Sex differences in anogenital distances and digit ratios in wild David’s rock squirrels *Sciurotamias davidianus*

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**Abstract** The prenatal hormonal environment plays an important role in organizing sex differences in the morphology, physiology and behavior of humans as well as other vertebrates. Currently, all related research on rodents has been focused on Mus. This study presents data on sex differences in the anogenital distance (AGD) and the ratio of the second to the fourth digit length (2D:4D) from 44 wild David’s rock squirrels *Sciurotamias davidianus* captured from the Qinling Mountains, China. This was the first study of a species from Sciuromorpha. The AGD as well as body mass are sexually dimorphic. There are no sex differences or lateral asymmetry in 2D:4D. Significant correlation was not found between AGD and 2D:4D for any paw. The findings indicate that 2D:4D may not be fixed or influenced by the prenatal steroidal environment in David’s rock squirrels [Current Zoology 60 (2): 180–185, 2014].

**Keywords** Anogenital distance, 2D:4D, *Sciurotamias davidianus*, Sex dimorphism

There is increasing evidence that the prenatal steroid environment plays an important role in organizing sexual differences in vertebrate phenotypes, such as the anogenital distance (AGD) and digit ratios (Williams et al., 2000; Manning, 2002; McFadden and Shubel, 2002; Thankamony et al., 2009; Zheng and Cohn, 2011; Dean and Sharpe, 2013). It is often impractical or unethical to manipulate intrauterine sex hormones when exploring the developmental influences of sex hormones on various traits, and thus, AGD and digit ratios are useful to be considered retrospective markers (Manning, 2002; Hotchkiss et al., 2007; Manno, 2008). AGD is sexually dimorphic in humans as well as some rodents: males have a greater AGD than females (Salazar-Martinez et al., 2004; Romano-Riquer et al., 2007; Manno, 2008). The recent evidence, however, indicates that AGD could be changed after birth via postnatal effects of sex steroids in rodents (Zheng and Cohn, 2011).

With regard to another phenotype influenced by prenatal hormonal effects, digit ratios are supposed to be fixed in utero, and finger lengths represent prenatal steroid levels (Manning et al., 1998). It is proposed that oestrogen promotes the growth of the index finger (2D) and testosterone boosts the growth of the ring finger (4D), the 2D:4D is highlighted as one observable adult-age marker of intrauterine exposure to steroids (Manning, 2002, 2008). In the human literature, some sexually differentiated traits reliably relate to 2D:4D (e.g. sexual orientation: Grimbos et al., 2010), while others do not (e.g. aggression and spatial cognition: Honekopp and Watson, 2011; Puts et al., 2008). The developmental link between 2D:4D and prenatal sex steroids remains unclear. Zheng and Cohn (2011) demonstrated that, in mice, the sexual dimorphism was determined by the proportion of testosterone and estrogen in the foetus acting on the growth of the phalanges. The 4D foetal digit contained many androgen (AR) and estrogen receptors (ER). Deletion of AR or blocking AR or adding estrogen to the fetus increased the 2D:4D. Deletion of ER or blocking ER or adding testosterone to the foetus decreased the 2D:4D. This effect was restricted to a narrow foetal developmental window and was subsequently fixed (Zheng and Cohn, 2011).

Numerous studies on 2D:4D in humans provide clear evidence for sexual dimorphism (Manning et al., 1998; Manning, 2002; Saino et al., 2006; Grimbos et al. 2010; Nelson et al., 2011; Zhao et al., 2012). Non-human studies conducted so far have, however, yielded inconsistent results, although sexual dimorphism in 2D:4D has been found in some of the tested species, including...
amphibians (*Oophaga pumilio*: Chang, 2008), reptiles (*Anolis carolinensis*: Chang et al., 2006; but see Lombardo and Thorpe, 2008; *Mabuya planifrons*: Rubolini et al., 2006; *Podarcis muralis*: Rubolini et al., 2006), birds (*Corvus corone*: Leoni et al., 2008; *Taeniopygia guttata*: Burley and Foster, 2004, but see Forstmeier, 2005; *Passer domesticus*: Navarro et al., 2007; *Phasianicus colchicus*: Romano et al., 2005; Saino et al., 2007), and mammals (rodents: Brown et al., 2002; Manning et al., 2003; Bailey et al., 2005; Lilley et al., 2009; Talarovicova et al., 2009; Yan et al., 2008, 2009; primates: Roney et al., 2004; McIntyre et al., 2009; Nelson and Shultz, 2010). These studies show that, in mammals, males tend to have a smaller 2D:4D on average than females, whereas in amphibians, reptiles and birds, males have higher 2D:4D on average than females (Manning, 2002, 2008; Lombardo et al., 2008; Nelson and Shultz, 2010; but see Roney et al., 2004). It is purported that sex differences on 2D:4D arise early in the foetus (in humans at the end of the first trimester) and thereafter the sex difference is more or less fixed (Manning, 2008).

Currently, all relevant research on rodents focus on Myomorpha. Laboratory samples were chosen in most studies (Brown et al., 2002; Manning et al., 2003; Bailey et al., 2005; Lilley et al., 2009; Talarovicova et al., 2009; Yan et al., 2008, 2009; Zheng and Cohn, 2011) whereas only one study used wild-captured individuals and just measured posterior paws (Leoni et al., 2005). Although laboratory rodent individuals were easy to obtain, it should be admitted that wild populations may potentially provide important insights into how these retrospective markers (e.g. digit ratios) are potentially influenced by natural environmental conditions and postnatal steroid environment (Manning, 2008). Given this background, the present study proposed to examine a member of the Sciuromorpha, the David’s rock squirrel *Sciurotamias davidianus*, a species endemic to China. The aim was to explore if wild *S. davidianus* show sex difference in 2D:4D of all four paws and in AGD, if the characteristic of 2D:4D supports the digit ratio theory namely that there is a relation between these two measures.

### Materials and Methods

This study was conducted in the Zhouzhi National Nature Reserve that was established in 1985 to protect 52,931 km² of temperate forest on the northern slope of the Qinling Mountains, in Shaanxi province, China. The Reserve experiences a semi-humid montane climate, and the composition of the forest varies with altitude, from deciduous broad-leaf forest at low elevations to mixed coniferous broad-leaf forest above 2,200 m and coniferous forest above 2,600 m. Rock squirrels are widespread in the reserve. Forty-four squirrels (24 males, and 20 females) were live-captured by use of stainless wire cages with peanut baits.

After sacrifice, the paws were surgically removed above the wrists and ankles (the method utilised in most relevant studies, e.g. Lilley et al. 2009). Following the method of Manno (2008), this study examined 2D:4D for all four paws: left-anterior, right-anterior, left-posterior, right-posterior (Table 1). Each animal’s paw was placed on a level surface and the digits were measured directly with vernier calipers (0.01 mm precision). Digit measurement was done by placing the zero point of the caliper on the basal crease proximal to the palm and then measuring to the tip of the digit, excluding toenails (Manning et al., 2005). The AGD was measured as the length of the perineum from the center of the genital papilla to the proximal end of the anus (Graham and Gandelman, 1986). Given that interobserver error is larger than intraobserver error (Voracek et al., 2007), digit measurements were made two times for each digit and AGD by one author (i.e. Dapeng Zhao). Re-measurement reliability was significantly high for the first and second measurement (intra-class correlation coefficients: all $P < 0.05$), and mean values were used in evaluation. In addition, body mass was also measured, on the grounds that it is often correlated with the measurement of AGD (e.g. Hotchkiss and Vandenbergh, 2005; Manno, 2008).

Statistical analysis was performed using SPSS for Windows (version 16.0), with a level of significance of $P \leq 0.05$. Sexual differences in AGD and body mass

### Table 1  Mean (±SD) of AGD and digit ratio (2D:4D)

<table>
<thead>
<tr>
<th>Sex</th>
<th>Body mass (g)</th>
<th>AGD (mm)</th>
<th>Digit ratio (2D:4D)</th>
<th>Cohens d value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>left-anterior paw</td>
<td>right-anterior paw</td>
</tr>
<tr>
<td>Males</td>
<td>230.96±22.544</td>
<td>33.97±4.098</td>
<td>1.32±0.121</td>
<td>1.31±0.131</td>
</tr>
<tr>
<td>Females</td>
<td>198.60±18.222</td>
<td>5.40±1.829</td>
<td>1.31±0.132</td>
<td>1.29±0.111</td>
</tr>
<tr>
<td>Cohen d</td>
<td>1.600</td>
<td>8.937</td>
<td>0.024</td>
<td>3.548</td>
</tr>
</tbody>
</table>
were tested with the Mann-Whitney U-test. A general linear model was applied to explore whether sex, anterior-posterior asymmetry or left-right asymmetry explains variation in 2D:4D. The correlation between measures was determined by a Pearson's correlation test. Given the limited sample size, we analyzed the effect size (Cohen d value) of sex difference for each measure based on Cohen (1988).

2 Results

The mean score of AGD, body mass and 2D:4D across 44 squirrels can be seen in Table 1. Body mass was significantly correlated with AGD ($r = 0.614$, $P < 0.001$). There was a significant sex difference in body mass ($U = 61.00$, $n_1 = 24$, $n_2 = 20$, $Z = -4.228$, $P < 0.001$) and AGD ($U = 0.00$, $n_1 = 24$, $n_2 = 20$, $Z = -5.657$, $P < 0.001$). Males showed significant higher scores of body mass and AGD than females.

The mean 2D:4D in males is similar to that in females for each paw (Fig. 1): there is no sex difference in 2D:4D ($r^2 =0.005$, $F = 0.581$, $P = 0.447$) (Table 2). The 2D:4D in wild *S. davidianus* shows significant anterior-posterior asymmetry ($r^2 = 4.614$, $F = 493.336$, $P < 0.001$) rather than left-right asymmetry ($r^2 = 0.023$, $F = 2.426$, $P = 0.121$) (Table 2).

No significant correlation was found between AGD and 2D:4D for each paw (Table 3).

3 Discussion

This study appears to be the first to present evidence of AGD and 2D:4D in a species of wild Sciuromorpha. We examined 2D:4D for all four paws in the present study. The results should be treated with caution due to limited sample size ($n = 44$, Table 1). The main findings of our study were that: 1) both AGD and body mass are sexual dimorphic in David’s Rock Squirrels; 2) no significant correlation was found between AGD and 2D:4D; 3) wild *S. davidianus* does exhibit anterior-posterior asymmetry rather than left-right asymmetry of 2D:4D.

The digit ratio hypothesis proposes that the 2D:4D is determined by sex steroids in utero and so should differ between the two sexes (Manning et al., 1998). To date, although sexual dimorphism has been reported in some tested species, there are still inconsistent results across studies, ranging from reptiles (*Anolis carolinensis*: Lombardo and Thorpe, 2008), birds (*Taeniopygia guttata*: Forstmeier 2005; *Hirundo rustica*: Dreiss et al., 2008), to Myomorpha rodents (*Microtus agrestis*: Lilley et al., 2009; mice: Bailey et al., 2005; Yan et al., 2009; Zheng and Cohn, 2011; Wistar rats: Talarovicova et al., 2009). This study found no significantly sex difference in 2D:4D of wild *S. davidianus*, which were very similar in males and females for each paw. Four possible explanations exist for the absence of sexual dimorphism: the first explanation is that variation on sex difference in

Table 2  GLM analysis for effects of sex, left-right, and anterior-posterior differences on digit ratio (2D:4D)

<table>
<thead>
<tr>
<th></th>
<th>$F$</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex</td>
<td>0.581</td>
<td>0.447</td>
</tr>
<tr>
<td>Left-right</td>
<td>2.426</td>
<td>0.121</td>
</tr>
<tr>
<td>Anterior-posterior</td>
<td>493.336</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Sex × Left-right</td>
<td>0.023</td>
<td>0.881</td>
</tr>
<tr>
<td>Sex × Anterior-posterior</td>
<td>0.048</td>
<td>0.826</td>
</tr>
<tr>
<td>Left-right × Anterior-posterior</td>
<td>0.330</td>
<td>0.566</td>
</tr>
<tr>
<td>Sex × Left-right × Anterior-posterior</td>
<td>0.051</td>
<td>0.822</td>
</tr>
</tbody>
</table>

Table 3  Correlation test between AGD and digit ratio (2D:4D) for each paw

<table>
<thead>
<tr>
<th>Paw</th>
<th>$r$</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Males (n = 24)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male left-anterior</td>
<td>0.195</td>
<td>0.362</td>
</tr>
<tr>
<td>Male right-anterior</td>
<td>0.277</td>
<td>0.189</td>
</tr>
<tr>
<td>Male left-posterior</td>
<td>0.082</td>
<td>0.704</td>
</tr>
<tr>
<td>Male right-posterior</td>
<td>-0.073</td>
<td>0.735</td>
</tr>
<tr>
<td>Females (n = 20)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female left-anterior</td>
<td>-0.059</td>
<td>0.811</td>
</tr>
<tr>
<td>Female right-anterior</td>
<td>0.146</td>
<td>0.552</td>
</tr>
<tr>
<td>Female left-posterior</td>
<td>-0.221</td>
<td>0.350</td>
</tr>
<tr>
<td>Female right-posterior</td>
<td>0.317</td>
<td>0.173</td>
</tr>
<tr>
<td>Total (n = 44)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Left-anterior</td>
<td>0.035</td>
<td>0.822</td>
</tr>
<tr>
<td>right-anterior</td>
<td>0.107</td>
<td>0.496</td>
</tr>
<tr>
<td>left-posterior</td>
<td>0.137</td>
<td>0.376</td>
</tr>
<tr>
<td>right-posterior</td>
<td>0.112</td>
<td>0.470</td>
</tr>
</tbody>
</table>
2D:4D may in part arise from pregnancies which include both male and female foetuses; testosterone may diffuse from males foetuses into female foetuses (Zheng and Cohn, 2011). If this happens early in development of S. davidianus it may obscure the sex difference in 2D:4D among litter mates. The second is that different species may simply behave differently. Current evidence shows that sex hormones affect digit growth during puberty as well as in utero in mammals (McIntyre et al., 2005; Perry et al., 2008), so absence of sexual dimorphism in S. davidianus may be interpreted as the potential influence of further steroid effects after birth on the determination of digit ratios. The third is that inconsistent results on 2D:4D may be due to variation in measurement methods (e.g. vernier caliper: Manning et al., 2004; photocopy machine prints: McIntyre et al., 2007; photos: Hurd et al., 2008; scans: Weis et al., 2007; X-rays: Lilley et al., 2009). For example, the soft tissue in animals may cause the differences in photocopy measurements compared with direct assessments. The last explanation is that daily scratch-digging behavior of S. davidianus may induce morphological adjustment of forepaws, as reported in other squirrel species (e.g. Spermophilus citellus: Lagaria and Youlatos, 2006), so as to obscure possible sexual dimorphism in 2D:4D.

The AGD is determined by prenatal testosterone concentrations in utero (Ophir and Delbarco-Trillo, 2007; Thankamony et al., 2009), so that it is likely that any prenatal phenotype related to androgens would be correlated with AGD. The current study finds there was no significant correlation between AGD and 2D:4D in S. davidianus and corroborates results from other rodent studies (Hurd et al., 2008; Manno, 2008). It suggests that these two measures (2D:4D and AGD) do not reflect a common influence of androgen exposure in this squirrel, which raises some doubt on the validity of 2D:4D as the retrospective marker in the prenatal hormone levels (Dresseler and Voracek, 2011; Snihur and Hampson, 2011; Voracek, 2011; Dean and Sharpe, 2013).

Manning (2008) proposed that population differences of digit ratios may be linked to mating systems. Nelson and Shultz (2010) found that 2D:4D varied across anthropoid groups such that it was lower in polygynous species and higher in monogamous species. The 2D:4D of males in nonpair-bonded species is significantly lower than that of females whereas there is no significant sex difference in pair-bonded species (Nelson and Shultz, 2010). Unfortunately, reports on the mating system are not available in S. davidianus although large sexual dimorphism in body mass to some extent suggests this species may be polygynous. Further studies should investigate this issue so as to explore its potential influences on digit ratios in this species.

In conclusion, this study demonstrates for the first time that David’s Rock squirrels are sexual dimorphic in AGD as well as body mass, while 2D:4D may not be fixed or influenced by the prenatal steroidal environment. This study further broadens our knowledge on sexual differentiation, particularly in regard to anogenital distance (AGC) and digit length ratios in vertebrates. It should be admitted that body mass is sexually dimorphic, and tightly correlated with AGD. Such character of body mass is likely to confound measurement and lower the power to detect effects in the AGD analysis. Further investigation with larger samples in this species as well as in other non-human species are required to illuminate the true relationship between developmental variables and digit ratios.

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