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Direct and radiographic digit ratio (2D:4D) measurements of Tuvan children and adolescents from Southern Siberia: Sex differences and skeletal maturation

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ABSTRACT

Background: In Mongolian-origin ethnic groups digit ratio (2D:4D; a proxy for prenatal sex-steroids) is sexually dimorphic (males < females), as reported for other ethnicities. Most studies measured 2D:4D from soft tissue (directly from the digits, or indirectly from hand scans), or from radiographs. Evidence on the correspondence of 2D:4D measurements from soft tissue with measurements from radiographic images is scarce and has not been reported for a Mongolian-origin sample. In addition, previous research has not considered relationships between 2D:4D and measures of skeletal maturity.

Aim: To examine (i) associations between 2D:4D measured directly from the palms with those obtained from radiographic images of the same individuals in a sample of children and adolescents from the Tuvan population in Siberia (Russian Federation), and ii) associations between 2D:4D measurements with chronological and skeletal age.

Subjects and methods: Participants were Tuvan boys and girls aged 7 to 18 years. 2D:4D of the right and left hand was measured from soft tissue (directly from the palm) and compared with radiographic images (left hand only). In addition to finger length 2D:4D, we examined 2D:4D of the phalanges from measurements of radiographs. Skeletal age was assessed using the Tanner-Whitehouse method.

Results: Sex differences (boys < girls) in measurements of 2D:4D from soft tissue and radiographs were found for total finger length and phalanges. In addition, 2D:4D measurements from radiographs correlated positively and significantly with those obtained from soft tissue. Sex predicted 2D:4D measurements from soft tissue and radiographs, but no effects of chronological/skeletal age and body height were detected. In girls (but not in boys), earlier skeletal maturity (relative to chronological age) was associated with higher 2D:4D in soft tissue measurements of both hands, radiographic 2D:4D, and 2D:4D of the proximal phalanges.

Conclusion: Consistent with reports from other ethnic groups, 2D:4D in young Tuvans was sexually dimorphic, with boys having lower 2D:4D than girls. For girls, higher 2D:4D was found for participants whose skeletal age was more advanced than chronological age. This finding was obtained from direct soft tissue and indirect radiographic measurements. Age and body height were not associated with 2D:4D, which suggests differences in hormone developmental trajectories for 2D:4D and height.

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1. Introduction

The relative length of the 2nd and 4th fingers (digit ratio; 2D:4D) in healthy humans is sex-differentiated, with males having lower 2D:4D than females from the same population [1–7]. The evidence for this sex difference is robust across populations and has been documented in studies with large samples (i.e., the BBC internet study; n > 250,000; [7,8]. However, not every digit ratio study has detected a sex difference [1,9–13]. Whether the latter findings reflect adaptive processes in particular populations or are due to differences in protocols across studies, including statistical power issues, remains to be understood. Large negative relationships with 2D:4D are often found with masculinity [14–18] and physical performance, both in men [19–22] and women [23,24].

Manning et al. [6] proposed that 2D:4D is a negative correlate of foetal testosterone levels in males and a positive correlate of foetal oestrogen levels in females. Testosterone facilitates the growth of the ring finger (4D), whereas oestrogen facilitates the growth of the index finger (2D) [17,25]. Thus, 2D:4D may be determined prenatally and remain relatively stable throughout development [5,6,17,26,27]. Concentrations of testosterone and oestrogen may reflect prenatal gonadal differentiation, specified by members of the *Homeobox* gene family (*Hoxa* and *Hoxd*), and these genes also may influence skeletal growth [28,29]. Studies of congenital adrenal hyperplasia (CAH; mostly due to 21-hydroxylase deficiency) corroborate this hypothesis. Females with CAH (compared to female controls) showed masculinized 2D:4D similar to male controls [30–33], an effect attributable to greater androgenic action (but see [34]).

Research on the effect of age on 2D:4D has produced mixed results [4,35,36]. Some studies have reported positive correlations between 2D:4D and age [35,36], and others negative [37,38] or null relationships [4,6,17]. 2D:4D may undergo minor changes during postnatal development, particularly in the prepubertal period. An increase in 2D:4D with age has been demonstrated in longitudinal studies of both sexes [39-41]. In a cross-sectional study, age-related changes in 2D:4D were observed for females only [42]. These findings suggest that 2D:4D is only fully established once digital growth is complete. Assessing participants ranging in age from 4 to 60 years, Gillam et al. [35] noted that 2D and 4D displayed biphasic growth with the inflexion occurring between 13 and 20 years of age. Females entered puberty earlier than males (12-15 vs. 17-20 years), and 2D and 4D reached their maxima earlier in females than in males. Moreover, digit ratios were perturbed in females around 9-12 years of age, and 2D in dextral females was longer in the right hand, whereas no such effects were observed in males. In females, 4D length and 2D:4D showed evidence of age-dependent lateral asymmetries at 11-18 years of age.

Height is not robustly associated with 2D:4D [4,17,43]. However, some studies suggest that 2D:4D predicts male body size [44,45]. Correlations between testosterone and height also have been observed [46–49].

The universally observed sex difference in maturation rate [50–53] deserves special attention in the context of the current study. According to data from the Paediatric Bone Mineral Accrual Study (1991–2017), girls mature approximately two years earlier than boys (11.8 vs. 13.4 years), and they are shorter and have greater fat mass [54]. Long bone growth and epiphyseal closure depend on estradiol production [55–60]. Higher oestrogen levels in females compared to males lead to earlier bone fusion in females, resulting in sex differences in adult height and mass [61]. Higher oestrogen levels during development may result in broadened pelvises and more successful birthing [62,63].

The goal of the present study was to investigate the association of 2D:4D from soft-tissue measurement and radiographic images with sex, age, and "delta age" (i.e., the difference between chronological and skeletal age). Testing the effect of maturation speed (e.g., skeletal age) on 2D:4D in boys and girls during development may be of special interest, given the sex difference in growth spurts. Analysis of 2D:4D for

individuals with different delta ages may inform the role of skeletal maturation trajectories in the production of sex-specific differences in digit ratio.

We hypothesized that i) girls with earlier skeletal maturation (i.e. negative delta age) will have a higher 2D:4D than later-maturing girls; ii) this effect will be more visible for the radiographic composite 2D:4D than for the soft-tissue ("direct") right and left 2D:4D, and iii) these associations will be sex-specific.

2. Methods

2.1. Study sample

The present study was cross–sectional. The sample comprised of 389 (180 boys) Tuvan children and adolescents. Chronological age ranged from 6.9 to 18.1 years, with a mean of 12.0 ± 2.7 years. The data were collected in Kyzyl (N $51^{\circ}43'$, E $94^{\circ}27'$), the capital of the Republic of Tuva (Russian Federation), in 2018. The Tuvans are one of the two most numerous ethnic groups in Siberia. The population of Tuvans is estimated at $\sim\!260,000$ in Russia. Most of the children who participated were born in rural areas and their families only recently settled in urban areas. The Tuvan language belongs to the Sayan group of Turkic origin of the Altai family, and most Tuvans today speak both Tuvan and Russian, with Russian especially common in the younger generation (see [64]).

2.2. Anthropometric measurements

The 2nd and 4th fingers were measured directly from the right and left hands, and also from radiographic images (left hand only) recorded during fieldwork. Direct measurements of right and left 4D from the tip of the digit to the metacarpophalangeal crease were conducted with a Vernier calliper (Emil Lux GmbH & Co. KG, Germany) with an accuracy of 0.01 mm. All measurements were conducted twice for each hand. Participants who reported injuries or deformities of 2D or 4D were excluded from the analysis. Based on two measurements of the right-and left-hand 2D and 4D, the means of the two measurements, respectively, were calculated (R2D:4D and L2D:4D). The interclass correlation coefficient (ICC) for each hand was high: right-hand ICC = 0.914, n = 385, p < .0001; left hand ICC = 0.952, n = 388, p < .0001. Thus, we used the mean ratios in subsequent analyses. Along with direct measurements of fingers, body height was measured with an anthropometer (GPM Instruments GmbH, Switzerland) with an accuracy of ± 0.1 cm.

2.3. Radiographic assessment

Single radiographs of the left hand were obtained from each participant in the posteroanterior position with the X-ray source located 90 cm above using the radiographic technique described by Pavlovsky [65]. The hand was exposed for $1.5{\text -}2~\text{s}$ at $100{\text -}150~\text{mA}$, without intensifying screens, at 75 kV.

2.4. Skeletal maturity assessment

Radiographs were used to measure the lengths of proximal, middle, and distal phalanges from the proximal head to the distal base, respectively, affording assessments of skeletal maturity. Left-hand 2D and 4D were measured with a digital tool on the screen (in mm) (the "Mashtab" program, http://antropol.narod.ru/scalehlp.html). For the analysis, the lengths of the three phalanges including the interphalangeal joints were summed and the digit ratio from radiographic images ("OST 2D:4D") was calculated. Skeletal maturity was estimated based on the Tanner—Whitehouse system of bone ageing, 2nd edition (TRW2; [66]). The 20 bones of the left wrist, hand, and fingers from radiographs were compared with X-ray images in a standard atlas of bone development [67], and a composite index was calculated. According to consensus (e.

g., [67]), there are several reasons for using left (rather than right) hand and wrist radiographs for bone age assessment. Most people are right-handed and, therefore, the right hand is more likely to be injured than the left. The hand and wrist were used because it is easy to identify the ossification centres which afford accurate assessment of skeletal maturity.

Because the time of ossification can be accurately estimated for all participants (given the provision of an X-ray), we were able to assess finger growth completion. Thus, in the analysis, we used three age parameters: chronological age, skeletal age, and "delta age" (i.e., the difference between chronological and skeletal age). Delta age may be useful as it can differentiate slow-, normal-, and fast-maturing individuals and indicate whether an individual's development corresponds with members of their peer group.

The protocol was approved by the Scientific Council of the Institute of Ethnology and Anthropology of the Russian Academy of Sciences (protocol N° 1, dated 19 February 2015). All participants provided written or verbal consent for participation. The local school administrations and parents of children were informed about the purpose of the study and provided consent as well.

3. Results

3.1. Descriptive statistics

Skeletal age ranged from 5.1 to 18.1 years, with a mean of 12.3 \pm 3.0 years, and body height ranged from 1.14 to 1.80 m, with a mean of 1.45 \pm 0.14 m. None of these measures showed a sex difference (see Table 1).

The measurements of 2D and 4D (range, mean \pm SD) from hand soft tissue in boys were as follows: R2D 47.33–77.72 (61.33 \pm 6.77), R4D 50.81–82.44 (64.44 \pm 7.33), L2D 47.35–78.29 (61.46 \pm 6.94), L4D 50.91–82.20 (64.96 \pm 7.37); and for girls: R2D 47.35–74.52 (60.87 \pm 5.79), R4D 49.68–74.43 (62.11 \pm 5.96), L2D 47.31–75.85 (60.32 \pm 5.89), L4D 49.73–76.87 (62.51 \pm 5.96). The summed measurements (phalanges including interphalangeal joints) from radiographs were: for boys, 2D 48.46–88.74 (66.65 \pm 8.50), 4D 55.34–98.83 (74.08 \pm 9.54); for girls, 2D 47.22–78-79 (65.04 \pm 7.22), 4D 54.41–86.66 (71.65 \pm 7.64).

The mean 2D:4D of soft-tissue measurements from right and left hands, 2D:4D (left hand) from radiographic proximal, middle, and distal phalanges, and the composite 2D:4D of three phalanges including joint spaces ("OST 2D:4D") are reported in Table 1 together with sex differences assessed by independent-samples *t*-test. All 2D:4D measures were sexually dimorphic in the expected direction (boys < girls) (Table 1).

3.2. Correlations of digit lengths and digit ratios from soft-tissue and radiographic measurements

Correlations (Pearson r) of (left-hand) digit lengths and digit ratios from soft-tissue and radiographic measurements in the total sample were as follows: L2D $r=0.957,\,p<.001,\,$ L4D $r=0.964,\,p<.001,\,$ L2D:4D $r=0.558,\,p<.001.$ For soft tissue measurements, 2D was positively correlated with 2D:4D $(r=0.139,\,p<.01)$ and negatively correlated with 4D $(r=-0.171,\,p<.001)$. For radiographic measurements, 2D was positively correlated with 2D:4D $(r=0.148,\,p<.01)$ but no significant correlation was found for 4D with 2D:4D $(r=-0.059\,p=.248)$.

3.3. Correlations of age and body height with digit lengths and digit ratios from soft-tissue and radiographic measurements

In both boys and girls, there were positive correlations (Pearson r) between chronological age and body height, respectively, and soft tissue 2D and 4D length, but not with 2D:4D either from soft tissue (right and left hand) or from radiographs (left hand) (Table 2). In boys, body height correlated positively with chronological age (r=0.904, p<.001) and skeletal age (r=0.937, p<.001) and similar relationships were found in girls (r=0.865 and r=0.935, both p<.001).

3.4. The effects of chronological/skeletal age and body height on digit ratios from soft-tissue and radiographic measurements

We performed a multivariate analysis of covariance (MANCOVA) with digit ratios as dependent variables (right and left 2D:4D from soft-tissue measurement, left 2D:4D of phalanges, and composite 2D:4D from radiographs), sex of participant as a between-subjects factor, and chronological/skeletal age and body height as covariates (see Table 2). Multivariate tests showed an effect of sex (*Wilks'* $\lambda = 0.877$, F(6,375) = 8.801, p < .001, $partial\ eta^2 = 0.123$) but no effects of chronological age (*Wilks'* $\lambda = 0.989$, F(6,375) = 0.708, p = .643, $partial\ eta^2 = 0.011$), skeletal age (*Wilks'* $\lambda = 0.995$, F(6,375) = 0.333, p = .919, $partial\ eta^2 = 0.005$), or height (*Wilks'* $\lambda = 0.983$, $F(6,375) = 1.109\ p = .356$, $partial\ eta^2 = 0.017$). The results indicated a sex difference (boys < girls) for all 2D:4D measures and no effects of age variables or height on the dependent variables (see Table 3).

3.5. Associations between digit ratios and skeletal maturation in boys and girls

Neither chronological age nor skeletal age showed a sex difference (see Table 1) but were highly correlated in the total sample (r = 0.927, p < .001) and separately for boys (r = 0.926, p < .001) and girls (r = 0.935, p < .001). However, delta age showed a sex difference [t(386) = 0.935, p < .001).

Table 1
Descriptive statistics and tests for sex differences for chronological age and skeletal age (both in years), height (m), and 2D:4D ratios. R2D:4D and L2D:4D refer to measurements from soft-tissue ("direct"). 2D:4D from phalanges and the length of three phalanges together including the interphalangeal joints ("OST 2D:4D") were measured from radiographs.

	N Males	N Females	Mean (SD) Males	Mean (SD) Females	T	df	P	95 % CI
Chronol.	180	209	12.17 (2.63)	11.77 (2.79)	1.43	383.77	0.154	[-0.148, 0.934]
Skeletal age	179	209	12.40 (3.12)	12.29 (2.84)	0.35	363.75	0.728	[-0.493, 0.705]
Body height	180	209	1.47 (0.15)	1.44 (0.13)	1.91	355.17	0.057	[-0.0008, 0.056]
R2D:4D	178	208	0.958 (0.032)	0.980 (0.030)	-6.72	367.57	< 0.001	[-0.028, -0.015]
L2D:4D	180	209	0.947 (0.030)	0.965 (0.030)	-6.11	377.40	< 0.001	[-0.024, -0.013]
Phal. dist. 2D:4D	180	209	0.885 (0.039)	0.894 (0.041)	-2.06	383.70	< 0.05	[-0.016, 0.0001]
Phal. med. 2D:4D	180	209	0.856 (0.036)	0.865 (0.035)	-2.27	375.21	< 0.05	[-0.015, -0.001]
Phal. prox. 2D:4D	180	209	0.934 (0.026)	0.941 (0.025)	-2.42	371.44	< 0.05	[-0.011, -0.001]
OST 2D:4D	180	209	0.900 (0.023)	0.908 (0.022)	-3.43	380.17	< 0.05	[-0.012, -0.003]

Table 2Pearson correlations (*r*) between chronological age and body height, digit length and digit ratios for boys and girls. R2, R4, L2, L4, R2D:4D and L2D:4D refer to measurements from soft-tissue ("direct"). 2D:4D from phalanges and the composite 2D:4D including the interphalangeal joints ("OST 2D:4D") were measured from radiographs.

	Boys			Girls			
	Chronol. Age	Skeletal Age	Body height	Chronol. Age	Skeletal Age	Body height	
R2D	r = 0.826	r = 0.854	r = 0.914	r = 0.754	r = 0.825	r = 0.900	
	p < .001	p < .001	p < .001	p < .001	p < .001	p < .001	
R4D	r = 0.813	r = 0.850	r = 0.905	r = 0.789	r = 0.841	r = 0.911	
	p < .001	p < .001	p < .001	p < .001	p < .001	p < .001	
L2D	r = 0.841	r = 0.866	r = 0.924	r = 0.755	r = 0.822	r = 0.909	
	p < .001	p < .001	p < .001	p < .001	p < .001	p < .001	
L4D	r = 0.822	r = 0.858	r = 0.911	r = 0.793	r = 0.842	r = 0.920	
	p < .001	p < .001	p < .001	p < .001	p < .001	p < .001	
R2D:4D	r = -0.064	r = -0.101	r = -0.088	r = -0.107	r = -0.055	r = -0.040	
	p = .395	p = .181	p = .234	p = .124	p = .427	p = .562	
L2D:4D	r = 0.060	r = 0.014	r = 0.036	r = -0.068	r =0.008	r = 0.026	
	p = .423	p = .850	p = .633	p = .331	p = .907	p = .713	
Phal. dist. 2D:4D	r = 0.136	r = 0.097	r = 0.097	r = 0.087	r = 0.104	r = 0.062	
	p = .069	p = .196	p = .196	p = .210	p = .134	p = .373	
Phal. med. 2D:4D	r = -0.044	r = -0.057	r = -0.031	r = 0.065	r = 088	r = 0.100	
	p = .561	p = .449	p = .684	p = .353	p = .206	p = .152	
Phal. prox. 2D:4D	r = 0.026	r = -0.016	r = -0.007	r = -0.073	r = 0.024	r = 0.063	
	p = .730	p = .829	p = .926	p = .294	p = .733	p = .367	
OST 2D:4D	r = 0.037	r = -0.012	r = 0.011	r = 0.021	r = 0.093	r = 0.102	
	p = .622	p = .873	p = .882	p = .762	p = .181	p = .140	

Table 3
Summary of the ANCOVA results for dependent variables 2D:4D ratios and independent variables sex of participant and covariates chronological/skeletal age and body height.

Source	Dependent variable	df	F	p	Partial eta
Sex	R2D:4D	1,380	42.739	< 0.001	0.101
	L2D:4D	1,380	37.669	< 0.001	0.090
	Phal. dist. 2D:4D	1,380	4.542	< 0.05	0.012
	Phal. med. 2D:4D	1,380	5.778	< 0.05	0.015
	Phal. prox. 2D:4D	1,380	5.488	< 0.05	0.014
	OST 2D:4D	1,380	11.972	< 0.001	0.031
Chronol. age	R2D:4D	1,380	0.858	0.355	0.002
	L2D:4D	1,380	0.738	0.391	0.002
	Phal. dist. 2D:4D	1,380	0.947	0.331	0.002
	Phal. med. 2D:4D	1,380	0.016	0.899	0.000
	Phal. prox. 2D:4D	1,380	2.608	0.107	0.007
	OST 2D:4D	1,380	0.333	0.564	0.001
Skeletal age	R2D:4D	1,380	0.007	0.933	0.000
	L2D:4D	1,380	0.231	0.631	0.001
	Phal. dist. 2D:4D	1,380	0.263	0.608	0.001
	Phal. med. 2D:4D	1,380	0.252	0.616	0.001
	Phal. prox. 2D:4D	1,380	0.002	0.964	0.000
	OST 2D:4D	1,380	0.015	0.904	0.000
Body height	R2D:4D	1,380	0.188	0.665	0.000
	L2D:4D	1,380	2.404	0.122	0.006
	Phal. dist. 2D:4D	1,380	0.972	0.325	0.003
	Phal. med. 2D:4D	1,380	0.857	0.355	0.002
	Phal. prox. 2D:4D	1,380	2.692	0.102	0.007
	OST 2D:4D	1,380	1.125	0.290	0.003

2.392, p < .05]; the mean difference (\pm SD) for boys was -0.248 ± 1.20 and for girls, it was -0.518 ± 1.02 . Thus, boys and girls showed signs of advanced maturation as skeletal age was – on average – greater than chronological age and this was particularly so for girls. Delta age in boys was negatively associated with height (r = -0.449, p < .001), chronological age (r = -0.222, p < .01), and skeletal age (r = -0.534, p < .001). Negative correlations with delta age also were found for girls, although these were smaller than for boys (body height r = -0.137, p < .05; skeletal age r = -0.148, p < .05), and the correlation with chronological age was not significant (r = 0.131, p = .059).

To examine the link between digit ratios and skeletal maturation, we performed linear regressions with the dependent variables(s) digit ratio [right/left 2D:4D from soft-tissue measurement, left 2D:4D of phalanges, and composite 2D:4D from radiographs, respectively] and delta age as

the independent variable.

For boys, there were no significant regression models for 2D:4D and delta age associations, either for soft-tissue or radiographic 2D:4D [soft-issue: R2D:4D $R^2=0.012$, F(1,175)=2.204, p=.139; L2D:4D $R^2=0.008$, F(1,177)=1.438, p=.232; radiographs: distal phalanges $R^2=0.003$, F(1,177)=0.486, p=.487; medial phalanges $R^2=0.003$, F(1,177)=0.538, p=.464; proximal phalanges $R^2=0.012$, F(1,777)=2.197, p=.140; composite 2D:4D $R^2=0.015$, F(1,777)=2.711, p=.101] (see Table 4).

For girls, the regression models were significant for (soft-tissue measurements) R2D:4D [$R^2=0.012$, F(1,206)=4.00, p<.05], L2D:4D [$R^2=0.027$, F(1,207)=5.560, p<.05], radiographic proximal phalanges [$R^2=0.071$, F(1,207)=15.825, p<.001], and the composite 2D:4D [$R^2=0.041$, F(1,207)=8.762, p<.01], but not for the medial phalanges [$R^2=0.005$, F(1,207)=0.976, p=.324] or distal phalanges [$R^2=0.003$, F(1,207)=0.555, p=.457] (see Table 4).

Fig. 1 presents scatterplots with regressions lines, separately for boys and girls, to illustrate associations between delta age and digit ratios – here with left-hand 2D:4D from radiographic images. The common finding from regression analyses is a positive slope for boys and a negative slope for girls (see Table 4). However, these slopes are non-

Table 4Summary of linear regression models for dependent variables (2D:4D) and independent variables (difference between chronological and skeletal age) in boys and girls.

	В	SE B	beta	T	p
Boys					
R2D:4D	0.003	0.002	0.112	1.485	0.139
L2D:4D	0.002	0.002	0.090	1.199	0.232
Phal. dist. 2D:4D	0.002	0.002	0.052	0.697	0.487
Phal. med. 2D:4D	0.002	0.002	0.055	0.734	0.464
Phal. prox. 2D:4D	0.002	0.002	0.111	1.482	0.140
OST 2D:4D	0.002	0.001	0.123	1.647	0.101
Girls					
R2D:4D	-0.004	0.002	-0.138	-2.00	< 0.05
L2D:4D	-0.005	0.002	-0.163	-2.377	< 0.05
Phal. dist. 2D:4D	-0.002	0.003	-0.052	-0.745	0.457
Phal. med. 2D:4D	-0.002	0.002	-0.068	-0.988	0.324
Phal. prox. 2D:4D	-0.007	0.002	-0.266	-3.978	< 0.001
OST 2D:4D	-0.004	0.001	-0.202	-2.960	< 0.01

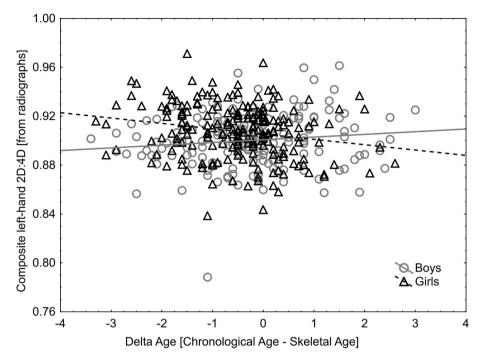


Fig. 1. Scatterplot of associations between "delta age" [chronological age – skeletal age] and the composite left-hand 2D:4D [the three phalanges together, including interphalangeal joints] from radiographs in boys and girls. Note: boys y = 0.9007 + 0.0022*x; girls 0.9054 + 0.0044*x.

significant in boys, and for girls, they are significant for right/left-hand 2D:4D from soft-tissue, from radiographs for the proximal phalanx, and the composite 2D:4D. Thus, girls for whom skeletal age > chronological age displayed higher 2D:4D. The results of the regression analysis together with Fig. 1 suggest that for boys, earlier skeletal maturation is associated with lower 2D:4D, although this effect was non-significant.

4. Discussion

Our data from Tuvan children and adolescents revealed sexual dimorphism (boys < girls) in 2D:4D measurements from soft-tissue (i.e., directly from the palm), corroborating results from European, African, and Asian samples [2–4] and from the majority of macro-ethnic groups [5,7,38]. Some studies reported larger sex differences in soft-tissue measurements for the right-hand 2D:4D than for the left-hand 2D:4D [68]; however, our study showed similar sex differences for right- and left-hand 2D:4D (see Table 1). We could not assess the sex differences in the radiographic 2D:4D of the right hand, because radiographic data were collected only for the left hand. Radiographic measurements of the phalanges showed sex differences for each phalanx 2D:4D and for 2D:4D comprising the three phalanges and interphalangeal joints.

Previous research using radiographs of Afro-Carribbean children's hands identified sex differences only for distal phalanges of the right hand [69]. The current findings from Tuvans are more comparable to those reported for the Fels Longitudinal Study samples (Caucasian US children; [70]), which reported sexual dimorphism of proximal and middle phalanges from left-hand radiographs. This also applies to the summed length of the three phalanges (including inter-phalanges joints), which was non-significant for the right hand in the Afro-Caribbean sample [69]. Trivers et al. [69] suggested that in 2D:4D research right-hand effects are larger than left-hand effects and, therefore, radiographic 2D:4D studies should include right-hand measurements. Although this suggestion is reasonable, it may be difficult to implement given the consensus favouring the use of left-hand radiographs in the assessment of bone age and development [67]. Sex (of participant) was a significant predictor for all tested (male vs. female) 2D:4D (including phalanges). The effect sizes for sexual dimorphism of the right- and left-hand measurements from soft tissue were medium, and small for the 2D:4D based on radiographic measurements.

Trivers et al. [69] suggested that in Afro-Caribbean children, the sex differences in the distal phalanx may have a signalling function. These authors speculated that sexual dimorphism in the tip of 4D (relative to other digits) may signal prenatal androgenization and is perceived when holding hands during courtship or shaking hands during social interactions. Such signals are more likely to be found in societies with stronger sexual selection such as those with polygynous mating systems [69]. In the present study of Siberian Tuvans, the effect sizes of 2D:4D of all phalanges were small; however, significant sexual dimorphism in 2D:4D was obtained for proximal phalanges only. We did not measure soft-tissue fingertips from radiographic images due to reliability concerns, as in many cases we were not certain about the endpoint of fingertips; therefore, a direct comparison with the finding from the Afro-Caribbean sample is not possible. However, we consider it unlikely that a signalling function of the 4D tip exists in Tuvans as their monogamous mating system [71], characterized by a sexual division of labour, may not have selected this trait.

The present study found that soft-tissue digit length and 2D:4D measured from the ventral surface of the hand are positively and significantly related to radiographic measures of 2D, 4D and 2D:4D. This is the first such finding in the literature. The proximal measurement point of the soft-tissue measurements is the flexion crease at the base of the digit. This is situated about the mid-point of the proximal phalanx. Despite this difference, digit lengths and 2D:4D from soft tissue and radiographs are significantly correlated and both are sexually dimorphic (males < females). Some studies have utilized dorsal (as opposed to ventral) measurements of digit length [72,73], suggesting that digit ratios derived from dorsal digit length (i.e., the distance between the tip of the finger and the dorsal base of the proximal phalanx) might better represent the sexual dimorphism in digit ratios derived from phalangeal bone length. The current study did not include dorsal digit measurements. However, it is noteworthy that dorsal 2D:4D in previous research was not found to be sexually dimorphic [73]. We have doubts about the accuracy of dorsal digit length measurements regarding their correspondence with phalangeal lengths (including the interphalangeal

joints) assessed from radiographs. Visual inspection of Fig. 1 in Kumar et al. [73] gives the impression that the head of the 2nd metacarpal bone (and the metacarpophalangeal joint) was included in the digit length measurement (that would be incorrect). The correlation of $r\sim 0.60$ between left-hand 2D:4D from radiographs and soft tissue in our study suggests that individual differences in "direct" ventral measurements correspond to bone lengths from the same individuals with acceptable accuracy. In addition, the relationship between radiographic 2D:4D and soft-tissue 2D:4D refutes the suggestion that 2D:4D is more likely a marker of sex differences in digit adiposity than a proxy for prenatal androgenization [74]. If that suggestion were true, one would expect no significant relationship between radiographic 2D:4D and assessments of 2D:4D from soft tissue. The findings of the present study suggest that this assumption is unlikely to be correct.

Concerning maturation, direct measurements (from soft tissue) of 2D and 4D of both hands showed that before the age of 12.5 years, 2D is longer for Tuvan girls than for boys; after that age, 2D is longer for boys. Regarding 4D length, boys exceed girls after the age of 10 years. Gilliam et al. (2008) reported greater lengths of 2D and 4D (both hands) for right-handed girls 4–11 years and for left-handed girls 4–12 years, and greater lengths for boys at later ages. Our study shows that compared to male digits, female digits reach their greatest length earlier, and this was exaggerated in left-handers compared to right-handers. For the radiographic composite digits (left-hand only), i.e. the three phalanges together including joints, girls' 2D lengths exceed those of boys before the age of 12 years, and boys had longer 2D after that age compared to girls; similar growth patterns were observed for 4D around the age of 11 years.

The results of the present study suggest higher 2D:4D in girls with an earlier maturation pattern whereas for boys the earlier skeletal maturation compared to chronological age did not result in differences in 2D:4D. Our findings are in line with knowledge about the dependence of long bone growth and epiphyseal closure on estradiol [55,57,58], according to which oestrogen accelerates the loss of progenitor cells in the resting zone of the long bone growth plate, which causes senescence in the growth plate and results in cessation of growth [56,59,60]. Higher oestrogen level in females results in earlier bone fusion compared to males, producing sex differences in adult height and mass [61]. From an evolutionary perspective, selection often favours earlier reproductive development in females compared to males; consequently, the sex difference in growth spurts is universally reported [50-53]. According to analyses of the Paediatric Bone Mineral Accrual Study (1991–2017), girls mature approximately two years earlier than boys (11.8 vs. 13.4 years), boys have greater bone mass than girls, and girls are shorter, have less lean mass, and have greater fat mass than boys [54]. Higher oestrogen levels in females during development may result in an expanded pelvis that facilitates more successful birthing [61-63].

Our study has several limitations. Following methodological tradition, our radiographic data were obtained for the left hand only. This limits the opportunity to compare the present results with those of several previous studies (e.g., [34,41,68,69]. However, we obtained effects of sexual dimorphism in the same direction for direct measurements from the right and the left hand, and for radiographic measurements of separate phalanges as well as their summed length. These findings may be useful for future research, especially research that aspires to incorporate data and/or results from previous radiographic studies. The current findings suggest that data on skeletal age may inform discussion of the nature of 2D:4D variation, between and within sexes. The data on skeletal maturity based on the Tanner-Whitehouse system of bone ageing [66], and the information contained in a standard atlas of bone development [67], may be useful when variations in 2D:4D have been separately presented for boys and girls. Of interest may be testing the differences in developmental processes of earlier and later maturation in girls, given potential variations in 2D:4D.

CRediT authorship contribution statement

CRediT roles: Marina Butovskaya: Conceptualization; Data Curation; Formal Analysis; Methodology; Resources; Roles/Writing – original draft; Supervision; Writing – review & editing; Yulia Adam: Data collection and data base creation; Valery Batsevich: Data collection; Methodology; Writing – review & editing; Todd Shackelford: Writing – review & editing; Bernhard Fink: Formal Analysis; Roles/Writing – original draft; Writing – review & editing.

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Informed consent

All participants provided written or verbal consent for participation.

Declaration of competing interest

The authors have no conflicts or competing interests to disclose.

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