

Digit length may reveal unusual breeding behaviour in a seabird

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This work is dedicated to Dr Xavier Ruiz, who died suddenly in April 2008.

The hormonal environment experienced during prenatal development may affect adult phenotype and behaviour. Digit lengths may provide an estimate of steroid levels encountered during embryonic development in humans and other vertebrates. Finger patterns in humans have been shown to reveal sexual orientation or cooperative behaviour. We explored individual breeding behaviour in a monogamous seabird, the Balearic shearwater *Puffinus mauretanicus* and unexpectedly detected some cooperative breeders. Furthermore, we show evidence of correlation between digit lengths and cooperative breeding in this species. Additionally, we suggest that the first digit could be a possible indicator of prenatal steroid levels. These results are the starting point for further tests of the hypothesis that first digit length is an indicator of prenatal hormone levels in other vertebrate species. Moreover, these results may offer practical use in wild populations to study the implications of the changes in prenatal environment for adult social behaviour.

Keywords: behaviour; bird; cooperative breeding; digit ratios; digit lengths; finger patterns

1. INTRODUCTION

Prenatal androgenic steroids influence future adult phenotype and may have important consequences on future adult behaviour in vertebrates (Phoenix *et al.* 1959; Ehrhardt & Meyer-Bahlburg 1981; Dufty *et al.* 2002; Groothuis *et al.* 2005, Komdeur 2007). Thus, changes in prenatal androgen levels trigger effects in: sexual orientation (Williams *et al.* 2000); sex-role identity (Csathó *et al.* 2003) and cooperative behaviour (Millet & Dewitte 2006) in humans; sexual behaviour in rats (Rhees *et al.* 1997); and rates of aggression and mounting behaviour in hyenas (Dloniak *et al.* 2006). Sex steroids have a primary effect on sexual differentiation and activation of sexual and agonistic behaviour in birds (Adkins 1978); however, little is known about how variation in steroid levels in the prenatal period affects avian reproductive behaviour (Rhen & Crews 2002).

The second and fourth digit length ratio (2 D : 4 D) is sexually dimorphic in humans (Williams *et al.* 2000)

and may provide an estimate of the steroid levels experienced during prenatal development (Manning *et al.* 1998). The link between digit development and prenatal exposure to steroids may be enforced by the simultaneous effect which some homeobox genes have on the development of both fingers and the urogenital system (Kondo *et al.* 1997; Manning *et al.* 1998). These genes are highly conserved across vertebrates and similar relationships between digit ratios and sex steroids have been documented in rodents (Brown *et al.* 2002), primates (Roney *et al.* 2004) and birds (Burley & Foster 2004; Romano *et al.* 2005; Saino *et al.* 2007). However, in birds, inconsistent patterns of variation appear (Romano *et al.* 2005; Garamszegi *et al.* 2007; Dreiss *et al.* 2008). Additionally, most studies analysing vertebrate digit patterns only involved 2 D : 4 D finger length ratios, but other digit lengths or ratios may be informative (McFadden & Shubel 2002), especially in (understudied) non-human species.

Most seabird species are monogamous, and cooperative breeding, when individuals additional to the male–female pair provide parental care, is rare (Stacey & Koenig 1990), occurring only in ground-nesting species (e.g. skuas: Hemmings 1994; gulls: Fitch & Shugart 1983). To our knowledge, cooperative breeding has never been documented in a burrow-nesting seabird species. In this study, we explored whether individual breeding behaviour (monogamy versus cooperative breeding) in the burrow-nesting Balearic shearwater *Puffinus mauretanicus* was linked with digit lengths or other morphometrics on this species. Determining whether breeding behaviour is reflected in digit lengths is of relevance to the debate about the effects of prenatal androgenic steroids on future adult behaviour, and may offer practical use in wild populations to study the implications in adult social behaviour.

2. MATERIAL AND METHODS

The Balearic shearwater is a long-lived, critically endangered, species that breeds colonially in the Balearic Islands (Mediterranean Sea; Oro *et al.* 2004; Genovart *et al.* 2007). During 2001–2004, we examined individuals attending nests in two colonies. We only considered as breeders those individuals either incubating or attending their chick in the nest, so the possibility of confounding residents with neighbours is neglected. Several body measurements were taken from breeders: head plus bill length; minimum bill depth; tarsus length; first digit length; middle-toe length (third digit); and wing and tail length (see Genovart *et al.* 2003). All measures were taken with Vernier callipers ($\times 0.02$ mm) except for wing and tail length which were measured with a ruler ($\times 0.5$ mm). From most captured birds, a small blood sample (approx. 25 μ l) was taken and stored in ethanol. DNA was extracted using the phenol–chloroform method, following digestion with proteinase K (Sambrook *et al.* 1989), and birds sexed using the polymerase chain reaction to amplify two *CHD* genes (Fridolfsson & Ellegren 1999). Those individuals from whom we had no blood sample were sexed by unequivocal scoring in the discriminant function specifically developed (Genovart *et al.* 2003). As this is an endangered species, visits were limited and we cannot guarantee the record of all breeding adults. Thus, as the most conservative assumption, we considered cooperative breeders as those individuals sharing nest attendance duties with a same-sex individual or with more than one individual, and the remainder were considered monogamous birds. Some individuals were recorded in different years but were included only once in morphometric analyses. Morphometric characters of monogamous and cooperative breeders were compared applying a general linear model with sex and breeding behaviour as factors and also testing for their interaction. We check for normality for all tested traits and for homoscedacity in the tests.

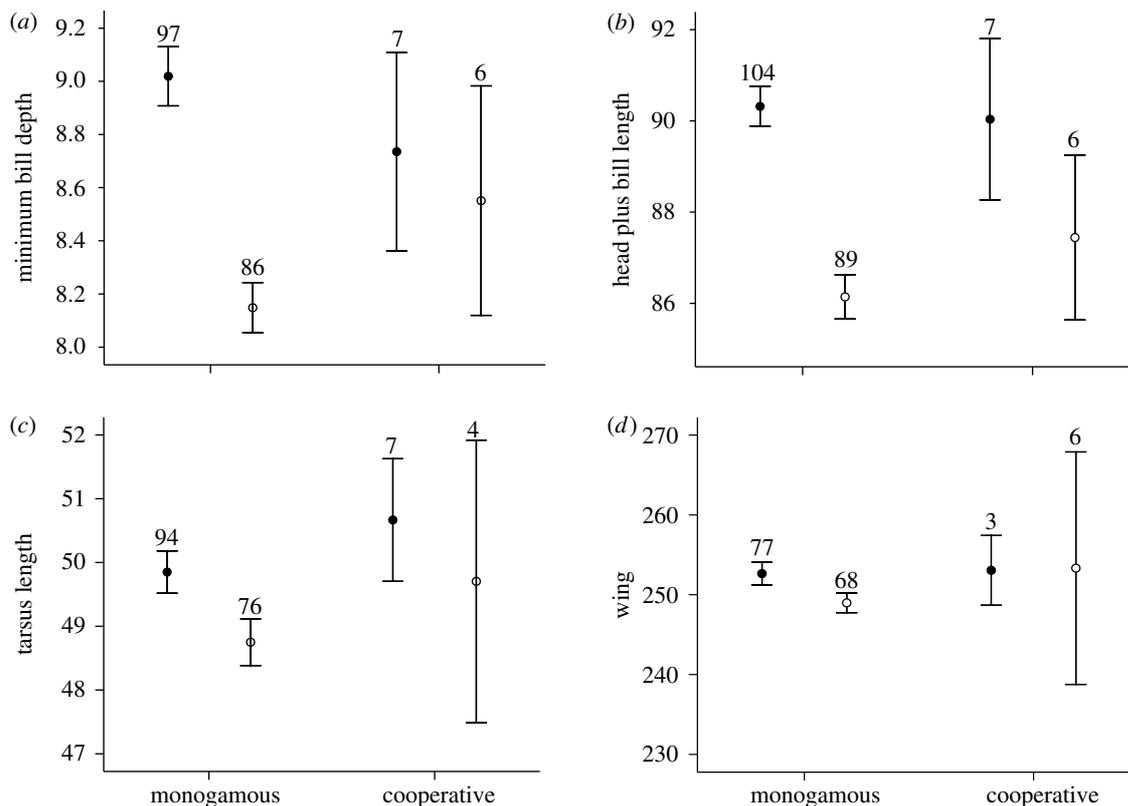


Figure 1. Morphometric comparisons of (a) bill depth, (b) head plus bill depth, (c) tarsus length and (d) wing, between males (solid circles) and females (open circles) and between monogamous and cooperative breeders. Bars represent confidence intervals (95%) for each measure (in mm) and sample sizes are shown above bars.

3. RESULTS

During the four consecutive breeding seasons, a total of 206 breeders (111 males and 95 females) from 162 different nests were measured and sexed. We captured more than one male (7 cases) and more than one female (2 cases) attending the same egg or chick among the 67 monitored active nests, thus getting evidence of cooperative breeding in 13.5 per cent of the breeding attempts.

As in previous studies of this species (Genovart *et al.* 2003), minimum bill depth and head plus bill length were the most sexually dimorphic characters ($F_{3,195} = 13.59$, $p < 0.001$; $F_{3,205} = 27.37$, $p < 0.001$; figure 1). Tarsus length showed slight sexual dimorphism ($F_{3,180} = 4.09$, $p < 0.05$; figure 1) but wing length did not show sexual dimorphism ($F_{3,153} = 0.664$, $p > 0.05$; figure 1). Despite having a small sample, cooperative breeders seemed to show lower sexual dimorphism in characters (figure 1), especially in minimum bill depth, the most dimorphic character: this character was found to be different between sexes in monogamous birds, but not between cooperative breeders ($F_{3,195} = 5.76$, $p < 0.05$). None of these morphometric characters differ significantly between cooperative breeders and monogamous birds (figure 1). When looking at digits, neither the first digit, the middle-toe, nor the 1 : 3 digit ratio showed sexual dimorphism ($F_{3,104} = 0.036$, $p > 0.05$; $F_{3,106} = 0.401$, $p > 0.05$; $F_{3,100} = 0.108$, $p > 0.05$; figure 2). Third digit length did not differ between breeding groups ($F_{3,106} = 0.967$, $p > 0.05$); however, despite having small sample sizes, cooperative breeders clearly differed from monogamous breeders in having

shorter first digits ($F_{3,104} = 7.72$, $p < 0.01$; figure 2) and greater 1/3 digit length ratios ($F_{3,100} = 4.71$, $p < 0.05$; figure 2).

4. DISCUSSION

Any character that provides an index of prenatal steroid levels could help us tease out the hormonal determinants of variation in social behaviour; for instance, cooperative breeding is one of the poorest known breeding systems in birds and examining the endocrine pathways could shed light on the occurrence of such behaviour. Several studies evidenced the link between hormonal environment experienced during prenatal development, digit lengths and adult behaviour in humans (Williams *et al.* 2000; Millet & Dewitte 2006). Interestingly, though our study lacks hormonal data, our results suggest that digit lengths may be related to breeding behaviour in birds, probably reflecting the hormonal environment experienced during their prenatal period. Our results also suggest that not only the 2 : 4 digit ratio but also other digit ratios or lengths may serve as indicators of hormone levels during early development. These results encourage further research, either on the hypothesis that first digit length is an indicator of prenatal hormone levels, as well as on the underlying endocrine processes related to certain social behaviour.

Previous studies have shown that variations in prenatal hormonal environment may play a role in body size differences between sexes on rats (Slob & Van Derr Werff Ten Bosch 1975; Cikos *et al.* 1992),

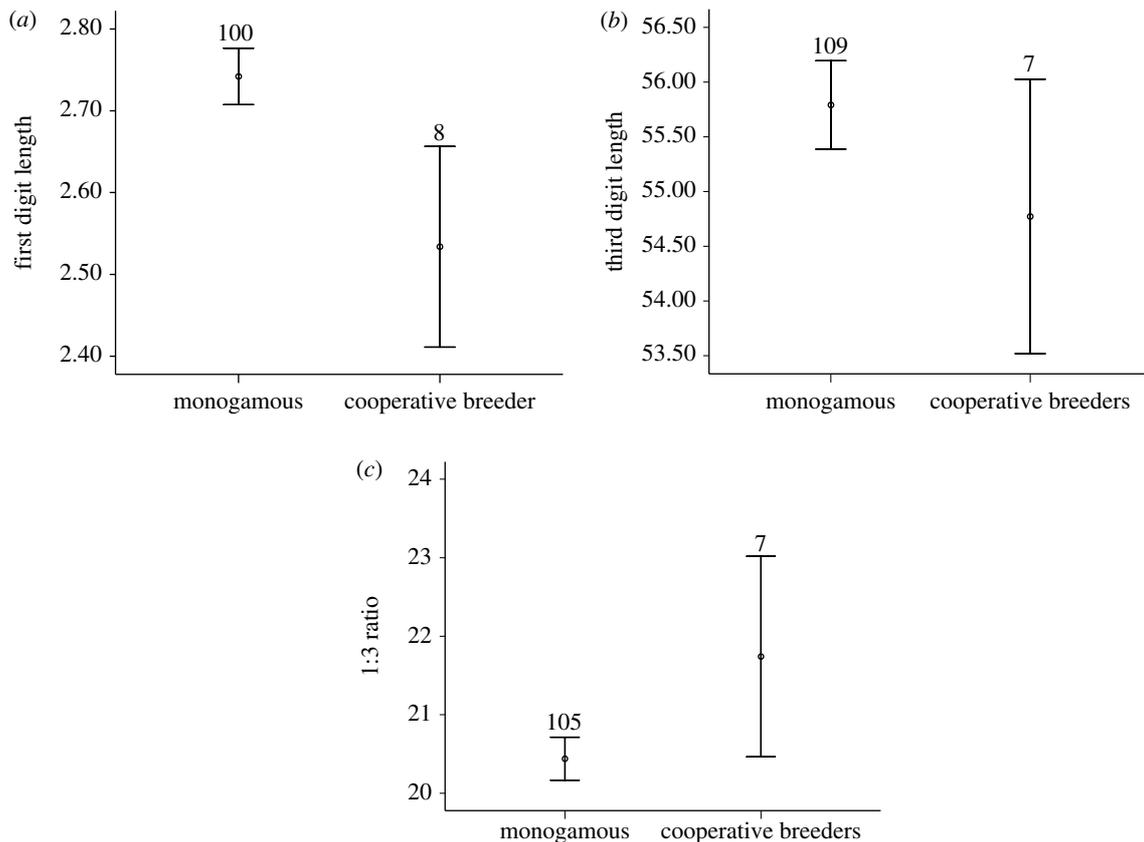


Figure 2. Confidence intervals (95%) for (a) first digit length, (b) third digit length and (c) 1:3 digit ratios, in monogamous and cooperative breeders. Sample sizes are shown above bars.

and von Engelhardt *et al.* (2006) showed that variation in maternal testosterone in birds enhanced growth of daughters and reduced growth of sons. In agreement with both studies, we show that most dimorphic characters, such as minimum bill depth (Genovart *et al.* 2003), are much less dimorphic in cooperative breeders than in monogamous birds, and also indicate that individuals showing cooperative breeding could have been subject to different prenatal hormone environment than monogamous birds.

In conclusion, this study could be the starting point for further tests on the hypothesis that first digit length is an indicator of prenatal hormone levels in this and other vertebrate species. Moreover, our results suggest that analysis of androgen-sensitive digit lengths and their correlates in wild populations of birds may enable us to trace important prenatal influences on individual social behaviour.

The relevant legal and ethical approval was given for this research.

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