

# Sexual Dimorphism in Digit-length Ratios of Laboratory Mice

WINDY M. BROWN,<sup>1</sup> CHRISTOPHER J. FINN,<sup>1</sup> AND S. MARC BREEDLOVE<sup>2\*</sup>

<sup>1</sup>Department of Psychology, University of California–Berkeley, Berkeley, California

<sup>2</sup>Neuroscience Program, Michigan State University, East Lansing, Michigan

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## ABSTRACT

The lengths of the index finger (2D) and ring finger (4D) are sexually dimorphic in humans, and men have a smaller 2D:4D ratio compared to women. Prenatal androgens appear to be important in the development of the 2D:4D sex difference, since it has been reported in children as young as 2 years old, and since humans exposed to supernormal prenatal androgen levels display a smaller 2D:4D ratio. We tested whether another mammalian species in which the process of peripheral sexual differentiation is androgen-dependent might also show a sex difference in digit ratios. The 2D:4D ratio of adult outbred mice was calculated for both the left and right rear paws. A sex difference was observed in the right rear paw: female mice had a larger 2D:4D ratio than did males. We also found this difference in prepubescent weanling mice. This sex difference is in the same direction as that observed in humans, and suggests that sexual dimorphism in digit length ratios is a feature common to many, if not all, mammals. The mouse may therefore be a useful animal model for studying the factors that influence finger length patterns, which have recently been correlated with several specific behaviors and disease predispositions in humans. *Anat Rec* 267:231–234, 2002. © 2002 Wiley-Liss, Inc.

**Key words:** digit ratios; sex difference; mice; adult; weanling

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A nineteenth-century anthropological report (Ecker, 1875) noted that when asked whether the index or the ring finger was longer, people tended to look at their own hand, and the answer depended on the sex of the person. Men generally reported that the ring finger was longer, whereas women more often stated that the index finger was longer. For over 50 years there were conflicting reports about whether there is truly a sex difference in the pattern of finger lengths, until George (1930) standardized a method of measuring fingers, gathered a large sample of subjects, and showed that the sex difference was statistically significant. This sex difference has recently been reformulated as the ratio of the length of the index finger (2D) to the length of the ring finger (4D). This ratio is indeed sexually dimorphic in humans: men have a smaller 2D:4D ratio on average than do women. The sex difference in the 2D:4D ratio is evident, and stable, from 2 years of age through adulthood (Manning et al., 1998).

Since the 2D:4D ratio sex difference appears to be unaffected by changing sex hormone levels at puberty, and since most other mammalian sex differences are androgen-dependent (Breedlove et al., 1998), prenatal androgen levels may play a role in digit length development. Supporting this supposition is the finding that individuals

with congenital adrenal hyperplasia (CAH) display masculinized finger length patterns (Brown et al., 2001). CAH is a disorder that causes the developing fetus to produce excessive adrenal androgens. Hormone levels are usually normalized at birth, so physical and behavioral differences between CAH and non-CAH individuals can primarily be attributed to the effects of supernormal prenatal androgens. This result further suggests that the human sex difference in the 2D:4D ratio reflects an organizational effect of androgens acting prenatally to permanently alter finger length patterns. In addition, the same subfamilies of genes that are involved in androgen-modulated genital

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\*Correspondence to: S. Marc Breedlove, Neuroscience Program, Psychology Research Building, Michigan State University, East Lansing, MI 48824. Fax: (517) 432-1674. E-mail: breedsm@msu.edu

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development, the *Hoxa* and *Hoxd* genes, also affect digit development in both humans and mice (Peichel et al., 1997), lending more support to the idea that prenatal androgens play a role in 2D:4D sexual dimorphism. Since the development of genital structures is influenced by androgens, it seems likely that the genes that code for this development are androgen-sensitive. Other structures (such as the fingers) that are regulated by the same genes might, therefore, also be influenced by androgens.

In the present study, we hypothesized that the 2D:4D sex difference seen in humans might also exist in other mammals for which prenatal androgens have been shown to be important in peripheral sexual differentiation, and that any sexual dimorphism discovered would be constant across natural hormonal fluctuations in animals after birth. As rodents are the traditional mammalian model in the study of sexual differentiation, outbred mice were examined to address this hypothesis. We found that the 2D:4D ratio of mice was also smaller in males than in females. Furthermore, just as the sex difference in the human 2D:4D ratio was present both pre- and postpubertally, we found that the sex difference in the mouse 2D:4D ratio was present both before and after puberty. Finally, just as the human sex difference in the 2D:4D ratio is greater on the right than on the left, we found that the sex difference in the mouse 2D:4D ratio was significant only on the right side.

## MATERIALS AND METHODS

### Experiment 1

All animals for this study were derived from our breeding colony of outbred laboratory mice, and were housed with food and water *ad libitum* in a 12-hr light:12-hr dark cycle. In the first study, the rear digits of 30-day-old weanling mice were measured. Animals were killed by carbon dioxide inhalation. A sticker with an identification number was placed around the tail of each animal so that the date of birth, litter number, and weight could be recorded and matched with digit ratios. The rear paws were removed and each pair was placed in a dish that was labeled with an identifying sticker matching that on the tail of each mouse. In this manner digit lengths could be measured without knowledge of the sex of the animal. The decision to measure rear paws rather than forepaws was based solely on size constraints. It is more difficult to take accurate measures of the forepaw digits in mice because they are much smaller than their rear-paw counterparts. Digits were measured to the nearest tenth of a millimeter under a dissection microscope with a transparent ruler. The zero mark of the ruler was placed at the base of the second or fourth digit, and the digit was aligned perpendicularly to the ruler markings and held in place between the ruler and the experimenter's finger with gentle pressure. A 2D:4D ratio was calculated for both the left and right rear paws of each animal by dividing the length of the second digit by the length of the fourth digit.

### Experiment 2

In the second study, data were collected from 20 male and 19 female adult, outbred mice aged 77–88 days old. Data were obtained following the first experiment's procedures, with the following exception. To improve measurement accuracy, a measuring device constructed especially for rodent digits (Fig. 1) was used to measure the

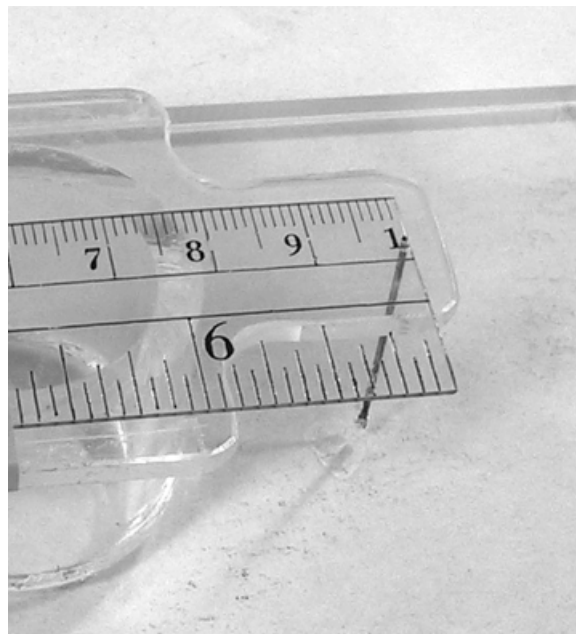


Fig. 1. The device used under a dissection microscope to measure the length of rodent digits. Paws are positioned, ventral side up, directly underneath the transparent plastic piece with the pin firmly pushed to the base of the skin between the second or fourth digit (the paw is repositioned to measure each digit) and the third digit. Constant pressure from below keeps the digits elongated and aligned perpendicularly to the millimeter ruler markings. A measurement is taken from the pin at the base of the second (or fourth) digit to the tip of the digit. A reading to the nearest tenth of a millimeter is taken for each digit from the millimeter ruler markings running alongside the digit.

lengths of the second and fourth digits to the nearest tenth of a millimeter under a dissection microscope. A 2.5-cm metal pin connected a rectangular piece of transparent plexiglass to a base. A transparent millimeter ruler was affixed to the top of the plexiglass with the zero mark directly above the pin. Paws were then positioned, with the palmar surface up (and therefore visible through the ruler markings and plexiglass), such that the pin was firmly pressed between the base of the second (or fourth) and third digits. The intersection between the second/fourth and third digit therefore corresponded to the zero millimeter marking of the ruler. The digits were then aligned parallel to a straight line running down the length of the ruler, and perpendicular to the millimeter markings. A reading to the closest tenth of a millimeter was taken at the tip of the digit.

## RESULTS

### Experiment 1: Weanlings

Two-tailed *t*-tests between males and females were conducted on calculated 2D:4D ratios. Group averages and standard errors are reported in Figure 2. At 30 days, body weight did not differ between males ( $14.8 \pm 0.67$  g) and females ( $13.6 \pm 0.41$  g;  $P = 0.12$ ). However, females had a significantly larger 2D:4D ratio on the right rear paw than did males ( $P < 0.03$ ). The difference in the 2D:4D ratio of females and males on the left was not significant ( $P < 0.88$ ). Asymmetry between the left and right 2D:4D ratios

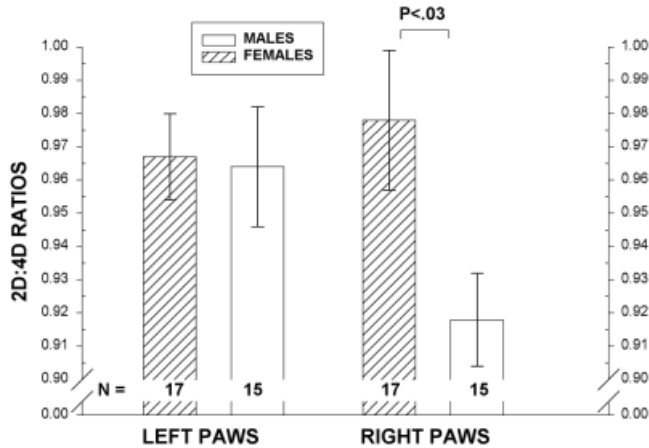


Fig. 2. Ratios of the lengths of the second and fourth digits (2D:4D) for male and female weanling mice. The female 2D:4D ratio is significantly larger than the male 2D:4D on the right side only.

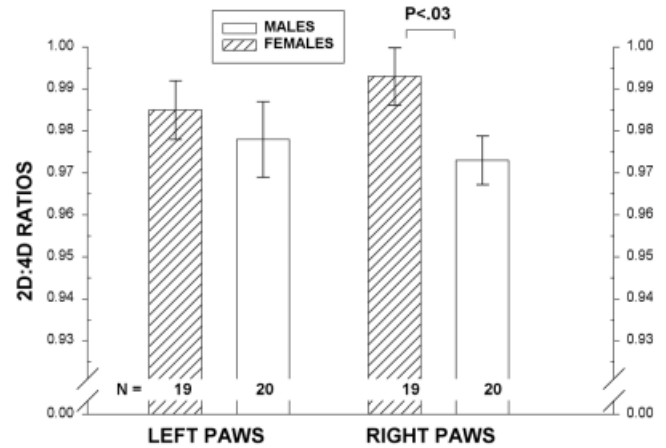


Fig. 3. Ratios of the lengths of the second and fourth digits (2D:4D) in male and female adult mice. The female right 2D:4D ratio is significantly larger than the male right 2D:4D ratio.

was also calculated by subtracting the right ratio from the left ratio for each animal. The difference in asymmetry between females ( $0.011 \pm 0.02$ ) and males ( $-0.046 \pm 0.02$ ) was significant ( $P < 0.05$ ). In other words, females tended to have a larger ratio on the right, and males tended to have a larger ratio on the left rear paw.

### Experiment 2: Adults

Two-tailed *t*-tests between males and females were conducted on calculated ratios. The group averages and standard errors are reported in Figure 3. Adult males had a significantly larger body weight ( $30.3 \pm 0.68$  g) than did females ( $22.9 \pm 0.41$  g;  $P < 10^{-10}$ ). Again, there was a significantly larger 2D:4D ratio in female mice than in male mice ( $P < 0.03$ ) on the right rear paw. No significant difference was seen between the female and male 2D:4D ratios on the left rear paw ( $P = .53$ ). The two sexes were not significantly different in terms of laterality (i.e., the difference between the left and right ratios;  $P = 0.32$ ).

### DISCUSSION

These results indicate that the sexual dimorphism in digit length patterns reported in humans is also present in mice. Female mice have a significantly larger 2D:4D ratio on the right rear paw than do males. This finding is consistent with human data, both in the direction of the sex difference (females have a larger 2D:4D ratio than do males), and in the laterality of the sex difference (more pronounced on the right than on the left (Manning et al., 1998; Williams et al., 2000)). The current findings are also in accord with finger length patterns recently measured in the metacarpals of baboons, in which again the 2D:4D ratio was greater in females than in males (D. McFadden, personal communication). An interesting point revealed by this work on digit ratios in baboons is the presence of other sex differences in digit length ratios. For instance, McFadden found that the 3D:5D ratio was more sexually dimorphic than the 2D:4D ratio. Since the primary purpose of the current set of studies was to test for the presence of a 2D:4D sex difference in mice homologous to that seen in humans, other digit ratios were not calcu-

lated. This leaves open the possibility that the 2D:4D ratio may not be the only sexually dimorphic digit length pattern in mice or humans.

In addition, the 2D:4D sex difference is evident in both 30-day-old weanlings and adult mice, suggesting that it is stable after birth. Again, this consistency across age parallels the human data, which show a stable sex difference from at least 2 years of age through adulthood. In humans, finger length patterns are thought to attain adult proportions by the seventh week of gestation (Garn et al., 1975). In conjunction with the masculinized ratios seen in CAH individuals, who are exposed to high levels of prenatal androgens, the stability in the mouse and human 2D:4D sex difference across age strongly implicates fetal androgens as the mechanism behind the human 2D:4D sex difference. The current data suggest that the same prenatal mechanism permanently alters digit length patterns during development in mice.

It is interesting that the patterns in 2D:4D sexual dimorphism are consistent on both the left and right paws of the mice, but are more pronounced on the right. A study examining the 2D:4D of heterosexual and homosexual men and women also reported more group differences in the right 2D:4D ratio than in the left ratio (Williams et al., 2000). The 2D:4D ratio of homosexual women was significantly smaller (i.e., more masculine) than the 2D:4D ratio of heterosexual women on the right hand only. These findings suggest that finger length patterns are asymmetrically affected by androgens during development, and that the right side is more sensitive to this influence.

Testosterone has been linked to asymmetrical development in numerous studies. A recent review of cortical asymmetry concluded that men exhibit more cortical asymmetry than do women, that the asymmetry usually favors the right side, and that testosterone is the most likely candidate to produce these asymmetries (Wisniewski, 1998). In male cats, asymmetry in brain weight (R-L) is positively correlated with overall body weight (Tan and Kutlu, 1993), suggesting a common mechanism (testosterone) underlying both brain asymmetry and increased body weight. Similarly, we found that a sexual dimorphism in the mouse hippocampal formation



was due to an asymmetry in males only (the dentate gyrus granule cell layer volume was greater on the right than on the left (Tabibnia et al., 1999)). A higher incidence of left-handedness has also been reported among CAH individuals, and is thought to reflect asymmetrical brain development brought about by above-average prenatal androgens (Kelso et al., 2000). Particularly interesting in relation to prenatally determined digit ratios is the evidence for fetal cortical asymmetry reported in a previous study (de Lacoste et al., 1991). Those researchers found an asymmetry in the striate and extrastriate cortices of male fetuses, with larger volumes on the right side than on the left. They suggested that this is due to androgens that either increase growth of the right hemisphere or retard growth of the left hemisphere. The primarily right-sided sex difference in digit ratios may reflect asymmetrical effects of testosterone on tissues outside of the central nervous system.

Early steroid hormone levels are thought to play a role in the development of many sexually dimorphic human behaviors. Although this role is difficult to distinguish from postnatal environmental factors that also influence behavior, sometimes a clear effect of hormones can be established. For instance, differences in childhood play behavior between CAH girls and non-CAH girls have been attributed to differences in levels of prenatal testosterone (Berenbaum and Hines, 1992). Prenatal hormones are suspected to play a predisposing role in some sex-specific disease processes as well (Rubinstein, 1993; Phillips, 1998; Newbold et al., 2000). Recently, large 2D:4D ratios have been correlated with a significantly higher likelihood of myocardial infarction in men (Manning, 2001). Manning's report suggests that low levels of prenatal androgens may predispose men toward heart attacks later in life. In addition, below-average finger length ratios have been reported among children with autism and their immediate relatives (Manning et al., 2001), again suggesting that prenatal hormone levels somehow influence disease predisposition later in life. It is particularly interesting that in the case of autism the disorder is approximately four times more common in males than in females (Fombonne, 1999). This too suggests that androgens, which underlie most biological sex differences in mammals, play a role in susceptibility to autism.

In the search for postnatal markers of prenatal hormone levels that may correlate with behavior or disease predisposition, one could employ a well-established, sexually dimorphic measure such as childhood play. However, such a measure is also open to influence from environmental variables such as parenting style, peer role models, and the mood of the child during testing. Although sex differences in the 2D:4D ratio are subtle, they are unique in that they provide an opportunity to examine an easily accessible sexually dimorphic characteristic that is uninfluenced by social variables. They are therefore a relatively direct retrospective marker of hormonal events in the womb. As a common marker of early androgen exposure shared by both mice and humans, finger length pat-

terns may aid in our understanding of the developmental events underlying variables related to these digit ratios. An animal model such as the sexually dimorphic mouse 2D:4D ratio may prove to be particularly useful in this endeavor.

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