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Directional Asymmetry in the Limbs, Skull and Pelvis of the Silver Fox (*V. vulpes*)

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Abstract

Directional asymmetry (DA) is a characteristic of most vertebrates, most strikingly exhibited by the placement of various organs (heart, lungs, liver, etc.) but also noted in small differences in the metrics of skeletal structures such as the pelvis of certain fish or sauropsids. We have analyzed DA in the skeleton of the fox (*V. vulpes*), using ~1,000 radiographs of foxes from populations used in the genetic analysis of behavior and morphology. Careful measurements from this robust data base demonstrate that: 1) DA occurs in the limb bones, the ileum, and ischium and in the mandible; 2) regardless of the direction of the length asymmetry vector of a particular skeletal unit, the vectorial direction of length is always opposite to that of width; 3) with the exception of the humerus and radius, there is no correlation or inverse correlation between vectorial amplitudes or magnitudes of bone asymmetries. 4) Postnatal measurements on foxes demonstrate that the asymmetry increases after birth and continues to change (increasing or decreasing) during postnatal growth. 5) A behavior test for preferential use of a specific forelimb exhibited fluctuating asymmetry but not DA. None of the skeletal asymmetries were significantly correlated with a preferential use of a specific forelimb. We suggest that for the majority of fox skeletal parameters, growth on the right and left side of the fox are differentially biased resulting in fixed differences between the two sides in either the rate of growth or the length of the period during which growth occurs. Random effects around these fixed differences perturb the magnitude of the effects such that the magnitudes of length and width asymmetries are not inversely correlated at the level of individual animals.

Keywords

fox; *V. vulpes*; skeleton; directional asymmetry; pelvis; mandible; limb bone

INTRODUCTION

Skeletal directional asymmetry (DA) is the consistent difference between a pair of skeletal structures such that the larger metric consistently occurs on one side (the smaller on the

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other). This phenomenon has been observed in a large number of taxa (Palmer, 1996), striking examples of which are the horn of the narwhale or the claw of the fiddler crab. Less striking examples of DA have been found in many vertebrates: e.g., the hind limbs of a lizard (Seligmann, 1998), the pelvis of a toad (Robins and Rogers, 2002) and of stickleback fish (Shapiro et al., 2004, 2006; Bell et al., 2007), the limb bones of harbor porpoise (Galatius, 2005), macaque (Falk et al., 1988) and humans (Auerbach and Ruff, 2006; Kujanová et al., 2008) and the mandible of the mouse (Leamy et al., 2000).

Palmer (2004) has reviewed earlier work on the evolution of DA. More recently, a number of experiments in *Drosophila* failed to demonstrate selection for DA (e.g., patterning of wing veins; Carter et al., 2009), whereas DA of jaw laterality in cichlids was shown to be under control of a selectable genetic locus (Stewart and Albertson, 2010). Cichlid fish may represent one of the rare examples where it is possible to demonstrate segregation and selection of DA.

In a search for genetic loci affecting DA, Leamy et al. (2000), identified three QTL that were associated with mandibular DA in mice. Analysis of the molecular basis of DA evolution in chordates identified a role of *Pitx2* (Boorman and Shimeld, 2002; Zhao, 2003) and subsequent analysis of DA involved in pelvic reduction in stickleback fish (Shapiro et al., 2006; Bell et al., 2006) has implicated *Pitx1*. Analysis of lower limb malformations in humans (Gurnett et al., 2008) also implicated a role of *Pitx1*. Thus, there exists a body of evidence implicating a genetic basis for regulation of DA.

We investigate DA in skeletal metrics of the silver fox (*V. vulpes*). For this investigation, we have analyzed a robust data base of radiographs comprising 1,000 foxes that had been characterized for differences in morphology (Kharlamova et al., 2007). In the examples cited above, different species were used to investigate DA focusing in each study on a particular skeletal system (limbs, pelvis, or mandible). In the fox, we had the opportunity to answer the following questions: 1) Does DA occur in different parts of the skeleton and if so, is the direction of asymmetry different between skeletal systems (e.g., the limbs, pelvis, or mandible)? 2) Does DA differ between different aspects of bone morphology (e.g., length and width)? 3) Is there any correlation between DA of different bones and if so is there a functional or developmental relationship? 4) In the fox populations examined, variation in skeletal metrics was heritable (Kharlamova et al., 2007). Is variation in DA also heritable? 5) Could we identify DA in the use of the forelimbs that might be correlated with, and therefore explain, skeletal DA? 6) Does DA change during postnatal growth?

Answers to these questions should provide a basis for relating DA to the regulation of skeletal growth.

METHODS

Animals and Animal Maintenance

We used silver-fox populations selectively bred and maintained at the experimental farm of the Institute of Cytology and Genetics, Novosibirsk, Russia. Populations selected for tame (436 foxes) or aggressive (161 foxes) behavior have been described (Trut, 2001), as well as segregant populations derived from crosses between tame and aggressive parents (Kukekova et al., 2008). The latter included two separate backcrosses to tame populations (143 and 132 segregant foxes), one backcross to aggressive population (139 segregant foxes) and one intercross (F2) segregant population (160 foxes).

All foxes in any given year were born March–April. Foxes are raised under consistent farm conditions and have similar interactions with people (mostly limited to maintenance

procedures). Pups are caged with their mothers until they are 1.5 months old. Subsequently, all littermates are housed together without their mother until 2.5–3 months of age, after which each pup is moved to its own cage (for more details see Trut, 2001, Kukekova et al., 2008).

Radiographic Data

Radiographs of foxes from various populations and ages were taken as described previously (Kharlamova et al., 2007). Specific, easily recognized, landmarks were used to define bone dimensions. These landmarks were accurately recognized by three different people. All points were recorded using the paths tool of Adobe Photoshop. Points were then exported using the “export to illustrator” option and read into R (R Development Core Team, 2006). Lengths of path were adjusted for measurement error (removal of extreme outliers) and checked for systematic bias by any investigator. Supporting Information Figure 1 presents radiographs showing the measurements taken.

To investigate early growth, radiographs were taken from a selected set of 45 foxes at 2 months, 3 months and 7 months of age. At each time point, 6 different views were taken: left foreleg including skull, right foreleg including skull, left hind-leg, right hind-leg, dorsal ventral skull and hips.

Behavioral Data

Forelimb preference—Tests for forelimb preference were carried out as follows: food was placed in a container that could be rotated into a fox’s cage by manipulating it with the fore-paws. Foxes were observed to see whether they used the left, right, or both forelimbs. For each fox, 10 such trials were carried out. For each trial, a fox received a forelimb preference score: 0 if it used only the right forelimb; 0.5 if it used both; 1 for the left only. Forelimb preference was estimated as the mean over the 10 trials.

Statistical Tests

For each metric a paired t-test for the difference between the left and right was performed using the R call “t.test (L, R, paired = T)” where L was the vector of values for the left metric and R was the vector of values for the right metric. (These t-test results matched results with a nonparametric test, e.g., permutation tests where left and right values are randomly swapped.) Pearson product correlation coefficients were calculated with the “cor” function. Significance was determined from 100,000 permutations.

Heritability

Asymmetry is the difference between a value on the left side and the right side (L–R). These values show considerable variation in this population. Because the mean asymmetry value was significantly different from zero we referred to this as DA. We estimated the heritability of phenotype y as the additive genetic variation divided by the total variation (σ_A^2/σ_T^2), using $y = \sigma_A^2 A + \sigma_E^2 I$. σ_A^2 , the additive genetic variation was estimated using the polygenic function of Sequential Oligogenic Linkage Analysis Routines (Almasy and Blangero, 1998) A is the additive genetic relationship matrix where $A_{ij} = 2\Theta_{ij}$; twice the coefficient of consanguinity (Falconer, 1996) estimated from four generation pedigrees (Kharlamova et al., 2007), σ_E^2 is the environmental variance and I is the identity matrix. Sex was not significant and thus not included in the model.

All methods were conducted in compliance with approved protocols by the appropriate institutional animal care committee and in adherence to the legal requirements of the country in which the research was conducted.

RESULTS

Directional Asymmetry Occurs in the Limbs, Pelvis and Mandible; and the Direction of Asymmetry Varies Between Bones and Between Bone Length and Width

Fox skeletal metrics, measured on radiographs of the limbs, pelvis and skull exhibited DA. Figure 1 presents examples from each of these structures: limbs—femur, humerus and radius; skull—mandible; pelvis—ileum. The data in Figure 1 comprise foxes segregating in three backcross populations (see “Methods”), but also is typical of all of the fox populations measured (e.g., the parental and F1 populations). It can be seen that for each example the direction of the length asymmetry (left column) is opposite to the width (right column).

Tables 1–3 summarize the DA observed in the limb bones (lengths, Table 1; widths Table 2; joints and levers, Table 3). The values are based on data of the type presented in Figure 1. The magnitude of asymmetries are presented as the percent of the mean value of the metric (e.g., in Table 1, the length of the right humerus exceeds the length of the left by 1.4% of half the value of the right + left humerus). For all of the limb long bones, the direction of asymmetry for length was opposite to the direction of asymmetry for the cortical thickness. Table 4 summarizes DA observed in the pelvis and skull. Again, the vector of asymmetry for length is opposite to width. It should be noted that the DA vectors for the lengths of the ileum and ischium are oppositely oriented.

With the Exception of the Forelimb, There is No Correlation Between DAs of Different Bones

Table 5 presents significant correlations in the forelimb. As might be expected, the DA of the radius was highly correlated with that of the Ulna. There was also a significant inverse correlation between the DA of the humerus and DAs of the radius and ulna, tending to maintain a comparable length between the forelimbs.

Correlations between the DA of limb bones were restricted to the lengths of forelimb bones. Other asymmetries were not correlated. In particular, the opposing vectorial asymmetries of length and width were neither correlated nor inversely correlated (e.g., the data in the bottom half of Table 5). As with the limb bones, no correlation (nor inverse correlation) was observed between the magnitudes of length and width asymmetry of the mandible or of the ileum and ischium nor between the DA of the lengths of the ileum and the ischium.

Heritable Variation in DA Could Not be Detected

Several fox populations were used in this study. These included parental, F1, and backcross populations. From pedigrees of the parental, F1, and segregant populations it has been possible to demonstrate heritability of morphological phenotypes. Although segregation of morphological metrics of limb bones had demonstrated that the variation observed in many limb metrics was highly heritable (Kharlamova et al., 2007), a similar analysis using these same populations failed to implicate any heritable component for the variation in directional asymmetries described in Figure 1 and Tables 1–3 and 5. In our analysis, heritability of variation greater than 25% would have been detected.

Behavior—Preferential Use of Right or Left Forelimb

In humans and some other vertebrates, handedness, or lateralized behavior has been related to DA (Falk et al., 1988; Galatius, 2005; Kujanová et al., 2008). We therefore tested whether

foxes were laterally biased in a behavioral test using their forelimbs. Food (pieces of liver) was placed in a container that could be rotated into the cage by a fox using its forelimbs. For each fox, 10 trials were recorded. The results for these tests are presented in Figure 2, in which the 243 foxes tested are ranked according to the number of trials for which they used their left paw. Ten thousand Bootstrap trials over the 10 replicates were used to establish standard errors for individual fox means and for population means. There was a slight bias toward left forelimb preference (mean = 0.51, SE = 0.0041). Analysis of variance shows a significant forelimb preference for individual foxes (P -value < 0.0001). There was no significant difference between the sexes and no significant heritability. While there was no large bias in the population toward the use of either right or left forelimb individual foxes often tend to use one forelimb preferentially (either right or left). However, there was no significant correlation between preferential use of a forelimb and limb asymmetry.

Changes in Asymmetry Occurred During Postnatal Growth

DA might be fixed during prenatal development or continue to change after birth. To test this, radiographs of 45 pups were taken at 2, 3, and 7 months. Left and right fore and hind limbs were measured and the means compared. The results (Table 6) show that asymmetry continues to change during this growth period: DA is evident at 2 months in the lengths of the femur and radius and continues more or less proportional to the subsequent growth of these bones. Although DA is present at 2 months in the length of the ulna, the magnitude of the difference was very small, becoming larger at 3 months. In the humerus and tibia, DA could not be detected at 2 months but was apparent at 3 months in the humerus and at 7 in the tibia. DA could be detected in the lengths of the metatarsals at 2 and 3 months but became insignificant at 7 months. Thus length DA changed in magnitude during the first 7 months of growth. Width measurements were much less reliable due to the imprecision resulting from the very small measurements involved. Asymmetry in the two-month measurements of the humerus and radius cortical thickness could not be determined with significance. However, for the femur and ulna the direction of width asymmetry was opposite to that of length at 2 months. At 7 months, the vector of the cortical thickness of the femur and tibia, while not significantly different from zero, remained opposite to that of length, consistent with the finding from the much more robust adult data set. In contrast, the vector for cortical thickness of the radius and ulna differed significantly with the values from the adult data set. It seems clear that variation in postnatal growth in limb length is accompanied by variation in DA. Variation in the cortical thickness was often too difficult to measure due to the small differences in the inner and outer diameters of bones in the young animal. However, where it could be determined, changes in cortical thickness DA were much more dramatic than changes in length DA as could be seen in the DA of the femur, radius and ulna when compared to adult values.

DISCUSSION

We have demonstrated the existence of DA in the several skeletal systems of the silver fox. Left-right asymmetric values were obtained for the limb bones, the mandibles and both the ileum and ischium in the pelvic girdle. Because of the size of the populations analyzed, we were able to establish significant asymmetries involving small differences between left and right metrics. Our data demonstrate that skeletal metrics in the fox populations vary around mean values that differ for each metric. For some metrics, the mean value is greater on the left than on the right, for others greater on the right than on the left. Hence, we observe DA.

We analyzed foxes from different populations, as well as progeny from crosses between these populations. These populations had been shown to be genetically different and progeny developed from crosses between these populations display heritable variation in skeletal morphology (Kharlamova et al., 2007). Nevertheless, we were unable to

demonstrate heritable variation in skeletal DA. Variation around these values can be due to a number of environmental effects, which may obscure minor differences in the DA values. If so, the genetic signal must be smaller than 25% which is the limit of our power to detect heritability in this population. (In the mouse, Leamy et al. (2000) found that heritable variation of mandibular DA was very low, well below our limit of detection.) Alternatively, the failure to detect segregation of DA would be expected if values of DA were genetically fixed in the fox (see discussion below).

Not surprisingly, the magnitudes of asymmetry of the radius and ulna were highly correlated. There also was significant correlation between the asymmetric properties of the humerus and those of the radius and ulna (Table 4). These correlations extended to the olecranon and elbow joint. Thus, at some level, the asymmetries in growth of the forelimb appear to be coordinated. However, no such correlation (or inverse correlation) could be found for the magnitude of the inverse DA vectors for the length and width metrics of the limb bones, the ileum and ischium, or of the mandible.

The consistent inverse directional relationship between length and width is in striking contrast to observations of DA in porpoise (Galatius, 2005), human (Kujanová et al., 2008), or macaque (Falk et al., 1988), in which lengths and widths of the bones of the forelimb have the same DA vector ($R > L$ except for the radius of the macaque in which length and width are both $L > R$). In all of those cases, it was argued that DA derived from a behavioral bias that resulted in greater usage of the directionally favored limb (handedness). We tested for behavioral bias by presenting foxes with the need to rotate food containers into their cage, which they could do using either the right or left forelimb. Although there were significant preferences by individual foxes to use either the right or the left forelimb, the population was not heavily biased toward one side or the other (i.e., this behavior presents as fluctuating rather than DA). Although there may be a behavioral bias that affects the directional asymmetries described here, we have not observed such behavior. Because movement of these animals is restricted within their cage behavioral differences may have less of an impact on skeletal loading. However, the consistent inverse vectorial relationship of length to width observed in the skull, and pelvis as well as the limb bones is difficult to attribute to a behavioral bias. Moreover, any such behavioral explanation would have to account for the inverse vectorial DA of the ileum and ischium.

We would like to propose an alternative to behavior as a basis for the observed directional asymmetries: The most important component of canine skeletal shape (other than size) is the inverse correlation between length and width of various bones observed in both foxes and dogs (Carrier et al., 2005; Kharlamova et al., 2007). This inverse relationship is heritable and in dogs, genetic loci regulating this variation have been identified (Carrier et al., 2005; Lark et al., 2006; Parker et al., 2009; Quignon et al., 2009). Previous results analyzing the pelvis of the dog (*C. familiaris*) have demonstrated a shape component in which the lengths of the ilium and ischium also were inversely correlated. That relationship was heritable and loci regulating that relationship also were identified (see Fig. 2 in Carrier et al., 2005).

Here, we have seen a striking similarity that governs the vectorial relationship of the asymmetries of the limbs, skull, and pelvis whereby the DA vector of length is always opposite to that of width. This inverse vectorial relationship of length/width DA may be related to the inverse relationship between overall length and width observed in previous studies. For example, if growth on the right and left sides of the animal are differentially biased, such that one either terminates slightly ahead or after the other, then a longer bone would necessarily also be thinner (because of the inverse relationship between overall length and width), giving rise to opposing asymmetries for length and width. Lack of correlation in the amount of asymmetric growth (length vs. width) would represent random variation

(noise) in the growth process (e.g., in the supply of nutrients to the tissues during growth). The results in Table 6, comparing asymmetries during postnatal growth, suggest that such inequalities between growth rates (or of periods of growth) on the left and right sides change in magnitude and direction with age, a result previously observed in macaques (Helmkamp and Falk, 1990).

This concept of differential bilateral skeletal growth is supported by genetic evidence that in dogs, the laxity of the right coxofemoral joint is regulated by one locus whereas the left is regulated by another (Chase et al., 2004; Todhunter et al., 2005). A similar explanation of vectorial asymmetries could apply to the ilium and ischium, i.e., as a result of regulation of growth of the ilium and ischium, when the ilium was longer the ischium would necessarily be shorter and as a consequence, asymmetries of the two would be reversed.

In summary, our data suggest that for the majority of skeletal growth parameters, growth on the right and left side of the fox may be differentially biased, resulting in fixed differences between the two sides in either the rate of growth or the length of the period during which growth occurs, giving rise to DA. Random effects around these fixed differences (i.e., noise) perturb the magnitude of the effects such that length and width asymmetries are not inversely correlated at the level of individual animals.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Fig. 1. Directional asymmetry of fox bone metrics. Cumulative distribution graphs of scaled metrics from the left (o) and right side (+). Values are expressed as a fraction of the mean, e.g., $x_r/\text{mean}(x_r, x_l)$. Length metrics (left column) are shown for the femur, humerus, ulna, ilium, and mandible. Width metrics (right column) are shown for the cortical thickness of the femur (Fcortt), humerus (Hcortt), as well as the ulna (Ucortt) and for the ilium width (Ilwd) and vertical height of the mandible (Manht). See Supporting Information Figure 1 for radiographs showing the endpoints used for each measurement.

Fig. 2.

Fluctuating asymmetry in the choice of forelimb used for rotating food into the cage. Foxes are ranked on the *Y* axis according to their average preference score. For each trial, a fox received a preference score: 0 if it used only the right forelimb; 0.5 if it used both; 1 for the left only. The abscissa shows the average score for ten trials. Horizontal lines indicate ± 1 SE of the mean for each fox across the 10 replications.

TABLE 1

Directional asymmetry of limb bone lengths

Bone	Length		
	Vector	Diff %	P value
Fore leg			
Humerus	R > L	1.4	***
Radius	L > R	1.6	***
Ulna	L > R	1.6	***
Metacarpal	R > L	0.7	***
Phalanges	R > L	0.7	***
Hind leg			
Femur	L > R	1.2	***
Tibia	L > R	0.2	**
Metatarsal	L > R	0.2	**
Foot phalang.	L > R	1	***

P value:

**
 $P < 10^{-7}$

 $P < 10^{-20}$

The vector column indicates the direction of the magnitude difference. The Diff % column shows the Left/Right differences as a percentage of the mean: $(\text{abs}(x_l - x_r) / \text{mean}(x_l, x_r)) \times 100\%$. Two levels of paired t-test significance are indicated. Phalanges and metacarpals are averages from the middle three digits. See Supporting Information Figure 1 for radiographs showing the endpoints used for each measurement.

TABLE 2

Directional asymmetry of limb bone widths

Bone	Inner diameter			Outer diameter			Cortical thickness		
	Vector	% Diff	P value	Vector	% Diff	P value	Vector	% Diff	P value
Fore leg									
Humerus	R > L	1.1	**	L > R	1.5	***	L > R	4.9	***
Radius	R > L	2.3	**	R > L	1.3	**	R > L	0.7	ns
Ulna	R > L	8.2	***	R > L	5.1	***	R > L	3.9	***
Hind leg									
Femur	L > R	12	***	L > R	3.2	***	R > L	8.9	***
Tibia	L > R	4.1	***	R > L	1.1	**	R > L	6.3	***

P value: ns: not significant

**
 $P < 10^{-7}$

 $P < 10^{-20}$

Three aspects of bone width are presented: inner diameter, outer diameter, and cortical thickness (outer diameter – inner diameter). The vector column indicates the direction of the magnitude difference. The Diff % column shows the Left/Right differences as a percentage of the mean: $(\text{abs}(x_l - x_p) / \text{mean}(x_l, x_l)) \times 100\%$. Three levels of paired t-test significance are indicated. See Supporting Information Figure 1 for radiographs showing the endpoints used for each measurement.

TABLE 3

Directional asymmetry of other limb metrics (joints and levers)

Hind limb	Vector	Diff %	P value	Fore limb	Vector	Diff %	P value
Femur head	R > L	0.47	*	Olecranon	R > L	0.8	***
Patella	R > L	2.6	***	Elbow Joint	R > L	3.4	**
Tibia head	R > L	1.3	***	Pisiform	R > L	2.4	***
Heel height	L > R	0.5	*				
Tarsal joint	R > L	0.8	**				
Calcaneus	L > R	0.5	***				

P value:

* $P < 0.002$

** $P < 10^{-7}$

*** $P < 10^{-20}$

Left/Right differences for various joint and lever metrics. The vector column indicates the direction of the magnitude difference. The Diff % column shows the Left/Right differences as a percentage of the mean: $(\text{abs}(x_l - x_r) / \text{mean}(x_l, x_r)) \times 100\%$. Three levels of paired t-test significance are indicated. See Supporting Information Figure 1 for radiographs showing the endpoints used for each measurement.

TABLE 4

Directional asymmetry of pelvis and mandible

Bone	Length			Width		
	Vector	Diff %	P value	Vector	Diff %	P value
Pelvis						
Ilium	L > R	1.8	***	R > L	4.7	***
Ischium	R > L	1.7	***	L > R	1.4	***
Acetabulum	—	—	—	L > R	2	***
Skull						
Mandible	L > R	0.9	***	R > L	5.2	***

P value:

 $p < 10^{-20}$.

Mandible thickness was measured as in Figure 1.

Left/Right differences for metrics of the hip and skull. The vector column indicates the direction of the magnitude difference. The Diff % column shows the Left/Right differences as a percentage of the mean: $(\text{abs}(x_l - x_r) / \text{mean}(x_l, x_r)) \times 100\%$. The significance of the paired t-test is indicated. See Supporting Information Figure 1 for radiographs showing the endpoints used for each measurement.

TABLE 5

Correlations

Trait 1	Trait 2	<i>r</i>
Significant		
Radius	Ulna	0.75
Humerus	Radius	0.44
Humerus	Ulna	0.41
Olecranon	Ulna	0.28
Humerus	Olecranon	0.26
EJNT	Humerus	0.22
Not significant		
Length	Cortical thickness	
Femur	Femur	0.05
Humerus	Humerus	0.04
Radius	Radius	0.03
Tibia	Tibia	0.01
Ulna	Ulna	0.06

Correlations between asymmetries: Pearson correlations between trait asymmetries are presented for the only significant correlations. No other correlations were significant e.g., the examples of length–width correlations shown.

TABLE 6

Asymmetry during early postnatal growth age

Limb metric	2 Months	P value	3 Months	P value	7 Months	Adult
Femur						
Length	1.2 ^a		0.9 ^b	**	1.5 ^a	1.2
Cortical thick.	-15 ^a		ns		ns	-8.9
Tibia						
Length	ns		ns	***	2.1	0.2
Cortical thick.	-7 ^c		-11 ^a	*	ns	-6
Metatarsal						
Length	-1.7 ^b		-1.9 ^b	*	ns	0.2
Humerus						
Length	ns	***	-2.3 ^a		-1.4 ^a	-1.4
Cortical thick.	ns		ns		ns	4.8
Radius						
Length	1.4 ^a		1.1 ^a	**	1.5 ^a	1.6
Cortical thick.	ns	**	11 ^b		15 ^a	-0.4
Ulna						
Length	0.6 ^b		1.2 ^a		2 ^a	1.6
Cortical thick.	-12 ^a	***	6 ^c		16 ^a	-3.6

** Asymmetry values derived from radiographs of 45 foxes at ages two months, three months and seven months. Values for limb length and cortical thickness are presented and compared with adult values from Tables 1 and 2. %LR = $(x_1 - x_2) / \text{mean}(x_1, x_2) \times 100$. The sign of the %LR indicates the direction of the asymmetry as Left minus Right (+, L>R; -, R>L). The significance of the paired T-test was:

^a $P > 0.001$;

^b $P < 0.01$;

^c $P < 0.05$;

ns not significant (DA could not be demonstrated). Adult values were all significant (see Tables 1 and 2). Three levels of significant change in asymmetry between the time intervals 2-3 months and 3-4 months are indicated (* <0.05 , ** <0.01 , *** <0.001).