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Early constraints in sexual dimorphism: survival benefits of feminized phenotypes

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Abstract

Sexual dimorphism (SD) has evolved in response to selection pressures that differ between sexes. Since such pressures change across an individual's life, SD may vary within age classes. Yet, little is known about how selection on early phenotypes may drive the final SD observed in adults. In many dimorphic species, juveniles resemble adult females rather than adult males, meaning that out of the selective pressures established by sexual selection feminized phenotypes may be adaptive. If true, fitness benefits of early female-like phenotypes may constrain the expression of male phenotypes in adulthood. Using the common kestrel *Falco tinnunculus* as a study model, we evaluated the fitness advantages of expressing more feminized phenotypes at youth. Although more similar to adult females than to adult males, common kestrel fledglings are still sexually dimorphic in size and coloration. Integrating morphological and chromatic variables, we analysed the phenotypic divergence between sexes as a measure of how much each individual looks like the sex to which it belongs (phenotypic sexual resemblance, PSR). We then tested the fitness benefits associated with PSR by means of the probability of recruitment in the population. We found a significant interaction between PSR and sex, showing that in both sexes more feminized phenotypes recruited more into the population than less feminized phenotypes. Moreover, males showed lower PSR than females and a higher proportion of incorrect sex classifications. These findings suggest that the mechanisms in males devoted to resembling female phenotypes in youth, due to a trend to increase fitness through more feminized phenotypes, may provide a mechanism to constrain the SD in adulthood.

Introduction

Given their different reproductive roles and life histories, males and females show different phenotypes. Sexual dimorphism (SD) is the result of sex differences in optimum trait values under natural, and particularly sexual, selection pressures (Lande, 1980; Hedrick & Temeles, 1989; Chenoweth *et al.*, 2008). Since such pressures change across individual life stages, the optimal value of traits is expected to change accordingly (Schluter *et al.*, 1991). For example, sexual selection may not exert influence on SD at juvenile stages but directly affect after sexual maturity (Chippindale *et al.*, 2001). Furthermore, the final divergence of adult sexual traits may be influenced by sex-specific genetics, maternal effects and environmental factors during development (Badyaev, 2002).

Our knowledge about the ontogeny of SD comes mainly from the study of the quantitative degrees of size differences between sexes, such as those found on body mass or skeletal measurements (Fairbain, 2007). In turn, sexual dichromatism, when present, is expressed by different and discrete values of sex-specific characters (e.g. secondary sexual traits), for which the classical approach to the study of their variation has not been ontogenetic, but phylogenetic and based on adult phenotypes (Badyaev & Hill, 2000; Oliver &

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Monteiro, 2011; Bell & Zamudio, 2012). Sexually immature individuals exhibit dimorphism in coloration, colour patterns and designs to varying degrees that have been scarcely investigated within populations (Johnsen *et al.*, 2003; Fargallo *et al.*, 2007a, 2014), even considering their importance in understanding the evolution of SD under natural selection pressures outside of the sexual context (Vegara & Fargallo, 2008; Vergara *et al.*, 2010; Tringali & Bowman, 2012). Moreover, our knowledge about the ontogeny of SD comes mainly from the study of a single trait variation, however phenotypes are complex as a result of functional, developmental and genetic covariation among traits in a given organism ('phenotypic integration'; Pigliucci & Preston, 2004). The study of single traits and its relationship with fitness has limited evolutionary interpretations of theoretical models of evolution, which explicitly emphasize the importance of multivariate relationships among traits (McGuigan *et al.*, 2011). The study of SD from an integrated perspective of phenotypes is appropriate because patterns of covariation among functionally related traits can differ between sexes (Roulin *et al.*, 2010; Kim *et al.*, 2013; Fargallo *et al.*, 2014;) and promote different selection pressures between males and females (Roulin *et al.*, 2010; Kim *et al.*, 2013), thus favouring multivariate evolution of given phenotypic combinations of different traits in each sex.

Sexually dimorphic traits are often expressed discontinuously or bimodally with sexually intermediate morphs within each sex at the same life stage (Fairbain, 2007; Roulin *et al.*, 2010). An extreme case of particular interest is that of female-like males in which males of several taxa resemble females either in morphology, behaviour or endocrine functions (e.g. Dominey, 1980; Rohwer *et al.*, 1980; Forsyth & Alcock, 1990; Slagsvold & Saetre, 1991; Zamudio & Sinervo, 2000; Hanlon *et al.*, 2005). In the context of sexual selection, female-like males may have evolved as an alternative mating tactic allowing access to breeding resources while avoiding confrontations with other males (Dominey, 1980; Rohwer *et al.*, 1980; Zamudio & Sinervo, 2000; Hanlon *et al.*, 2005). In other cases, however, female-like males may result from ecological constraints such as predator avoidance (Slagsvold *et al.*, 1995; Shine *et al.*, 2001) and thermoregulation (Shine *et al.*, 2001). A further possibility is the one we examine here. Recent evidence suggests that selection may operate differentially between sexes, with female traits resulting from competition for resources rather than through competition for mates (LeBas, 2006; Tobias *et al.*, 2011, 2012). Under this scenario, it is likely that more female-like individuals will benefit in intra-specific competition for ecological resources (Tobias *et al.*, 2012). This may be why males tend to resemble females in early life stages when selective pressures for mate competition and mate choice are not yet established. Thus, the mechanisms in males devoted to resembling female phenotypes during development may constrain the final phenotypic divergence of the sexes.

Different to the SD exhibited by adults, and with an adult female appearance, common kestrel *Falco tinnunculus* fledglings do still exhibit SD in size and coloration (Fig. S1). However, from a human perspective, the phenotypic sexual differentiation is not unequivocal, with some individuals intermediate in size that are also intermediate in chromatism, thus suggesting a phenotypic relationship between colour and size. Here, we investigated, from a multivariate perspective, whether SD in size and coloration may be adaptive in juvenile common kestrels. For this, we first examined phenotypic integration between coloration and morphology. We used the axes (PCs) extracted from a principal components analysis combining several colour variables and tested for potential relationships between PCs and morphological variables in relation to sex. Second, we analysed the phenotypic divergence between sexes by estimating the individual phenotypic sexual resemblance (PSR) as a measure integrating morphological and chromatic variables, in order to know how much each individual looks like the sex to which it belongs. Finally, we tested the fitness benefits associated with PSR by means of the probability of recruitment in the population, which reflects post-fledging survival in this species (Kim *et al.*, 2013; Terraube *et al.*, 2015). If PSR during development affects fitness, adaptive strategies developed under ecological pressures in early life may imply an evolutionary constraint in the sex phenotypic divergence in adult stages and under sexual selection pressures in traits, such as skeletal size, that cannot be reverted.

Materials and methods

Study species and general procedures

Adult common kestrels (hereafter kestrels) show marked SD in size and plumage coloration with males being smaller and more conspicuously coloured than females (Massemin *et al.*, 2000). As in many dimorphic birds, fledglings are more similar to adult females than to adult males. Yet, within such a female appearance, kestrel fledglings also exhibit SD in size and coloration with males being smaller and showing greyer coloration in their rump and lesser blackish appearance than female fledglings. These phenotypic differences in size and coloration have served to traditionally assign sex to fledglings (e.g. Village *et al.*, 1980; Dijkstra *et al.*, 1990). Nonetheless, there are individuals that are difficult to sex due to their sexually intermediate phenotypes in both size and plumage coloration (Village *et al.*, 1980). Plumage coloration in kestrels is melanin based (Fargallo *et al.*, 2007a) and has been found to genetically covary with body mass in males but not in females,

indicating sexual differences in phenotypic trait integration (Kim *et al.*, 2013).

The study was performed during the breeding seasons of 2004–2012, in Segovia (central Spain), where most kestrels breed in nest boxes (Fargallo *et al.*, 2009). Nests were frequently visited to determine laying date (the day the first egg was laid), clutch size (range 3–7), hatching date (of the first chick in each brood) and brood size at fledging (range 1–7). Morphological and chromatic variables for determining SD were recorded at the age of 26 days when fledglings were measured (tarsus length, wing length and body mass), plumage coloration was estimated (see below) and a blood sample was extracted for sex determination (see below). Also, on this day fledglings were individually marked with metallic rings in order to identify them in the following years as recruits into the population. To estimate local recruitment adults were captured using nest-box traps during the chick-rearing phase.

Fledgling coloration

Plumage coloration of fledglings was measured over 7 years (2004–2007 and 2009–2011) in 324 females and 337 males ($n = 661$). An experiment carried out in 2004 affected coloration (Fargallo *et al.*, 2007a), for which reason only fledglings from the control group were considered in this study. coloration was assessed following previously published procedures (Fargallo *et al.*, 2007a,b). Briefly, the greyness of rump and uppertail coverts (hereafter rump greyness; RG) was estimated as the percentage of grey with respect to brown covering the whole rump (0% = completely black-barred brown and 100% = completely black-barred grey, Fig. S2a). The repeatability of this measurement is high (Fargallo *et al.*, 2007a,b). Digital photographs were taken from the back side of the bird while it was held with the wing and the tail outstretched (i.e. at an angle of 90° between the objective of the camera and the surface of the body) using a Nikon D70 camera (objective: 18–70 mm AF-S Nikkor DX). All photographs were taken under a sunshade in the morning. Images were imported into Photoshop CS3, where four colour trait variables were measured: (i) width of black terminal tail bands (TTB, Fig. S2b), (ii) width of black superior tail bars (TB, Fig. S2b), (iii) width of black rump bands (RB, Fig. S2b) and (iv) wing blackness (WB, Fig. S2c). All measurements were conducted by the same person (ILR). In a subset of 30 photographs belonging to 30 individuals, all colour variables were measured twice to determine repeatability. A metric scale was set for all pictures prior to measurements by equalizing pixel distance to measurement units (cm). All measurements were performed at real scale. To compute bar and band widths the 'ruler tool' was used. Measurements of the TTB were performed in the two central tail feathers, whereas TBs and RBs were measured in four different feathers on the left side of the body. Band and bar widths were calculated as the mean value of these feather measurements and were highly repeatable in all cases (TTB: $R = 0.95$, $F_{1,60} = 40.40$, $P < 0.0001$; TB: $R = 0.90$, $F_{1,60} = 18.00$, $P < 0.0001$; RB: $R = 0.91$, $F_{1,60} = 20.38$, $P < 0.0001$). To estimate WB, the proportion of black coloration (spots and bars) was calculated in the covert feathers of a known area of the wing. Black spots and bars are easily distinguishable from the brown background of the wing plumage, and thus, they were outlined and their area determined as the number of pixels occupied using the 'magic wand tool' (Fig. S2c). WB measurements were highly repeatable ($R = 0.97$, $F_{1,60} = 58.53$, $P < 0.0001$). As WB and RG are proportions, they were transformed using arcsine transformation.

Molecular sex determination

Fledglings were sexed using a polymerase chain reaction (PCR) amplification and agarose electrophoresis based on the technique used by Fridolfsson & Ellegren (1999). The method is based on the detection of a constant size difference between CHD1W and CHD1Z introns. DNA was isolated from the red blood cells using the Qiagen QIAamp kit and the manufacturer's protocol (Qiagen, Hilden, Germany). PCR amplification was run using a particular set of primers (2550F and 2718R) as proposed by Fridolfsson & Ellegren (1999).

Phenotypic sexual resemblance

Phenotypic sexual resemblance (PSR) was defined as the probability that an individual will be correctly sexed according to its phenotype. PSR was calculated first using a discriminant analysis in order to identify the best combination of phenotypic traits distinguishing sex. Variables used were both morphological (tarsus length, wing length and body mass) and chromatic (RG, WB, TTB, TB and RB). After that, posterior classification probabilities for each group was computed based on Mahalanobis distances of a given case (individual) to each group centroid, that is, the point that represents the means for all individuals for a group (sex in our case as determined by molecular sexing) in the multivariate space defined by the variables in the model. In general, the nearer a case is from the centroid of its group and the further away from the other group centroids, the more likely it is that it belongs to that group. Therefore, the higher the value of PSR, the more likely it is that the fledgling was correctly sexed according to these phenotypic variables.

Local recruitment

As a proxy of survival we used post-fledgling survival, which is calculated as the binary probability of

recruitment and has been previously used for the study of common kestrels (Kim *et al.*, 2013; Terraube *et al.*, 2015). We also recorded age at recruitment defined as the age at first-time initiation of reproduction. Recruits were monitored during the 3 years following the birth of chicks, as almost all kestrels in our population recruit between the ages of 1 and 2 years, but some individuals recruit in their third year after fledging. For this reason, local recruitment was based on the ring codes of breeding kestrels captured from 2005 to 2012 of the individuals born between 2004 and 2009 (excluding 2008).

Statistical procedures

SD and integration of sexually dimorphic traits

To test for SD in fledgling biometry (tarsus length, wing length and body mass) and coloration (RG, WB, TTB, TB and RB), univariate linear mixed models (LMM) were carried out using each of the three body and five colour traits as dependent variables. WB and RG were arcsine transformed prior to analysis. Sex was included as a fixed factor, and nest and year were random factors. First, to understand the effect of sex in the relationship between colour and size, the five colour variables were combined in a principal components analysis that resulted in two main axes showing eigenvalues > 1 (Table S1). The first axis (PC1) explained 43% of the variance and represented a gradient from high values of WB, RB and TB and low values of RG to the opposite. The second axis (PC2) explained 24% of the variance and described a gradient of width in the TTB. The relationship between PCs as dependent and morphological variables in relation to sex were tested using univariate LMMs with sex as a fixed factor, the three morphological traits as covariates and nest and year as random factors. The variance inflation factor (VIF) when the three morphological variables were included in the model was adequate (tarsus length VIF = 1.22, wing length VIF = 1.17 and body mass VIF = 1.58), indicating that the model does not suffer from significant bias due to multicollinearity. Both analyses started with all candidate variables (including interactions with sex) then followed a backward stepwise procedure, testing the exclusion of each variable using the Akaike's information criterion (AIC), deleting the variable that improved the model the most by being excluded and repeating the process until no further improvement is possible. The best model was the one with the lowest AIC value with a difference > 2 from the second best model.

Probability of correct sex classification and probability/age of recruitment

The probabilities of correct sex classification (1 = correct classification and 0 = wrong classification), recruitment (1 = recruited and 0 = unrecruited) and age of recruitment (0 = recruited at age 1 and 1 = recruited at age 2 or 3) in relation to PSR were analysed using generalized linear mixed models (GLMM; binomial error, logit function). In all models sex was included as fixed factor and nest and year as random factors. PSR was arcsine transformed and used as a continuous covariate in the case of recruitment.

PSR and SD

PSR distribution was skewed to the right, bounded $0 \leq \times \leq 1$ and followed a beta distribution (Van Hauwermeiren & Vose, 2009) as follows: $F(x) = 661 \times 0.1 \times \text{beta}$ (X; 1.4822; 0.3179). Thus, sex difference in PSR was analysed using a GLMM with beta error and logit link function structure. Nest and year were included as random factors and sex as a fixed factor. PSR was arcsine transformed when used as an independent variable.

All analyses were performed in SAS 9.3 statistical software (SAS Institute Inc., Cary, NC, USA) and STATISTICA. Discriminant analyses, LMMs and GLMMs in SAS were implemented using 'proc discrim', 'proc mixed' and 'proc glimmix' respectively. All tests were two-tailed and the values are given as means ± SD.

Results

SD and phenotypic integration of sexually dimorphic traits

The degree of SD varied quantitatively among different characters of coloration and size (Table S2). Both plumage pattern coloration (PC1) and the width of the black TTB (PC2) differed significantly between sexes (Table 1). Overall, females presented blacker coloration in wings, wider bars in the rump and tail and less greyness in rumps than males (higher values of PC1), while males presented wider TTBs (higher values of PC2) than females. Furthermore, PC1 was significantly explained by tarsus length (Table 1). Individuals with longer tarsi also showed higher values of PC1, that is, a more female-like phenotypic coloration (Table 1). PC1 was not significantly explained by wing length and body mass and no significant interactions with sex were observed (all $P > 0.2$). PC2 was significantly dependent on wing length and tarsus length (Table 1). Individuals having longer tarsi also showed wider TTBs, with this effect being more pronounced in males as concluded from the significant interaction between sex and tarsus length (Table 1). PC2 was not significantly explained by body mass or the interactions sex × body mass and sex × wing length (all $P > 0.2$).

Phenotypic divergence between sexes: PSR

Except for tarsus length ($P = 0.36$), the remaining variables (wing length, body mass and the five colour traits) contributed significantly to discriminating the sexes

Table 1 Phenotypic integration of chromatism and size in common kestrel fledglings. Results of univariate linear mixed models shows the effect of sex, tarsus length and wing length on principal components of colour (PC1 and PC2) in common kestrel fledglings. Estimates, standard errors (SE), 95% confidence intervals (95% CI), degrees of freedom (d.f.), F and *P* values are shown.

Effect	Estimate	SE	(95% CI)	d.f.	*F*	*P*
PC1						
Sex	1.3304	0.05	(1.43, 1.23)	1,595	613.5	< 0.001
Tarsus length	0.0557	0.02	(1.10, 0.01)	1,595	6.4	0.011
PC2						
Sex	5.7230	2.39	(10.41, 1.04)	1,593	5.8	0.017
Tarsus length	0.1380	0.04	(0.21, 0.06)	1,593	7.1	0.008
Wing length	0.0207	0.00	(0.03, 0.001)	1,593	26.8	< 0.001
Sex × tarsus length	−0.1299	0.05	(−0.03, −0.22)	1,593	7.0	0.008

PC1: initial model AIC = 1439.4, final model AIC = 1402.1 (AIC for the second best model = 1410.6, this model included the nonsignificant term wing length $F = 0.93$, $P = 0.335$); PC2: initial model AIC = 1650.1, final model AIC = 1625.1 (AIC for the second best model = 1630.8, this model included a nonsignificant sex × wing length interaction $F = 2.30$, $P = 0.130$).

(Table 2). The discriminant function correctly classified 89% of cases, being correct in 90.43% of females and 86.65% of males (Fig. 1). This difference was statistically significant (GLMM, $F_{1,596} = 6.4$, $P = 0.012$, estimate ± SE = 0.6514 ± 0.26): males were more likely to be incorrectly classified than females. Similarly, PSR also differed between sexes, with females having a higher PSR (mean ± SD, females = 0.87 ± 0.21, males = 0.83 ± 0.25; GLMM, $F_{1,596} = 5.1$, $P = 0.024$, estimate ± SE = 0.3271 ± 0.11).

PSR and local recruitment

Recruitment probability was higher in males than in females (males = 12.1% and females = 9.5%; Table 3). The relationship between probability of recruitment and PSR differed significantly between sexes as concluded by the significant PSR × sex interaction (Table 3). More female-like females (higher PSR) showed higher probabilities of recruitment than less female-like females (Table 3, Fig. 2). In addition, recruited males tended to be more female-like (lower PSR) than nonrecruited males (Table 3, Fig. 2). When working only with recruited individuals the model showed that the age of recruitment was higher in males than in females (Table 3). More feminized individuals in both sexes tended to recruit at older ages, as concluded by the significant interaction observed between PSR and sex (Table 3, Fig. 3).

Table 2 Summary of the discriminant function analysis for sex of common kestrels.

	Wilks' λ	Partial λ	*F*	d.f.	*P*	Tolerance
Body mass	0.410	0.911	64.07	1,653	0.000	0.887
Wing length	0.380	0.989	7.55	1,653	0.006	0.859
% Wing blackness (WB)	0.391	0.962	25.98	1,653	0.000	0.957
Terminal tail band (TTB)	0.391	0.962	25.68	1,653	0.000	0.831
Tail bars (TB)	0.410	0.917	58.82	1,653	0.000	0.743
Rump bars (RB)	0.380	0.988	7.76	1,653	0.005	0.736
% Rump greyness (RG)	0.486	0.772	192.35	1,653	0.000	0.967

Model: Wilks' $\lambda = 0.375$, $F = 154.95$, d.f. = 7, 653, $P < 0.00001$.

Discussion

Juveniles of many dimorphic bird species resemble adult females rather than adult males (Moreno & Soler, 2011), suggesting that feminized phenotypes may be adaptive at youth. Here, we provide evidence of the fitness benefits of female-like phenotypes in juvenile common kestrels. We found that male and female

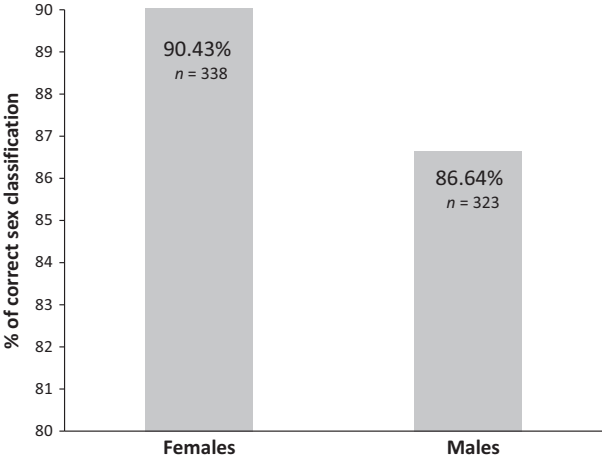

Fig. 1 Results from the discriminant function showing the percentage of correct sex classification in relation to morphological (tarsus length, wing length and body mass) and colour variables (rump greyness, wing blackness, width of black terminal tail bands, width of black superior tail bars and width of black rump bands).

Table 3 Results of the generalized linear mixed model showing the effect of phenotypic sexual resemblance (PSR) and sex on post-fledging recruitment (0 = unrecruited and 1 = recruited; binomial error, dispersion = 0.92, $N = 491$), and recruitment by age in recruited individuals (0 = recruited at age 1 and 1 = recruited at ages 2 or 3; binomial error, dispersion 0.93. $N = 53$). In all models nest and year were included as random factors.

Effect	Estimate	SE	(95% CI)	d.f.	*F*	*P*
Recruitment						
PSR	−0.0127	0.01	(−0.03, 0.00)	1,430	1.7	0.194
Sex	−4.7194	2.04	(−8.73, −0.71)	1,430	5.4	0.021
PSR × sex	0.0585	0.03	(0.01, 0.11)	1,430	5.2	0.023
Only recruited individuals						
PSR	−0.0127	0.02	(−0.06, 0.03)	1,19	3.5	0.077
Sex	−20.3006	9.12	(−39.41, −1.19)	1,19	4.9	0.039
PSR × sex	0.1105	0.11	(0.00, 0.46)	1,19	4.4	0.049

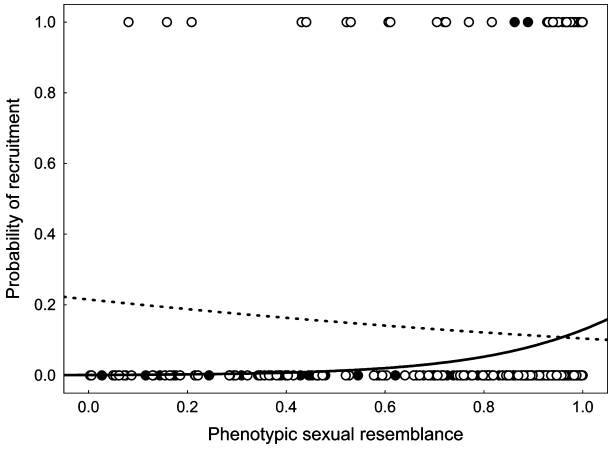

Fig. 2 Relationship between the probability of post-fledging recruitment and phenotypic sexual resemblance (PSR) in males [white dots and dotted line; $y = \exp(-1.295 + (-0.852) * x)/(1 + \exp(-1.295 + (-0.852) * x)$] and females [black dots and solid line; $y = \exp(-6.769 + (4.859) * x)/(1 + \exp(-6.768 + (4.859) * x)$].

fledglings adopt two different phenotypic strategies that integrate dimorphism in size and coloration and strongly influence individual survival.

Early SD from an integrated perspective

At 26 days old, 89% of fledglings express their sex through an integration of at least seven traits including body size and plumage coloration. In both sexes, individuals with longer tarsi showed a more female chromatic appearance. In the case of the TTB, larger individuals presented wider bands. Such phenotypic integration may have evolved as a result of natural selection favouring certain combinations of different quantitative traits rather than a single trait (Pigliucci & Preston, 2004). Similar results have been shown in several taxa, including common kestrels, in which genetic covariation has been identified (Armitage & Siva-Jothy, 2005; Kim *et al.*, 2013; Kim & Velando, 2015), thus reinforcing the evidence that melanin-based coloration covaries with other phenotypic traits such as size, body mass, morphology, physiology and behaviour. Our results also evidence that morphological traits can be differently integrated with colour traits within sexes as it has been found in this and other bird species (Kim *et al.*, 2013; Fargallo *et al.*, 2014). In general, larger, but not heavier, individuals showed more feminized chromatic phenotypes, indicating a correlation between chromatism and size in which individuals of intermediate sexual chromatism (11%) were also intermediate in body measurements. Interestingly, these intermediate sexual phenotypes were more frequently observed in males than in females.

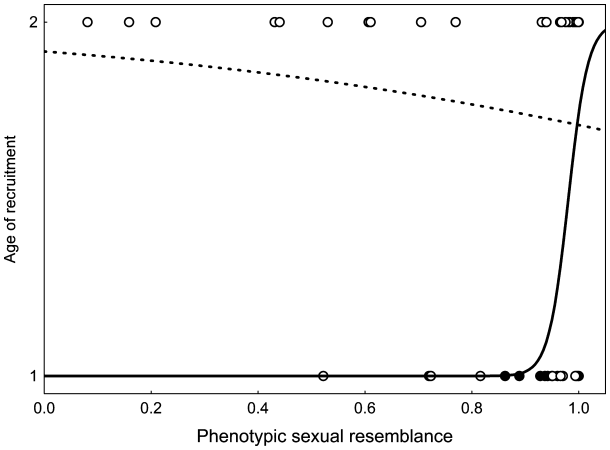

Fig. 3 Relationship between the probability recruitment at ages 1 and 2 and phenotypic sexual resemblance (PSR) in males [white dots and dotted line; $y = \exp(-2.405 + (-1.512) * x)/(1 + \exp(2.405 + (-1.512) * x)$] and females [black dots and solid line; $y = \exp(-53.317 + (54.380) * x)/(1 + \exp(-53.317 + (54.380) * x)$]. Note that values 1 and 2 in the '*y*' axis correspond with values 0 and 1, respectively, in the statistical model.

Fitness benefits associated with phenotypic sexual resemblance

The main finding of our study is that individual survival is related to the extent to which an individual phenotype resembles the sex to which it belongs (PSR). In common kestrels it has been reported that most individuals do not exceed 50 km of dispersal distance (Terraube *et al.*, 2015), therefore, leading local recruitment as a good estimator of individual survival. Our results show that although more feminized females achieved higher survival, the higher values in males were exhibited by less masculinized individuals, suggesting a

selection on juvenile males towards more feminized phenotypes. In agreement with this, male fledglings showed lower PSR values than females and a higher proportion of incorrect sex classifications based on phenotype. Moreover, age of recruitment was also influenced by PSR in both sexes with more feminized phenotypes recruiting at older ages. A longer period of time and more kestrel cohorts are required to complete data on the individual lifetime reproductive success of our population. In other species, however, recruitment at early ages can influence individual fitness positively (Charmantier *et al.*, 2006; Dugdale *et al.*, 2011; Kim *et al.*, 2011; Zhang *et al.*, 2015), negatively (Auld & Charmantier, 2011; Kim *et al.*, 2011) or have no effect (Froy *et al.*, 2013; Pardo *et al.*, 2013). Such data in our population would give us information on whether different reproductive tactics could be phenotypically identified during the fledging period and how adult SD is derived from juvenile phenotypes.

Two classical hypotheses highlight the fitness benefits associated with a female-like appearance, although only one has demonstrated this phenotype to affect fitness (Berggren *et al.*, 2004). The first posits that more cryptic female-like phenotypes increase survival by reducing predation risk (Selander, 1965), which may be especially significant for juveniles during their first winter (Rohwer & Butcher, 1988). The second states that when male–male competition involves aggressive interactions, female-like individuals avoid agonistic behaviour of males and gain access to resources during winter, thus increasing the probability of survival (Berggren *et al.*, 2004). These two hypotheses are not mutually exclusive and they may both explain our results. Although the winter biology of common kestrels has been scarcely investigated, they are known to be territorial and aggressive against conspecific intruders during this period (Wiklund & Village, 1992). Information on their sexual hierarchy of dominance when competing for hunting territories is also lacking. In the closely related American kestrel *Falco sparverius*, it has been recorded that adult females (which are also the larger and more cryptic sex) obtain better hunting territories than males and maintain them over the winter, indicating a higher capacity than males in scramble competition (Ardia & Bildstein, 1997). Also, in the common kestrel, female nestmates show a capacity superior to males when fighting for food (Fargallo *et al.*, 2003). We posit that if female phenotypes signal higher competitive faculties, juveniles with female-like phenotypes may benefit when occupying hunting territories during winter, since diminishing costly interactions via signalling should be adaptive (Rohwer, 1982).

Is early SD adaptive in kestrels?

Since in both sexes more feminized phenotypes achieved higher survival, why does early dimorphism exist in kestrels? Sexual dichromatism in juveniles has been scarcely studied and its adaptive significance is unknown. It has been suggested to play a signalling role in offspring–parent interactions to gain parental investment (Johnsen *et al.*, 2003), or within juvenile flocks to mediate encounters between unfamiliar individuals (Tringali & Bowman, 2012). Although appealing, neither of these mechanisms may be at work since both predict individual variation in the expression of coloration, but not sexual dichromatism. Moreover, there is no evidence supporting differential parental investment related to offspring sexual phenotype in common kestrels (Fargallo *et al.*, 2003; Laaksonen *et al.*, 2004; Vegara & Fargallo, 2008), and this species does not form juvenile flocks. In relation to size, smaller males are more efficient hunters and are preferred by kestrel females (Hakkarainen *et al.*, 1996). However, before small size can play a favourable role in adult mate selection and foraging, males must compete for territories and survive as juveniles during winter. A female-like appearance could provide competitive benefits to males during this period. Therefore, males would face a trade-off between enhancing survival by exhibiting more feminized phenotypes (larger size) or enhancing reproductive success by producing less feminized phenotypes (smaller size).

Interestingly, some chromatic characteristics of the fledgling male phenotype are similar to that expressed by adult females. For example, male fledglings showed wider TTBs than female fledglings and greyer coloration on the rump. These characters are associated with indicators of quality in fledglings and adult females (Vegara & Fargallo, 2008; Vergara *et al.*, 2009). Furthermore, smaller males (more male-like fledglings) are those showing a higher chromatic similarity (greyer rumps) with adult females. Therefore, we propose that an adult female plumage appearance could provide benefits to smaller males within this hierarchical system of dominance. The question remains as to why juvenile females do not adopt this phenotypic strategy. The chromatic phenotype produced by adult females and juvenile males may entail costs. In common kestrels, the expression of two heritable characters, grey coloration and immune response, are negatively correlated (Kim *et al.*, 2013). Selection on the immune response is stabilizing, with individuals showing intermediate immune responses selected, however in the case of rump coloration the selection was disruptive, favouring the greyest and brownest phenotypes, although the greyest individuals with the lowest immune responses were those with the highest survival rates (Kim *et al.*, 2013). We therefore propose that larger and more competitive female fledglings do not incur the costs of producing expensive chromatic signals of quality (grey coloration), since size is in itself a signal that can be evaluated by individuals in contest situations (Maynard Smith & Parker, 1976). On the other hand, male fledg-

lings may adopt two different strategies: being larger and browner (more fledgling female-like) or being smaller and greyer (more fledgling male-like). Although the benefits of the former strategy seem to be a trend for better winter survival, the benefits of the latter strategy remain to be investigated. We propose that males adopting the second strategy might obtain benefits during reproduction due to female preference for smaller males with higher hunting abilities.

In summary, our study raises the possibility that outside of sexual selection, selective pressures acting in youth may constrain the sexual divergence of adult traits due to a trend to increase fitness through more feminized phenotypes at juvenile stages. We propose that variation in adult SD may be influenced by differential phenotypic strategies adopted in early life. This is therefore an essential perspective from which to focus investigations on SD that highlights the importance of the ontogenetic studies on size and chromatism. Moreover, winter behaviour in the acquisition of resources including territories and the change in adult SD over time at the population level are two main questions that should be investigated to determine the true influence of juvenile phenotypes on the evolution of SD.

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Supporting information

Additional Supporting Information may be found in the online version of this article:

Table S1 Factor coordinates of principal components analysis on the five colour variables measured in common kestrel fledglings.

Table S2 Results of the LMM showing sexual differences in body measurements and plumage coloration (mean ± standard error) in fledglings after controlling for year and nest.

Figure S1 Colour sexual dimorphism in common kestrel adults (a) and fledglings (b).

Figure S2 Colorimetric variables of fledglings: (a) - rump greyness, (b) - (1) width of black terminal tail band, (2) width of black tail bars, (3) width of black rump bars and (c) - wing blackness.

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