



COMMENTARIES

Phenotypic correlates of digit ratio in a wild bird: implications for the study of maternal effects

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Digit ratio, the relative length of the second digit (2D) to the fourth digit (4D), as measured in childhood and adulthood, is generally assumed to mirror exposure to prenatal steroids in humans (Manning 2002). Specifically, high prenatal androgens and/or low prenatal oestrogens may result in longer 4D relatively to 2D, as expressed by 2D:4D. Although much recent research focused on the phenotypic correlates of digit ratio, providing promising results, including a correlation between digit ratio and the ratio of fetal testosterone and fetal oestradiol in humans (Lutchmaya et al. 2004), findings are often contradictory and the role of prenatal testosterone in the development of digits is far from proven (Putz et al. 2004; Cohen-Bendahan et al. 2005; McIntyre et al. 2006).

In birds, prenatal hormone regimes may involve components of maternal effects due to androgens (Schwabl 1993). Such maternal effects via maternal hormones are of central interest in evolutionary biology, because yolk testosterone can have short-term or long-term organizational effects on offspring, and thus mothers can actively generate phenotypic plasticity in an adaptive way (Gil 2003; Groothuis et al. 2005). Maternal hormones are thus subject to different selection pressures both at the maternal and offspring sides, which often involve evolutionary conflicts. Such trade-off provides a proximate basis for a key concept in evolutionary ecology (Gil 2003; Groothuis et al. 2005).

Given its plausible mechanistic link with prenatal steroids, digit ratio has been suggested to be used for

studying the effects of early maternal hormones (Burley & Foster 2004). The measurement of digit ratio in the field would provide an easy way to get insights into how an individual's behaviour is shaped by its maternal environment. For example, variance in digit ratio is associated with variance in reproductive success probably due to differences in attractiveness and mating success (Manning et al. 2000; Putz et al. 2004; Forstmeier 2005; Saino et al. 2006), which may have obvious evolutionary implications for maternal effects. The use of digit ratio offers practical benefits as, in wild populations, it is often impractical to measure or manipulate testosterone levels in an egg, and then to follow the offspring hatched from it to study reproductive performance in adulthood. An experimenter may have little chance to recapture the individuals of interest when adult, because of dispersal and frequently high juvenile mortality rate (in fact, if an egg is used for testosterone measurement, it cannot produce offspring). Because these difficulties do not apply to digit ratio, it may be expected that subsequent field studies in birds will prefer using digit ratio that can be measured in adults instead of directly assessing yolk testosterone levels in the egg. However, it is dangerous to adopt this preference without justifying the link between digit ratio and maternal testosterone level, because correlations between behaviour and digit ratio may be falsely interpreted in the context of maternal effects (Forstmeier 2005).

In fact, hitherto studies have provided conflicting evidence for the underlying assumption linking digit ratio to maternal effects in birds. Originally, Burley & Foster (2004) showed in a laboratory population of the zebra finch, *Taeniopygia guttata*, that digit ratio varies systematically between sexes, with laying order and with the

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strength of sexual preference in females. While these findings imply that digit ratio and maternal hormones may be subject to similar selection pressures, another study on the same species reached opposing conclusions. In a quantitative genetic study, Forstmeier (2005) showed that digit ratio is sexually monomorphic, it does not covary with laying or hatching order, and it has very high heritability leaving very small variation for environmental effects. Additionally, it was unlikely that these environmental effects were caused by maternal effects deposited into the egg (Forstmeier 2005). A unique experimental study in captive ring-necked pheasants *Phasianus colchicus* indicated that testosterone-injected eggs produce females with larger 3D:4D (Romano et al. 2005). However, this was not the case for other digit ratios and for males, and digit ratios were similar in the two sexes (Romano et al. 2005). Hence, the determination of digit ratio in birds appears to be different for different digits with inconsistent patterns among populations and species. Accordingly, at present, any assumption regarding the link between avian maternal steroids and digit ratio should be made with caution (Forstmeier 2005).

Our knowledge about the relationship between digit ratio and prenatal hormone exposure in birds originates exclusively from studies of captive animals. Laboratory animals may be useful to uncover developmental pathways, and to establish experimentally a mechanistic link between digit ratio and maternal hormones, as elegantly done in the pheasant study (Romano et al. 2005). However, the evolutionary implications of variation in digit ratio among individuals could be better assessed in wild populations, as they consist of animals that respond to natural selection forces. The adaptive value of maternal effects allowing females to adjust the phenotype of the offspring may substantially differ in captive populations, because they have been artificially selected (Clutton-Brock 1989). Therefore, the evolutionary importance of digit ratio, if there is any, may be different in different zebra finch populations that underwent different selection regimes.

Unfortunately, it is difficult to establish a mechanistic link between yolk testosterone and digit ratio in wild animals by measuring hormone levels in the egg, because these approaches necessarily involve low or no recapture probability in adulthood. However, by studying variation in digit ratio in the wild, indirect questions can be addressed about the potential evolutionary role of the trait. Findings for digit ratio can be compared with results on maternal hormones, and parallel findings may suggest similar adaptive mechanisms. For example, Burley & Foster (2004) tested whether observed variation in digit ratio was consistent with the previously reported relationship between androgen allocation and laying order (Gil et al. 1999). In addition, theory and data on directly measured yolk testosterone levels suggest that females may allocate hormones depending on the quality of the mate (Gil et al. 1999, 2004, 2006; von Engelhardt 2004; Tanvez et al. 2004; Michl et al. 2005; Uller et al. 2005); thus, similar patterns should exist for digit ratio. Therefore, offspring may be expected to express 2D:4D according to the quality of their father. Furthermore, the deposition

of androgens into egg yolk can have long-term organizational effects on offspring (Gil 2003; Groothuis et al. 2005) including survival (Sockman & Schwabl 2000; Navara et al. 2005), development of secondary sexual characters (Strasser & Schwabl 2004; Rubolini et al. 2006b) and potentially future reproductive success (von Engelhardt 2004; Groothuis et al. 2005). If digit ratio and maternal steroids are shaped by the same selection pressures, they should similarly correlate with survival, expression of sexually selected traits and breeding success.

Here we present phenotypic correlations between male 2D:4D, age, survival, and secondary sexually selected plumage and song traits in a natural population of the collared flycatcher, *Ficedula albicollis*. We evaluate the suggestion of Burley & Foster (2004), and we examine whether earlier conclusions based on measurements of yolk testosterone levels can be reproduced when our analyses are based on digit ratio as measured on the right foot. We assess whether variation in digit ratio and maternal testosterone have the same implications for fitness, which would be necessary for digit ratio to become a useful indicator of maternal effects. Previously, we determined the most important correlates of yolk testosterone levels (Michl et al. 2005), which could be used for making explicit predictions in the current study of digit ratio. Accordingly, we predicted that digit ratio should be associated with the age of the father and the laying date of the nest of origin, because eggs with high yolk testosterone levels are known to originate from young fathers and from late nests (Michl et al. 2005). Moreover, if maternal hormones determine the quality of the offspring (Gil 2003; Groothuis et al. 2005), digit ratio should also be an individual-specific attribute that is associated with male quality. Hence, we also predicted that males receiving more maternal testosterone in the egg, and simultaneously having smaller 2D:4D, should have more elaborate secondary sexual traits and survive better than males with less maternal testosterone and larger 2D:4D. We also examined the effect of age, because maternal hormones may have consequences for survival (Sockman & Schwabl 2000; Navara et al. 2005). Accordingly, similar evolutionary pressures on digit ratio should produce differences between age classes, but this should be the result of phenotype-dependent survival, instead of consistent individual changes.

Methods

During the breeding seasons between 2004 and 2006, we measured the following variables. We estimated body size and body condition, as reflected by tarsus length and body mass corrected for body size, respectively. We measured the lengths of the beak, wing and tail. Based on plumage coloration, individuals were classified as yearlings or older birds. Recaptured males were considered as surviving males, while individuals not seen in subsequent breeding seasons were considered dead (survival data for birds measured in 2006 were not yet available). Relying on our long-term data set, we determined the age of the father, and the laying date of the nest of origin, if these could be identified. We characterized sexual traits in

males, such as the size of two white patches on the plumage (forehead patch and wing patch), and features of song (song was not available for four individuals). Details on the study species, capturing protocol and measurements are given elsewhere (Hegyí et al. 2002, 2006; Török et al. 2003, 2004; Garamszegi et al. 2004a, b, 2006).

Several methods have been described or suggested for the measurement of digits (Burley & Foster 2004; Forstmeier 2005; Romano et al. 2005). However, after having tried different approaches in the collared flycatcher, we decided to develop our own method that is easily and reliably applicable for the species at hand. First, we pressed the right foot against a paper that was fixed on a piece of polystyrene. The right foot was selected in preference to the left for practical reasons. A previous study in another passerine with similar size showed that digit ratio measurements on the two feet were highly ($r > 0.9$) correlated (Forstmeier 2005). Second, when we were sure that the toe was completely attached to the paper's surface, we inserted a needle in the junction of the digits and at the tip of the digits, thus excluding the claws. This procedure was repeated three times for each digit, after moving the toe with some millimetres between measurements. The distances between the needle holes representing independent assessments of the length of the digits were later measured with a digital calliper to the nearest 0.01 mm. To avoid memory effects, the distances were measured blindly to the digital display of the calliper, with each measurement being started with a closed tool. These measurements were done by a single observer (L.Z.G.) to avoid introduction of unwanted errors caused by different observers.

With the above approach, we reached high and significant repeatability (2D: $F_{69,138} = 11.01$, $P < 0.001$, $R = 0.77$; 4D: $F_{69,138} = 7.85$, $P < 0.001$, $R = 0.70$). Altogether, we measured digits in 70 different males. We averaged the three measurements for each digit and calculated 2D:4D. A paired t test revealed that within individuals, 2D length was consistently larger than 4D length ($t_{69} = 3.96$, $P < 0.001$). The lengths of the digits showed highly significant covariation ($r = 0.573$, $N = 70$, $P < 0.001$). Digit ratio and the length of second digit did not differ significantly between the 3 years of study (2D: $F_{2,67} = 0.43$, $P = 0.437$; 2D:4D: $F_{2,67} = 1.43$, $P = 0.247$), while we found a significant year effect on the length of the fourth digit ($F_{2,67} = 3.34$, $P = 0.041$). However, the inclusion of year as a cofactor in the subsequent analyses did not affect the conclusions. Hence, for simplicity, we report models that do not control for the potentially confounding effect of year.

In accordance with recent suggestions (Nakagawa 2004; Garamszegi 2006), we determined the strength and direction of the relationship between digit ratio and the investigated phenotypic traits by estimating effect sizes (such as Pearson's r sensu Cohen 1988), and the associated 95% confidence intervals (CI). We tested our predictions by using estimates for the combined 2D:4D ratio and also for the separate 2D and 4D estimates, because the amalgamation of 2D and 4D in a single measure may be misleading. The effects mediated by testosterone (as reflected by 4D) and oestrogens (as reflected by 2D) may be substantially different from each

other, and totally different biological mechanisms could be lumped together in digit ratio. In general, the use of ratios may be problematic, because a given value may arise from a small numerator, a large denominator, or a combination of the two. Note that if the lengths of digits involve fitness components independent of maternal hormone levels, the calculation of 2D:4D does not make biological sense.

All birds were caught by conventional nestbox traps widely used in long-term population studies. After capturing and measuring, all birds were released immediately. No bird died during handling. The majority of males captured were later observed displaying, and many of them were seen as chick-rearing fathers. The study was conducted under licences from the relevant authorities (Duna-Ipoly National Park: 2573/2/2004, and Középduna-Völgyi Környezetvédelmi, Természetvédelmi és Vízügyi Felügyelőség: KTVF:15951/2005) that permitted us to catch and ring birds with our standard binding protocol.

Results and discussion

The Effect of Origin

Although we do not know anything about the role of maternal oestrogens yet, yolk testosterone concentrations are known to be higher in eggs laid for younger than older males, but laying date also seems to correlate with yolk testosterone level (Michl et al. 2005). If digit ratio is favoured by the same evolutionary forces and thus reflects yolk hormone levels, this trait should be similarly related to father's age and also to the laying date of the nest of origin. However, we failed to detect strong effects in the same direction for these relationships (Table 1). Even when considering the wide confidence intervals of the estimated effects, effect sizes corresponding to digit measurements (Table 1) are considerably different from effect sizes in association with yolk testosterone (father's age: 0.639, lower CI = 0.319, upper CI = 0.831; laying date: 0.490, lower CI = 0.101, upper CI = 0.715) as obtained from Michl et al. (2005). Our estimations were statistically different from the hypothetical means as taken from the previous study on yolk testosterone (father's age: $t_{25} = 2.42$, $P = 0.023$; laying date: $t_{25} = 2.18$, $P = 0.038$). In particular, if females mated with young males produce eggs with high testosterone levels in the flycatcher (Michl et al. 2005), and if selection in association with egg testosterone levels also favours 4D, offspring with longer 4D should have younger fathers than offspring with shorter 4D. Despite this expected negative relationship, the associated positive effect size indicated the opposite tendency (Table 1). Therefore, in contrast to yolk testosterone levels, digit characteristics do not seem to be strongly favoured by father's age and laying date in the nest of origin.

Such different evolutionary roles may have implications for the use of digit ratios in field studies of maternal effects. Although we cannot exclude that digit ratio is related to yolk testosterone levels to some extent, the correlation seems to be weak or masked, thus making digit ratio impractical to use as a surrogate of yolk testosterone. If the true effect size is close to $r = 0.173$ (Table 1), an observer

Table 1. Effect sizes (Pearson's r) and the associated 95% confidence intervals (CI) for the relationship between male sexual and morphological traits and digit measurements. Effect size conventions: $r = 0.10$ small effect, $r = 0.30$ intermediate effect, $r = 0.50$ large effects (Cohen 1988). Effects that are statistically different from zero are highlighted. The signs of effect sizes are not adjusted to directional predictions, and are given as they appeared in the corresponding statistics.

	Effect sizes (Pearson's r) (95% CI: lower CI/upper CI)			N
	2D	4D	2D:4D	
Father's age (juvenile-adult)	0.369 (−0.021/0.662)	0.225 (−0.178/0.563)	0.173 (−0.230/0.525)	26
Laying date of nest of birth	0.168 (−0.219/0.509)	0.236 (−0.150/0.560)	−0.077 (−0.438/0.305)	28
Age (juvenile-adult)	−0.071 (−0.301/0.167)	0.209 (−0.027/0.423)	−0.302 (−0.501/−0.072)	70
Survival	−0.034 (−0.324/0.262)	0.042 (−0.255/0.331)	0.082 (−0.217/0.367)	45
Sexual traits				
Repertoire size	0.024 (−0.219/0.264)	−0.134 (−0.364/0.112)	0.182 (−0.063/0.406)	66
Song rate (strophes/min)	0.063 (−0.182/0.300)	−0.047 (−0.286/0.197)	0.124 (−0.122/0.355)	66
Tempo (figures/sec)	−0.020 (−0.261/0.223)	−0.059 (−0.297/0.186)	0.039 (−0.205/0.278)	66
Strophe length (sec)	0.097 (−0.149/0.331)	0.061 (−0.184/0.299)	0.028 (−0.215/0.268)	66
Complexity (%)	0.075 (−0.170/0.311)	0.059 (−0.186/0.297)	0.026 (−0.17/0.266)	66
Forehead patch size	0.070 (−0.168/0.300)	−0.035 (−0.268/0.202)	0.117 (−0.121/0.343)	70
Wing patch size*	−0.076 (−0.306/0.161)	−0.029 (−0.263/0.207)	0.038 (−0.270/0.199)	70
Morphological traits				
Tarsus length	0.288 (0.057/0.490)	0.159 (−0.079/0.380)	0.117 (−0.121/0.343)	70
Body mass†	0.119 (−0.119/0.344)	0.137 (−0.101/0.360)	−0.037 (−0.270/0.200)	70
Wing length	−0.276 (−0.480/−0.044)	0.055 (−0.183/0.286)	−0.345 (−0.537/−0.120)	70
Tail length	0.008 (−0.228/0.242)	−0.028 (−0.261/0.208)	0.039 (−0.198/0.271)	70
Beak length	0.040 (−0.196/0.273)	0.028 (−0.208/0.261)	0.012 (−0.224/0.246)	70

*Age corrected.

†Body size(tarsus length) corrected.

would need a sample size of 257 digit measurements to find a significant association between digit ratio and father's age at 80% power, which would necessitate ~30 years of study with the efficiency we had in the current study.

Survival and age

Increased levels of egg testosterone can have long-term consequences for survival (Sockman & Schwabl 2000; Navara et al. 2005). Consistently, birds from eggs with high testosterone levels should survive differently compared with those that developed in eggs with low levels. If similar survival effects are involved in the evolution of digit ratio, it should be associated with survival. However, we found a small effect size for this relationship (Table 1).

On the other hand, we detected intermediate effects for the relationship between digit ratio and age indicating that older males have smaller digit ratios than younger individuals. Given that there is only a weak, if any, relationship between digit ratio and survival, we infer that these age effects are more likely to result from consistent individual changes with ageing rather than phenotype-dependent mortality. In fact, the change of the length of digits and their ratio with ageing is well documented in humans. Although digit ratio was originally thought to stabilize soon after birth (Manning et al. 1998; Manning 2002), recent studies reported systematic changes in digit ratios during childhood (Manning et al. 2004; McIntyre et al. 2005; Trivers et al. 2006). The exact mechanism for the ageing of digits in birds is currently unknown, but other morphological traits including bones can change with age (e.g. Wagner & Morton 1997; Møller

& de Lope 1999; Yosef & Fornasari 2000; Yosef & Tryjanowski 2002; Loncarich & Kremenetz 2004; Childress et al. 2005; Koppl et al. 2005; Skrobanek et al. 2005). It seems that the change in 2D and 4D with age follows different schedules (Table 1), and these combined age effects are manifested in the digit ratio as a consequence of the mathematical formula. In any case, the evolutionary roles seem to be different for maternal hormones and digit ratio, as the former may affect survival, while the latter may show consistent changes with ageing.

From a practical perspective, if ageing effects differ between digits, this will mask the relationship between digit ratio and maternal hormones, which makes inferences about yolk testosterone levels difficult. Hence, if an experimenter intends to use digit ratio as a proxy for maternal effects in a field study, the control for individual age may be necessary, which further decreases the power of the analyses.

Sexual traits

Females may invest testosterone in eggs to modify the phenotype of their offspring including their future attractiveness (Gil 2003; Groothuis et al. 2005). Importantly, few studies characterized the potential link between maternal hormones and sexual characters, thus making generalization difficult at present (Strasser & Schwabl 2004; Rubolini et al. 2006b). If these effects are applied to the collared flycatcher, and if selective forces shaping yolk testosterone and male attractiveness also favour digit ratio, there should be covariation between sexual traits and digit measurements. Our study failed to find a strong

relationship between these traits (Table 1), for which we provide three possible explanations. First, the chosen plumage and song traits may be poor indicators of male quality. This explanation is unlikely, because many of the chosen characters are known to function in female choice or male–male competition (e.g. Gustafsson et al. 1995; Pärt & Qvarnström 1997; Sheldon et al. 1997; Török et al. 2003; Garamszegi et al. 2004a). Second, digit lengths may respond to selection pressures different from those acting on maternal testosterone levels in the species, which was already suggested above, and thus this remains a plausible explanation. Notably, in the zebra finch, digit ratio varied positively with song rate, a direction that also conflicts with the digit ratio/yolk hormone concept (Forstmeier 2005). Third, females of the collared flycatcher may not adjust the attractiveness of their male offspring by changing levels of yolk testosterone. Although experimentally increased testosterone levels in the egg can affect the expression of sexual traits in some species (Strasser & Schwabl 2004), this is not universal (Rubolini et al. 2006b). Therefore, it remains difficult to reject or accept the third alternative, and to compare the roles of digit ratio and maternal hormones under this theoretical scenario.

Morphological traits

Interestingly, some ordinary morphological traits showed associations with digit measurements with intermediate effect size (Table 1). Little is known about how maternal effects determine the development of morphological traits, but some studies reported that yolk testosterone levels may influence chick body mass (Eising et al. 2001; Tschirren et al. 2005; Rubolini et al. 2006a). If such effects are applicable to other morphological traits and are maintained throughout life, it may imply a link between maternal hormones and morphology. Moreover, there may be an indirect relationship between morphology and yolk hormones, if high-quality offspring expressing morphological traits at a certain level originate from eggs with high hormone levels. Such speculations would allow covariation between digit measurements and morphology, if digit ratio evolves parallel to maternal effects. However, less speculative explanations can be applied if the length of digits is regarded as a simple morphological trait, and evolves independently of maternal hormones. Digit lengths appeared to scale allometrically, as shown by the association between 2D and tarsus length (Table 1) and by their correlation with each other (see Methods). Although the effect of body size was weakened when calculating 2D:4D ratio, this is the consequence of the mathematical formula rather than the elimination of these phenomena. If the allometric slopes are different for different digits (which seems to be the case), this may produce false biological conclusions arising from the use of digit ratio. Wing length was negatively correlated with 2D, but not with 4D. These inconsistent patterns resulted in a negative correlation with digit ratio, when these traits were combined in 2D:4D (Table 1). It is difficult to disentangle the mechanisms behind these relationships, but these findings may at least indicate effects acting

differently for different digits. These results also suggest that completely different selection pressures for digit characteristics and maternal hormones may arise from morphological constraints.

Practically, the digit-dependent effect of morphology on digit ratio may inflate any conclusion, if it is treated as a proxy for maternal effects.

Conclusion

This study cannot exclude that digit ratio correlates with the amount of maternal hormones, which could be achieved by experiments or direct hormone measurements only. However, we showed that selective regimes arising from a set of phenotypic traits may have different evolutionary consequences for maternal effects and digit ratios. The available evidence concordantly suggests that the proximate and ultimate determination of digit ratio does not necessarily follow the concept of maternal effects. These may involve the strong genetic determination of digits (Forstmeier 2005), possibly due to the effect of developmental genes such as Homeobox (HOX) genes (Manning et al. 1998; Manning 2002), measurement errors and biases (e.g. we found lower repeatability than in human studies), digit-dependent allometric scaling (this study), age effects (this study), and adaptations to stabilizing selections acting on the shape of the toes (as speculated by Romano et al. 2005). Given these obvious differences between the role of yolk testosterone and digit ratio, any result based on digit ratio should be interpreted with caution for the study of maternal effects in birds.

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