

## RESEARCH ARTICLE

# Phenotypic selection on an ornamental trait is not modulated by breeding density in a pied flycatcher population

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## Abstract

Most studies of phenotypic selection in the wild have focussed on morphological and life-history traits and looked at abiotic (climatic) variation as the main driver of selection. Consequently, our knowledge of the effects of biotic environmental variation on phenotypic selection on sexual traits is scarce. Population density can be considered a proxy for the intensity of intrasexual and intersexual competition and could therefore be a key factor influencing the covariation between individual fitness and the expression of sexual traits. Here, we used an individual-based data set from a population of pied flycatchers (*Ficedula hypoleuca*) monitored over 24 years to analyze the effect of breeding density on phenotypic selection on dorsal plumage colouration, a heritable and sexually selected ornament in males of this species. Using the number of recruits as a fitness proxy, our results showed overall stabilizing selection on male dorsal colouration, with intermediate phenotypes being favoured over extremely dark and dull individuals. However, our results did not support the hypothesis that breeding density mediates phenotypic selection on this sexual trait. We discuss the possible role of other biotic factors influencing selection on ornamental plumage.

## KEYWORDS

breeding density, *Ficedula hypoleuca*, plumage colouration, sexual selection

## 1 | INTRODUCTION

Sexual selection is a major driver of phenotypic variation and plays a major role in speciation (Andersson, 1994; Andersson & Iwasa, 1996; Darwin, 1871; Maynard Smith, 1991). In many species, one or both sexes exhibit conspicuous traits that are sexually selected. The evolution of sexual traits is maintained because only high-quality individuals can afford to pay the costs of producing and/or maintaining the most elaborate traits (Grafen, 1990; Zahavi, 1975). Importantly, the costs and expression of sexual traits, rather than being constant,

are modulated by environmental variation (Martínez-Padilla et al., 2014a; Vergara et al., 2011, 2012). Changes over time and/or space in the strength and form of environmentally driven selection (Bell, 2010; Evans & Gustafsson, 2017; Garant, 2020; Lehtonen et al., 2009a; Wade & Kalisz, 1990) will result in an evolutionary change observed by a temporal change in the phenotype and its genetic value (Evans & Gustafsson, 2017). Nevertheless, very few studies have examined how the (local) environment shapes the evolutionary trajectories of sexual traits in wild populations (Evans & Gustafsson, 2017; Head et al., 2007).

Jesús Martínez-Padilla and David Canal contributed equally to this work.

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Terrestrial vertebrates, especially birds, are a classic model for the study of sexual ornaments (Ducrest et al., 2008; McGraw, 2006; McNamara et al., 2021). Melanin-based colourations are one of the most common forms of ornamentation in birds, and there is abundant evidence that such ornaments are involved in sexual selection through display and aggression (Jawor & Breitwisch, 2003; Kingma et al., 2008; Lopez-Idiáquez et al., 2016; Meunier et al., 2011; Roulin, 2009). Melanocortin, and its antagonist, the agouti signalling protein (ASIP), are involved in the production of eumelanin and pheomelanin, respectively, which are the pigments responsible for the dark and brownish colourations of the integument of birds (Ducrest et al., 2008; McGraw, 2006). In addition, melanocortin is involved in the expression of agonistic behaviours, such as aggressiveness and territoriality, as proposed by the Hypothesis of Pleiotropy in the Melanocortin System or HPMS (Ducrest et al., 2008; López-Idiáquez et al., 2019; Nicolaus et al., 2016). Consequently, darker melanin-based ornaments are often associated with behavioural traits that are advantageous in male–male competition and mate acquisition (Kingma et al., 2008; Sirkkiä & Qvarnström, 2021; Tarof et al., 2005), including extra-pair paternity (Lehtonen et al., 2009b; Canal et al., 2011). However, a number of studies have also shown that the expression of exaggerated signals, including eumelanin ornaments, can result in increased costs and reduced fitness (Fargallo et al., 2007a; Vergara & Fargallo, 2008; Vergara et al., 2009; Weaver et al., 2017), particularly under harsh environmental conditions (Lopez-Idiáquez et al., 2016; Siva-Jothy, 2000).

Environmental factors may vary in space and time, predictably or erratically, influencing phenotypic selection (Garant, 2020). Abiotic factors, such as precipitation and temperature, have been suggested as environmental drivers of selection at the phenotypic and genetic level (Evans & Gustafsson, 2017; Garant et al., 2004; Gienapp et al., 2008; Merilä & Hendry, 2014; Parmesan, 2006; Remacha et al., 2020; Siepielski et al., 2017; Sirkkiä et al., 2010; Visser et al., 2015). Biotic influences, such as intensity of predation (Huhta et al., 2003; Reimchen & Nosil, 2002), parasitism (Martínez-Padilla et al., 2019; Potti et al., 2002; Siva-Jothy, 2000), breeding synchrony (Spottiswoode, 2004) and population density (Hayward et al., 2018), may also impose strong, but temporally variable, selection pressures on a number of traits. Nevertheless, the selective effects of biotic factors on sexual traits have rarely been quantified (Evans & Gustafsson, 2017; Head et al., 2007).

Population density largely determines the degree of intersexual and intrasexual competition, especially the variability and availability of potential mates and competitors. Hence, the intensity of sexual selection may ultimately depend on population density (Alatalo & Lundberg, 1984; Conner, 1989; Head et al., 2007; Kokko & Rankin, 2006; Lehtonen & Lindström, 2008; Martínez-Padilla et al., 2014a, 2014b; McLain, 1992). Further, changes in breeding density may alter the mating strategies of individuals (Byrne & Roberts, 2004; Kokko & Rankin, 2006). For example, high breeding densities may encourage high-quality individuals to increase their investment in extra-pair relationships, whereas low-quality individuals may prioritize mate guarding (Thusius et al., 2001). Breeding density is therefore

expected to influence the evolutionary trajectories of sexual traits (Hayward et al., 2018). Several studies have investigated the role of population density in mate choice in birds (Head et al., 2007; Kokko & Rankin, 2006), but only one has explored the influence of population density on selection on a secondary sexual trait—the white forehead patch of males—in a wild population (Evans & Gustafsson, 2017). Studies exploring the role of density variation over time are therefore needed to provide a comprehensive perspective on the evolutionary trajectories of sexual traits and melanin-based traits in particular.

Here, we used a long-term (1988–2018) individual-based data set from a population of pied flycatchers (*Ficedula hypoleuca*) to investigate the mediating role of breeding density in phenotypic selection on a melanin-based sexual trait, the black dorsal colouration of the male plumage. The pied flycatcher is a long-distance migratory songbird that exhibits sexual dimorphism in plumage colour during the breeding season. Males arrive earlier on the breeding grounds than females (Lundberg & Alatalo, 1992; Potti, 1998; Potti & Montalvo, 1991a, 1991b), establish a territory around a nesting site and try to attract a female. Dorsal plumage colouration varies substantially among males, both within and between populations (Laaksonen et al., 2015; Lehtonen et al., 2009a; Potti & Montalvo, 1991c), ranging from completely black males to brown, female-like individuals (Camacho et al., 2018; Drost, 1936; Lundberg & Alatalo, 1992).

Dorsal plumage colouration is a heritable, sexually selected and reliable indicator of the overall quality, attractiveness and future parental effort of male pied flycatchers (Alatalo et al., 1994; Canal et al., 2011; Lehtonen et al., 2009a). For instance, darker individuals establish their breeding territories earlier (Lundberg & Alatalo, 1992; Siitari & Huhta, 2002), produce more complex song repertoires (Lampe & Espmark, 1994) and feed their fledglings at higher rates than brownish males (Saetre et al., 1995). Consequently, we expect overall positive directional selection favouring darker phenotypes. Furthermore, we expect density-mediated effects on selection on plumage colouration because (i) the costs of maintaining melanin-based traits are higher under poor environmental conditions (Fargallo et al., 2007a, 2007b; Galván et al., 2015; Vergara et al., 2009); (ii) breeding density can be considered a proxy for levels of intrasexual (Martínez-Padilla et al., 2014a, 2014b) and interspecific competition for nest sites (Camacho et al., 2013—see also Material and Methods); and (iii) in the study population, density has gradually increased over time and reached virtual saturation in recent years (Camacho et al., 2019). Specifically, we expect stronger negative selection on brownish males as population density increases. Brownish males are assumed to be inferior competitors relative to darker males and may therefore be out-competed in years of high breeding density, when intraspecific and interspecific competition are presumably more intense and the energy costs of acquiring and defending a territory increase accordingly. Consistent selection against brownish males is expected to change the frequency distribution of the phenotype over time, so we also explored temporal changes in the mean phenotype in the population.

## 2 | METHODS

### 2.1 | Study system

Data were collected between 1988 and 2018 in a population of pied flycatchers breeding in nest boxes in central Spain (La Hiruela, 41°04' N, 3°27' W). Sampling intensity was limited in the years 2002 and 2003, and therefore, these years were excluded from the analyses.

The study area and field protocols have been described in detail elsewhere (Camacho et al., 2018; Potti et al., 2018). Briefly, there are two study plots located in different habitats, a deciduous oak forest (*Quercus pyrenaica*) of 9.3 ha and a mixed coniferous plantation (mostly *Pinus sylvestris*) of 4.8 ha, located 1.1 km apart. The landscape surrounding each plot consists of unsuitable habitat types (e.g. rock outcrops and scrublands) due to the scarcity of nesting cavities for pied flycatchers. From 1988 to 1994, there were 200 nest boxes across the two study plots, a number that increased to 239 in 1995. Since then, the number and location of nest boxes have remained unchanged. In total, there are 156 nest boxes in the oak forest and 83 nest boxes in the pine forest, at a distance of  $20 \pm 9.2$  m (mean  $\pm$  standard error) from one another.

### 2.2 | Population monitoring and data collection

During the breeding season, from around the third week of April (when first males arrive from migration) to the first fortnight of July, all nest boxes were checked every 3 days to determine their occupation by pied flycatchers or other species (see Potti et al., 2021), and daily after occupation to ascertain laying date, clutch size (typically 5–6 eggs), hatching date and number of fledglings. Adults were captured with a nest-box trap (Friedman et al., 2008) while incubating (females) or feeding nestlings (males and females) on day 8 posthatching. Birds were marked with a numbered metal ring (males and females) and a unique combination of colour rings (only males). Unringed birds first caught as breeding adults were considered immigrants, whereas local recruits ringed as nestlings are hereafter referred to as 'residents'. Unringed (immigrant) individuals were aged as first year (hereafter, yearling) or older based on their plumage traits (Karlsson et al., 1986; Potti & Montalvo, 1991a). We took standard morphological measurements on each individual, including tarsus length ( $\pm 0.01$  mm), wing length ( $\pm 0.5$  mm), forehead patch size ( $\pm 0.01$  mm) and body mass ( $\pm 0.1$  g). Following previous studies on the pied flycatcher, dorsal colouration was visually estimated in the field as brownness, defined as the percentage of the head and mantle area covered by non-black feathers (brownish, grey or white), excluding the rump and the white forehead patch (Camacho et al., 2018; Drost, 1936; Laaksonen et al., 2015; Lehtonen et al., 2009a; Potti & Montalvo, 1991c). Despite dorsal colouration being quantified by several observers, there is consistency and agreement among observers in their colour estimates, as confirmed by the data from polygynous males that had been captured in different nest boxes and measured for brownness more than once in the same year

(repeatability = 0.81,  $p < 0.001$ ), as well as by independent scorings of the same males by different researchers (repeatability = 0.90,  $p < 0.001$ , respectively, see Camacho et al., 2018 for details).

### 2.3 | Fitness measurements

For each individual, we counted the annual number of recruits, defined as the total number of fledged offspring that returned to breed in our study plots and used this measure as a proxy for male fitness. Local recruitment is commonly used for estimating selection in birds, as it is a closer proxy for the actual genetic contribution of each individual to the population than, e.g. clutch size or fledging success (e.g. Blondel et al., 2006; Charmantier et al., 2004; Husby et al., 2009). Local recruitment is considered a good measure of fitness in the study population because (i) recruitment rates are among the highest reported for the species (up to 22%, on average 14%; Canal et al., 2014; Potti et al., 2013; Potti & Montalvo, 1991a), (ii) the small number of ringed birds recorded during nonsystematic explorations of surrounding areas (~5–10 km, 14 recorded birds out of 4086 ringed adults and out of >20,000 ringed chicks) and the absence of ring recoveries in other intensively studied geographically close (<80 km) populations of pied flycatchers (A. Cantarero, pers. comm.) indicate that dispersal outside the study area is an extremely rare event, (iii) parent-offspring comparisons of dispersal probability in this population revealed no familiar resemblance that could potentially result in underestimation of dispersers' fitness (Camacho et al., 2015) and (iv) mark-recapture analyses confirm that variation in the probabilities of survival (transition) and recapture do not vary over time (Santoro et al., 2022). For all these reasons, as in other similar long-term studies (Arcese et al., 1992; Pärn et al., 2009; Spagopoulou et al., 2020), we assume that local recruitment estimates are reliable and unbiased.

### 2.4 | Breeding density as an environmental factor

Here, we focused on breeding density because (i) population density is a reliable indicator of intersexual and intrasexual competition and, therefore, of their effects on individual fitness (Arcese & Smith, 1988; Both & Visser, 2000; Gustafsson, 1987; Wiggins, 1995); and (ii) the number of breeding pairs is the ultimate consequence of other biotic or abiotic influences, such as local climate, predation rates, habitat quality and food availability.

We estimated breeding density as the annual proportion of nest boxes occupied by pied flycatchers in relation to the total number of nest boxes. In addition, to examine the effects of interspecific competition on selection, we conducted a complementary analysis where we considered all species using our nest boxes (pied flycatchers; tits, *Paridae*; treecreepers, *Certhia brachydactyla*; and nuthatches, *Sitta europaea*) when estimating breeding density (Potti et al., 2021). The results of this complementary analysis remained qualitatively similar to those considering pied flycatcher density alone (see Table S1).

## 2.5 | Statistical analyses

To analyze patterns of selection on male dorsal colouration, we fitted generalized linear mixed models (GLMMs) to the data on the annual number of recruits (as a surrogate of fitness). Using annual values instead of the lifetime number of recruits allowed us to maximize the sample size by keeping all individuals regardless of annual gaps in their capture histories. As predictors, we considered dorsal colouration (centred and standardized by subtracting the mean annual brownness in the population divided by its standard deviation), its squared term (to explore nonlinear associations with fitness), breeding density and its quadratic term, and the two-way interactions between dorsal colouration and breeding density (in both its linear and quadratic forms). Because of the progressive increase in breeding density throughout the study period, we did not include a temporal component (i.e. year as a continuous variable) in the analyses of selection to avoid collinearity problems (see also Camacho et al., 2013, 2019). In addition, we included three controlling variables in the model: (1) habitat type (two-level class variable: oak or pine) to account for differences in habitat quality (Camacho et al., 2015); (2) breeding (egg laying) date because it is a key predictor of reproductive success in this (Canal et al., 2012) and many other temperate-zone bird species (Newton, 2008); and (3) male age (two-level class variable: yearling or older, since exact age can only be determined for immigrants captured for the first time as yearlings), to account for age-related differences in performance, as described in this and other bird species (Evans et al., 2011; Lundberg & Alatalo, 1992; Nol & Smith, 1987). We included male and female identities as random intercepts to account for repeated observations of birds appearing multiple times in the dataset (ca. 41% of males and 43% of females), as well as to control for the potential effects of female mates on the reproductive success of males. Breeding season identity (i.e. categorical year effect) was also included as a random intercept to account for the nonindependence of samples from the same year and for stochastic variation among years (see e.g. Evans & Gustafsson, 2017, for a similar approach). The effect size was calculated for these parameters (Table S2).

Before investigating the determinants of selection on dorsal colour, we examined the goodness-of-fit of several candidate models using different error distributions and random structures (see Tables S3 and S4) and sequentially compared them using the corrected Akaike Information Criterion corrected for small samples (AICc, Burnham et al., 2002). These analyses showed that the most supported model had a Conway–Maxwell–Poisson distribution, a random intercept structure (but not random slope) and zero inflation; therefore, we present the results based on this model structure.

Extra-pair paternity occurs in this population (Canal et al., 2011, 2012) and might affect selection patterns. However, it is unlikely that extra-pair paternity causes a severe bias in our selection estimates for three reasons. First, a two-year molecular study indicated that the frequency of extra-pair young (EPY) in the study population amounted to 11–20% (Canal et al., 2012), of which only males (ca.

50%) are considered in the present analysis. Second, the number of recruits per nest in the study population is on average 0.5 (Le Vaillant et al., 2021), further reducing the potential impact of EPY. Specifically, in the two-year study mentioned above, the total number of male recruits being EPY per year was  $n = 5$  and  $n = 7$ , representing 12% and 6% of all recruits. Given the annual number of breeding males in the population (up to 160 males), the influence of these few individuals on our analyses is expected to be small to negligible. Third, an analysis based on the recruits included in the molecular study suggests that selection patterns are similar for EPY and non-EPY male recruits (Table S5).

Male pied flycatchers may postpone their first breeding attempt until their second year of life (Potti & Montalvo, 1991b). Thus, to avoid underestimating the recruitment rates for the 2000 and 2001 cohorts due to the limited field effort in 2002 and 2003, we excluded those years from the selection analysis. For the same reason, all birds born in the last two years of study (2017 and 2018) were also excluded from the analysis. In addition, we excluded data from individuals involved in experiments conducted during the long-term study (e.g. cross-fostering; Camacho et al., 2016,  $n = 724$  birds) due to the potential effect of the treatments on annual reproductive success.

We fitted a linear mixed model (LMM) to analyze the trend in mean dorsal colouration of the male population over time. In this model, we included brownness as the dependent variable and, as explanatory terms, year (continuous variable), age (yearling or older), habitat type (oak or pine) and tarsus length (continuous variable) to control for potential size-dependent variation in the trait. In addition, to test for temporal differences in brownness between resident and immigrant males, we modelled the interaction between year and philopatric status (class variable: resident vs. immigrants). The effect sizes of the model parameters are also given in the supplementary material (Table S6). We used male identity and year as random intercepts to account for the nonindependence of samples between individuals and breeding seasons, respectively.

Statistical analyses were performed in R version 4.0.1 (<https://www.r-project.org>). GLMMs were performed using the package 'lme4' version 1.1–26 (Bates et al., 2015). The significance of the fixed effects was calculated with Type II Wald Chi-square tests (Zuur et al., 2009) using the function ANOVA in the car package (Fox & Weisberg, 2011). We followed a backward method, in which we started from a full model and nonsignificant interactions were progressively removed according to  $p$  values to analyze the first-order effects. The full model with all interactions is shown in Tables S7 and S8 for the selection and temporal trend models respectively. The package HLMdiag, version: 0.5.0 (Loy & Hofmann, 2014) and the VIF function (car package; Fox & Weisberg, 2019) version 0.99.40 were used for model diagnostics. To model the second polynomial of the variables, we use the function 'poly' (stats package R Core Team, 2020) version 4.0.3, which creates two orthogonal vectors for the first and second polynomial of the original variable. The package ggeffects (Lüdtke, 2018) was used to convert parameter estimates of the models to effect sizes.

### 3 | RESULTS

There was a quadratic effect of dorsal colour on male fitness indicating nonlinear selection on male plumage colour (Table 1; Figure 1); so that individuals with intermediate phenotypes produced more recruits than individuals at either the blacker or the browner extremes of the colour distribution (Figure 1). Neither breeding density ( $\beta = 0.007$ ,  $SE = 0.048$ ,  $p = 0.781$ ) nor its interactions with dorsal colour ( $\beta = 0.031$ ,  $SE = 0.020$ ,  $p = 0.125$ ) or with its quadratic term ( $\beta = 0.003$ ,  $SE = 0.011$ ,  $p = 0.766$ ) had any effect on reproductive success, suggesting that breeding density did not influence selection on dorsal colouration.

Plumage colouration of male pied flycatchers became progressively darker over the 24 years of study (Table 2; Figure 2). Neither philopatric status (resident vs. immigrant,  $p = 0.637$ ) nor its interaction with year ( $p = 0.475$ ) were statistically significant, suggesting no differences in the temporal trends of plumage colour between immigrant and resident males.

### 4 | DISCUSSION

Using individual-based data from a long-term study of pied flycatchers, we have examined the mediating effect of breeding density in selection on male plumage colouration. Breeding density is a biotic environmental factor assumed to have strong implications for intrasexual and intersexual interactions and, therefore, for the phenotypic trajectory of sexually selected traits. We found nonlinear stabilizing selection on the dorsal colouration of male pied flycatchers, as individuals with intermediate phenotypes had the highest fitness compared with any of the extremes of the colour distribution, either very dark or very dull plumages. Contrary to our predictions, we found no evidence for density-mediated selection on male plumage colouration. Moreover, males became darker over the study period despite negative selection acting on both extremes of the dorsal colouration.

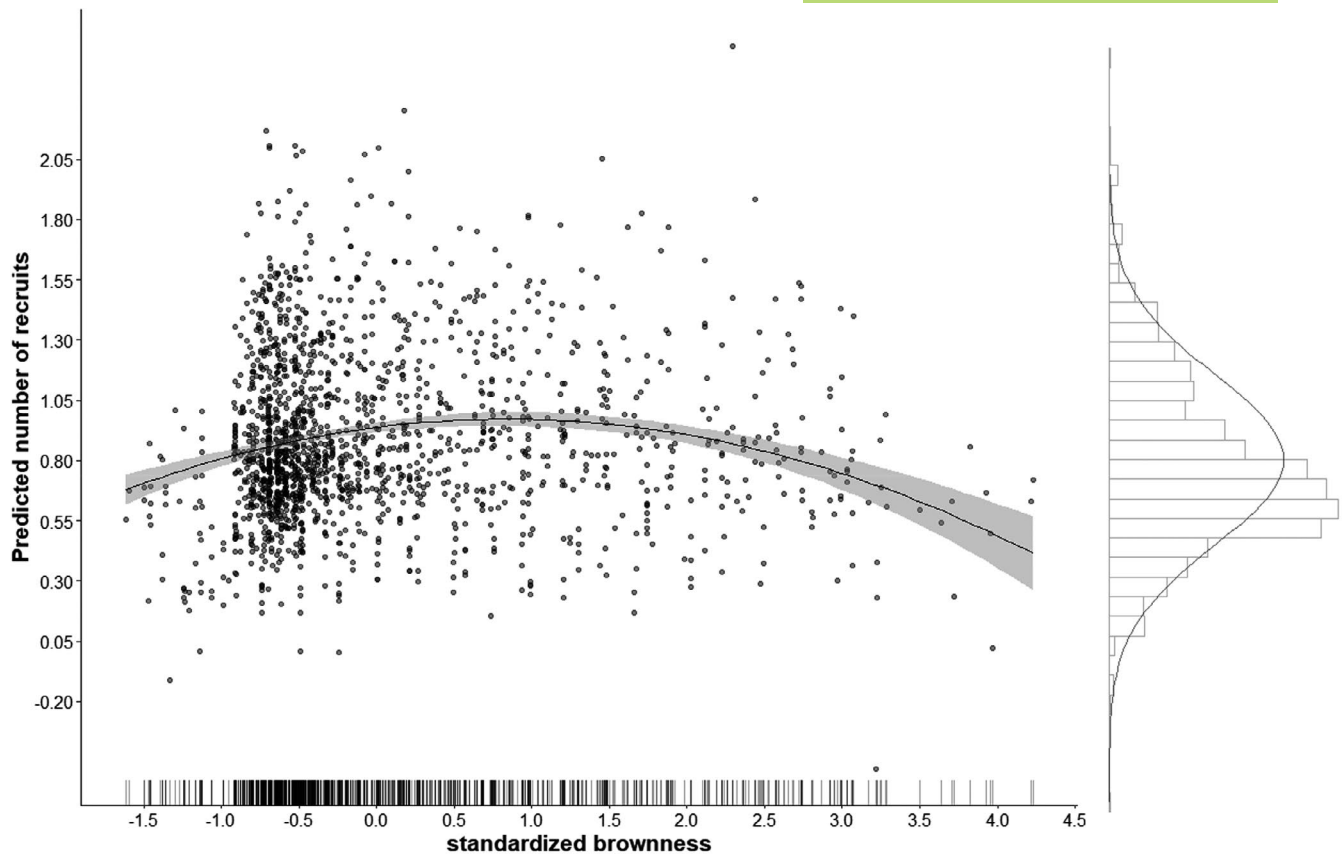
Eumelanin-based ornaments are increasingly recognized as reliable indicators of individual quality (Jawor & Breitwisch, 2003; Meunier et al., 2011; Roulin, 2009; Sirkiä & Qvarnström, 2021). In particular, in pied flycatchers, dark males are thought to be of

superior quality relative to brownish individuals (Galván & Moreno, 2009; Lampe & Espmark, 2002; Lundberg & Alatalo, 1992; Potti & Montalvo, 1991c; Sirkiä & Laaksonen, 2009; Slagsvold & Lifjeld, 1992). However, constraints to reproductive performance also exist to dark males in the form of environmental and social challenges. For instance, dark males tend to settle earlier than brownish males in the breeding areas, when environmental conditions are still adverse (e.g. harsh weather and food scarcity). Dark males also often face intense intrasexual and intersexual competition for territories and females (Canal et al., 2021; Lundberg & Alatalo, 1992; Potti, 1998; Potti & Montalvo, 1991c). Exposure to energy demanding environmental conditions and highly competitive social contexts may carryover to impact parental investment and thus explain the lower reproductive success of the darkest males in our population. In agreement with these ideas, a number of observational and experimental studies have also found that individuals carrying exaggerated ornaments or armaments may suffer increased fitness costs (Fitzpatrick et al., 1995; Hayward et al., 2018; Möller, 1989; Qvarnström, 1997; Sanz, 2001).

Environmental conditions play an important role in selection on phenotypic traits in natural populations (Evans & Gustafsson, 2017; Hayward et al., 2018; Head et al., 2007). Studies of phenotypic selection have traditionally been based on climatic variables, and thus, our current knowledge of the selective effect of biotic factors on phenotypic traits is rather limited (Head et al., 2007; Sirkiä & Qvarnström, 2021). However, climatic variables per se may not be the causal agents of selection but may mediate an array of other biotic factors acting as true environmental constraints on the evolution of phenotypic traits. On the basis of the progressive increase in breeding density observed over time in the study population (Camacho et al., 2019), we expected breeding density to have a prominent role in the evolution of sexual traits due to its effects on male-male competition and patterns of mate choice by females (Chek et al., 1996; Holveck et al., 2015; Jirotkul, 1999; Lundberg et al., 1987). Indeed, a recent study on pied flycatchers found that black males from a Swedish population had greater success in male-male competition for nesting sites than dull males, so that in densely occupied habitats, black phenotypes are presumed to be favoured over lighter ones (Sirkiä & Qvarnström, 2021). However, we found that selection on plumage darkness was not sensitive to breeding density,

Random effects	Variance	Std. Dev.		
Male identity	0.058	0.241		
Female identity	0.123	0.248		
Year	0.123	0.350		
Fixed effects	Estimate	Std. Error	Type II Wald X2	p value
Intercept	1.124	0.090		
Dorsal colour	0.073	0.025	8.325	0.004
Dorsal colour <sup>2</sup>	-0.044	0.014	10.051	0.002
Habitat type [Pine]	0.171	0.041	17.123	<0.001
Breeding date	-0.011	0.002	21.519	<0.001

**TABLE 1** Results of the generalized linear mixed model testing for selection on male dorsal plumage colour. The model considered a random intercept, with a Conway-Maxwell-Poisson distribution and zero inflation. Breeding (egg laying) date, breeding density, habitat type (pine vs. oak) and bird age (yearling vs. adult (>2 years)) were considered in the model. Effect sizes and full model including nonexplanatory terms are given in the SM (Tables S2 and S7)



**FIGURE 1** Nonlinear selection on the dorsal colouration of male pied flycatchers. Values in the x-axis correspond to the standardized (see methods) values of dorsal colouration: negative values indicate darker plumages, while positive values indicate browner plumages in relation to the average population each year. Black vertical lines below the x-axis represent individually measured individuals. The shaded area around the curve depicts the confidence interval

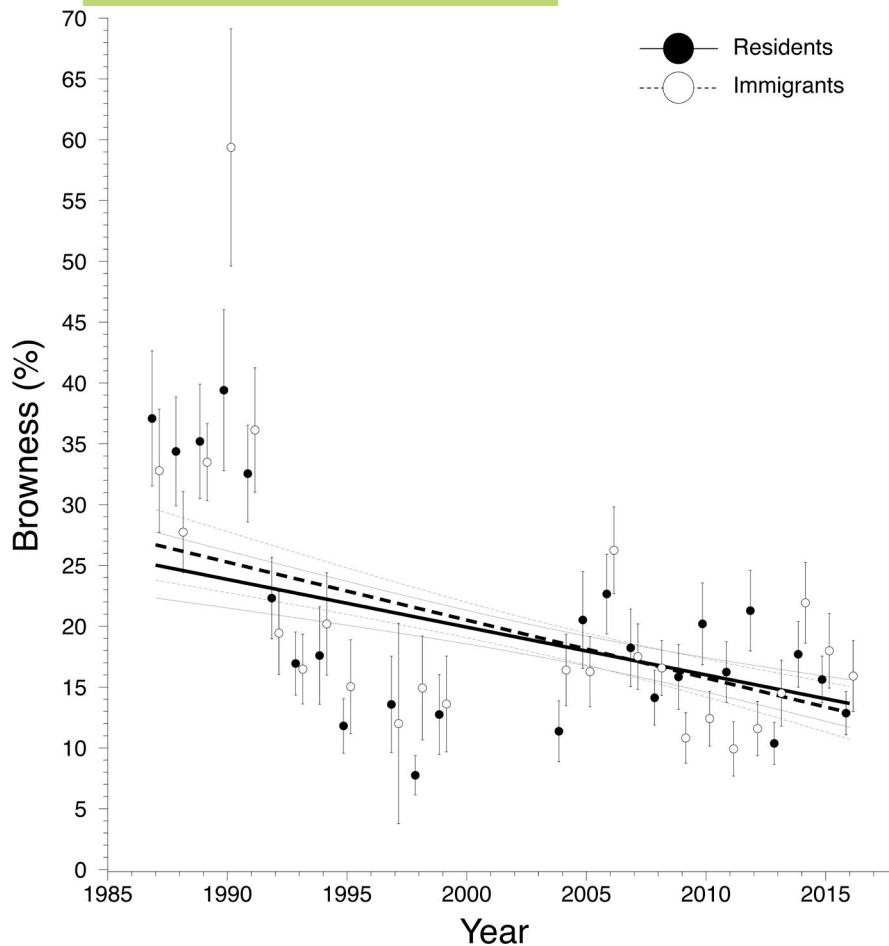
**TABLE 2** Results of the linear mixed model (LMM) analyzing the overall trend of dorsal colouration of male pied flycatchers over time. The model included a random intercept. Parameter estimates and SE were calculated using REML models (see main text). Effect sizes and full model including nonexplanatory terms are given in the ESM (Tables S6 and S8)

Random effects	Variance	Std. Dev.		
Male identity	210.40	14.505		
Year	32.09	5.665		
Fixed effects	Estimate	Std. Error	t	p
Intercept	85.824	16.692	5.142	<0.001
Year	-4.246	1.289	-3.292	<0.001
Male age (adult)	-8.082	1.149	-7.034	<0.001
Tarsus length of males	-3.092	0.856	-3.612	<0.001

evidencing the controversies on this issue still lingering today. One might argue that the breeding density of either the pied flycatchers or the bird community (see Table S1) using the nest boxes does not accurately reflect the real density in the study area. However, even if underestimated, the dramatic, progressive increase in breeding density in our study area since the installation of the nest boxes (from <0.15 pairs/ha to >11.5 pairs/ha; see Camacho et al., 2019) should have been large enough to trigger detectable biological responses, as has been documented for other density-dependent processes (e.g. dispersal; Camacho et al., 2018, 2019). It is also possible that other unmeasured environmental variables (e.g. predation risk, food availability and sex ratio variation; see below) experienced by

individuals in the breeding and nonbreeding areas are more important than breeding density in shaping selection on dorsal colouration (Slagsvold & Lifjeld, 1992). Unfortunately, our knowledge of the myriad environmental factors that male pied flycatchers experience throughout their annual cycle is scarce.

Male colour changed over time in the population from duller to darker males, although the reason for this shift is puzzling because (i) melanin-pigmented traits (Kim et al., 2013) and, specifically, of the dorsal colour of pied flycatchers are highly heritable (Lehtonen et al., 2009a) and (ii) the peak of fitness does not fall on blacker-than-average scores each year, but on duller-than-average ones (Figure 1). Nevertheless, there are some possible explanations for this pattern.



**FIGURE 2** Temporal trend from browner to darker dorsal colouration of male pied flycatchers at the population level. The black solid line represents resident birds, and the dashed line shows immigrants. Error bars indicate the standard error of the percentage of brown each year

First, as discussed above, environmental factors other than density may play a more important role in selection on male colour or they may influence selection in opposite directions or during different life stages (Garant et al., 2004; Hayward et al., 2018; Sirkkä et al., 2010). For example, in the case of laying date, perhaps the best-known life-history trait in wild birds from an evolutionary perspective, multiple biotic and abiotic environmental factors can influence selection on breeding time, as indeed occurs in our study species (Both & Visser, 2001; Goodenough et al., 2010, 2011; Le Vaillant et al., 2021; Visser et al., 2015). Second, indirect selection could influence phenotypic changes over time in the population. The pleiotropic nature of the expression of melanin-pigmented traits is well known (Ducrest et al., 2008; Kim et al., 2013), so it is possible that the expression of male darkness is associated with other key traits in pied flycatchers, such as morphological and personality traits (Camacho et al., 2018; Ducrest et al., 2008). A third possibility is that changes in the age structure or composition of the population due to immigration have driven the observed phenotypic change. However, this is unlikely because the analyses exploring potential temporal changes controlled for the age of individuals (see statistical analysis); also, the temporal trend in blackness did not differ statistically between residents and immigrant males (see results and Figure 2). Fourth, it is possible that the fitness loss of individuals showing slightly darker phenotypes than the average (around  $-0.5$  of standardized colour; Figure 1) may be compensated for by the higher frequency of this

phenotype in the population, which would push the phenotype of the population to the darker extreme of the distribution. In summary, understanding the dissociation between the observed temporal trend in a highly heritable trait, such as the one studied here, and that expected from the patterns of selection on it would require the exploration of multiple factors that are beyond the aims of this study (Pujol et al., 2018).

Overall, we found stabilizing selection on the dorsal colouration of male pied flycatchers. Our results do not support a major role of breeding density in selection on this sexual ornament. Future research may consider other environmental factors that may influence selection on blackness in this species during adulthood. Particularly promising are factors related to temperature, as darker individuals could be positively selected during episodes of warm temperatures because they can better cope with the costs associated with an enhanced expression of the trait (Delgado Sánchez et al., 2019; MacLean et al., 2019; Côte et al., 2018). While tackling multiple climatic or biotic environmental influences in selection on sexual traits is an appealing and promising field, hypothesis-driven studies need to be developed to determine the most relevant agents of selection acting on melanin-based traits.

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## PEER REVIEW

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