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Digit ratios, secondary sexual characters and condition in barn swallows *Hirundo rustica*

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The second and fourth digit length ratio (2D:4D) is sexually dimorphic in many vertebrates. This ratio has been suggested to provide an estimate of steroid levels encountered during prenatal development, which may have organizational consequences for physiology and behavior of adults. However, recent studies showed that the relation between digit ratio and steroids seems inconsistent and may vary among species and populations. We tested the hypothesis that digit ratios would be correlated with the expression of secondary sexual characters, using the barn swallow (*Hirundo rustica*) as a model system. This was done by testing whether variation in 2D:4D ratio was correlated with tail length and features of song, which are important secondary sexual characters positively correlated with circulating steroid concentration in adult birds. Furthermore, we examined the prediction that male and female digit ratios would correlate with body mass in an antagonistic way. There was no significant sexual dimorphism in digit ratio, which may be due to low levels of sexual selection in this population. Adult right 2D:4D ratio was negatively linked to tail length but not to male song output. Moreover, right 2D:4D ratio was negatively correlated with body mass in male and positively in females. These results are consistent with high digit ratios reflecting low levels of testosterone in this bird species. *Key words:* bird song, digit ratio, *Hirundo rustica*, morphology, secondary sexual characters. *[Behav Ecol]*

The hormonal environment experienced during prenatal development has important consequences for future adult phenotype (vom Saal 1989; Dufty et al. 2002; Groothuis et al. 2005). Early androgen exposure has long-lasting consequences for sexual differentiation of physiology, brain, and behavior of both sexes (Groothuis et al. 2005), and it can shape the phenotype within one sex (Clark and Galef 1998). A marker of prenatal hormonal exposure would thus be a powerful tool for studying the consequences on life history of prenatal environment. Several recent studies suggest that the 2D:4D ratio (relative length of the second and fourth fingers) is a good candidate in humans and may be a pointer to prenatal steroid levels. There is indeed correlational evidence that sex steroids differentially stimulate prenatal growth of the 2nd and 4th digits (Manning 2002; Lutchmaya et al. 2004), and men commonly have lower 2D:4D ratios than women (Phelps 1952; Manning et al. 1998). An increasing number of studies use this marker to examine the role of early steroids in sexual attractiveness (Firman et al. 2003; Neave et al. 2003), health (Brown, Hines, et al. 2002; Walder et al. 2006), or fertility (Manning et al. 1998; Koehler et al. 2004).

In nonhuman species, dimorphism in digit ratio has been reported in some, but not all species, and the sex difference varies among species (even within a taxon) and between populations. Like in humans, 2D:4D finger ratio (forelimb) tends to be lower in males than females in mice (Brown, Finn, Breedlove, et al. 2002), but this dimorphism is reversed in baboons (Roney et al. 2004) and varies between 2 lizard species (Rubolini et al. 2006). A sexual dimorphism on hindlimb digit ratio opposite to that recorded in human fingers but in the same direction than human toes (McFadden and Shubel 2002), has also been reported in bird species, but it is inconsistent among studies (Burley and Foster 2004; Forstmeier 2005; Romano et al. 2005; Navarro et al. 2007; Saino et al. 2007). Moreover, Burley and Foster (2004) found that 2D:4D increased with egg laying order in the zebra finch, *Taeniopygia guttata*, suggesting that it may be sensitive to variation in yolk hormonal levels (but see Forstmeier 2005). Experimental studies on pheasants, *Phasianus colchicus*, found no effect of testosterone injection on offspring 2D:4D but an impact on female 2D:3D ratio (Romano et al. 2005), whereas injection with estradiol induced a significant decrease in male 2D:4D (Saino et al. 2007). In zebra finches, 2D:4D correlated positively with female preference for an attractive male trait (Burley and Foster 2004) and with male song rate, a secondary sexual character (Forstmeier 2005). Similarly, 2D:4D was positively with male visible badge size in house sparrows, *Passer domesticus* (Navarro et al. 2007).

These recent studies suggest that digit ratios in birds may be affected by sex steroids. However, no consistent pattern appears across species and populations, and empirical data are needed given that no clear predictions are currently possible. There is still very little research on the relationship between 2D:4D ratio, sexual characters, and physiology in nonhuman species. It is the aim of the present study to determine whether 2D:4D ratio may be a marker of the organizational effect of prenatal testosterone levels in a passerine bird, the barn swallow, *Hirundo rustica*, using extensive information obtained from a natural population in the field. First, we investigated whether the digit ratio was sexually dimorphic. Second, we investigated whether digit ratio is correlated with adult male and female phenotypes and whether this relation is consistent with the hypothesis of digit ratio being related to prenatal testosterone. This included examining the correlation between song features of reproducing male barn swallows

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and 2D:4D ratios of left and right feet. Bird song is an important sexually selected character (Darwin 1871; Andersson 1994), and its production is linked to circulating levels of testosterone (Catchpole and Slater 1995; Schlinger 1997; Pinxten et al. 2002). Furthermore, Ward et al. (2001) suggested that within-clutch variation in prenatal testosterone in canaries, *Serinus canaria*, has consequences for adult song quality. We also determined whether the expression of an other secondary sexual character, tail length of male barn swallows (Møller 1994a), was predicted by digit ratio and whether this relationship between digit ratio and tail length differed between the sexes. If testosterone level and digit ratio development are related, digit ratio is expected to be related to tail length in the same way in both sexes because testosterone influences the expression of both male and female secondary sexual traits (De Ridder et al. 2002; McGraw 2006). Finally, we tested whether body condition was related to digit ratio in a sex-antagonistic way because high levels of prenatal testosterone is predicted to be beneficial for sons but not for daughters in this species (Saino et al. 2006). These predictions were tested for both the right and the left feet, as some studies have found the expected effect on the right but not on the left 2D:4D ratio (reviewed in Manning 2002).

METHODS

General field procedures

The study was carried out in March–April 2004, in a farm near Badajoz (38°50′N, 6°59′W, Extremadura, Spain) on a population of barn swallows that had been subject to a long-term study. We studied a total of 89 adult barn swallows (44 males and 45 females), and we recorded digit ratios and morphological characters from all of these but were, for logistic reasons, only able to obtain detailed information on song for a total of 34 males.

All adults were captured with mist nets at dawn and provided with a numbered aluminum ring and a unique combination of color plastic rings. Birds were also marked on the belly with water-resistant pens for focal identification. For all birds, we recorded body mass with a Pesola spring balance to the nearest 0.5 g, at first capture that occurred between arrival and 1 week before the first egg was laid. We measured tail length (as the mean of the left and right outermost tail feathers) with a ruler to the nearest 0.5 mm.

We identified all individuals and the composition of pairs from observations of color-ringed birds. All nests were visited regularly, at least once per week, and nestlings were ringed when 14 days old.

Digit measurements

Digit measurements were performed by C.N., by gently pressing one foot on a horizontal sheet of paper over a cardboard. Once the toes were straight, the positions of the extremity of the toes and the point between 2 toes were marked by a hole in a sheet of paper with a thin insect pin. Each foot was recorded twice. All these recordings were made blindly with respect to tail length and song recordings, thereby preventing intentional or unintentional bias in relationships among variables. D2 and D4 were subsequently measured on the sheet of paper with a digital caliper to the nearest 0.01 mm. The 2 measurements were subsequently averaged and used in the analyses.

Recordings and song analysis

Singing performance of 34 males that had settled in a breeding territory was recorded. Recordings were carried out in the morning (from 07:30 AM to 12:30 PM) close to the nest or on outside perching places, using shotgun microphones (Audio Technica AT835B) connected to a Marantz PMD101 cassette recorder.

Male barn swallows display successions of strophes (song units separated by a pause) of 2 different types (see Galeotti et al. 1997). Strophe type A is composed of varied and complex syllables arranged in various sequences, whereas strophe type B consists of a succession of simple contact calls. For each male, we estimated syllable repertoire size of type A strophes, as did Galeotti et al. (1997). For other song variables, we analyzed all strophes recorded (types A and B).

We only considered complete strophes, which are characterized by the presence of a terminal rattle (a rapid succession of impulses sung at high frequency). Song was analyzed with the computer program Avisoft-SASlab Pro (Specht 1998) (bandwidth: 200 Hz; frequency resolution: 80 Hz; time resolution: 32 ms). For each male, we measured the following variables:

1. Strophe duration (mean duration of all complete strophes, to the nearest 0.001 s).
2. Syllable repertoire size of type A strophes. Syllable types were classified visually in 19 categories by A.N.D. A second inspection of all strophes was done blindly with respect to the first inspection and gave exactly the same results. Repertoire was estimated by the count of syllable types in more than 3 strophes, which is expected to give a reliable estimate of the real repertoire size (Galeotti et al. 1997).
3. Rattle duration (mean duration of the terminal rattle of all strophes, to the nearest 0.001 s).
4. Song rate (proportion of time during which the male sang within half an hour of presence). For 20 males, 2 measurements were done on 2 different mornings.

The mean number of complete strophes analyzed per individual was 22.7 ± 9.7 standard deviation (SD). Multiple estimates of the song variables of individuals were averaged for the subsequent analyses.

Statistical analyses

Analyses were performed using SAS (1999). Sample values are given as mean ± SD. The distributions of the variables did not differ significantly from normality using the Kolmogorov–Smirnov test.

The repeatability of measurements was assessed with analysis of variance procedures with individual as a factor. Digit ratio was highly repeatable (right: $F_{80,79} = 2.69$, $R^2 = 0.73$, $P < 0.0001$; left: $F_{79,77} = 2.66$, $R^2 = 0.73$, $P < 0.0001$). Song rate repeatability was evaluated by comparing measurements from 2 observation sessions on different mornings ($F_{19,20} = 21.41$, $R^2 = 0.95$, $P < 0.0001$). Repeatability of strophe and rattle duration was estimated among days (strophe duration: $F_{33,81} = 2.01$, $R^2 = 0.54$, $P = 0.01$; rattle duration: $F_{33,82} = 2.92$, $R^2 = 0.64$, $P = 0.0002$). As syllable repertoire size was only measured once, repeatability was not estimated for this variable.

We analyzed the link between variables with Pearson correlations. Multiple regression analysis, with a stepwise backward procedure of elimination of nonsignificant independent terms ($P > 0.05$), was used to describe variation of male song traits and adult biometrics (body mass and tail length). Explanatory variables were right and left 2D:4D and sex (the latter only for the analysis of adult biometrics). Second-degree interactions were included in the models. Final models only contained significant effects and main effects involved in significant interactions (McCullagh and Nelder 1989). A few missing observations for some of the birds explain slight variation in sample sizes among tests.

RESULTS

Strophe duration and syllable repertoire size were positively correlated ($r = 0.56$, $P = 0.0006$, $N = 34$). We found no other significant correlation between male song features and tarsus length, tail length and body mass, respectively, nor did we find any between female body mass and tail length.

Sexual dimorphism

There was no significant sexual dimorphism in digit ratios (right: $t = 0.27$, $P = 0.79$, $N = 85$; female: 1.060 ± 0.069; male: 1.053 ± 0.081 [mean ± SD] and left: $t = -0.32$, $P = 0.75$, $N = 86$; female: 1.094 ± 0.075; male: 1.096 ± 0.076). On the other hand, right and left digit ratios were significantly different (females: $t = -2.88$, $P = 0.006$, $N = 44$; males: $t = -2.46$, $P = 0.018$, $N = 42$) but positively correlated ($r = 0.23$, $P = 0.031$, $N = 86$). Variance did not differ significantly between right and left feet (females: $F = 1.18$, $P = 0.58$, $N = 44$; males: $F = 1.22$, $P = 0.53$, $N = 41$).

Male song, adult biometrics, and digit ratios

We found no significant relationship between digit ratios and male song output (Table 1).

As expected, adult tail length differed between sexes ($F_{1,83} = 93.28$, $P < 0.0001$; Table 1), with males having longer tails than females, but it was also significantly negatively related to right 2D:4D ($F_{1,83} = 8.44$, $P = 0.005$). The final model was highly significant ($F_{2,83} = 51.74$, $R^2 = 0.55$, $P < 0.001$; Figure 1). The interaction between right digit ratio and sex was not significant ($F_{1,83} = 0.67$, $P = 0.42$), but the slope tended to be slightly higher for males.

Adult body mass was related to the interaction between sex and right 2D:4D ($F_{1,84} = 8.24$, $P = 0.005$; Table 1). The right 2D:4D ratio was significantly negatively correlated with body mass before reproduction in males but positively related to body mass in females (Figure 2). We found no significant relationship with left digit ratio (Table 1).

DISCUSSION

The main findings of this study were that 1) sexual size dimorphism in 2D:4D digit ratios was not statistically significant; 2) right but not left digit ratio was significantly related to body mass before reproduction in a sex-antagonistic way; and 3) a major secondary sexual character (tail length) was negatively related to right digit ratio in adult barn swallows. Measurements of yolk steroid levels are needed to assess whether these relationship resulted from a hormonal control of adult traits and digit ratio simultaneously. However, our results are consistent with the hypothesis that early androgen levels affect 2D:4D negatively in this population, which is contrary to most results in other bird species previously studied.

Dimorphism in digit ratio

Sexual dimorphism of 2D:4D has been found in some species, but not in others, and sexual differences are not biased in the same direction in all species. This implies the evolution of either quantitative sex differences in hormone levels or sensitivity to sex steroids or different sex-specific development of digit ratio.

We found no significant sex difference in digit ratios in the barn swallow, which were very similar in males and females. In our barn swallow population, dimorphism in tail length, a secondary sexual character, is also relatively small compared with other populations of the same species (Møller 1995), and so the intensity of sexual selection as reflected by the frequency of extrapair paternity is relatively low (Møller et al. 2003). This may be due to the high prenatal testosterone concentration in our Spanish study population (in comparison with an Italian barn swallow population, Gil et al. 2006; Saino et al. 2006), which may induce development of relatively similar tail length in both sexes. As we found that digit ratio was negatively correlated with tail length, the absence of dimorphism in digit ratio may be related to the low level of tail length dimorphism. High prenatal testosterone may stimulate similar tail length and digit ratio in this population, or the development of tail length and digit ratio may be controlled by the same pleiotropic genes.

Right and left digit ratios

The relationships between 2D:4D ratio and adult phenotype was only observed for digit ratios of the right, but not the left foot, and the right and left ratios were significantly different,

Table 1
Models of the relationship between secondary sexual characters and body mass, respectively, and digit ratios, sex, and their interactions

	Independent effect				Model result				
Dependent variable	Term	SS	F	P	Degrees of freedom	Res SS	R^2	F	P
Strophe duration	R 2D:4D	0.133	0.65	0.43	2, 30	6.105	0.02	0.72	0.33
	L 2D:4D	0.005	0.02	0.88					
Syllable repertoire	R 2D:4D	2.682	0.82	0.37	2, 30	97.921	0.04	0.61	0.55
	L 2D:4D	1.382	0.42	0.52					
Rattle duration	R 2D:4D	0.0001	0.07	0.80	2, 30	0.067	0.01	0.10	0.91
	L 2D:4D	0.0003	0.14	0.71					
Song rate	R 2D:4D	0.052	1.91	0.18	2, 28	0.766	0.09	1.39	0.27
	L 2D:4D	0.022	0.81	0.38					
Tail length	R 2D:4D	307.582	7.42	0.008	3, 82	3399.922	0.56	51.74	<0.0001
	L 2D:4D	5.649	0.14	0.71					
	Sex	3830.641	92.39	<0.0001					
Body mass	R 2D:4D	0.431	0.29	0.59	4, 80	119.041	0.24	6.43	0.0002
	L 2D:4D	0.280	0.19	0.67					
	Sex	9.957	6.69	0.011					
	R 2D:4D × sex	12.244	8.23	0.005					

In addition to right (R) and left (L) 2D:4D, only significant independent terms are reported. SS, sum of squares; Res SS, residual sum of squares.

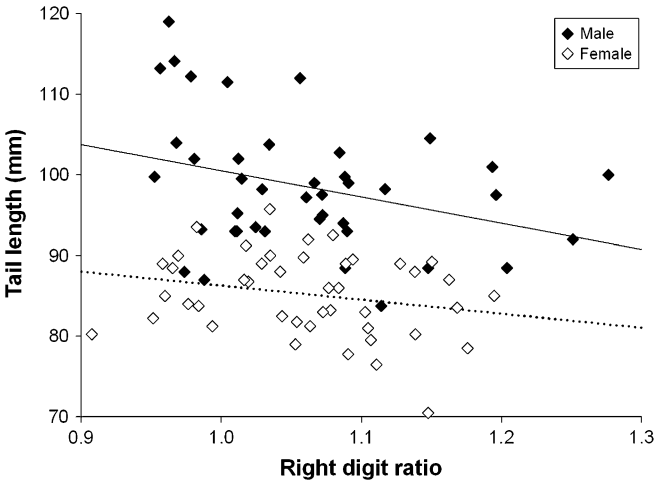

Figure 1
Tail length (mm) in relation to right 2D:4D ratio of male (open squares) and female (filled squares) barn swallows. The regression line for males is $y = -32x + 133$, $R^2 = 0.12$ and for females it is $y = -17x + 104$, $R^2 = 0.06$.

but also weakly positively correlated. Digit ratio is consistently more dimorphic on the right than the left side in humans (Manning et al. 1998; McFadden and Shubel 2002), baboons (McFadden and Bracht 2003), mice (Brown, Finn, Breedlove, et al. 2002), and zebra finches (Burley and Foster 2004). Moreover, the right digit ratio is a stronger predictor of physiological traits than the left one in a number of studies (Brown, Finn, Cooke, and Breedlove 2002; Csatho et al. 2003; Manning, Trivers, et al. 2000; Williams et al. 2000). Absence of difference of variance between right and left feet does not suggest a difference in degree of constraint. This asymmetry between right and left feet could be due to a higher sensitivity to steroids of the right side (Manning 2002; McFadden and Shubel 2002; Williams et al. 2000), being a structural consequence of other functional asymmetries, like androgen receptor asymmetry in the cerebral cortex leading to cerebral lateralization (Sandhu et al. 1986). These findings stress the importance for further work to separate left and right sides in analyses rather than using the mean of right and left 2D:4D.

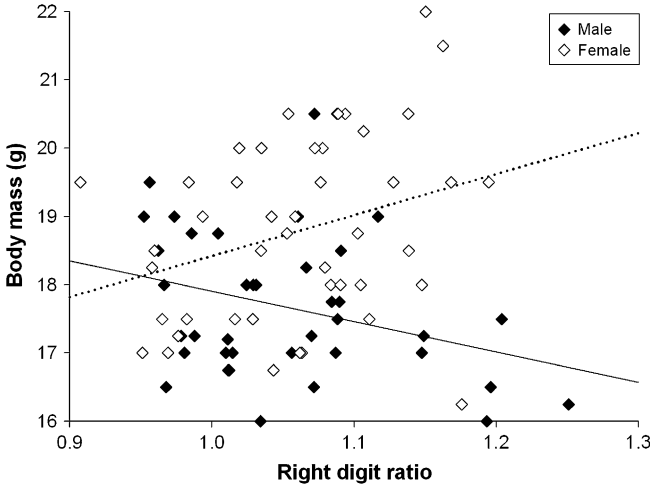

Figure 2
Body mass (g) before reproduction in relation to right 2D:4D ratio of male (open squares) and female (filled squares) barn swallows. The regression line for males is $y = -4.4x + 22.3$, $R^2 = 0.09$ and for females it is $y = 6.0x + 12.4$, $R^2 = 0.09$.

Secondary sexual characters and digit ratio

The right digit ratio of adults was negatively related to tail length (Figure 1). This suggests that digit ratio and adult phenotype develop under the same hormonal control or the same pleiotropic genes. Male tail length in the barn swallow is involved in female mate choice (Møller 1988), reliably signaling viability (Møller 1994a). Male secondary sexual characters develop under prenatal testosterone control in general (Groothuis et al. 2005), and early testosterone exposure of males has been shown to influence subsequent female preferences (Clark and Galef 1998). Similarly, adult male house sparrows, *P. domesticus*, injected with testosterone while in the egg produced larger sexual badges when adults than controls (Strasser and Schwabl 2004). Our result is therefore consistent with the hypothesis that early sex steroid levels affect future development of tail length and 2D:4D simultaneously in the barn swallow. The direction of the relationship found here between male secondary sexual character and digit ratio was opposite to that found in zebra finches (Forstmeier 2005) and house sparrow (Navarro et al. 2007). This suggests a high degree of variability among bird species in genetic determinism of digit development or in the interaction between genes and the environment.

Right 2D:4D was also negatively related to tail length in females, which is expected if this character develops under the control of testosterone in both sexes. Relatively long female tails in our population may be a by-product of a high general level of testosterone found in this population (Møller 1995; Gil et al. 2006).

Digit ratios were not significantly related to male song features. Bird song develops and is maintained under the action of testosterone (Catchpole and Slater 1995), and androgens also mediate early organizational effects on the brain that influence adult song production (Gurney and Konishi 1980). The lack of correlations between 2D:4D ratios and song output in the barn swallow may be due to a problem of statistical power that prevents detecting a slight influence of prenatal hormones, or it may represent a true absence of an effect.

Body mass before reproduction and digit ratio

Body mass was predicted by 2D:4D ratio in a sex-antagonistic manner in the barn swallow. Body mass is a reliable predictor of important fitness components in male barn swallows (Møller 1994b). For example, Spanish barn swallow males in prime condition have few extrapair offspring in their nest (Møller et al. 2003). In female birds, body mass before reproduction is positively related to egg size and brood size (Ankney et al. 1991; Sedinger et al. 1995), as in the barn swallow (Møller 1993).

Male body mass correlated negatively with digit ratio, whereas the correlation was positive in females (Figure 2). Other studies have found similar sexual antagonistic correlations between 2D:4D and adult health, attractiveness, or fertility. For example, in humans, low digit ratios are related not only to high attractiveness and high sperm production in men but also to high efficiency of the cardiovascular system and blood glucose control (Manning, Barley, et al. 2000; Manning 2002). In contrast, in women, low digit ratios are related not only to low attractiveness and reduced fertility but also to increased probability of cardiovascular disease and elevated incidence of diabetes (Manning, Barley, et al. 2000; Manning 2002;). These patterns are consistent with the hypothesis that prenatal sexual hormones affect both digit

ratio and adult condition and that they have sexually antagonistic effects on adult condition. Several studies have found direct or indirect evidence of antagonistic effects of prenatal hormones on offspring phenotype (but see von Engelhardt et al. 2006). In Mongolian gerbils (*Meriones unguiculatus*), female embryos benefit from being positioned during development between 2 female embryos, in a low testosterone environment. In contrast, males that have developed between 2 brothers, in high testosterone conditions, are preferred as mates over males that have developed between 2 sisters (Clark and Galef 1998). Likewise, an experimental study in the barn swallow found that male nestlings from eggs injected with androgens were larger, whereas their female siblings were smaller than controls, providing direct evidence for sex-antagonistic effects of testosterone in our study species (Saino et al. 2006).

Conclusion

Our findings on digit ratios in the barn swallow are consistent with the hypothesis that digit ratio reflects early exposure to sex steroids in the egg. Digit ratio in birds may be a useful tool for investigating the effects of the prenatal steroid environment, although patterns differ among bird species. The correlation between sexually selected traits in adults and right digit ratio may be the result of the prenatal sex steroid environment or control by pleiotropic genes during development. Under this hypothesis, general body condition of adults measured as body mass may be influenced by the hormonal environment encountered during early development.

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