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The correlation between coloration and exploration behaviour varies across hierarchical levels in a wild passerine bird

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heritability;
melanocortin;
Parus major;
personality;
phenotypic plasticity.

Abstract

In vertebrates, darker individuals are often found to be more active and willing to take risks (representing characteristics of a ‘proactive’ coping style), whereas lighter individuals are instead more cautious and less active (representing characteristics of a ‘reactive’ coping style). It is thus generally expected that melanin-based coloration and proactivity form a suite of positively integrated traits at the among-individual level. Here, we use a multi-generational pedigree of free-living great tits (*Parus major*) to partition variation in, and the correlation between, melanin-based breast stripe (‘tie’) size and exploration behaviour (a proxy for coping style) into its among- and within-individual components. We show that both traits harbour heritable variation. Against predictions, tie size and speed of exploration were negatively correlated at the among-individual level due to the combined influences of permanent environmental and additive genetic effects. By contrast, the two traits were weakly positively correlated within individuals (i.e. individuals increasing in tie size after moult tended to become more explorative). The patterns of among-individual covariance were not caused by correlational selection as we found additive and opposite selection pressures acting on the two traits. These findings imply that testing hypotheses regarding the existence of a ‘syndrome’ at the among-individual level strictly requires variance partitioning to avoid inappropriate interpretations as the negative ‘unpartitioned’ phenotypic correlation between exploration and tie size resulted from counteracting effects of within- and among-individual correlations. Identifying sources and levels of (co)variation in phenotypic traits is thus critical to our understanding of biological patterns and evolutionary processes.

Introduction

Many vertebrates exhibit dark colour patterns based on melanin pigments. Melanin pigmentation differs greatly among individuals of the same species, and there is accumulating evidence that this variation covaries with other phenotypic traits (Ducrest *et al.*, 2008). For example, darker individuals are usually bigger, have high

metabolic rates, and express high levels of sexual activity and aggressiveness (e.g. Roskaft *et al.*, 1986; West & Packer, 2002; Bókony *et al.*, 2006; Kingma *et al.*, 2008). Phenotypic associations between coloration and other functional traits lead to the question of how and why such correlations have emerged. Phenotypic correlations can arise for many different reasons but affect evolutionary trajectories only if they have a genetic basis. Therefore, characterizing the genetic architecture of complex correlated phenotypes can provide important information about how traits evolve in response to selection (Lande & Arnold, 1983).

Genetic integration of melanin-based coloration and behaviour may arise from various processes. From an

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ultimate perspective, particular combinations of coloration and behaviours may have been favoured by correlational selection (Brodie *et al.*, 1995). This would occur when natural (or sexual) selection acts on the integration of traits (Brodie *et al.*, 1995; Kingsolver *et al.*, 2001; Sinervo & Svensson, 2002). Empirical support for the presence of correlational selection is surprisingly scarce because it requires large sample sizes (Kingsolver *et al.*, 2001) but is expected whenever the functional integration between such selected traits is beneficial (Sinervo & Svensson, 2002). For example, viability selection induced by predation has been shown to favour certain combinations of prey coloration and behaviour (Brodie, 1992; Forsman & Appelqvist, 1998). In the context of this study, positive correlations between dark coloration and aggressiveness are expected to be (sexually) selected as darker ornaments should represent honest signals of aggressiveness and resource holding potential (e.g. Johnstone & Norris, 1993).

From a proximate perspective, covariance between coloration and behaviour can arise through various distinct mechanisms (whether or not favoured by correlational selection). For example, positive state-dependent (social) feedback mechanisms (*sensu* Sih *et al.*, 2015) may generate long-term trait associations when individuals with higher energetic needs are more aggressive, therefore win contest over food, which in turn allows them to produce costly ornaments that signal and reinforce their dominance (for further examples, see Jawor & Breitwisch, 2003). Trait covariance may also occur because of pleiotropic effects of hormones (Ketterson & Nolan, 1999) or genes (Sinervo & Svensson, 2002). For example, elevated testosterone levels can increase both aggressiveness and the expression of dominance signals, that is dark ornaments (Vergara & Martinez-Padilla, 2012). Genes controlling melanogenesis (*POMC* gene), that is the synthesis of dark pigmentation, also presumably up-regulate aggressiveness, sexual behaviour and stress responsiveness (Ducrest *et al.*, 2008). Finally, (genetic) covariance between traits can also arise due to linkage disequilibrium (Sinervo & Svensson, 2002).

We may expect from both a proximate and ultimate viewpoint that melanin-based coloration and aggression, sexual- or stress-related behaviours form a suite of integrated traits that covary positively among individuals. Behavioural differences associated with melanin-based coloration are thus expected to be closely related to the ‘proactive–reactive’ axis described by stress physiologists, with individuals with proactive coping styles being generally more active and willing to take risks compared with individuals with more reactive coping styles (Coppens *et al.*, 2010). The study of ultimate and/or proximate factors explaining the existence of coping styles or behavioural syndromes (i.e. among-individual behavioural correlations; Sih *et al.*, 2004) is important because correlations among functionally distinct traits

are commonly observed in animal populations (Garamszegi *et al.*, 2012) yet poorly understood from an evolutionary perspective (Wolf & Weissing, 2012; Dochtermann & Dingemanse, 2013).

This study aims at describing the hierarchical structuring of, and selection acting on, the integration of a melanin-based colour trait and exploration behaviour in free-living great tits (*Parus major*). For this species, it is well established that speed of exploration is heritable in the wild (Dingemanse *et al.*, 2002; Quinn *et al.*, 2009; Nicolaus *et al.*, 2012b; Korsten *et al.*, 2013), that it represents a proxy for coping style (Stuber *et al.*, 2013; Nicolaus *et al.*, 2015) and is associated with social dominance (Dingemanse & de Goede, 2004). That is, fast-exploring individuals are more aggressive, less responsive to environmental change and bolder (characteristics of a proactive coping style), whereas slow-exploring individuals are less aggressive, responsive to environmental changes and shy (characteristics of a reactive coping style) (e.g. van Oers *et al.*, 2004; Stuber *et al.*, 2013; Cole & Quinn, 2014; Nicolaus *et al.*, 2015). This species is also sexually dimorphic with males exhibiting a larger black breast stripe (or ‘tie’) compared with females. Previous studies have shown that tie size correlates (at the phenotypic level) with dominance, aggressiveness (Järvi & Bakken, 1984; Poysa, 1988; but see Radford & Blakey, 2000) and boldness in males (Quesada & Senar, 2007) and predicts female fitness (Remeš & Matysioková, 2013). Tie size is often considered to represent an honest signal of an individual’s reproductive potential and is assumed to evolve under natural and sexual selection (Norris, 1993; Quesada & Senar, 2009), although evidence for its heritable nature is surprisingly scarce (Norris, 1993; Quesada & Senar, 2009). Moreover, its cross-year repeatability is relatively moderate (0.41; Kölliker *et al.*, 1999). Using quantitative genetics and phenotypic selection analyses, we aim here at (i) identifying sources of variation in tie size, (ii) formally quantifying the presence of heritable variation in this trait, (iii) partitioning the correlation among tie size and exploration behaviour into among- and within-individual variance components and (iv) testing whether tie size and speed of exploration are indeed positively genetically correlated at the among-individual level and whether this pattern of trait covariance is favoured by selection.

Materials and methods

Study site

The study was carried out between 2005 and 2009 in a nest-box population of great tits inhabiting a mixed deciduous forest in the Lauwersmeer area, the Netherlands (53°20’N, 06°12’E). The study site consisted of 12 woodlots (plots) fitted with 50 boxes each (Nicolaus *et al.*, 2012a).

Catching and housing

In all years, individuals were captured when roosting in nest boxes in February/March and again in November. At capture, all birds were weighted, transported to the laboratory within 1.5 h and unbanded birds ringed. The birds were then housed individually overnight with *ad libitum* access to food and water (see details in Dingemanse *et al.*, 2012a). The following morning, exploration behaviour was scored for each individual in a 'novel environment room' following standard procedures established for this species (Dingemanse *et al.*, 2002). Exploration scores were calculated as the total number of flights and hops within the first 2 min after arrival in the room (Dingemanse *et al.*, 2002, 2012a). After the test, we measured the birds (body mass, tarsus length and wing length) and aged them based on plumage characteristics (Svensson, 1992). They were then released at their place of capture. In 2009, a picture of individual tie size was taken additionally when measuring morphometric traits.

For quantitative genetics analyses of tie size, we included 437 observations of 366 great tits of known pedigree that were caught in February/March ($n = 193$ observations) and November ($n = 244$ observations) 2009. Seventy-one of those individuals were caught and photographed twice. The majority of birds at first capture were birds within their first or second year of life (1- to 2-year-old birds: $n = 289$, older birds: $n = 77$). For quantitative genetics analyses of exploration scores, we included 1790 assays of 1243 individuals of known pedigree collected over 5 years (2005–2009). Within these 5 years, surviving individuals were tested once each year (Dingemanse *et al.*, 2012a). Therefore, for the correlational selection analysis detailed below, we used the individual's first exploration score (i.e. its first test) as a measure for its 'personality'. We used the first score rather than a mean over all assays for several reasons including the fact

that exploration score increases within individuals over repeated observations (Dingemanse *et al.*, 2012a) which would affect mean scores and thus potentially bias estimates of selection patterns (see also Nicolaus *et al.*, 2016).

Measurement of tie size

Birds were positioned on their back on top of a millimetric grid and held extended, with the crown in contact with the background (Fig. 1). Belly feathers were gently smoothed before taking a picture of the breast with a Nikon Coolpix S610 camera (Nikon, Nikon Instruments Europe BV, Amsterdam, The Netherlands). The camera was attached to a tripod at a fixed distance (circa 20 cm). Photographs were analysed using the free software ImageJ 1.44b (National Institutes of Health, Bethesda, MD, USA). We calculated tie size as the black surface comprised in a 3-cm-long rectangle starting at the lowest point of yellow on the chest and as broad as the width of the belly (Fig. 1). To test for the reliability of our measurement, we took two photographs of 112 haphazardly chosen birds (63 males and 49 females). After the first picture, we repositioned the bird again over the millimetric grid. We then calculated the repeatability of tie size over those two pictures. Within-day repeatability was high (repeatability r (95% credible interval) = 0.93 (0.91, 0.94); within-males: $r = 0.77$ (0.70, 0.81); within females: $r = 0.75$ (0.67, 0.80), indicating that our procedure was relatively well standardized. We did not correct tie size for individual structural size because 'corrected tie size' (measured proportionally to tarsus size) was positively and significantly correlated to 'raw tie size', used here, ($r = 0.94$, $P < 0.001$, $n =$ subset of 19 individuals) and including tarsus as fixed effect into the models may remove among-individual variation of interest because tie size and body size were significantly correlated ($r = 0.35$, $P < 0.01$, $n = 437$ observations). All

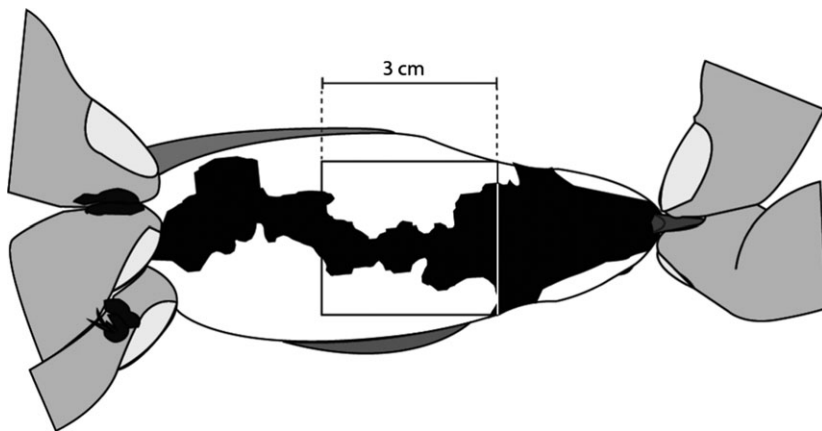


Fig. 1 Area used to measure tie size in great tits. Tie size was measured as the surface of black pigmentation within a 3-cm-long rectangle as broad as the width of the belly. Note that the rectangle starts at the lowest point of yellow on the chest (left-hand side of the bird). Birds were held stretched on their back with the head on the right side.

measurements of tie size were taken by RP; all photographed birds were also scored for exploration.

Quantitative genetic analyses

We used models with a pedigree based on social matings to estimate variance components of tie size and exploration score. Such ‘animal models’ allow for the decomposition of the phenotypic variance into random and fixed effect variance components by comparing phenotypes of known relatives also using pedigree information and facilitate quantitative genetic analyses of unbalanced data sets (Kruuk, 2004). We first used univariate models of repeated measures of exploration scores and tie size using a fixed effect structure detailed below. Exploration scores were square-root-transformed (to reach normality) and standardized over all individuals (by subtracting the population mean from each individual’s observation and dividing it by the population’s standard deviation), whereas tie size was standardized within sex to control for sexual dimorphism (by subtracting the population mean for a given sex from each individual’s observation and dividing it by the population’s standard deviation of a given sex). We included 1790 assays of 1243 individuals collected over 5 years (2005–2009) for exploration scores and 437 measurements of 366 individuals for tie size (2009). We did not include within-day repeated measurements of tie size in these analyses and instead only used each day’s first measure. We ran the animal models for a maximum pedigree depth of 5 and 3 generations for exploration behaviour and tie size, respectively (for an overview of relevant pedigree statistics see Appendix S1). The social pedigree was built using all information about individuals marked in our nest boxes between 1993 and 2011 and based on the assumptions that (i) immigrants are unrelated to each other and to resident birds (ii), the social parents are also the genetic parents, and (iii) the occurrence of extra-pair paternity (about 10% for this population; Brommer *et al.*, 2010) has little effect on the estimate of heritability (Charmantier & Réale, 2005). Pedigree summary statistics were generated using the R package ‘pedantic’ (Morrissey & Wilson, 2010). We calculated (phenotypic, permanent environmental, genetic and residual within individual) variances in, and covariances between, exploration score and tie size in a bivariate model where both traits were included as response variables.

We adopted a statistical modelling and inference approach advocated by Wilson *et al.* (2010). Starting with univariate models, we first included the fixed effects detailed below (models 1A and 1B, Table 1) and breeding area (‘plot’) and natal brood identity (‘brood’; immigrants were assigned a unique arbitrary brood number) as random effects to control for potential spatial heterogeneity and early environmental effects, respectively. As a second step (models 2A and 2B,

Table 1), we included random intercepts for individual identity, thereby partitioning the phenotypic variance not explained by fixed effects (V_P) into among-individual (V_I) and within-individual residual (V_R) variance components, that is $V_P = V_I + V_R$. As a third step (models 3A and 3B, Table 1), we decomposed V_I into its additive genetic (V_A) and permanent environmental (V_{PE}) components, that is $V_P = V_A + V_{PE} + V_R$. This third model was constructed by including additive genetic merit (or ‘breeding value’) as an additional random effect. As a fourth step (models 4A and 4B, Table 1), additive genetic merit was included as the only random effect to specifically test for the significance of the permanent environment effect. Adjusted ratios r , pe^2 and h^2 were calculated as the proportion of V_P corrected for fixed effects and explained by V_I , V_{PE} and V_A , respectively. In the bivariate models, we further estimated the covariances for among-individual (COV_I), within-individual residuals (COV_R), additive genetic (COV_A) and permanent environment (COV_{PE}) terms. This information was used to subsequently calculate the among-individual (r_I), within-individual residual (r_R), additive genetic (r_A) and permanent environment (r_{PE}) correlations between exploration score and tie size. (Co)variance components, r , pe^2 , h^2 and correlations were calculated using the package ‘MCMCglmm’ (Hadfield, 2010) in R v.3.2.3 (R Development Core Team 2015). Following Wilson *et al.* (2010), we specified the priors by splitting the observed phenotypic variance evenly between the random and residual effects. Univariate models of exploration behaviour were ran with 65 000 iterations (thinning interval = 10, burn-in period = 15 000). Univariate models of tie size and bivariate models were ran with more iterations to reduce autocorrelation (number of iterations = 650 000, thinning interval = 100, burn-in period = 150 000). The statistical significance of r , pe^2 , h^2 , correlations and fixed effects were directly assessed based on values of the posterior distribution of the model parameters and 95% credible intervals (CrI). We consider an effect to be significant in the frequentist’s sense when its associated 95% CrI does not overlap with 0. About 95% CIs were defined as the 2.5 and 97.5% quantiles of the posterior distribution of parameter estimates (Gelman *et al.*, 2012).

In all of our models, we included fixed effects known to affect either exploration scores or tie size in West European populations of great tits (Hegyi *et al.*, 2007; Quinn *et al.*, 2009; Dingemanse *et al.*, 2012a). In models analysing variation in exploration score, we included log-transformed time of year (the days from July 1 or Julian date), log-transformed intertest interval (in days) between subsequent tests of the same individual, test sequence number (first tests coded as 0; subsequent tests coded as 1) and the factor year (from breeding season_{*t*} to breeding season_{*t*+1}; e.g. year ‘2006’ would run from May 2006 until end of April

Table 1 Results of univariate animal models of exploration score and tie size. (a) Estimates of variance components: among-individual variance (V_I), additive genetic variance (V_A), permanent environmental variance (V_{PE}), among-study plot variance (V_{PLOT}), among-brood variance (V_{BROOD}), and residual within-individual variance (V_R) and estimates of repeatability (r), heritability (h^2) and permanent environmental effects (pe^2). (b) Estimates of the fixed effects of models 2A and 2B (β). All estimates are given with their 95% credible interval (CrI) in parentheses, and significance of fixed effects is assessed directly from their 95% CrI.

(a) Variance components									
	V_I	V_A	V_{PE}	V_{PLOT}	V_{BROOD}	V_R	r	h^2	pe^2
Exploration									
1A	0.34 (0.27, 0.40)	–	–	0.03 (0.01, 0.07)	0.03 (0.02, 0.06)	0.43 (0.38, 0.48)	0.44 (0.37, 0.50)	–	–
2A	–	0.09 (0.05, 0.16)	0.24 (0.18, 0.32)	0.02 (0.01, 0.07)	0.02 (0.01, 0.05)	0.43 (0.38, 0.48)	–	0.11 (0.06, 0.18)	0.30 (0.21, 0.37)
Tie size									
1B	0.22 (0.05, 0.43)	–	–	0.04 (0.02, 0.16)	0.07 (0.03, 0.22)	0.56 (0.39, 0.76)	0.31 (0.08, 0.51)	–	–
2B	–	0.07 (0.02, 0.23)	0.09 (0.03, 0.37)	0.05 (0.01, 0.14)	0.05 (0.02, 0.18)	0.52 (0.37, 0.71)	–	0.09 (0.02, 0.23)	0.09 (0.03, 0.37)
(b) Fixed effects									
Exploration	β	(95% CrI)	Tie size			β	(95% CrI)		
Intercept	–0.65	(–0.82, –0.46)	Intercept			0.02	(–0.19, 0.19)		
Time of year	0.61	(0.50, 0.76)	Mean age			–0.18	(–0.32, –0.07)		
Interval	–0.11	(–0.17, –0.05)	Age deviation			0.28	(0.03, 0.63)		
Sequence	1.32	(0.98, 1.60)							
Year									
Year 2	0.07	(–0.04, 0.18)							
Year 3	0.50	(0.35, 0.61)							
Year 4	0.21	(0.06, 0.36)							
Year 5	0.52	(0.38, 0.69)							

2007) as fixed effects. In the tie size model, we controlled for age effects that can exist both at the among- and within-individual level (since 71 individuals were measured twice). Therefore, we used within-individual centring to separate the within-individual effects from the among-individual effects of age (van de Pol & Wright, 2009). For each individual, we calculated mean age over all of its observations and the deviation for this mean for each observation. An effect of age deviation should inform us on whether individuals exhibit plastic adjustments of tie size with age across moults. Great tits moult generally at the end of the breeding season (Jenni & Winkler, 1994). Hence, birds photographed both in February/March and in November 2009 experienced one moulting cycle. Age deviation would be 0 (and thus statistically noninformative) for individuals sampled once. We present results of analyses that include individuals with one observation because doing so is known to improve parameter estimation (Martin *et al.*, 2011). We did not include time of year, year (surviving birds are sampled in the breeding year 2008 (February/March catches) and in the breeding year 2009 (November catches), see explanation above) or maximum age in the models where tie size was the dependent variable because the first two variables are highly correlated with within-individual age effects and the latter with among-individual age effects.

Correlational selection analyses

We calculated correlational selection gradients to quantify the strength of selection acting on the combination of tie size and exploration behaviour. This was achieved by analysing variation in relative lifetime offspring recruitment and relative longevity in relation to standardized tie size and standardized square-root-transformed exploration score (to obtain the linear selection gradient, β), their interaction (to obtain correlational selection gradients) and their quadratic effects (to obtain nonlinear selection gradients, γ). Quadratic selection gradients and their 95% credible interval were doubled following Stinchcombe *et al.* (2008). Lifetime offspring recruitment was calculated as the total number of recruits produced by an individual during its lifetime divided by the mean of the data set. Longevity was calculated as the number of years an individual lived divided by the data set mean. Tie size and exploration score were both standardized over all individuals for which we had both exploration score and tie size ($n = 299$ birds). We focused on the total number of recruits and their longevity as these two components should jointly explain an individual's contribution to the gene pool of the population. We assigned an exact longevity estimate for locally born individuals (longevity = last year caught – birth year) and a minimum estimate for immigrants (assuming they were

entering the breeding population in their second calendar year). All birds used in this analysis are presently dead, implying that our measure of longevity is not biased towards short-lived birds. Standardization of traits was carried out by subtracting the mean of the data set from each individual's observation and dividing it by the data set's standard deviation. To further explore whether the strength or form of (correlational) selection acting on exploration behaviour and tie size differ between sexes, we also ran the same analyses including sex and sex interactions as fixed effects (see Appendix S3).

We used a generalized linear model (GLM) with Gaussian errors for all selection analyses. All models were constructed in R v.3.2.3 using the `glm` function of the 'lme4' package. Estimates of fixed (β) are given with their 95% confident intervals (CI). The statistical significance of selection gradients and correlational selection gradients were assessed based on these 95% CIs (as detailed above).

Results

Decomposition of phenotypic variance into within- and among-individual components

Exploration score

Quantitative genetics analyses confirmed previous findings in this population showing that exploration score harboured both significant permanent environmental and additive genetic (i.e. heritable) variation (pe^2 (95% CrI) = 0.30 (0.21, 0.37), h^2 (95% CrI) = 0.11 (0.06, 0.18); Table 1, part (a), model 2A; Nicolaus *et al.*, 2012b). Speed of exploration further varied positively with date and sequence, negatively with interval between tests and exhibited annual variation (see Table 1, part (b)) as previously documented (Dingemanse *et al.*, 2012a; Nicolaus *et al.*, 2012b).

Tie size

Tie size was significantly repeatable over the half-year period considered in this study (r (95 CrI%) = 0.31 (0.08, 0.51), Table 1, part (a), model 1B) with an adjusted repeatability of same magnitude to those previously reported for great tits (e.g. $r = 0.41$ in Kölliker *et al.*, 1999). Decomposition of the among-individual variance revealed significant permanent environment (pe^2 (95% CrI) = 0.09 (0.03, 0.37), Table 1, part (a), model 2B) and additive genetic effects, resulting in an adjusted heritability (h^2 (95% CrI)) of 0.09 (0.02, 0.23) (Table 1, part (a), model 2B).

The relationship between tie size and age differed depending on the level of variation considered. Consistent with previous studies (e.g. Hegyi *et al.*, 2007), tie size increased significantly with age within individuals (within-individual effect: age deviation: β (95% CrI) = 0.28 (0.03, 0.63), Table 1, part (b)). However, tie

size instead decreased significantly with age among individuals (among-individual effect: mean age: β (95% CrI) = -0.18 (-0.32 , -0.07), Table 1 part (b)). In other words, individuals characterized by large tie sizes were underrepresented in older age classes.

Correlations between exploration behaviour and tie size

We predicted a positive correlation between speed of exploration and tie size at the among-individual level (see Introduction). In contrast, bivariate model results showed that the phenotypic correlation between exploration score and tie size did not deviate significantly from zero; in fact, it tended to be overall negative instead of positive (r_R (95% CrI) = -0.14 (-0.23 , 0.02), Table 2, model 1). Variance partitioning revealed that this correlation was significantly negative at the among-individual level (r_I (95% CrI) = -0.56 (-0.85 , -0.16), Table 2, model 2), whereas it did not deviate significantly from zero at the within-individual level (r_R (95% CrI) = 0.13 (-0.14 , 0.29), Table 2, model 2), where the point estimate was positive. These apparent opposite patterns at the within- and among-individual levels are depicted in Fig. 2. Visual inspection of the raw data split per sex showed that these patterns were similar in females and males (Appendix S2). The decomposition of r_I into additive genetic (r_A) and permanent environment (r_{PE}) correlations further showed that these coefficients did not deviate significantly from zero but tended to be negative (r_A (95% CrI) = -0.65 (-0.90 , 0.04), r_{PE} (95% CrI) = -0.32 (-0.79 , 0.35), Table 2, model 3). We were thus not able to formally identify the source of variation responsible for the negative among-individual correlation (both r_A and r_{PE} are accompanied with relatively large CrI) probably due to low statistical power. Notably, the placement of the 95% CrIs of our genetic correlation estimate implied that there was strong evidence for the absence of a moderate to strong positive correlation.

Correlational selection

To investigate whether the tight negative among-individual correlation between tie size and speed of exploration was in line with patterns of selection, we performed correlational selection analyses. We found evidence for strong positive (nonlinear) and significant selection on exploration behaviour based on analyses of lifetime offspring recruitment and longevity, and negative significant selection on tie size based on longevity (Table 3 and Fig. 3): birds with relatively fast exploration scores and/or small tie size lived longer and produced more recruits. Selection on exploration behaviour appeared to be significantly nonlinear with faster exploring individuals at the extreme end enjoying relatively higher fitness than other individuals

(Table 3 and Fig. 4). This nonlinearity was not caused by square-root-transformations applied to our exploration data as nonlinear significant effects were also found when not transformed (Results not shown). Correlational selection gradients were not significant, that is they did not deviate significantly from 0 (Table 3). We did not further detect significant sex-specific patterns of selection (Appendix S3). Therefore, it seems unlikely that pooling the sexes in the main analysis could explain discrepancies with previous studies where positive patterns of trait covariation were often reported for males only (see references in Ducrest *et al.*, 2008).

Discussion

Our aims were to identify levels and sources of variation in tie size and to quantify the presence of heritable variation in this trait. We found opposite relationships between tie size and age depending on the level of variation considered: within individuals, tie size increased with age which may reflect changes in individual age-related 'status' (e.g. increase of social dominance (Järvi & Bakken, 1984) or territoriality (Wilson, 1992)), whereas among individuals, tie size decreased with age suggesting, selective disappearance of older birds with large ornaments that are presumably more costly to produce and maintain (Mcgraw, 2008). The phenotypic selection analysis corroborates the interpretation that birds with large tie size lived relatively shorter than birds with small tie size. Quantitative genetic analyses further revealed significant heritable variation in this colour ornament. The adjusted heritability estimate of tie size (h^2 (95% CrI) = 0.09 (0.02 , 0.23)) was significantly lower than estimates previously reported in the literature (0.77 ± 0.33 in Quesada & Senar (2009) and > 1 in Norris (1993)). The discrepancy with previous published estimates may be explained by statistical method: unlike the parent-offspring regression methods used in previous papers, the animal models used here provide more reliable heritability estimates because they are not restricted to assessments of relatedness between subsequent generations. This leads to estimates that are less biased by environmental effects; at the same time, the animal model accommodates for effects of selection, assortative mating and inbreeding (Kruuk, 2004; Postma & Charmantier, 2007).

Melanin-based coloration in animals is generally hypothesized to be positively correlated with level of dominance and aggressiveness via pleiotropic effects of the *POMC* genes involved in melanogenesis and/or hormones (Ducrest *et al.*, 2008). Therefore, another important aim of this study was to test whether black tie size and exploration behaviour were positively correlated among individual great tits. We found instead that tie size and exploration behaviour were negatively correlated at the among-individual level (as were the point estimates of the underlying genetic and permanent

Table 2 Results of bivariate animal models where exploration behaviour and tie size were fitted as the two response variables. (a) Estimates of variance components: among-individual variance (V_I), additive genetic variance (V_A), permanent environmental variance (V_{PE}), among-study plot variance (V_{PLOT}), among-brood variance (V_{BROOD}), and residual within-individual variance (V_R). (b) Estimates of covariances for among-individual (COV_I), within-individual residual (COV_R), additive genetic (COV_A) and permanent environment (COV_{PE}) terms, and among-individual (r_I), within-individual residual (r_A), additive genetic (r_A) and permanent environment (r_{PE}) correlations between exploration score and tie size. All estimates are given with their 95% credible interval (CrI) in parentheses, and significance of fixed effects is assessed directly from their 95% CrI.

(a) Variance components										
Trait	Model	V_I	V_A	V_{PE}	V_{PLOT}	V_{BROOD}	V_R			
Exploration	1	–	–	–	0.07 (0.03, 0.19)	0.13 (0.04, 0.21)	0.57 (0.45, 0.67)			
Tie size	1	–	–	–	0.09 (0.03, 0.22)	0.13 (0.06, 0.28)	0.76 (0.60, 0.90)			
Exploration	2	0.22 (0.09, 0.34)	–	–	0.05 (0.02, 0.16)	0.06 (0.03, 0.16)	0.37 (0.27, 0.50)			
Tie size	2	0.22 (0.10, 0.44)	–	–	0.06 (0.02, 0.18)	0.13 (0.04, 0.22)	0.51 (0.38, 0.72)			
Exploration	3	–	0.07 (0.03, 0.24)	0.12 (0.03, 0.25)	0.05 (0.02, 0.14)	0.04 (0.02, 0.12)	0.37 (0.27, 0.49)			
Tie size	3	–	0.15 (0.05, 0.34)	0.12 (0.04, 0.31)	0.05 (0.02, 0.17)	0.05 (0.02, 0.16)	0.51 (0.38, 0.67)			

(b) Covariance components										
Model	COV_I	COV_A	COV_{PE}	COV_R	r_I	r_A	r_{PE}	r_R		
Exploration – tie size	–	–	–	–0.07 (–0.16, 0.01)	–	–	–	–0.14 (–0.23, 0.02)		
Exploration – tie size	–0.11 (–0.23, –0.02)	–	–	0.06 (–0.06, 0.13)	–0.56 (–0.85, –0.16)	–	–	0.13 (–0.14, 0.29)		
Exploration – tie size	–	–0.06 (–0.18, 0.02)	–0.02 (–0.15, 0.05)	0.02 (–0.05, 0.13)	–	–0.65 (–0.90, 0.04)	–0.32 (–0.79, 0.35)	0.06 (–0.12, 0.30)		

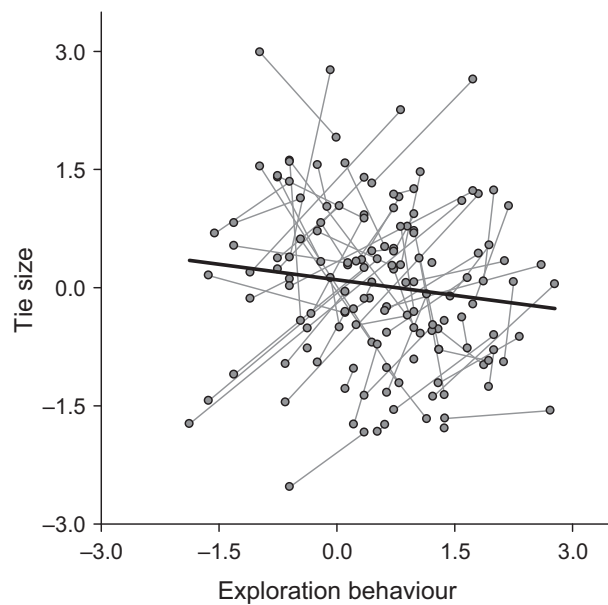


Fig. 2 Patterns of within- and among-individual correlations between exploration behaviour and tie size. In the Lauwersmeer great tit population, these two traits are negatively correlated among individuals (bold line) but tend to be positively correlated within individuals (66% of the within-individual slopes are positive; each grey line is one individual). Exploration behaviour was standardized over the whole data set, whereas tie size was standardized over the whole data set within each sex to control for sexual dimorphism (raw data, $n = 71$ individuals with 2 observations).

Table 3 Correlational selection analyses of exploration behaviour and tie size. Relative lifetime offspring recruitment (LOR) and relative longevity were studied in relation to exploration behaviour and tie size (both standardized over the whole data set), their quadratic terms (doubled following REF.) and their interaction ($n = 299$ observations). Estimates of fixed effects (β) are given with their 95% confidence intervals. Significant linear and nonlinear selection gradients are denoted in bold face.

Fixed effects	LOR β (95% CI)	Longevity β (95% CI)
Intercept	0.70 (0.45, 0.94)	0.88 (0.76, 1.00)
Exploration	0.45 (0.27, 0.63)	0.18 (0.09, 0.28)
Tie size	-0.07 (-0.26, 0.12)	-0.12 (-0.22, -0.02)
Exploration ²	0.40 (0.014, 0.66)	0.18 (0.06, 0.32)
Tie size ²	0.120 (-0.02, 0.42)	0.06 (-0.06, 0.16)
Exploration \times tie size	-0.08 (-0.26, 0.11)	0.04 (-0.06, 0.13)

environmental correlations) with slow-exploring individuals exhibiting larger tie sizes than fast-exploring ones. Although pleiotropic effects of genes may still cause the negative among-individual correlation, our results contradict the specific hypothesis of Ducrest *et al.* (2008) and suggest that tie size and exploration

behaviour are thus not under the joint control of the *POMC* gene in a way predicted by literature. A recent study in great tits, notably, also failed to find a polymorphism at the melanocortin-1 receptor (*MCR1*), another important gene known to have a function in melanin coloration, and could not detect an association with tie size (Riyahi *et al.*, 2015). The authors thus suggested that variation in tie size may be regulated through genetic variation in other genes or via modification of the gene expression inside the melanocortin system and melanogenesis (Riyahi *et al.*, 2015). As for the role of hormones, testosterone levels in great tits covary positively with tie size (Galván & Alonso-Alvarez, 2010) and negatively with speed of exploration (van Oers *et al.*, 2011) which suggests that antagonistic pleiotropic effects of hormones constitute a possible explanation for the existence of coloration-behavioural syndromes.

Regardless of the exact proximate mechanism underlying the negative among-individual correlation between tie size and exploration behaviour, the correlational selection analyses for the 2009 bird cohort revealed that selection acted in opposite directions on these traits. We are aware that due to our relatively small sample size, our correlational selection gradients were associated with large uncertainty (i.e. they did not deviate significantly from zero) and thus we cannot firmly conclude that correlational selection was indeed operating. However, our analyses showed that both fast speed of exploration and small tie size conferred the highest fitness. Therefore, the adaptive nature of the observed among-individual correlation remains unclear. An important related question is how genetic variation in behaviour and coloration is maintained in this population. We recently showed that exploration behaviour is under strong density-dependent survival selection and therefore fluctuations in the social environment may represent a key ecological factor maintaining genetic variation in personality (and indirectly in tie size) in this population (Nicolaus *et al.*, 2016). In this recent study, we showed that fast-exploring individuals survived better in low density years, whereas slow-exploring individuals instead survived best in high density years (Nicolaus *et al.*, 2016). As our selection analysis reported in the current paper was performed only for birds caught in 2009 (a low density year), the finding that fast-exploring individuals lived relatively longer is consistent with our previous findings. Interestingly, our finding that fast-exploring individuals with small tie sampled in 2009 enjoyed higher fitness (a low density year with presumably low competition) contradicts the general assumption that tie size represents an appropriate proxy for competitive ability and is thus typically under positive directional selection (e.g. Johnstone & Norris, 1993). It instead suggests that this assumption at best depends on social context: benefits of signalling high competitiveness may outweigh its

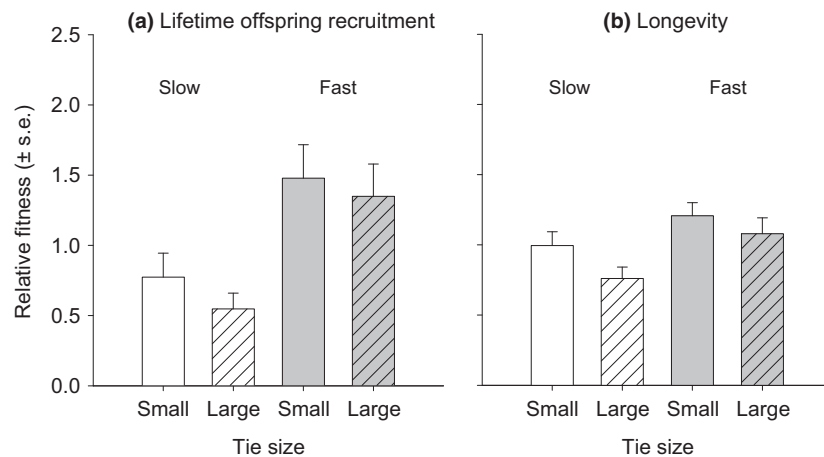


Fig. 3 Correlational selection acting on the integration of exploration behaviour and tie size. Phenotypic selection analyses revealed opposite patterns of selection acting on exploration behaviour and tie size, but lack of significant correlational selection: individual great tits with small tie and individuals with fast exploration scores enjoyed a relatively higher fitness. This pattern was found for both lifetime offspring recruitment (a) and adult longevity (b). For graphical purpose only, individuals have been categorized in 4 groups based on the each trait's median with 'slow' and 'fast' explorers being depicted in white and grey, respectively, and individuals with 'small' and 'large' tie with plane and stripped filling, respectively. Means are shown with their standard error (raw data, $n = 299$ individuals).

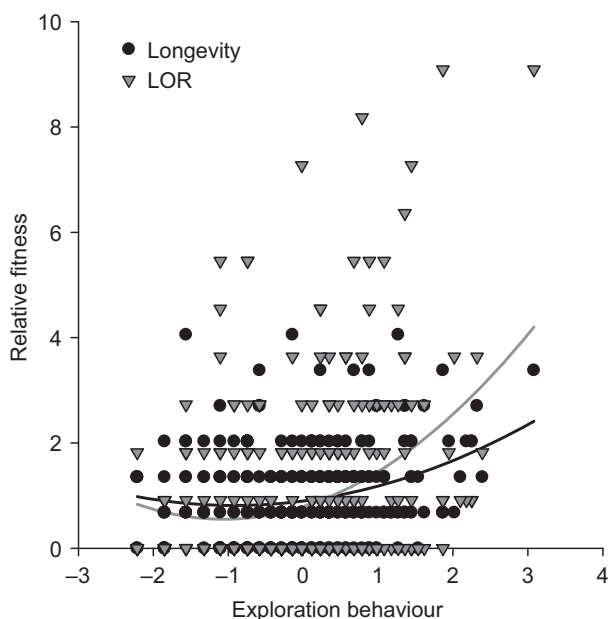


Fig. 4 Nonlinear selection acting on exploration behaviour. The phenotypic selection analyses revealed that fast-exploring individuals at the extreme of the range enjoyed higher fitness: they lived relatively longer and produced relatively more recruits (LOR). Here, square-root-transformed exploration scores are standardized over the whole data set (raw data).

costs at low but not at high densities, for example, because of potential detrimental physiological aspects (e.g. enhanced negative effects of social defeats or prolonged high testosterone levels, Dufty, 1989) or socially

induced costs (e.g. enhanced agonistic interactions with conspecifics, McGraw *et al.*, 2003; Nakagawa *et al.*, 2007). Note that because body size and tie size are positively correlated, it remains a possibility that the weak selection patterns on tie size were partly caused by selection acting on body size. Another puzzling aspect of our results is the absence of sex-specific selection patterns on tie size despite strong sexual dimorphism in this species. That is, we would have expected our selection analyses to show sex-specific optima. This apparent inconsistency may result from how effects of natural and sexual selection on tie size combine when directional natural selection favours smaller ties (our results) but stabilizing sexual selection favours two different optima for males and females (larger and smaller tie, respectively) (Owens & Hartley, 1998).

Our study did not demonstrate convincing evidence for the integration of phenotypic plasticity in tie size and exploration behaviour within individuals, although birds growing larger tie tended to also increase speed of exploration across subsequent observations. This within-individual correlation cannot be caused by correlated measurement errors (*sensu* Dingemanse & Dochtermann, 2013): the two traits were measured using different protocols and equipment, which implies that there was little scope for correlated measurement errors. Within-individual correlations are also unlikely to solely reflect effects of age because age was included as a fixed effect in our models. Although not significant, the tendency for this within-individual correlation to be positive instead indicates that there may be a trade-off between the allocation of limited resources into exploration behaviour vs. tie size. Indeed, previous

studies have demonstrated that black ornaments are involved in social dominance and resource acquisition (Järvi & Bakken, 1984; Poysa, 1988) and that their production is presumably energetically costly (e.g. McGraw, 2008; Galván *et al.*, 2015). Additionally, nutritional stress during ontogeny gives rise to fast exploration (Carere *et al.*, 2005), implying that producing slow-exploring phenotypes are more costly to produce. Hence, the notion of a positive within-individual correlation is consistent with the idea that birds have to trade-off the investment in tie size vs. investment in reactivity (i.e. slow exploration). Formal experiments are now needed to experimentally reveal the presence of such trade-offs.

Our analyses showed that the sign of the correlation between exploration score and tie size differed at the among- vs. the within-individual level. We were able to reveal this architecture because we used models that allowed for the partitioning of variation in, and correlations among, phenotypic traits into among- and within-individual variance components (Wilson *et al.*, 2010; Dingemanse & Dochtermann, 2013). Most past studies that reported a phenotypic association between melanin-based coloration and behavioural traits in wild animal populations (see Table S2 of Ducrest *et al.* (2008)) have, unfortunately, not partitioned correlations across levels. If not partitioned, reported correlations consequently represent a mix between correlations occurring at the among- and within-individual levels (Dingemanse *et al.*, 2012b; Brommer, 2013; Downs & Dochtermann, 2014). This implies that positive ‘unpartitioned’ phenotypic correlations between dark coloration and behaviours may actually reflect within- rather than among-individual correlations; the former correlation can, of course, not be used to test the focal hypothesis. Similarly, the appearance of uncorrelated traits may result from counteracting effects of within- and among-individual correlations of opposite signs, particularly for traits with moderate repeatability (as detailed in Dingemanse *et al.*, 2012b; Dingemanse & Dochtermann, 2013). This echoes recent discussions among behavioural ecologists on whether or not taking the ‘individual gambit’ is a valid approach in syndrome research (Brommer, 2013), that is whether or not one can assume that phenotypic correlation between traits is a valid proxy for among-individual correlations across traits (Dingemanse *et al.*, 2012b; Brommer, 2013; Dingemanse & Dochtermann, 2013).

Overall, our findings highlight the importance of identifying sources of and levels of (co)variation between phenotypic traits to capture the complexity of biological patterns and increase our understanding of evolutionary processes. By revealing a negative individual-level correlation between exploration behaviour and tie size and patterns of selection that do not suggest adaptive integration between these traits, this study implies that melanin-based coloration and animal

personality can evolve in concert, and potentially independently, in response to selection, and their integration may affect how quickly populations can adapt to environmental changes.

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Supporting information

Additional Supporting Information may be found online in the supporting information tab for this article:

Appendix S1 Descriptive statistics of the pruned pedigree of the Lauwersmeer great tit population; the pruned pedigree only including individuals that are informative with regards to the quantitative genetics of exploration behaviour (A) and tie size (B).

Appendix S2 Sex-specific patterns of within- and among-individual covariance between exploration behaviour and tie size.

Appendix S3 Sex specific correlational selection analyses of exploration behaviour and tie size.

Data deposited at Dryad: doi: 10.5061/dryad.4nj8t

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