

Digit ratios (2D:4D), secondary sexual characters and cell-mediated immunity in house sparrows *Passer domesticus*

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Abstract Homeobox genes regulate development of digits, and it has been suggested that the ratio of length of second to length of fourth digit reflects such genetic effects in a sex-specific manner. We show that digit ratios in the sexually dichromatic house sparrow *Passer domesticus* differ between sexes, with males having higher ratios than females, and that individuals produce consistent ratios on the two feet. If Homeobox or other genes had pleiotropic effects on development of digits, behavior, and physiology of males and females, we would expect secondary sexual characters and immunity to be related to digit ratio in a sex-specific manner. The size of the visible part of the black badge in February (a secondary sexual character), but not total badge size, was positively correlated with digit ratios, suggesting that males with more male-like digit ratios had larger visible badges. Because of sex-specific effects of development on secondary sexual characters and immunity, we predicted sex-specific differences in immune

response to be related to digit ratio. House sparrows with large digit ratios had weaker T cell-mediated immune response than individuals with small digit ratios, particularly in females, implying that females with more male-like digit ratios had weak immune responses. These findings are consistent with the hypothesis that early development, as reflected by digit ratios, and genetics affect the expression of adult characters that are supposedly strongly contributing to fitness.

Keywords Development · Homeobox genes · Immunity · Sexual selection

Introduction

Early maternal effects have attracted considerable attention because of their potential influence on subsequent development (Mousseau and Fox 1998). The fact that females can manipulate early embryonic environment and thereby influence conditions under which their offspring develop (e.g., Gil et al. 1999; Dufty et al. 2002; Groothuis et al. 2005b) raises the possibility that females can fine-tune the developmental environment for their offspring.

A particularly important family of genes that plays a crucial role in embryonic development is Homeobox (HOX) genes (Goodman and Scambler 2001), with different families of HOX genes affecting different suites of characters. The development of fingers and toes and the urogenital system occurs under the influence of HOXA and HOXD genes (see reviews in Mortlock et al. 1996; Kondo et al. 1997), with mutations in these genes producing phenotypes inferior in terms of viability (Muragaki et al. 1996; Mortlock and Innis 1997; Peichel et al. 1997). Because both digits and gonads develop under the influence

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of the same HOX genes, morphology of digits and their ratios may be directly related to gonadal function (Manning et al. 1998; review in Manning 2002). The ratio of the length of the second and the fourth digit (2D:4D) shows significant sexual size dimorphism in humans, nonhuman primates, mice, and birds (Ecker 1875; Brown et al. 2001, 2002a,b; Manning 2002; Burley and Foster 2004; Roney et al. 2004), although others have failed to show a statistically significant sexual size dimorphism (Forstmeier 2005; Romano et al. 2005). While studies of mammals consistently have shown lower values in males than in females (Ecker 1875; Brown et al. 2001, 2002a,b; Manning 2002; Roney et al. 2004), studies of birds have shown higher values in males than in females (Burley and Foster 2004; Møller, unpublished data) or no significant difference (Forstmeier 2005; Romano et al. 2005). Males with more male-like digit ratios have been shown to have higher levels of circulating testosterone than males with less exaggerated digit ratios in both humans and baboons *Papio cynocephalus* (Manning et al. 1998; Roney et al. 2004). Manning et al. (1998) have suggested that the sex difference in 2D:4D may reflect an interaction between gonad and digit differentiation. If the 2D:4D ratio is established already in utero, this ratio may be modified due to an interaction between effects of HOX genes, maternal effects, and prenatal levels of testosterone produced by fetal testes.

Maternal effects can have extensive influence on development and organization of entire suites of phenotypic traits in adults (Mousseau and Fox 1998; Dufty et al. 2002). Such programming effects of early maternal hormone exposure could influence the development of secondary sexual characters and immunity, with antagonistic effects on these two suites of characters (Dufty et al. 2002; Groothuis et al. 2005b). These effects also extend to digit ratios that have been hypothesized to develop under the influence of the maternal hormone environment (review in Manning 2002), as supported recently by experimental evidence from birds (Saino et al. 2006b). Such maternal effects and their organizational role could create positive covariation between digit ratios, on one hand, and other phenotypic characters such as secondary sexual characters and immunity, on the other, because all these characters may develop under the organizational influence of the same maternal hormones. If such relationships could be demonstrated, this would enhance the possibility of studying the long-term consequences of maternal effects by measurement in adults of digit ratios that would have developed in response to the maternal hormone environment created in the egg.

Many secondary sexual characters and sexual behaviour develop under the influence of sex steroids, and size differences between sexes and other kinds of sexual differentiation may appear already during early development (Dufty et al. 2002; Groothuis et al. 2005b; Rubolini et

al. 2006). Usually, secondary sexual characters are absent in juveniles and develop immediately before reproduction. This does not preclude early maternal effects from having profound effects on development of secondary sexual characters later in life (e.g., Manning et al. 1998). Thus, we predicted a positive relationship between expression of secondary sexual characters and digit ratios.

Maternal hormones may produce antagonistic effects on offspring of the two sexes because the benefits of a specific developmental environment for sons will be traded against the detrimental effects of such an environment for daughters (Groothuis et al. 2005b; Saino et al. 2006a). Immunity may be negatively affected by sex steroids, while secondary sexual characters are positively affected by sex steroids (Folstad and Karter 1992; Martin 2000; Roberts et al. 2004), potentially creating a conflict of interest between sons and daughters in terms of optimal developmental environment for immunity and secondary sexual characters. Some experimental studies have shown evidence of negative effects of elevated testosterone in eggs on subsequent immunity in offspring (Andersson et al. 2004; Groothuis et al. 2005a; Uller and Olsson 2003), while others have not (Rubolini et al. 2006; Saino et al. 2006a). If such a mechanism existed, we would expect early exposure to high levels of sex steroids to impair subsequent development of immunity, particularly in females which is the sex supposedly impaired by exposure to high levels of testosterone, thereby partially accounting for sex-specific associations between digit ratios and disease condition (Manning et al. 1998; Manning 2002). Because females of oviparous animals allocate hormones to eggs (e.g., Gil et al. 1999; Groothuis et al. 2005b), such allocation could negatively affect the ability to subsequently raise efficient immune responses once reaching adulthood.

Many studies have suggested that digit ratios in humans predict ability to perform in sports, music, and other activities in a sex-specific way (review in Manning 2002). Burley and Foster (2004) showed that digit ratio varied with laying order and predicted female mate preferences in the zebra finch *Taeniopygia guttata*, while a second study on the same species could not repeat several of these relationships, although it did show that song rate was related to digit ratio (Forstmeier 2005). Furthermore, digit ratio was related to spur length in the pheasant *Phasianus colchicus* (Rubolini et al. 2006). In addition, various disease conditions are linked to digit ratio in a sex-specific manner in humans (review in Manning 2002), including breast cancer (Manning et al. 2001), age at myocardial infarction (Manning and Bundred 2001), and autism (Manning et al. 2001). Manning et al. (1998) suggested that the sex difference in 2D:4D may reflect an interaction between gonad and digit differentiation. This interpretation rested on the assumption that digit ratios would develop under the

influence of developmental genes such as HOX genes, but also under the influence of maternal endocrine environment during gestation and the production of hormones by the developing embryo. However, quantitative genetic studies on humans and birds have suggested that there is a strong effect of quantitative genetics on digit ratios (Ramesh and Murty 1977; Forstmeier 2005; Paul et al. 2006) accounting for 58, 72, and 69% of the phenotypic variance, respectively. Recently, Romano et al. (2005) experimentally injected pheasant eggs with testosterone, but found no evidence of an effect on 2D:4D ratio despite a very large sample size, while a subsequent study that injected estradiol into eggs significantly changed the 2D:4D ratio (Saino et al. 2006b). The latter result is consistent with a study of 2D:4D ratios in humans showing a significant relationship with the ratio of fetal testosterone to fetal estradiol in amniotic fluid (Lutchmaya et al. 2004), and with the observation that men and women with congenital adrenal hyperplasia (a trait that is associated with high prenatal testosterone) have lower digit ratios than controls (Brown et al. 2002b; Okten et al. 2002). These results suggest that digit ratio is an indicator of both maternal effects and genetic constitution of an individual, with long-lasting predictive effects on performance in a number of different domains including sports, arts, and disease for humans, with important implications for studies of animals as well.

The objectives of this study of the house sparrow *Passer domesticus* were threefold. First, we investigated whether digit ratios differed between sexes, with males having higher ratios than females, as reported by Burley and Foster (2004). Second, we tested whether development of a secondary sexual character increased with digit ratio, as predicted because males have higher ratios than females (Burley and Foster 2004), suggesting that more male-like ratios (i.e., larger ratios) should be associated with larger secondary sexual characters. Previous experimental evidence based on testosterone injection into eggs has shown that badge size in male house sparrows develops directly under the influence of testosterone (Strasser and Schwabl 2004), although this finding does not preclude the possibility that genetic effects during development may play an additional role. Third, we tested whether a component of immunity decreased with digit ratio, particularly in females, as predicted if more male-like digit ratios in females were indicative of a more adverse developmental environment for females. Previous studies of digit ratios in humans and animals alike have found strong relationships between digit ratios on the right hand or foot compared to the left one (e.g., Manning 2002; Burley and Foster 2004), suggesting a significant effect of “handedness” on these relationships. Therefore, to avoid making unnecessarily many tests, we chose explicitly to restrict tests to patterns for the digit ratio of the right foot.

Materials and methods

Study site and general procedures

We captured house sparrows in mist nets during February 2004 at two rural sites outside Badajoz (38°50'N, 6°59'W), Spain.

Body mass was recorded with an electronic balance to the nearest 0.01 g. We measured length of tarsus and keel at capture with a digital calliper to the nearest 0.01 mm. Wing and tail length were measured to the nearest millimeter with a ruler. Badge size of males was estimated from length and width of total badge (including the part that was hidden by white feather tips) and the visible part of the badge (the part not covered by white feather tips) with a digital calliper to the nearest 0.01 mm after pressing feathers against the body (González et al. 1998). Digit ratio was recorded by placing one foot at a time with out-stretched toes on a piece of white paper on top of cardboard. The position of tips of all toes and the angle between toes was marked with an insect pin inserted in a pencil. These landmarks made with a pin were recorded independently twice for each foot. Distances between these landmarks were subsequently recorded using a digital calliper to the nearest 0.01 mm. Digits were numbered as indicated by Romer (1955). All measurements were made by CN, which reduced variance due to interobserver variability. Measurements were made blind with respect to badge size and immune response.

We estimated T cell-mediated immune response to a challenge with phytohemagglutinin (PHA) (Goto et al. 1978). Injection with PHA results in local activation and proliferation of T cells, followed by recruitment of inflammatory cells and major histocompatibility complex molecules (Goto et al. 1978). House sparrows were injected with 0.05 ml of 0.2 mg phytohemagglutinin in one patagium and 0.05 ml physiological water in the other.

We measured thickness of the patagium injected with PHA and with physiological water before injection and after 6 h in captivity (Navarro et al. 2003), using a pressure-sensitive calliper (Digimatic Indicator ID-C Mitutoyo Absolute cod. 547-301, Japan), with an accuracy of 0.01 mm. This measure had high repeatability (Falconer and Mackay 1996), as assessed from a one-way analysis of variance (ANOVA) with individual as a factor and three repeat measurements as the observations (R (SE)=0.95 (0.02), $F=37.47$, $df=68,138$, $P<0.0001$), as shown previously for the same species (González et al. 1998; Navarro et al. 2003, 2004).

Statistical analyses

All analyses were performed with JMP (2000).

We calculated repeatability of digit lengths and ratios using one-way ANOVA with individual as a factor (Falconer

and Mackay 1996). This procedure tests for significance of the between-individual component of variance; a prerequisite for further analyses because individuals cannot be considered to differ in the absence of a significant test statistic for a one-way ANOVA. Consistency in digit ratios between feet was recorded by correlating digit ratio of the right foot with digit ratio of the left foot.

We tested for relationships between mean digit ratio from the two measurements (ratio of length of second digit to length of fourth digit) and badge size, and between mean digit ratio, T-cell response and badge size, respectively, using linear regression.

We tested for the independent effect of a number of different predictor variables on badge size and T-cell response by stepwise regression analysis. We used forward and backward elimination procedures to test for stability of conclusions, and in no case did the choice of procedure change conclusions.

We tested for a sex-specific relationship between T cell-mediated immune response and digit ratio using digit ratio, sex, and digit ratio by sex interaction as predictors. We retained sex as a predictor variable despite it not being statistically significant because the interaction between sex and digit ratio was statistically significant.

Sample sizes differ slightly between tests because one individual had a broken, but healed toe due to an old accident.

Values reported are means (SE).

Results

Digit lengths were measured repeatably as shown by statistically significant one-way ANOVAs (eight digits on the two feet: $F > 29.29$, $df = 68, 69$, $P < 0.0001$, $R(SE) > 0.90$ (0.03)). Likewise, the ratio between length of second and fourth digit was repeatable (right foot: $F = 3.32$, $df = 68, 69$, $P < 0.0001$, $R(SE) = 0.54$ (0.12); left foot: $F = 7.57$, $df = 68, 69$, $P < 0.0001$, $R(SE) = 0.77$ (0.07)). The difference in digit ratio between right and left foot was statistically significant (mean difference (SE) = 0.057 (0.011), paired t test, $t = 5.12$, $df = 68$, $P < 0.0001$), with no significant difference between the sexes ($F = 0.87$, $df = 1, 67$, $P = 0.36$). The mean digit ratio (SD) for right foot of males was 1.12 (0.07), left foot of males 1.06 (0.06), right foot of females 1.11 (0.08), and left foot of females 1.07 (0.07). Finally, digit ratios on the right foot were positively correlated with digit ratios on the left foot ($r = 0.27$, $df = 68$, $P = 0.025$). There was a significant sex difference in digit ratio ($F = 5.63$, $df = 1, 67$, $P = 0.02$, males: 1.084 (SE = 0.012), $N = 49$, females: 1.033 (SE = 0.016), $N = 20$).

Size of the visible badge was on average 276 mm² (SD = 41), while the proportion of the badge that was visible was

on average 0.80 (SE = 0.02) in 49 males. Visible badge size was significantly positively correlated with right digit ratio (Fig. 1a; $F = 9.18$, $df = 1, 47$, $R^2 = 0.16$, $P = 0.004$, slope (SE) = 272 (90)). Likewise, the proportion of the badge that was visible was significantly positively related to right digit ratio (Fig. 1b; $F = 9.76$, $df = 1, 47$, $R^2 = 0.17$, $P = 0.003$, slope (SE) = 0.903 (0.289)). In contrast, the relationship with total badge size was not significant ($F = 1.48$, $df = 1, 47$, $R^2 = 0.03$, $P = 0.23$). The correlation between digit ratio and visible badge size was independent of body mass, and length of keel, wing, or tail, which did not enter as significant predictors in stepwise regression models.

T cell-mediated immune response was on average 0.339 mm (SD = 0.108) among 69 adult house sparrows,

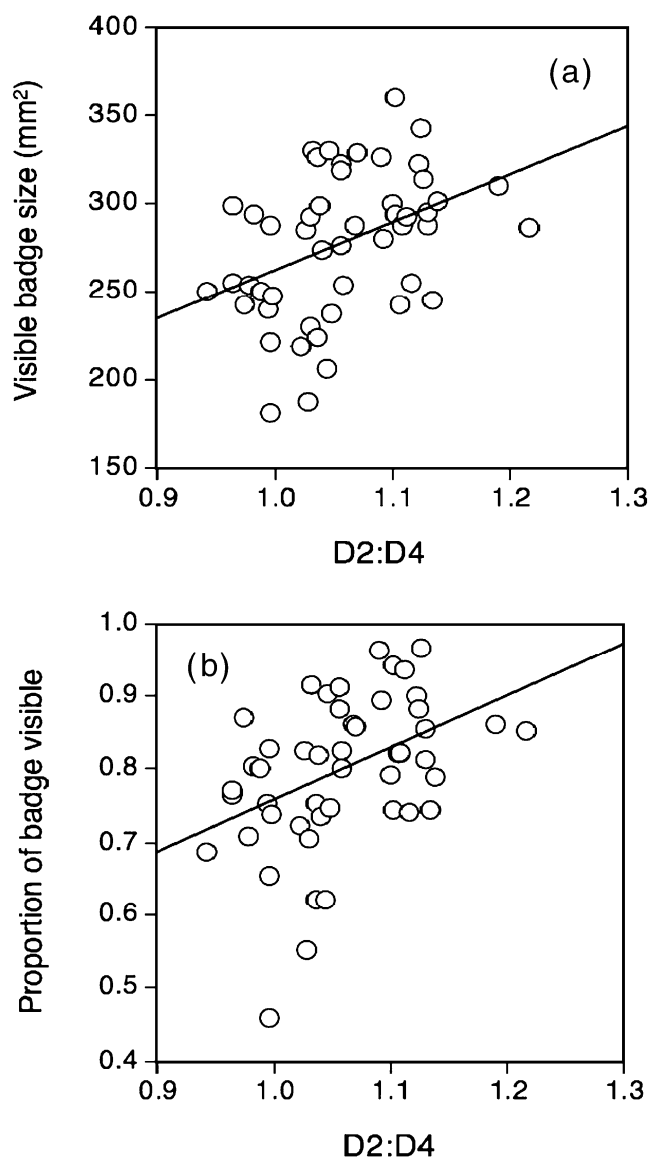


Fig. 1 Visible badge size (a; mm²) and proportion of badge size being visible (b) in male house sparrows in relation to right 2D:4D digit ratios. The lines are linear regression lines. $N = 49$ males

similar to previous results (González et al. 1998, 1999; Navarro et al. 2003, 2004). T-cell response was significantly correlated with right digit ratio ($F=5.04$, $df=1,67$, $R^2=0.07$, $P=0.028$, slope (SE)=-0.381 (0.170)), independent of body mass, and length of tarsus, keel, wing, or tail, which did not enter as significant predictors. Entering digit ratio of the right foot, sex, and digit ratio by sex as predictors produced a significant model accounting for 12% of the variance in T-cell response (Table 1). There was a negative relationship between immune response and digit ratio, while the sex difference was not significant (Table 1). The relationship for digit ratio differed significantly between the sexes, as shown by the significant sex by digit ratio interaction (Table 1). A model based on mean digit ratio of the two feet produced qualitatively similar results (results not shown).

Discussion

The main findings of this study were: (1) digit lengths and digit ratios were highly repeatable (right foot 0.54, left foot 0.77), and in addition ratios in the two feet were weakly positively correlated. There were significant sex differences in digit ratios; (2) males with a large visible badge size had a large digit ratio; (3) adults with a stronger T cell-mediated immune response to a challenge with the mitogenic phytohemagglutinin had smaller digit ratios, particularly in females. We briefly discuss these findings.

Digit ratios in humans differ significantly between sexes (Ecker 1875; Manning 2002), and differences between the sexes have also been reported for zebra finches, showing that males have higher ratios than females (Burley and Foster 2004), opposite to the pattern in human hands. We also found that male house sparrows had significantly higher ratios than females, and a similarly directed sex difference occurs in barn swallows *Hirundo rustica* (Møller, unpublished data). The mean difference between male and female zebra finches amounted to 6%, and we found a mean difference of 5% in the house sparrow. Early

developmental conditions may influence ontogeny of phenotypes thereby affecting fitness (Mousseau and Fox 1998; Dufty et al. 2002; Groothuis et al. 2005b). Consistent with our initial predictions, we found a significant positive association between digit ratio and the expression of a secondary sexual character in male house sparrows. Males with large digit ratios had larger visible parts of their badges in February than males with small ratios (Fig. 1). This effect was statistically independent of a number of other potentially confounding variables such as body size or body condition. This finding is consistent with our hypothesis that early embryonic conditions affect subsequent development of secondary sexual characters, as shown experimentally for the house sparrow by Strasser and Schwabl (2004). Males developing from eggs with experimentally injected testosterone had larger badges than males from a control treatment group (Strasser and Schwabl 2004). Males originating from testosterone-treated eggs were also more dominant than control males in standard dominance tests (Strasser and Schwabl 2004). Rubolini et al. (2006) also showed a differential effect of injection of testosterone in eggs of pheasants on development of spurs, but not on secondary sexual characters that are not used in male-male interactions. Male house sparrows have a badge that is partially covered by light feather tips in fall and winter, with males with large total badges developing a large visible badge earlier than other males (Møller and Erritzøe 1992). The visible badge remains larger for such males throughout spring and summer (Møller and Erritzøe 1992), and this development of the showy part of the plumage is under control of testosterone as shown by experimental manipulation of circulating testosterone (González et al. 2001). The relationship between digit ratio and visible badge size reported in this study could potentially affect male mating success because males with large visible badges develop badges earlier in spring and have larger overall badges (including the part that is not yet visible) than other males (Møller 1988, 1990; Møller and Erritzøe 1992; Veiga 1993; González et al. 2001). If large digit ratios reflect a specific embryonic environment with respect to sex steroids (Brown et al. 2002b; Manning 2002; Okten et al. 2002; Lutchmaya et al. 2004; Saino et al. 2006b), then males with small digit ratios should produce small visible badges, while males with large digit ratios should produce large badges. This is the pattern that we found. Early exposure to sex steroids could reduce the ability to produce efficient immunity; hence, only males with large digit ratios would be able to produce large secondary sexual characters without compromising immunity. Therefore, digit ratios may reveal constraints on development of secondary sexual characters as influenced by early maternal effects. Experimental enhancement of environmental conditions during molt should differentially benefit males with small digit

Table 1 Analysis of covariance with T cell-mediated immune response as the dependent variable and right digit ratio, sex, and digit ratio by sex as predictor variables

Variable	SS	df	F	P	Slope (SE)
Sex	0.013	1	1.25	0.27	0.015 (0.014)
Digit ratio	0.074	1	6.87	0.01	-0.761 (0.290)
Sex × digit ratio	0.054	1	5.00	0.03	-0.649 (0.290)
Residual	0.701	65			

The overall model is statistically significant; $F=3.09$, $df=3,65$, $R^2=0.12$, $P=0.033$

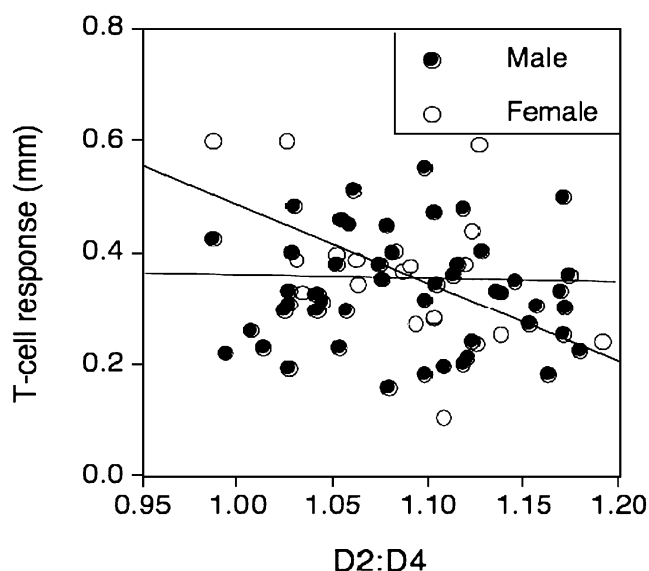


Fig. 2 T-cell mediated immune response (mm) of house sparrows in relation to right 2D:4D digit ratios. The lines are the linear regression lines

ratios because such males should differentially increase badge size under environmental improvement.

Immunity is generally strongly affected by condition as shown by numerous studies of wild and domesticated animals and humans (reviews in Chandra and Newberne 1977; Gershwin et al. 1985; Møller et al. 1998; Alonso-Alvarez and Tella 2001). Because the immune system may interact with the endocrine system to optimize the trade-off between sexual signaling and parasitism, as originally predicted by Folstad and Karter (1992), we predicted that specific embryonic steroid environments may be detrimental to immune function, as suggested by the literature (Martin 2000) and some recent experimental studies (Andersson et al. 2004; Groothuis et al. 2005a; Uller and Olsson 2003), but not by others (Rubolini et al. 2005; Saino et al. 2006a). We also predicted that the relationship between digit ratio and immunity should vary between the sexes because an embryonic environment high in testosterone may benefit sons while simultaneously negatively affecting daughters (Saino et al. 2006a). In particular, we have shown that when females have more male-like digit ratios, they also have reduced T cell-mediated immunity (Fig. 2, Table 1), independent of confounding factors such as body size. The negative relationship differed between the two sexes, with a significant negative relationship in females, but not in males, as shown by the significant sex by digit ratio interaction (Table 1). This pattern suggests that the relationships reported in this study are not due to circulating levels of androgens in adults. Because adult female house sparrows have low circulating levels, while adult males have high levels, we should expect a negative

relationship in males, but not in females, if adult androgen levels accounted for the association between T-cell response and digit ratio in Fig. 2. Obviously, these effects would also be affected by sex differences in sensitivity to testosterone. Likewise, associations between digit ratios and circulating testosterone in humans and baboons have been interpreted as both traits being caused by early exposure to embryonic testosterone rather than adult testosterone levels directly affecting digit ratios (Manning et al. 1998; Roney et al. 2004). Maternal effects may affect sons and daughters differently, selecting for maternal effects dependent on the sex of the offspring, as sometimes observed for maternal testosterone and other components of eggs (Petrie et al. 2001; Saino et al. 2003; but see Eising et al. 2003). Flegr et al. (2005) have recently shown that humans with low 2D:4D are more likely to have toxoplasmosis, probably due to a compromised immune response (Flegr et al. 2005). We hypothesize that several disease conditions are mediated by the early embryonic environment (Manning et al. 2003b) and this may ultimately control development of disease in a sexually antagonistic way.

In conclusion, digit ratios in free-living house sparrows differed between sexes. A secondary sexual character was positively related to digit ratios, while one component of immunity was negatively related to digit ratios in females.

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