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Barn swallow personality covaries with melanic coloration and predicts survival

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Lay summary

Conspecific individuals may differ in “personality traits”, including boldness, and individual fitness variation can depend on personality. In this study, barn swallows were subjected to a restraint-handling protocol and their behavioral response was recorded. Individuals were consistent in boldness upon repeated manipulations, reflecting boldness in response to predation. Bolder females, but not males, had larger survival until the next breeding season and, in both sexes, bolder individual showed darker ventral plumage coloration.

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Barn swallow personality covaries with melanic coloration and predicts survival

Abstract

Conspecific individuals often consistently differ in their behavioral responses to specific exogenous stimuli. Such individual differences in 'personality' have been shown to be heritable, suggesting that selection maintains variation in personality traits. However, survival selection on a major personality trait, boldness, and its sex-dependency, has been seldom measured in the wild. Here we found that yearling barn swallows (*Hirundo rustica*) showed consistency in boldness upon repeated exposure to a restraint-handling protocol, likely reflecting boldness in response to predation, and that males were bolder than females. Females that exhibited bolder behavior were more likely to survive until the next breeding season with no variation among three study years nor across seven breeding colonies, suggesting that variation in boldness is not maintained by spatio-temporal variation in viability selection on boldness. In both sexes, the intensity of melanin-based ventral plumage coloration positively covaried with boldness, consistently with previous observations from diverse vertebrate species. In some barn swallow populations, male coloration is targeted by directional intersexual selection, suggesting that melanization signals boldness to prospecting females and that sexual selection for boldness, or other correlated personality traits, can drive the evolution of sexual color dimorphism in barn swallows. Thus, we showed that viability selection occurs on boldness in response to predation and that spatio- temporal variation in selection has no major role in promoting variation in boldness. More melanized individuals were bolder, implying that melanization may signal personality in a sexual communication context in a sexually dimorphic species where male coloration is an epigamic trait.

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Keywords: Boldness, *Hirundo rustica*, melanin-based coloration, personality, plumage color, survival.

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Introduction

Individuals do not often exhibit the full range of behavioral phenotypic trait values that exist in the population to which they belong (Réale and Dingenmanse 2010). Independently of age, sex and previous experience, limits to individual behavioral flexibility thus normally result in within-individual consistency in the expression of the same behavioral traits upon repeated exposure to the same environmental or social stimuli. Individuals are therefore said to differ in 'personality' or 'temperament' (Dall et al. 2004; Sih et al. 2004; Groothuis and Carere 2005; Réale et al. 2007; Dingemanse et al. 2010; e.g. Thys et al. 2017). Personalities have been observed along several behavioral axes of variation, such as aggressiveness, risk-taking, activity and exploration, and sociality (Bell and Sih 2007; Réale et al. 2007; Koolhaas et al. 1999), and has been documented in a broad diversity of vertebrate and invertebrate taxa (see Gosling 2001; Bell et al. 2009; Dall and Griffith 2014 for reviews). Variation in personality is often shown to possess a heritable genetic component, as suggested by correlational and experimental (e.g. artificial selection) studies (e.g. Dochtermann et al. 2015), although epigenetic processes may contribute to observed transgenerational variation (Verhulst et al. 2016).

Personality is often assumed to cause individual fitness variation, as differences in traits like exploration, aggressiveness, risk-taking when confronted with predators or sociality are expected not to be selectively neutral. Several studies have addressed the issue of how variation in personality traits is translated into variation in important traits like natal dispersal, habitat use, and social and sexual behavior (Réale et al. 2007; Biro and Stamps 2008; Smith and Blumstein 2008; Schuett et al. 2010), under the assumption that these life-history traits affect individual fitness. However, to date, studies showing a relationship between personality as measured in the field (i.e. not on individuals maintained in artificial captivity conditions) and major fitness components, such as reproductive success and survival, in the wild are still relatively rare, and even fewer studies have tested for temporal and spatial variation in the relationship between personality and major fitness

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components (Dingemanse et al. 2004; Dingemanse and Réale 2013; Mutzel et al. 2013; Patrick and Weimerskirch 2014; Niemelä et al. 2015; Habig et al. 2017; Wetzel 2017; but see Bijlveld et al. 2014).

Heritability of personality traits raises the question of the mechanisms that contribute to maintenance of their genetic variation within populations (Carere and Mastripieri 2013; Dochtermann et al. 2015). Genetic variation in personality may persist, among other processes, because of individual-level variation in optimal solutions to life-history trade-offs (McElreath and Strimling 2006; Stamps 2007; Wolf et al. 2007), under condition- (or state-) dependent selection (Gross, 1996), or because of negative frequency-dependent selection, whereby specific personalities are favoured as they tend to become rare in the population. Selection on personality traits can also occur in sexual contexts, and sexual selection has been suggested also to promote individual level variation in personality (Schuett et al. 2010). Furthermore, genetic variation in personality can be maintained by spatial and temporal environmental heterogeneity, because selection on specific personality traits can vary in direction during time or between habitats in local demes that are connected by gene flow (Quinn et al. 2009; Dingemanse and Réale 2013; Nicolaus et al. 2016a).

Personality traits have also been often found not to vary independently from one another, rather showing covariation at the individual level, extending the idea of individual differences in personality to constellations of functionally different traits like aggressiveness, risk-taking and exploration, and sociality (Réale et al. 2007; Sih et al. 2004; Wolf and Weissing 2012; Corti et al. 2017). Thus, personality traits appear to be organized in 'behavioral syndromes' that vary along complex behavioral *continua*, like the so-called proactive-reactive axis (Koolhaas et al. 1999: Sih et al. 2004; Groothuis and Carere 2005; Réale et al. 2007; Wolf and Weissing 2012). Proactive individuals are generally bolder and more aggressive than reactive ones, tending to dominate and outcompete them in a stable environment (Koolhaas et al. 1999; Reale and Festa-Bianchet 2003). The existence of behavioral syndromes strongly suggests that different traits share underlying

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genetic and physiological mechanisms of control, that are expressed in different contexts (Koolhaas et al. 1999), and the hypothalamo-pituitary-adrenal axis of response to stress has been advocated as a potential major mechanism causing associations between personality traits (Carere et al. 2003; Cockrem 2007).

A further level of complexity in the organization and evolution of personalities and behavioral syndromes has been suggested by the observation that particular personality traits covary with morphological traits including for example melanin-based coloration. In vertebrates, darker individuals are often observed to show more proactive (as oppose to reactive) behavior (Saino and Scatizzi 1991; Roulin 2004; Ducrest et al. 2008). Mostly positive correlations have been found between melanic coloration and personality traits, including aggressiveness and boldness, exploratory and anti-predator behavior, in fishes, tortoises and lizards, and birds (Quesada and Senar, 2007; Kittilsen et al., 2009; Mafli et al., 2011; van den Brink et al., 2011; Mateos-Gonzalez and Senar, 2012; van den Brink et al., 2012; Williams et al., 2012; Da Silva et al., 2013; Mònus et al., 2016; Nicolaus et al., 2016b; Winters and Jawor, 2017; but see e.g. Kingma et al., 2008) (see Roulin 2004; Ducrest et al. 2008). The observation that the covariation among traits extends beyond behavior to encompass other sets of traits discloses the possibility that personality traits evolve in conjunction with morphological traits that are relevant in natural selection contexts (e.g. predator-prey interactions) or in socio-sexual communication contexts. For example, personality traits may evolve under selection on signaling mediated by coloration, if coloration signals temperament in social interactions or during the mate choice process (Schuett et al. 2010).

From an ultimate perspective, the association between coloration and behavior may arise because of correlational selection (Broodie et al. 1995). At a proximate, mechanistic level, covariation between personality traits and melanic coloration could be mediated by the melanocortin system, because post-transcriptional products of the proopiomelanocortin (POMC) gene interact not only with the melanocortin 1 receptor (MC1R), which is involved in melanogenesis, but also with four other

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receptors (MC2R-MC5R) which are involved in the regulation of other traits including the HPA-axis, aggressiveness, physical activity and immunity (Ducrest et al. 2008). We may therefore expect that melanin-based coloration and personality traits form a suite of integrated traits that covary within individuals (Nicolaus et al. 2016a).

The first main aim of the present study was therefore to test for a relationship between behavioral response to a standardized restraint-handling protocol by adult (1-year old) free-living barn swallows (*Hirundo rustica*) upon capture at their breeding colony and survival. Behavior during handling, as measured in terms of agitation, vocalization and aggressiveness towards the handler's was assumed to reflect boldness towards a predator (hereafter 'boldness' for brevity). A restraint-handling protocol has been used to measure handling aggression in previous studies on other species (Brommer and Kluen 2012; Fresneau et al. 2014; Kluen et al. 2014; see also Réale et al. 2000). Within-individual consistency in boldness was assessed by testing a subsample of individuals upon repeated captures. We expected that boldness positively predicted the odds of annual survival, i.e. survival from the breeding season when boldness was measured till the next breeding season, that could be estimated with high accuracy thanks to the extremely high breeding philopatry recorded in our study population (Møller 1994). In addition, we tested if the relationship between boldness and survival varied across three annual selection episodes and across seven breeding colonies with different densities and micro-ecological conditions.

The second main aim of the present study was to test if boldness of adult barn swallows after the restraint-handling protocol is related to melanic coloration. Both male and female adult barn swallows considerably vary in melanic coloration, particularly of the ventral plumage, that varies from white to chestnut (Turner 2006). Coloration is determined by both pheo- and eumelanin, and is consistent within individuals between years, i.e. after consecutive molts (Costanzo et al. 2017; our unpubl. data). In the same barn swallow population that we studied here, natal dispersal, which is considered a reflection of personality along the exploration behavioral axis, has been shown to

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positively correlate with ventral plumage melanic coloration (Saino et al. 2014). In addition, coloration of barn swallows has been shown to covary both with circulating corticosterone levels and with change in corticosterone levels after acute restraint stress, suggesting that an association may exist between coloration and personality traits that are proximately controlled by the HPA stress response axis (Saino et al. 2013a). Hence, there is evidence that adult barn swallow coloration covaries with personality and that it is related to one of the main physiological mechanisms that putatively control the expression of personality. Based on previous observations on the covariation between personality and coloration in other species (see above), we expected boldness to increase with the extent of melanic coloration, with no specific prediction on differences in this relationship between males and females.

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Methods

We studied 1-year old barn swallows breeding at a total of seven colonies (= farms) in an area located approximately 50 km West of Milan (Northern Italy) during 5 years. In all study years (2013-2017) we captured all the breeding individuals by two capture sessions per farm, conducted by placing, in the early morning (04:00 a.m.-05:00 a.m.), mist nets at all exits of the rural buildings where barn swallows breed and spend the night. Breeding adults are highly philopatric and do not switch to other farms to breed in consecutive years (Møller 1994; Saino et al. 1999), whereas the young usually disperse from their natal to other colonies (Møller 1994; Turner 2006); thus, exhaustive capture of the breeding adults every year allowed us to assign age 1 year to the individuals that had not been captured in the previous year, and to consider dead the individuals that were not recaptured during a particular year (Romano et al. 2016).

Individual colonies varied in several ecological factors, including presence and number of cattle heads and other domestic animals, which are known to affect barn swallow breeding habitat choice (Ambrosini et al. 2002, 2012), presence and disturbance by humans working inside or living nearby the rural buildings where colonies are settled (our personal observation), quality of the agricultural habitats where barn swallows forage (Ambrosini et al. 2002, Sicurella et al. 2012), presence of bird and mammalian predators (our personal observation), as well as in the number of pairs breeding in the colony.

Individuals that were caught were removed as soon as possible (usually within 3 min) from the net and placed individually in cloth bags. Individuals were then processed sequentially to ring them, measure morphological variables, estimate ectoparasite abundance, collect a blood sample by puncturing the brachial vein and collect a sample of feathers for coloration analyses. All the individuals were subjected to the same measurements and handled always by the same person for approximately the same amount of time (ca. 5 min). During handling, the birds showed different

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levels of agitation apparently in an attempt to escape from the handler. At the end of the handling procedure, before releasing the bird, the handler scored boldness into the following ordinal levels.

0: the bird stayed still in the hand;

1: the birds only moved the legs;

2: the birds moved its legs and tried to move its body and flap its wings, as could be sensed directly by the handler's hand, and grasped the handler's fingers;

3: the birds frequently moved its legs and tried to move its body and flaps the wings;

4: the bird vigorously moved its body and tried to escape by flapping the wings; it also shook its head and whole body in an attempt to escape;

5: as in 4 but the bird also vocalized and pecked at the handler's hand.

Boldness was also measured a second time on some birds at a later recapture (mean time elapsed between the first and the second measurement for males: 31.8 days (3.8 SD); females: 32.3 days (2.9 SD)), to test for within-individual consistency in boldness score.

The order in which the birds were processed was independent of coloration, as the birds were extracted from the bags haphazardly with respect to their coloration and we have no reason to speculate that the order in which the bags had been hang was associated with coloration of the bird that they contained. We are therefore convinced that the order in which the birds were processed, and thus the time that they spent in the bag before being processed, which could affect their behavior during handling, did not produce any bias in the results and could only produce 'white' noise.

The sample for the analysis of boldness in relation to coloration was collected over four years (2014-2017; range of yearly sample sizes pooled across colonies: 90-109) and across 7 colonies (range of sample sizes for individual colonies pooled across years: 1-110). The sample for the analysis of survival in relation to boldness was collected over 3 annual intervals (2014-2015, 2015-

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2016, 2016-2017; range of yearly sample sizes: 72-108) and across 7 colonies (range of sample sizes for individual colonies: 1-65). Large variation in sample sizes among colonies merely reflects the fact that colonies considerably differed in size.

Coloration analysis

Reflectance of one, randomly chosen, ventral feather, was recorded by using an Avantes DH-2000 spectrometer as described in Saino et al. (2013b, c). Coloration was quantified by processing reflectance data according to the tetrahedral color space model (Goldsmith 1990) using TetraColorSpace program (Version 1a; Stoddard and Prum 2008) implemented in MATLAB 7 (MathWorks, Natick, MA). Each color vector in the tetrahedral color space is described by the spherical coordinates ϑ and ϕ, representing the human-visible and the ultraviolet components of color, respectively, and rA, representing color saturation (Stoddard and Prum, 2008). In the range of color of barn swallow ventral feathers higher ϑ values represent a paler coloration (Saino et al. 2013 b, c). Estimates of tetrahedral coloration components are highly repeatable within feathers, as assessed by measuring twice the same feather ($r > 0.73$, $n = 45$ individuals). In addition, coloration is also repeatable between different feathers from the same ventral region ($r > 0.74$, $n= 10$ individuals) (Saino et al. 2013b). Finally, coloration estimates obtained from a single plucked feather is strongly correlated with coloration estimates obtained by overlapping three ventral feathers and directly on the bird's body. This was assessed by first measuring coloration on a sample of specimens that were found dead in the field and then measuring coloration on the feathers collected from the same region where coloration on the specimen had been measured (correlation coefficients between the color variable values obtained by the three measurements: ϑ: $r > 0.88$, $n = 14$ individuals; ϕ and rA: $r > 0.91$, $n = 14$ individuals, Romano et al. 2015).

Statistical analyses

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Consistency of boldness scores between the two captures was analyzed using a non-parametric correlation approach (Kendall's τ analysis); dependency of behavioral consistency according to sex was tested by z-test after transformation of τ into Pearson's r-values (Walker 2003).

Survival of 1-year old individuals to the following breeding season was analyzed in generalized linear mixed models while assuming a binomial error distribution and a logit link-function as implemented in PROC GLIMMIX of SAS 9.3 statistical package. Survivors were scored as 1 while non-survivors were scored as 0. In the models, we included sex as a factor, boldness as a continuous covariate and their interaction effect. In addition, colony or year were included as random effect factors in the models. To test for the effect of the random terms, we applied Laplace approximation to estimate likelihoods and then applied likelihood ratio tests comparing the model containing either random effect (to avoid convergence problems in estimation procedures) with the model that only contained the random intercept effect. Because boldness was in fact an ordinal variable, we also repeated the analysis of the relation to survival using a non-parametric correlation approach (Kendall's τ analysis) on each sex separately. Comparison between Kendall's τ values was performed by z-test after transformation of τ into Pearson's r-values according to Walker (2003).

To test for among-years and among-colonies variation in the slope of the relationship between survival and boldness, we applied random slopes models on either sex separately by also including boldness as a random effect. We tested for significance of variation in the slopes by comparing the likelihood (estimated with Laplace approximation) of model including the random term of boldness with that including only the random intercept effect.

To model the relationship between boldness score recorded during handling and coloration we applied logistic regression analysis for ordinal data as implemented in PROC LOGISTIC of SAS 9.3, while adopting a proportional odds model and a cumulative logit link function. In the models, we initially entered year, farm and sex as factors and each tetrahedral coloration component at a time as covariates. In addition, we included the interaction term between sex and individual

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coloration components. Thus, one separate model was run for each coloration variable. The non-significant interaction was then removed from the models as its effect was never found to attain statistical significance. The assumptions to apply a proportional odds model were always met, as shown by the score test (χ^2 values associated to $P > 0.33$ in all cases). This implies that all the response functions had a common parameter associated to the individual coloration variables. This analysis considers the factors (year, farm and sex) as fixed-effect terms. We did not rely on a mixed modelling approach, while considering year and farm as random-effect factors, because ordinal multinomial mixed-effects modelling of boldness (using PROC GLIMMIX in SAS 9.3) invariably led to converge problems in likelihood estimation.

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Results

Boldness scores at first capture were strongly positively correlated with those at a later recapture, as assessed in a subsample of the birds that were used for analysis of the association of boldness with survival or coloration (males: $\tau = 0.54$, $N = 31$, $P = 0.0003$; females: $\tau = 0.67$, $N = 29$, $P < 0.0001$). The strength of the correlation between boldness scores recorded at consecutive tests did not differ between males and females ($z = 1.36$, $P = 0.17$).

Boldness and annual survival

Survival from the breeding season when boldness was assessed to the next breeding season was differentially predicted by boldness scores in either sex (Table 1; Fig. 1). Survival significantly positively covaried with boldness in females whereas the relationship for males was not statistically significant (Table 1; Fig. 1). In the same model, survival significantly varied among the seven study colonies. A model where the effect of year rather than that of colony was included as a random effect confirmed the differential effect of boldness on survival of individuals of either sex (interaction between boldness and sex: $F_{1,261} = 4.18$; $P = 0.0419$) and also disclosed a significant effect of year ($\chi^2_1 = 4.42$, $P = 0.036$). Kendall's τ correlation analysis confirmed the significant relationship between survival and boldness for females ($\tau = 0.29$, $N = 129$, $P = 0.0008$) but not for males ($\tau = 0.03$, $N = 138$, P = 0.699). The difference in the correlation coefficients recorded for females and males was highly significant ($z = 3.42$, $P = 0.0006$). Consistency of among-years and among-farms relationships between survival and boldness scores was tested in random slopes binomial linear mixed models on either sex separately (see *Statistical analyses*). The slope of the relationship did not vary among farms for both males ($\chi^2_1 = 0.00$, $P = 0.99$) and females ($\chi^2_1 = 0.52$, $P = 0.471$). Similarly, there was no significant variation in the slopes of the relationship among

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years for males ($\chi^2_1 = 0.00$, $P = 0.99$) or females ($\chi^2_1 = 0.87$, $P = 0.351$). Hence, the relationship between survival and boldness was consistent among years and breeding ecological conditions.

Boldness and melanic coloration

Boldness was greater in individuals that had darker ventral plumage coloration in the 'visible' spectrum (Table 2, Fig. 2), had larger reflectance in the UV (Table 2), and also had larger color saturation (Table 2). In addition, independently of coloration, boldness was found to be larger in males as compared to females (Table 1; Fig. 3). The statistical effect of coloration on boldness did not vary with sex, as shown by the invariably non-significant sex-by-coloration interaction terms ($\chi^2_1 < 1.18$, $P > 0.27$ for all coloration variables) that were excluded from the models (see *Statistical analysis*). Hence, a unit increment in coloration variables translated into a similar change in the log odds of boldness level in either sex. In these analyses, the effect of colony was never found to be statistically significant and was therefore excluded from the models. However, we found a significant effect of year on boldness (Table 2). When included in the model, the effect of the interaction between year and coloration was invariably statistically non-significant ($\chi^2_1 < 1.32$, $P > 0.72$ for all coloration variables). Thus, the relationship between coloration and boldness was similar across all four study years.

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Discussion

Boldness of individual adult barn swallows in response to restraint and handling, which we interpret as related to proactivity in anti-predator behavior, was consistent over time within the same breeding season. This suggests that individuals consistently differ in a behavioral trait relevant in an ecological context, because predation is likely to be a major source of mortality (Møller 1994; Turner 2006; Newton 2008; our unpublished observation) . Variation in this personality trait has been documented also in other species (e.g. Sih et al. 2004; Mafli et al. 2011; Zao et al. 2016). In addition, our present study showed that consistency in behavior, measured as the strength of the correlation between behavioral scores at consecutive tests, did not differ between the sexes. However, boldness *per se* was larger in males than in females. Moreover, consistency in the individual behavioral response to stressful conditions is coherent with the findings of a previous experimental work carried out on nestlings of the same barn swallow population focus of the present study (Corti et al. 2017).

Sex-dependent variation in personality has been shown to differ considerably between species and traits (Carere et al. 2005; Saino et al. 2014; Devost et al. 2016; Edwards et al. 2016; Lermite et al. 2017; see Schuett et al. 2010). Proximately, sex-differences in personality can depend on sex-differences in androgen profiles (see Hau and Goymann 2015). Larger boldness of males may be selected for if more proactive males are favoured in intra- or in inter- sexual selection. Female mate preference for darker, more melanized males observed in some barn swallow populations (Turner 2006; Romano et al. 2017), including the population where the present study was performed (Parolini et al. 2017) and the relationship between melanization and boldness may suggest that boldness, or other correlated personality traits of males, as signaled by melanization, are targeted by directional female mate preference, which drives the evolution of color sexual dimorphism in this species (see also below).

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Boldness positively predicted annual survival in females but not males. Few studies of personalities have specifically looked at sex-dependent variation in the relationship between personality and major fitness traits. For example, in wandering albatrosses (*Diomedea exulans*), boldness differentially predicted the decline in reproductive success with age (Patrick and Weimerskirch 2015). In black browed albatrosses (*Thalassarche melanophrys*) personality traits have been shown to have sexually antagonistic effects on fitness (Patrick and Weimerskirch 2014) (see also Biro and Stamps 2008; Smith and Blumstein 2008; Fresneau et al. 2014). These studies have mainly focused on fitness traits that inherently differ between the sexes, like clutch and thus brood size. Few studies have reported a differential association between survival and personality between the sexes. For example, in great tits (*Parus major*) the relationship between survival and exploratory behavior differed between males and females, with the difference being reversed between good and poor years (Dingemanse et al. 2004). The observation that in barn swallows boldness only predicted survival in females but not males was rather unexpected because there is no published evidence that either sex incur different mortality costs from predation. Such apparent sex-dependency of the relationship between boldness and survival is unlikely to depend on power of the statistical tests because the analysis of males involved a large number of individuals, that was even slightly larger than for females, and the statistical test of the association between survival and boldness in males was far from significance. In addition, variance in aggression scores did not seem to be smaller for males than for females (see number of males or females with different aggression scores in Figure 1). Thus, lack of statistical effect of boldness on survival does not appear to be due to reduced variance in the predictor variable among males compared to females.

One possibility is that boldness covaries with other personality traits, i.e. that it concurs to a behavioral syndrome, and that these other traits are actually under sex-dependent selection during the annual life cycle. Boldness has been shown to covary with other personality traits along the reactive-proactive behavioral axis of variation like aggressiveness and exploratory behavior

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(Verbeek et al. 1996; Sih et al. 2004; Groothuis and Carere 2005). In the barn swallows there is indirect evidence that this could also be the case, because melanization of the ventral plumage positively covaries with both boldness (present study) and dispersal propensity (only males; Saino et al. 2014), which may be a proxy for exploratory behavior. In addition, females have larger dispersal propensity than males (Møller 1994; Turner 2006; Saino et al. 2014). We may thus speculate that sex-dependency in the relationship between survival and boldness rests on sex-dependent variation in the relationship between exploratory behavior, or other personality traits, and survival.

Alternatively, males and females may differ in the resolution of trade-offs between boldness and other life-history traits. For example, bolder 1-year old females may have larger odds of annual survival but lower reproductive success, whereas the relationship between boldness and reproductive success may be weaker in males (see Patrick and Weimarskirch 2015).

Independently of the mechanisms that cause sex-dependency in the association between survival and personality, the present results are relevant to the interpretation of the mechanisms that maintain variation in personality traits because they suggest that selection may have divergent or even antagonistic effects on personality in either sex and therefore promote genetic variation at these traits. To the best of our knowledge, this is the first time that this hypothesis has been made explicit.

Indeed, one common theme in evolutionary studies of personality traits is what mechanisms are involved in the maintenance of individual variation. Some studies have shown that individual variation in personality is at least partly genetic in nature, and both additive and non-additive genetic variation in personality traits has been documented both in parent-offspring resemblance analyses and in artificial selection studies (e.g. Koolhaas et al. 1999; Dingemanse et al. 2002; Drent et al. 2003; Dochtermann et al. 2015; see Baugh et al. 2017). Such genetic variation may be maintained under temporal and spatial variation in selection on personalities (Réale and Festa-

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Bianchet 2003; Dingemanse and Réale 2005). At present, whether variation in personality of barn swallows has a genetic component still has to be elucidated. However, the present study shows that the relationship between annual survival, which is a major determinant of lifetime reproductive success via its effect on longevity and thus on the lifetime number of reproductive events (Costanzo et al. 2017), and boldness did not vary among study years when annual survival was shown to differ. Hence, the relationship between survival and boldness did not differ among years when ecological conditions varied to an extent that caused variation in survival. In addition, the relationship between survival and boldness did not vary among study colonies that differed in size and also in breeding habitat. The effects of both year and colony on the slope of the relationship between survival and boldness were far from statistical significance. Hence, the present study does not lend support to the hypothesis that variation in personality is maintained by temporal and spatial variation in selection, although we cannot exclude that a study based on an even larger number of years and colonies could disclose conditions under which this relationship is markedly affected.

Melanization positively predicted boldness, and this relationship was similar in either sex. Several studies have shown an association between personality traits and melanin-based coloration (Roulin 2004; Ducrest et al. 2008; Roulin and Ducrest 2011; see also *Introduction*). In most instances, darker, more melanized individuals have been shown to have more proactive as compared to reactive behavioral syndromes (e.g. Koolhas et al. 1999; Sih et al. 2004; Mateos-Gonzalez and Senar 2012, and references therein). The present results were therefore expected based on existing knowledge on diverse vertebrate species.

One mechanism potentially linking melanization to personality traits is mediated by the melanocortin system, which has pleiotropic physiological effects on melanin biosynthesis and with the expression of behavioral traits, including aggressiveness and sexual behavior, via an interaction between the post-transcriptional products of the POMC gene and proopiomelanocortin receptors (Ducrest et al. 2008). The observation that darker individuals are bolder and more aggressive (i.e.

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proactive) has been considered to be consistent with the observation that darker individuals are better able to cope with social stress, as reflected by lower circulating corticosterone levels and by lower activation of the HPA-axis following a stressful event (Almasi et al. 2010). However, in the barn swallow, paler, rather than darker individuals have lower basal corticosterone levels and provide smaller response to acute stress, although the relationships between coloration and corticosterone levels were found to depend on experimentally altered parental effort (Saino et al. 2013). We may therefore expect that darker individuals, being more susceptible to activation of the HPA-axis under stress, pay a physiological cost for their proactive coping style.

In the barn swallow, natal dispersal has been also shown to be positively predicted by melanization, in that individuals that do disperse from their natal colony are darker than those that do not disperse (Saino et al. 2014; see also above). There is therefore evidence that also in the barn swallow, like in other species, dispersal and boldness concur to a behavioral syndrome that covaries with melanization, although the hypothesis of a relationship between behavioral traits in this species still awaits direct empirical testing. In addition, in some geographical populations of this species, which has a Holarctic distribution, melanin-based coloration has been shown to be under directional female mate preference (Turner 2006; Romano et al. 2017). The present results therefore suggest that plumage melanization may reliably reflect male personality traits to prospecting females, and that by preferring darker males females exert correlated selection on male personality (see Schuett et al. 2010).

In conclusion, we showed that boldness predicts survival of female but not male barn swallows, although the processes that contribute to such sex-dependency still remain to be elucidated. Spatial and temporal variation in selection on boldness is unlikely to maintain the observed variation in this personality trait. Finally, consistently with the general patterns observed in vertebrates, more melanized individuals of both sexes were bolder, suggesting that female mate preference for more

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melanized individuals may in fact reflect preference for boldness or other correlated behavioral traits of males.

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Figure Legends

Figure 1. Predicted survival of male and female barn swallows (surviving = 1, not surviving = 0) in relation to boldness. The numbers of overlapping data points is shown. Full squares represent predicted survival values according to the model in Table 1. Open squares indicate the 95% confidence limits of the predicted values. Females, but not males with larger boldness were significantly more likely to survive.

Figure 2. Boldness score of male and female barn swallows in relation to the 'visible' coloration of ventral plumage. Mean and standard error bar for coloration (θ value) are shown together with the number of overlapping data points.

Figure 3. Relative frequency distribution of boldness scores of male (n = 200) and female (n = 184) barn swallows. Males were on average bolder than females (see Table 1).

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Table 1. Binomial linear mixed model of survival of barn wallows in relation to boldness score (covariate), sex and their two-way interaction. The random model included the random effect of colony and its effect was tested by likelihood ratio test.

	χ^2	F	df	P	Coefficient (SE)	
Farm	5.27			0.0217		
Boldness		6.46	1,257	0.0116		
Sex		4.05	1,257	0.0453		
Boldness × Sex		4.65	1,257	0.0319	Males:	0.05 (0.18)
					Females:	0.61 (0.19)*

*: t = 3.22, P = 0.0014.

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Table 2. Separate multinomial regression models of boldness during handling on the tetrahedral components of plumage coloration of barn swallows. In the models we initially included sex, year and colony as factors, individual coloration components as covariates, and the interaction between sex and the coloration component. The statistically non-significant effects of colony and of the interaction between coloration and sex were then excluded from the model. The negative sign of the coefficient for the θ component implies that darker individuals were bolder because darkness decreases with θ.

	Wald χ2	*df*	*P*	Coefficient (SE)
Year	16.43	3	0.0009	
Sex	9.91	1	0.0016	
θ	12.61	1	0.0004	-6.23 (1.75)
Year	11.86	3	0.0079	
Sex	13.50	1	0.0002	
φ	4.56	1	0.033	2.74 (1.28)
Year	21.16	3	<0.0001	
Sex	12.27	1		
rA	9.18	1		4.97 (1.64)

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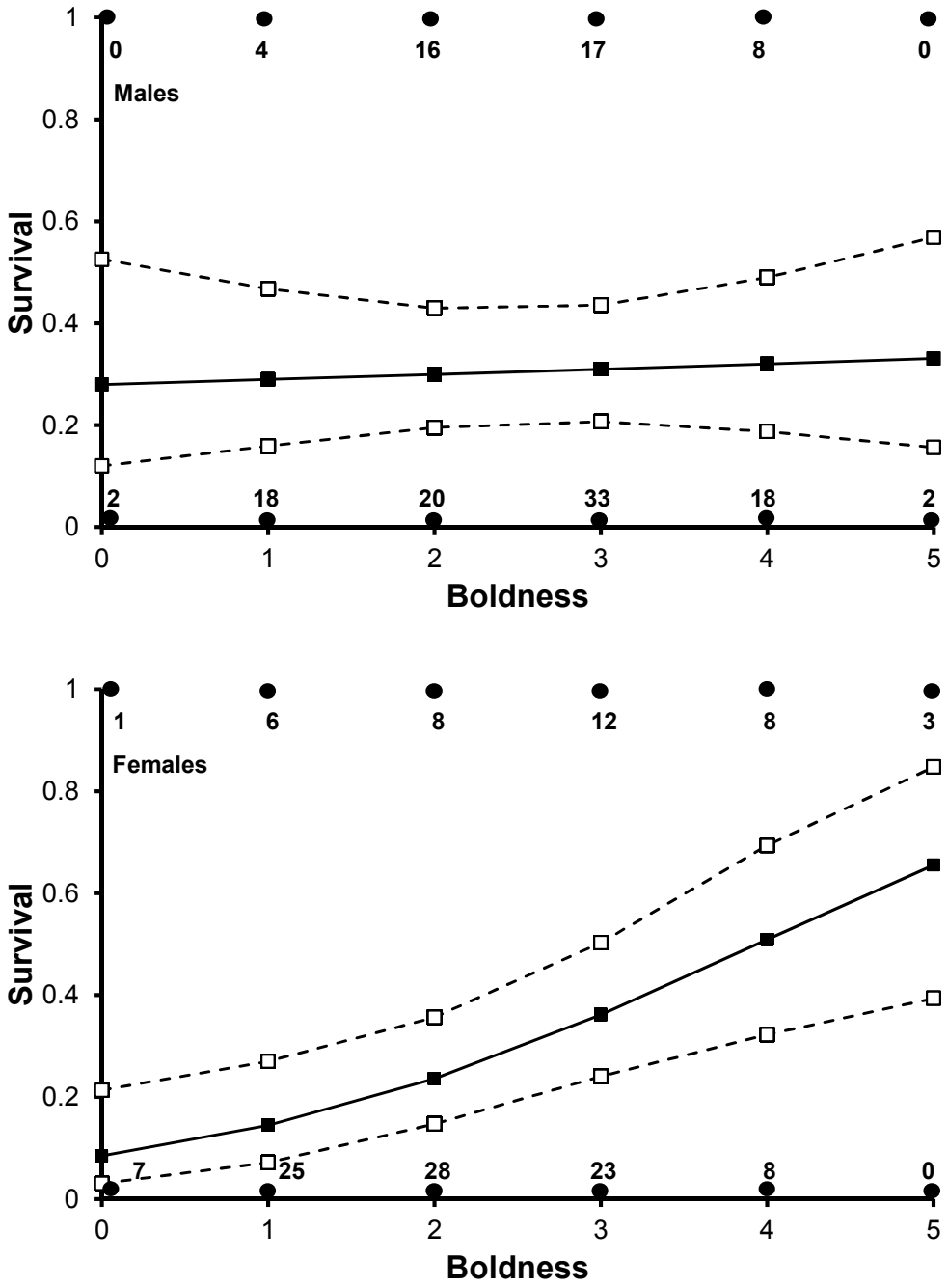
687 **Figure 1**



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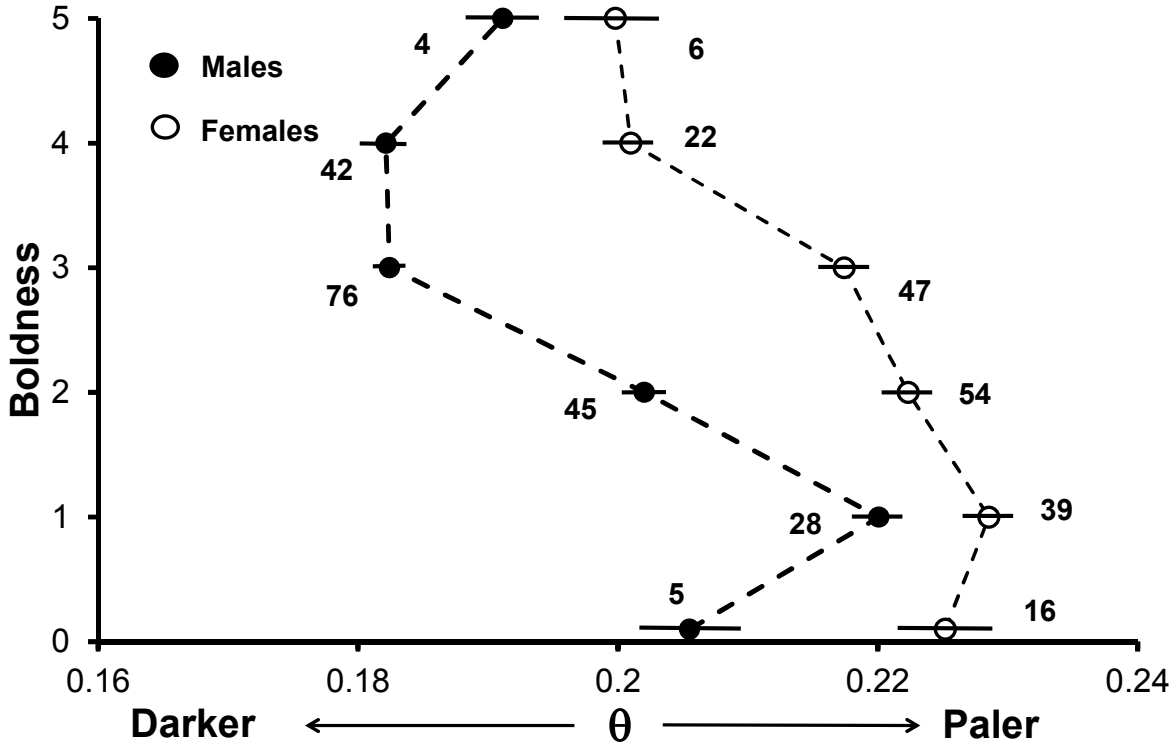
690 **Figure 2**



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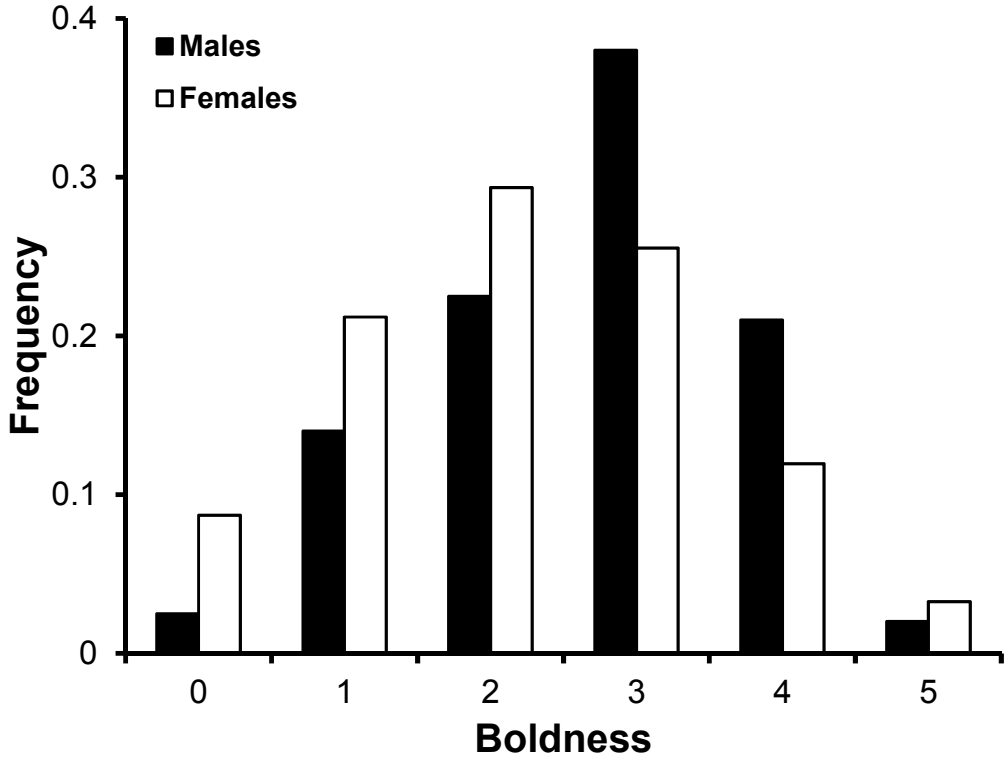
693 **Figure 3**



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