Letter to the Editor

Of mice and men – cross-species digit ratio (2D:4D) research: comment on Bailey, Wahlsten and Hurd (2005)

The human second-to-fourth digit ratio (2D:4D) is sexually dimorphic: on average, males present lower values than females. As a likely biomarker for prenatal androgen exposure and sensitivity and its associated organizational (permanent) effects on the brain and behavior, the 2D:4D ratio has generated much research interest recently and has emerged as a correlate of a multitude of sex-dependent, hormonally influenced traits (Manning 2002; Putz *et al.* 2004).

Bailey, Wahlsten and Hurd (2005) (hereafter BWH) investigated hindpaw digit ratio in eight inbred (i.e. genetically uniform) mouse strains. Although BWH found large interstrain differences, mirroring the population (geographical) differences seen in human 2D:4D (Manning 2002), in contrast to human 2D:4D studies, they failed to find within-strain sex differences. Furthermore, the direction of digit ratio–behavioral trait associations in mice appeared to be the opposite to those found for human 2D:4D and corresponding traits. Here, I review cross-species 2D:4D research and present other pertinent evidence, to offer possible explanations for these unexpected findings and to motivate future research efforts in this field.

Cross-species comparisons of digit ratios

BWH, investigating mouse hindpaws, actually measured toe ratios (2T:4T) rather than finger ratios (2D:4D). Recent accounts indicate that this represents important differences and that cross-species generalizability in this research area is uncertain.

McFadden and Shubel (2002) found (a) generally smaller sex differences in human toe ratios relative to finger ratios and (b) that ratios showing substantial sex differences in fingers were not exactly those showing substantial sex differences in toes. Both the findings are unsurprising. The direction of vertebrate embryonal development is rostro-caudaul (head-to-tail): toe ratios may therefore be less affected (i.e. are less sex-differentiated) by early gestational hormonal effects than finger ratios. Furthermore, anatomical axes in the human hand and foot differ. The hand axis is a line through the third metacarpal and the middle finger, whereas the foot axis goes through the

second metatarsal and the second toe. In contrast, other primate species' hand and foot axes alike pass through the middle digit (Cummins & Midlo 1961; p. 202). Corresponding digit ratios of human hands and feet thus are not homologous and may well belong to different, ray-related embryonal growth fields differently affected by prenatal androgens.

Regarding evidence from rodents, Brown *et al.* (2002) reported lower 2T:4T in male compared with female outbred laboratory mice. This sex difference was replicated in another study (Manning *et al.* 2003), except for that both group means were close to unity in the latter study whereas noticeably smaller than unity in the former one. By contrast, median 2T:4T values obtained by BWH (their Fig. 2a,b) invariably were larger than unity. Considering that 2T:4T is a unit-free ratio variable and not a length measurement, it appears unlikely that this consistent divergence in mean 2T:4T levels is attributable to different digit measurement techniques and tissue surface landmarks used across these studies. Consequently, BWH conjectured that inbred vs. outbred mice possibly differ in their typical 2T:4T. Relatedly, another report (McMechan *et al.* 2004) found a sex difference for mouse 2D:4D, too, again with both group means being smaller than unity.

As for avian species, sex differences in zebra finch 2T:4T (Burley & Foster 2004) were not replicated in a larger study (Forstmeier 2005) and lacked for pheasant 2T:4T (Romano *et al.* 2005). Similarly, skeletal studies on sex differences in metatarsal length or weight ratios of primate species (baboons, chimpanzees and gorillas) so far yielded inconsistent evidence (McFadden & Bracht 2003, 2005), and finally, contrary to the direction of the sex effect in human 2D:4D, in one *in vivo* study, male baboons presented substantially higher 2D:4D than females (Roney *et al.* 2004).

This brief overview indicates that patterns of sex differentiation in digit ratios across species are complicated and presently poorly understood that conclusions about finger (forepaw) ratios may not generalize to toe (hindpaw) ratios within the same species and that, for reasons currently unknown, the BWH findings on sex differences in inbred mouse 2T:4T diverge from other mouse evidence.

Validity threats of ecological correlations

BWH wondered 'whether smaller or larger ratios are more masculine in mice', because obtained associations of mouse 2T:4T and behavioral traits were 'the opposite to that of previous studies with human hand digit ratios' (p. 322). Some methodological notes are appropriate in this context.

To begin with, BWH calculated correlations between sex-specific, within-strain averages in 2T:4T (each taken as a separate data point) and retrievable strain-specific (but not

sex-specific) reference values for behavioral traits (obtained from http://www.jax.org/phenome; Mouse Phenome Database). In other words, BWH calculated ecological (group-level) correlations. This is an ingenious approach for a pilot study but also prone to ecological fallacies (i.e. aggregate-level findings not reflecting individual-level effects). Importantly, all known correlates of human 2D:4D are individual-level findings and typically have turned out to be sex-specific (e.g. 2D:4D is negatively associated with male, but not female, spatial ability level; Manning 2002).

Furthermore, BWH mostly had to rely on proxy variables for the cross-species comparisons (see their Table 1). The digit ratio–behavioral trait relation with (a) total daily activity in mice, taken as a stand-in variable, was compared with sporting success in humans, (b) average number of bites (mice) with physical aggression (humans), (c) systolic blood pressure with age at first myocardial infarction, (d) percentage of time spent in open quadrants with self-reported depression and only (e) body weight exactly with body weight. The puzzling results were digit ratio–trait relations (a) to (c) were positive in mice but negative in humans; relation (d) was negative in mice but positive in humans and relation (e) was weakly negative in mice but absent in humans (in aside, one study found human 2D:4D positively related to body-mass index, for men only; Fink et al. 2003). BWH rightly noted that 'some of these assays may not model the human traits well, and caution is warranted in interpreting the results' (p. 321), which is emphasized here again. Furthermore, due to the data aggregation, group-level effects overestimate individual-level effects and, in fact, the ecological digit ratio–trait correlations obtained by BWH were notably stronger than individual-level, sex-specific findings from human 2D:4D research (Manning 2002).

Most importantly, because of the real possibility of ecological fallacies having occurred here, it is entirely conceivable that individual-level correlations between digit ratio and target traits exist within mice strains, tallying to those found in humans but that the direction of these associations got reversed through the indirect, group-level analysis of BWH, due to interstrain 2T:4T differences and interstrain as well as sex differences in behavioral traits.

Future studies should thus directly analyze individual variation in mouse digit ratio with actually measured individual variation in target traits, with strains and the sexes considered separately. Preliminary evidence suggests only moderate heritability of human 2D:4D ($h^2 = 0.40$–0.50; Manning 2002). If this is also true for mice, even genetically uniform animals would present considerable individual variation in digit ratios.

Human–rodent differences in fetal developmental timing

BWH suggested inbred mice might be a promising system for further investigations into digit ratio–behavioral trait associations. – This is not necessarily the case, owing to human–rodent differences in the timing of prenatal developmental stages of the upper and lower extremities, including the dermatoglyphic features (epidermal creases) on their ventral surfaces.

Hand and foot differentiation onset occurs at 15% time passed in intrauterine life in human fetuses vs. 65% in rats. Finger pads and creases appear at 20 vs. 75%, fingers and toes separate at 25 vs. 80%, the majority of creases is present at 30 vs. 85%, respectively, and epidermal ridges are fully developed at 50% of human intrauterine time whereas only postnatally in rats (Durham et al. 2000; p. 212).

Interestingly, human dermatoglyphic traits, such as 2D:4D, are fixed prenatally, show numerous sexual dimorphisms, and therefore are also thought to be influenced by intrauterine androgen levels (Sorenson Jamison et al. 1993). There is also direct evidence for associations of dermatoglyphic traits with 2D:4D (Manning 2002; pp. 9–11).

Developmental timing of digit formation in rodents occurs much later than in humans. This corresponds to comparably late fetal androgen secretion in rodents relative to humans, but differences in the timing and type of sex-hormonal contributions to sexual differentiation between short-gestation species (rodents) and long-gestation species (humans) may still matter (Fitch & Denenberg 1998). It therefore remains to be seen whether the chosen animal model is valid, i.e. whether rodent digit ratios reflect gestational androgen effects to a similar degree than human digit ratios appear to do.

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No sex difference in mouse digit ratio: reply to Voracek

Voracek specializes in the study of human non-neural morphology (Voracek & Fisher 2002) and suicide (Voracek 2005), and he does not appear to appreciate the methods used in experimental mouse genetics. We (Bailey et al. 2005) investigated 2D:4D digit ratio in inbred mouse strains, because the findings can lead directly and fruitfully to a genetic analysis of factors that lead to higher and lower ratios (we follow the convention of using 2D:4D to refer to this digit ratio as have all previous published animal and human studies of hind limb

digit ratios. We know of no published work using Voracek's 2T:4T nomenclature. A PubMed search over the past 5 years uncovered not one paper using 2T:4T but 135 using 2D:4D, including all seven investigating hind limb digits). Indeed, our study detected substantial and interesting strain differences, although significant sex differences were not found. Voracek is evidently disappointed at the lack of a sex difference in our data and seeks to dismiss them by branding our work a 'pilot study' whose results 'diverge from other mouse evidence'.

The sum total of the 'mouse evidence' for the genus *Mus* is three studies. Brown *et al*. (2002) measured left and right hind paws of 32 weanling and 39 adult 'outbred lab mice' of unspecified origin, and they found a significantly lower 2D:4D ratio for males than females only for the right paw. Manning *et al*. (2003) measured only the left hind paw for 111 mice of unspecified age and strain. The poorly specified genetic composition of the two populations is appalling. Manning *et al*. did not even replicate the finding of Brown *et al*. for the right paw; hence, it is difficult to see how our data for a larger sample of 175 inbred mice from eight well-defined and easily replicable strains could 'diverge' from those two. Furthermore, for three strains with lower average digit ratios (BALB/cByJ, BTBR/T + tf/J and C3H/HeJ), we did find a lower ratio for males than females, although the sex difference was too small to achieve significance. While differences in measurement techniques (see discussion in Bailey *et al*. 2005) prevent rigorous comparisons of ratio values across studies, note that the absolute values from the Manning *et al*. study are almost exactly the same as the absolute values and sex differences of the C3H/HeJ strain in our study.

Voracek suggests that the evidence supporting a correlation between finger length ratio and developmental androgen exposure is very strong. We regard the evidence as circumstantial but suggestive. The Lutchmaya *et al*. (2004) study found a correlation between testosterone : estradiol and 2D:4D ratios in a small, combined sample of 18 males and 15 females. Differences in 2D:4D between human ethnic groups dwarf differences between the sexes, and sex differences also vary considerably across ethnic groups (Manning *et al*. 2000; McFadden *et al*. 2005). This suggests that the testosterone exposure story is at least an over-simplification. The only experimental study that manipulated developmental testosterone was Romano *et al*. (2005), which produced a change in hind limb digit ratio in pheasants.

More data are needed to illuminate the true relationship between developmental variables and digit ratio. For example, it would be interesting to study an F2 hybrid population, because extreme inbreeding might disrupt the regulation of developmental processes involving the digits. For the average effect size of the sex difference that we observed (d = 0.04), the research would need to be done with samples of at least 5000 per sex to achieve power of 90% when using Type I error probability = 0.05 (Wahlsten 1991).

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