more aggressive towards both the nest intruder and the researcher and less stressed during the nestling stage than during incubation. This implies that individual behaviours of females were not stable over the measured period. Thus, our findings extend the results of previous studies (Bell and Stamps, 2004; Lee and Berejikian, 2008) by demonstrating that a stable behavioural syndrome can coexist with unstable (plastic) individual behaviours within a breeding (nesting) period. This is particularly relevant in the context of nest defence when the costs of behavioural stability could outweigh the fitness benefits of behavioural plasticity.

Birds often use aggressive behaviours to defend their offspring or themselves. However, given that there are also different costs and

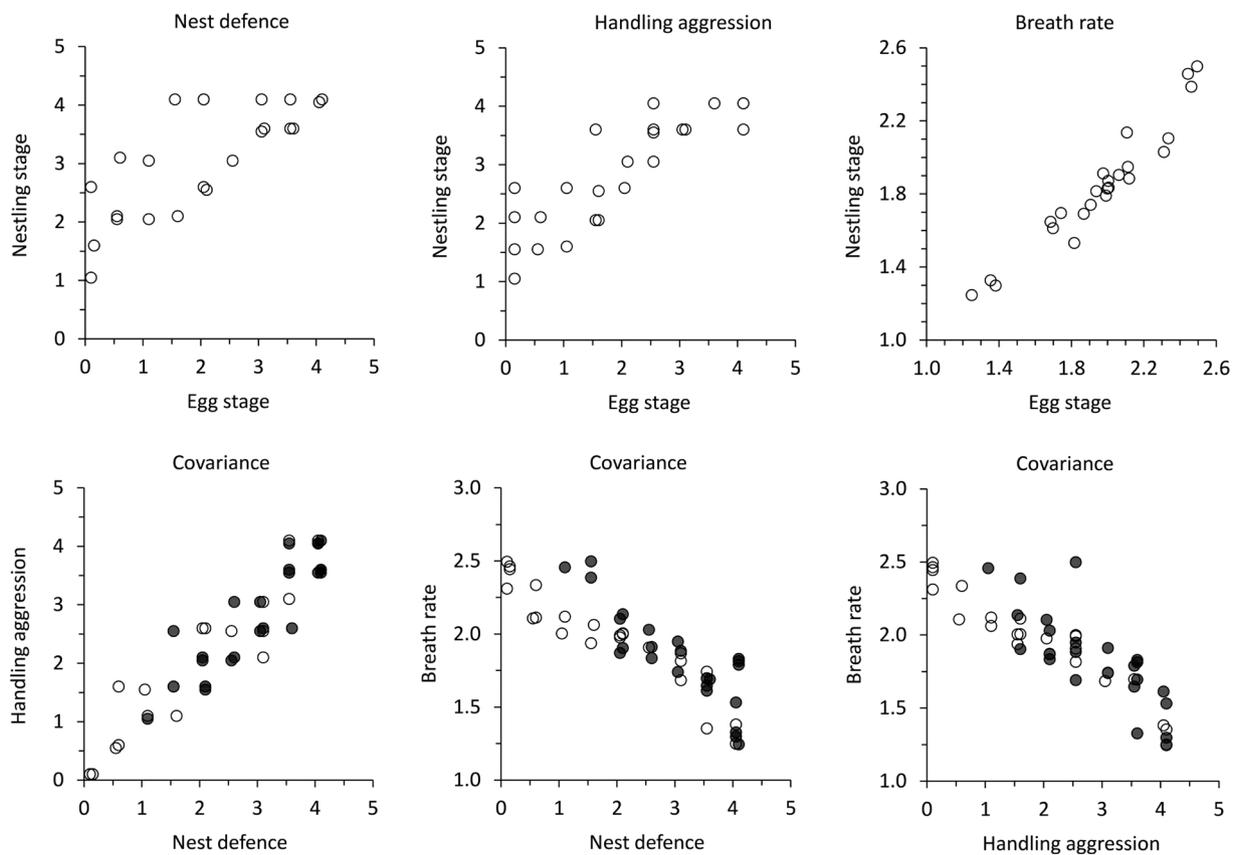


Fig. 1. Scatterplots of within-trait covariance between egg and nestling stages (upper row) and between-trait covariance (bottom row: egg stage = open circles, nestling stage = closed circles). Data points are slightly jittered to avoid complete overplotting of some points.

Table 2

Pair-wise correlations and covariances of three behavioural traits of great reed warbler females. We estimated correlations and covariances separately for the egg stage and the nestling stage. For simple between-trait correlations we used Spearman's correlation. We present simple correlation with their associated 95% confidence intervals and covariances with their 95% Bayesian credible intervals.

Trait	Simple correlation (95% CI)		Correlation of covariances (95% CI)	
	Egg	Nestling	Egg	Nestling
Nest defence vs. handling aggression	0.97 (0.93, 0.99)	0.89 (0.75, 0.95)	0.94 (0.89, 0.98)	0.86 (0.74, 0.95)
Nest defence vs. breath rate	-0.96 (-0.98, -0.91)	-0.86 (-0.94, -0.70)	-0.89 (-0.97, -0.80)	-0.73 (-0.90, -0.53)
Handling aggression vs. breath rate	-0.95 (-0.98, -0.89)	-0.84 (-0.93, -0.66)	-0.89 (-0.96, -0.81)	-0.79 (-0.92, -0.63)

benefits of expressing physical aggression within each of these contexts, individuals are assumed to modulate their aggressive behaviour depending on the current situation and/or external conditions (Andersson et al., 1980; Redondo, 1989; Duckworth, 2006). In agreement with parental investment theory and other “nest defence intensity” hypotheses (“reproductive value” and “re-nesting potential” hypotheses, Montgomerie and Weatherhead, 1988), great reed warbler females defended their nests more aggressively during the nestling period when the chance of offspring surviving until their own reproduction is markedly higher and, on the contrary, parental re-nesting potential is lower, than in the incubation period.

We also found a similar trend in female aggression during handling,

i.e., in self-defence, although nest defence and handling represent significantly different levels of risk. Handling is more risky than nest defence because the individual is already caught by a deadly predator, a human in this case, and thus, it risks not only its current reproductive value (eggs or nestlings) but also its future (residual) reproductive value (if the predator kills it; see also Fresneau et al., 2014; Trnka and Grim, 2014a).

Therefore, besides this ultimate explanation, these findings raise yet another question: what is a proximate factor that influences the observed patterns of female responses to handling? As our results and results of other studies have shown, aggressive behaviour of females during handling correlated significantly with their breath rate which is generally assumed to be directly linked to the acute physiological stress response in birds (Carere and van Oers, 2004; van Oers and Carere, 2007; Fučíková et al., 2009; Torné-Noguera et al., 2014; Karlíková et al., 2018). Hence, given that the physiological stress response typically involves the activation of endocrine mechanisms, mainly secretion of corticosterone (the main stress hormone in birds, Palme et al., 2005), and sympathetic or parasympathetic nervous systems (Wingfield, 2003; Angelier et al., 2018), the correlation between handling aggression and breath rate should also originate from the shared neuroendocrine pathways (Koolhaas et al., 1999; Groothuis and Carere, 2005; Réale et al., 2010). However, there may also be other receptors or downstream factors that could evolve independently to decouple behavioral syndromes.

Although there is a lack of studies directly investigating how birds respond hormonally to the immediate risk of nest predation, the same explanation as in the case of handling aggression and breath rate was also proposed for the positive correlation between increasing intensity of nest defence and aggression during handling. Thus, birds can actively modulate (either up- or down-ward) their hormone stress response to the reproductive value of the current brood (Heidinger et al., 2006;

Lendvai et al., 2007; Lendvai and Chastel, 2008). Such changes in stress hormone levels could also affect the dynamics of their nest defence and handling behaviours if they are both regulated by the same hormones. However, additional studies examining stress hormone concentrations concurrently with behavioural observations during the nesting cycle are needed to test this hypothesis.

Nevertheless, although our results are in line with the findings of most previous studies, they should be interpreted cautiously because measuring behavioural responses in birds has some limitations and many factors can unpredictably influence the accuracy of such measurements. We tried to eliminate or control for all known potential confounding factors (see 2.4. Confounding factors above), however, not all could be ruled out. An important methodological problem in behavioural syndrome or personality research is the habituation of individuals to the test due to repeated exposure to the same stimulus or stressor (Grim, 2005; van Oers and Carere, 2007; Class and Brommer, 2016). On the other hand, repeated presentation of the same type of nest predator can conversely lead to increased nest defence intensity because tested individuals become more familiar with a particular predator (Knight and Temple, 1986; Grim, 2005). We tested each individual only twice and the interval between the first and second experiment was relatively long: 16–21 days. As there is no evidence for habituation or sensitization in our study population at a much shorter time-scale (1 d, Trnka and Grim, 2013b), we believe that the higher intensity of nest defence aggression observed in great reed warbler females during the nestling stage was not biased by positive reinforcement.

Age is an additional factor that could influence defence behaviour in birds. Generally, older birds are expected to take more risks and defend their nests more aggressively than younger birds because of their lower residual reproductive value (Montgomerie and Weatherhead, 1988). However, we were not able to determine the age of all tested birds due to the difficulty of ageing adult great reed warblers after their complete moult and low return rate of birds ringed as nestlings in previous years. Thus we cannot rule out age as an influencing factor on our results. Although it seems unlikely that all tested birds in our study belonged to the same age group, additional studies focusing specifically on the effect of age on the temporal stability of nest defence behaviour would be valuable.

In conclusion, we found that great reed warbler females were highly consistent in their aggressive and fear behaviours over the breeding cycle and these behavioural traits, although each of them unstable through time, formed a stable behavioural syndrome. This provides direct evidence that stable behavioural syndromes do not inevitably constrain phenotypic plasticity in individual behaviours across the nesting cycle. We argue that observed behavioural correlations are presumably the product of interactions between behavioural and life history trade-offs, and that links between these traits are hormonally-mediated. Thus, we suggest that hormonal regulation represents a crucial proximate mechanism for the plasticity of nest defence and self-defence and for the correlations between these two behaviours in birds. However, further studies are needed to determine whether the observed pattern in great reed warbler females is species-specific or different between species with contrasting life-histories. Quantitative comparisons between temporal dynamics of stress hormone secretion and individual behavioural traits will allow to test whether there are correlations between hormone levels and behavioural responses of birds to simulated predation threat in different contexts and situations. Such an approach will disentangle proximate mechanisms behind personality or behavioural syndromes.

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Stability of a behavioural syndrome vs. plasticity in individual behaviours over the breeding cycle: ultimate and proximate explanations

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BEHAVIOURAL PROCESSES

ELECTRONIC SUPPLEMENTARY MATERIAL

Table S1 Outputs of the full and final models for the ordinal response variable of nest defence. "[incubation]" = incubation stage as a reference level of the predictor.

Predictor	Full model AIC _c = 213.2			Final model AIC _c = 200.8		
	χ^2	p	Estimate±SE	χ^2	p	Estimate±SE
Nest stage [incubation]	15.3	<0.0001	3.81±1.13	24.0	<0.0001	3.63 ± 0.89
Date	2.5	0.11	0.18±0.12	–	–	–
Daytime	0.3	0.56	0.23±0.40	–	–	–
Temperature	0.9	0.34	-0.17±0.19	–	–	–
Clutch size	1.5	0.22	-0.99±0.85	–	–	–
Body condition	0.8	0.38	-0.67± 0.76	–	–	–

Table S2 Outputs of the full and final models for the ordinal response variable of aggressiveness. "[incubation]" = incubation stage as a reference level of the predictor.

Predictor	Full model AIC _c = 210.6			Final model AIC _c = 194.9		
	χ^2	p	Estimate±SE	χ^2	p	Estimate±SE
Nest stage [incubation]	15.2	<0.0001	3.85±1.16	25.2	<0.0001	4.01 ± 1.01
Date	0.8	0.38	0.11±0.13	–	–	–
Daytime	0.3	0.61	-0.22±0.44	–	–	–
Temperature	1.0	0.31	0.18±0.18	–	–	–
Clutch size	0.0	0.95	-0.04±0.78	–	–	–
Body condition	0.9	0.35	-0.81± 0.89	–	–	–

Table S3 Outputs of the full and final models for the response variable of breath frequency. Model fits are summarized using marginal (R^2_m) and conditional (R^2_c) R-squared accompanied with AIC_c (Nakagawa and Schielzeth 2013). "[incubation]" = incubation stage as a reference level of the predictor.

Predictor	Full model			Final model		
	$R^2_m = 0.09, R^2_c = 0.97, AIC_c = 28.7$			$R^2_m = 0.03, R^2_c = 0.96, AIC_c = -15.0$		
	F	p	Estimate±SE	F	p	Estimate±SE
Intercept	–	–	1.91±0.20	–	–	1.96 ± 0.07
Nest stage [incubation]	15.4	0.0008	-0.11±0.03	40.4	<0.0001	-0.12 ± 0.02
Date	0.7	0.42	0.005±0.006	–	–	–
Daytime	0.9	0.37	-0.01±0.01	–	–	–
Temperature	0.3	0.59	0.003±0.005	–	–	–
Clutch size	0.9	0.34	0.02±0.02	–	–	–
Body condition	1.5	0.23	0.07± 0.06	–	–	–

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