Palaeontologia Electronica
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COMPARATIVE VARIABILITY OF INTERMEMBRANOUS AND ENDOCHONDRAL BONES IN PLEISTOCENE MAMMALS

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ABSTRACT

Study of the embryology and ossification of modern bones predicts that fossil intermembranous bones (which ossify from connective tissue) will exhibit greater size variability than endochondral bones (which are formed from embryological cartilaginous precursors), because intermembranous bones are less tightly constrained by joints and articular surfaces. To evaluate this hypothesis, we measured multiple dimensions of 989 intermembranous bones (patellae and other sesamoids) of the saber-toothed cat *Smilodon fatalis*, the Ice Age lion *Panthera atrox*, the bison *Bison antiquus*, the horse *Equus occidentalis*, the camel *Camelops hesternus*, the ground sloths *Paramylodon* (=*Glossotherium*) *harlani* and *Nothrotheriops shastensis* from Rancho La Brea and from the late Pleistocene San Josecito Cave in Nuevo Leon, Mexico. These were compared to measurements of 811 endochondral bones (primarily astragali) of comparable size. Through statistical analyses (coefficients of variation, ANOVA, modified Levene's test, and t-tests) we found slight evidence of higher variability in many of the intermembranous bones of these taxa (21 out of 27 CVs were higher for intermembranous bones than endochondral bones), although this trend is not found in all taxa. Using a modified Levene's test, only *Smilodon* and some of the dimensions of horse and bison patellae are significantly more variable than the corresponding dimensions of the astragali. Although the results are mixed, at least some data show that intermembranous bones are not as tightly constrained by growth and by adjacent tissues as are endochondral bones. This evidence of relative variability is important in assessing how much variability is typical of a single species, and thus has taxonomic implications.

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KEY WORDS: intermembranous bone; endochondral bone; sesamoid bone; variability; Pleistocene

PE Article Number: 13.1.4A

Submission: 19 December 2008. Acceptance: 25 January 2010

Raymond, Kristina R. and Prothero, Donald R., 2010. Comparative Variability of Intermembranous and Endochondral Bones in Pleistocene Mammals. *Palaeontologia Electronica* Vol. 13, Issue 1; 4A: 14p;
http://palaeo-electronica.org/2010_1/184/index.html

INTRODUCTION

In recent years, developmental biology has made enormous strides in understanding the growth and modification of bones, and the constraints on bone growth as well (Hall 2005; Currey 2006). Most paleontologists no longer view bones as static entities, but as dynamic three-dimensional objects that can vary in shape not only during ontogeny, but also due to changed biomechanical forces. Because of both ontogenetic and ecophenotypic factors, bone shapes and sizes can vary quite widely in a population (Yablokov 1974). Shape and size of fossil bones are important factors in making taxonomic decisions, and assessing the variability of a single population is critical in deciding how much variability in a fossil sample can be attributed to a single species, or requires other explanations.

In particular, variability due to different styles of growth between endochondral bones (which ossify directly from an embryonic cartilaginous precursor, often constrained by joints and articular surfaces) and less constrained intermembranous bones, is highly relevant to these issues. The topic of intermembranous and endochondral bone growth, size and variability is one that is not commonly touched upon, except briefly in passing, in paleontological literature. Generally, intermembranous bones are measured and discussed as only a slightly relevant topic in regards to larger studies of species or interspecific variation and sexual size dimorphism.

Intermembranous bones form directly from the connective tissue late in embryological development and after birth through intramembranous ossification. Some intermembranous bones, such as the kneecap (patella), are almost always ossified in adult mammals (with minor exceptions). Other intermembranous bones, known as sesamoids, occur only in areas where a tendon passes over a joint, and ossify in irregular and unpredictable patterns (Vickaryous and Olson 2007). The number and shape of intermembranous bones vary greatly within the Mammalia, and are highly taxon-dependent. Humans have only one sesamoid (the pisiform) in the carpus. In many mammals, such bones include the patella and large sesamoids in the manus and pes. In ungulates, on the other hand, the only large sesamoid element is the patella. The sesamoids in the manus or pes are small nodular ossifications in the digital flexor tendons, both at the metapodial-phalangeal joint and the distal interphalangeal joint; suids have as many as 13 sesamoids in the manus alone.

Vickaryous and Olson (2007) point out that although sesamoids receive little attention in paleontological literature, the majority of tetrapod lineages develop at least one sesamoid. "As a group, sesamoids and their ilk represent something of an anatomical enigma, with an enormous degree of variability in size, shape, and position both within and between taxa. Consequently, most skeletal descriptions relegate these elements to passages that summarize… bones and cartilages, predisposing them to continued marginalization" (Vickaryous and Olson 2007). This scientific neglect is largely because sesamoids are not as commonly preserved as more massive and larger bones of skeleton, or sometimes cannot be reliably associated with a known species.

Recent anthropological literature has commented on patellar variability in humans of both recent and Pleistocene age. Trinkaus (2000) concludes that all of his samples "exhibit considerable variability in these patellar proportions." Trinkaus and Rhoads (1999) and Ward et al. (1995) also discussed the variability of fossil hominid patellae, but in the context of functional morphological interpretations, rather than comparative variability. The literature cited above suggests that because modern lineages show patellar variability, the Pleistocene fossil record may also provide data to suggest that sesamoids have been variable throughout the history of life.

Walmsley (1940), Bland and Ashhurst (1997), and Bongers et al. (2005) discuss the development of the patella and its ossification from connective tissues and hyaline cartilage. Sarin et al. (1999) and Goldberg and Nathan (2004) analyzed the variability of human sesamoid bones, but without comparing this variability to that of endochondral bones or using it in a systematic context. Our own impetus for this research was stimulated when we noticed a similar high level of variability in ground sloth patellae (Figure 1), and the second author has seen many similar instances in the large collections of fossils he has examined over the past 40 years. Prothero (2005, tables 5.1-5.9) documented some of this variability in North American rhinocerotid patellae.

Based on these considerations, intermembranous bones are predicted to show a higher level of variability than endochondral bones because they have limited articulation with other bones and are formed through intramembranous ossification. Many intermembranous bones are referred to as 'free-floating." By contrast, endochondral bones are more constrained from unusual growth by artic-

FIGURE 1. Patellae (kneecaps) of the ground sloth, *Paramylodon harlani*, show apparent shape and size variability. Scale bar equals 1 cm.

ulations with other bones. Apparent variability may also be the result of ossification into the tendons because animals tend to replace minor tissue damage with bone at the intersection of bone and tendon as an inflammatory response. Therefore, older individuals or individuals who have suffered tendon or joint injury would display larger or oddly shaped sesamoids (Andrew Clifford, personal communication, 2007).

MATERIALS AND METHODS

One of the best ways to address this question is to measure a relatively large set of fossilized intermembranous and endochondral bones from a homogeneous population. Unfortunately, most fossil samples only rarely preserve sesamoid bones, especially the small sesamoids of the manus and pes, or else the taxonomic identity of the sesamoids cannot be established. Some exceptions to this rule are the large samples of bones (Figure 2) from late Pleistocene localities such as Rancho La Brea tar pits (Stock and Harris 1992) and cave deposits such as San Josecito Cave in Nuevo Leon, Mexico (Arroyo-Cabrales et al. 2003)

In this study, we measured samples of intermembranous and endochondral bones from nearly all the large mammals for which a sufficiently large sample (wherever possible, more than 100 of each element) exists. These include two ground sloths (*Paramylodon harlani* and *Nothrotheriops shastensis*), the extinct bison *Bison antiquus*, the extinct horse *Equus occidentalis*, the lamine camelid *Camelops hesternus*, the Ice Age lion *Panthera atrox*, and the saber-toothed cat *Smilodon fatalis*. The only common mammal not included in the study was the dire wolf (*Canis dirus*), which is currently under study by F.R. O'Keefe (personal commun., 2008).

Sloths not only possess patellae and sesamoids of the manus, but also a layer of dermal ossicles just under the skin, similar to an armadillo's armor, which may have been used as protection against predators (Stock 1925). The ossicles of the

FIGURE 2. Photographs of representative trays of the sample of *Smilodon fatalis* in the Page Museum. Each tray is one of many for each element, so there are typically more than 100 individual elements found in 3-7 separate trays. A. One of almost a dozen trays of patellae. B. One of many trays of astragali.

species *Paramylodon harlani* are the most common fossil found at the La Brea tar pits (S. Cox, personal commun.). Previous literature on giant ground sloths have commented on high levels of variability and sexual dimorphism amongst the larger species, *Eremotherium eomigrans* (De Iuliis and Cartelle 1999). However, our previous study found that the Rancho La Brea sloths did not exhibit significant size variability or sexual size dimorphism. Their bones did show some shape variability (Prothero and Raymond 2008), which is apparent in intermembranous bones such as the patella (Figure 1).

None of the specimens from Rancho La Brea and very few of those from San Josecito Cave are articulated or associated, so there is no way to relate individual bones to individual animals. In the case of Rancho La Brea bones, the radiocarbon dates for the samples we used are tightly clustered (Marcus and Berger 1984) so they appear to sample populations over a small interval of time and are not significantly time averaged. In addition, our work on most of the Rancho La Brea mammals and birds (Prothero and Raymond 2008; Prothero et al. 2009) demonstrates no significant size or shape change over the entire 40,000 years that the bones accumulated, so even if the samples spanned significant time intervals, they would not show increased size variation.

All of the bones measured in this study were unworn and pristine in condition, so there is no evidence of taphonomic abrasion or breakage that might affect the data set. As shown by Prothero and Raymond (2008), most postcranial bones show no evidence of sexual size dimorphism, so there is no way to segregate the bones into the two sexes. We eliminated bones that were poorly ossified or looked like they might belong to juveniles, so the samples we measured should contain only bones of mature adult individuals.

The La Brea felids, *Smilodon fatalis* and *Panthera atrox*, have been described in literature (Merriam and Stock 1932) quite thoroughly but very little research has been conducted specifically focusing on the variation of their intermembranous bones. Most research of these creatures involves their masticatory apparatus, paleodiet, paleoecology and systematic description. The osteology of *Camelops* was last reviewed by Webb (1965), and the systematics and osteology of North American Pleistocene bison was reviewed by McDonald (1981), but there has not been a lot of recent published research on the osteology of the La Brea horse (E. Scott, personal commun.)

Using dial calipers, the first author measured several dimensions of a grand total of over 1800 bones from these species and entered data into Microsoft Excel spreadsheets. Measurements of the 989 intermembranous bones were taken from the maximum lateral width, proximo-distal length and dorso-plantar depth of patellae and inner and outer carpal sesamoids. The endochondral bone selected for study was the astragalus, which was very abundant and could be measured in comparable dimensions and was comparable in size to the patella. Measurements of the 811 astragali were taken of the proximo-distal height, lateral width, and maximum dorso-plantar depth. In all cases, the measurements were taken along axes that represent the strain between articular surfaces, and thus should have functional significance.

Using Microsoft Excel, means and standard deviations of each dimensional measurement were calculated (Table 1). The statistical analyses followed the methods of Sokal and Rohlf (1994, 2009) and Plavcan and Cope (2001). These data were then used to calculate the coefficients of variation, which is used in paleontological and biological studies to determine the level of variability within a sample population. The coefficient of variation is defined as 100*(standard deviation/mean). Because the units of measurement in the standard deviation and the mean are the same, the CV becomes a unitless measurement. This is especially useful when comparing bones of significantly different sizes, since the standardization of the mean by the standard deviation makes the CV uniform, regardless of absolute size. The comparison of the coefficients of variation, which are derived from different distributions, is consistently valid and useful if the samples share a similar or related structure, position or value (Simpson et al. 1960; Lande 1977; Sokal and Rohlf 1994; Polly 1998; Dayan et al. 2002; Meiri et al. 2005). Fifty years of zoological research indicates that for most mammal populations, CVs will be less than 10 except for features that are extremely sexually dimorphic (Kurtén 1953; Simpson et al. 1960; Yablokov 1974).

Additional statistical analyses were conducted to test for significance of variability between intermembranous and endochondral bones. However, in order to determine which tests are appropriate to the study, we first had to determine whether the data samples are normally distributed. Using the f-statistic, probability plots were calculated by ranking the measurements and assigning a z-score (individual raw score minus population mean). Z-scores were plotted on bivariate plots (on the y-axis) against the ranked data (on the x-axis), and a regression line was added. In a normal distribution, the regression line crosses the x-axis at about the mean, and the slope is close to the reciprocal of the standard deviation. If all the data are normally distributed, certain parametric tests are then appropriate to analyze the variability of the samples.

The next test used to determine which statistical methods would be appropriate for this study is the f-test for equality of variances. Variance is defined as the average of the squared deviations from the mean, or the square of the standard deviation. Therefore, the variance is a measure of statistical dispersion from the sample mean and portrays the degree of distribution. The f-test measures whether the variances from two samples are statistically equal or unequal. The null hypothesis in this test states that the variances of the two normally distributed samples are equal and, therefore, comparable. The alternate hypothesis states that the variances are unequal. If the null hypothesis is accepted, t-tests for equal variances and Analysis of Variance (or ANOVA) are applicable to this study. If the null hypothesis is rejected, t-tests are available to assess the differences in means where the variances are assumed to be unequal. For an ANOVA to be valid, the samples involved must have a normal distribution, have equal variances, and variables must be independent. In this case there is one measurement variable and one nominal (or categorical) value. The purpose of the test is to analyze how much of the variation among the observations is due to variation in each factor (nominal variable) influencing the character (measurement value) being studied. If the F value is greater than the F-critical value, there is a statistically significant difference and the null hypothesis of equal means is rejected.

In addition to these traditional methods, we also used the modified Levene's test (Levene 1960; Lewontin 1966; Schultz 1985; Plavcan and Cope 2001) to determine whether the variabilities of two samples were significantly different. The modified Levene test uses natural logs to remove effects of relative size then calculates the absolute values of differences between each individual measurement and the median, which can then be analyzed for mean and standard deviation and tested for significance using ANOVA.

RESULTS

F-statistic for Normality

The f-statistic tests a given sample for normal probability distribution. Once all samples of this study were ranked, given a z-score (individual raw score minus population mean) and plotted on bivariate plots with regression lines, we determined that all samples were normally distributed. Because all of the distributions turned out to normal, coefficients of variation, t-tests, and ANOVA may be used if other standards are met.

TABLE 1. Raw statistics of specimens measured in this study. Number of specimens in parentheses. SD = standard deviation. CV = coefficient of variation. VAR = variance (SD squared). All measurements in mm.

Species	Element (#Specimens)	Dimension	Mean	SD	CV	VAR
Panthera atrox	Patella (64)	Max. Length	64.90	5.10	7.86	26.01
		Max. Width	45.35	3.55	2.43	12.60
		Max. Depth	27.70	7.82	8.76	61.15
	Astragalus (71)	Ant.-Post Length	64.60	4.05	6.27	16.40
		Max Width	41.85	5.00	11.95	25.00
		Max. Depth	37.80	2.69	7.11	7.24
Smilodon fatalis	Patella (367)	Max Length	53.10	4.03	7.59	16.24
		Max Width	40.61	2.96	7.28	8.76
		Max Depth	25.62	2.20	8.59	4.84
	Astragalus (373)	Ant.-Post Length	44 .00	7.07	5.26	49.98
		Max. Width	60 .00	4.95	6.11	24.50
		Max Depth	27.50	10.61	6.56	112.57
	Sesamoids (174)	Max Length	23.36	2.90	12.40	8.41
		Max Width	9.59	1.12	11.71	12.54
		Max Depth	9.75	1.18	12.11	1.39
Paramylodon harlani	Patella (55)	Max. Length	118.76	9.53	8.02	90.82
		Max. Width	105.20	6.64	4.28	44.09
		Max. Depth	58.76	4.28	7.28	18.31
	Astragalus (88)	Ant.-Post. Length	128.21	9.14	7.12	83.53
		Max. Width	134.23	9.04	6.73	81.72
		Max. Depth	85.93	7.24	8.42	52.42
	Inner sesamoid 3 (40)	Max. Length	46.20	3.07	6.65	9.42
		Max. Width	31.87	2.29	7.21	5.24
		Max. Depth	20.39	1.82	8.94	3.31
	Outer sesamoid 3 (38)	Max. Length	48.35	3.64	7.53	13.25
		Max. Width	31.87	2.68	8.40	7.18
		Max. Depth	20.61	2.00	9.71	4.00
Nothrotheriops shastensis	Astragalus (6)	Ant.-Post Length	103.83	3.65	3.52	13.32
		Max. Width	99.17	4.02	4.05	16.16
Camelops hesternus	Patella (37)	Max. Length	99.98	8.32	8.33	69.29
		Max. Width	54.91	5.87	10.67	34.50
		Max. Depth	43.28	4.21	9.74	17.79
	Astragalus (75)	Ant.-Post Length	78.65	10.95	13.91	119.85
		Max. Width	56.72	4.85	8.55	23.52
		Max. Depth	42.95	10.95	13.92	119.85
Bison antiquus	Patella (87)	Max. Length	73.26	6.41	8.75	41.13
		Max. Width	69.73	5.62	8.06	31.56
		Max. Depth	44.79	4.95	11.06	24.56
	Astragalus (96)	Ant.-Post Length	84.59	5.34	6.31	28.48
		Max. Width	52.48	4.28	8.15	18.31
		Max. Depth	45.76	3.64	7.94	13.21
Equus occidentalis	Patella (122)	Max. Length	80.48	3.91	4.86	15.31
		Max. Width	79.34	4.15	5.23	17.19
		Max. Depth	45.14	6.03	13.35	31.32
	Astragalus (101)	Ant.-Post Length	70.83	2.67	3.77	7.12
		Max. Width	65.65	3.94	5.99	15.51
		Max. Depth	58.81	3.03	5.16	9.20

F-test for Equal Variances

As previously discussed, the f-test for equal variances is necessary in order to determine which type of t-test (assuming equal or unequal variances) to use, and whether or not standards for ANOVA are met. Results of the f-tests (Table 2) indicate that the comparison of variances of astragali to patellae of *Paramylodon harlani* length, *Smilodon fatalis* depth, *Panthera atrox* depth, *Camelops hesternus* length, width and depth, and *Equus occidentalis* width meet qualifications for further analysis through ANOVA and t-tests assuming equal variances because the f-values are smaller than the f-critical values for these samples. Thus, in the aforementioned measurement comparisons, the null hypothesis is accepted. In addition, the p-values for the *Paramylodon harlani* length, *Panthera atrox* depth, *Equus occidentalis* width, and *Smilodon fatalis* depth are greater than the 0.05 significance level.

In all other sample measurements (*Paramylodon harlani* width and depth, *Smilodon fatalis* length and width, *Equus occidentalis* length and depth, *Bison antiquus* length, width, and depth, and *Panthera atrox* length and width), f-values are larger than the f-critical values. This forces us to reject the null hypothesis of equal variances. Therefore, ANOVA is not applicable to these measurements, and only t-test assuming unequal variances may be used to further analyze this data. Also, p-values for all remaining samples are less than the 0.05 significance level. This provides strong evidence that the null hypothesis is not true for these samples.

In some cases (*Equus occidentalis* length and depth, *Panthera atrox* length and width), the variances (Table 1) of the astragalus (an endochondral bone) are smaller than those of the patella (a intermembranous bone), which is consistent with our hypothesis. In other cases (*Paramylodon harlani* width and depth, *Bison antiquus* length, width, and depth, and *Smilodon fatalis* length and width), the variances of the astragalus are larger than those of the patella, which is not consistent with our hypothesis.

T-tests

Preliminary tests for equality of variances indicate that the variances between astragali and patellae in *Paramylodon harlani* length, *Smilodon fatalis* depth, *Camelops hesternus* length, width and depth, *Equus occidentalis* width, and *Panthera atrox* depth are statistically equal. Two-sample t-tests assuming equal variances were performed and results calculated (Table 3). All equal variance results except the *B. antiquus* depth and the *C. hesternus* width and depth have p-values that are less than the significance level of 0.05, and t-stat values fall outside of the range of t-critical values.

For the variances that were determined to be unequal (*Paramylodon harlani* width and depth, *Smilodon fatalis* length and width, *Equus occidentalis* length and depth, *Bison antiquus* length, width, and depth, and *Panthera atrox* length and width), two-sample t-tests assuming unequal variances were performed and results tabulated (Table 4). All results from t-tests assuming unequal variances fall outside of the range of t-critical values. All P-values for these taxa except for length and width in *Panthera atrox* and the depth measurement of *Bison antiquus* are less than the 0.05 significance level.

Analysis of Variance (ANOVA)

Analysis of variance was used to analyze how much of the variation among the observations is due to variation in each bone type influencing the character (measurement value) being studied. Most of the samples were determined to meet the standards for ANOVA: independent variables, normal distribution (calculated by f-stat), and equal variances (calculated by f-test). These samples, which include comparisons between the astragali and patellae of the *Paramylodon harlani* length, *Smilodon fatalis* depth, *Camelops hesternus* length, *Equus occidentalis* width, and *Panthera atrox* depth, yielded f-values higher than f-critical values in each case (Table 5), so the differences are significant. However, the depth and width dimensions of *Camelops hesternus* were below the f-critical value, so they are not significantly different.

Modified Levene's Test

As discussed above, the modified Levene test (Levene 1960; Plavcan and Cope 2001) is a popular method for testing for equal variability while using natural logs to correct for absolute size. Using ANOVA, we found that depth and length of bison and horse patellae were significantly more variable than their corresponding astragali, but not width (Table 6). None of the camel, sloth, or lion bones showed significantly more variability of patellae compared to astragali. However, all of the dimensions of *Smilodon* patellae were significantly more variable than those of the astragali.

TABLE 2. F-test for equal variance.

Species	Measurement	f	P(F<=f)	f-critical
Paramylodon harlani	Length	1.09	0.35	1.48
	Width	1.85	0.01	1.52
	Depth	2.86	<0.001	1.52
Smilodon fatalis	Length	1.68	<0.001	1.18
	Width	1.67	<0.001	1.18
	Depth	1.13	0.11	1.18
Panthera atrox	Length	1.59	0.03	1.5
	Width	1.9	0.003	1.5
	Depth	1.23	0.2	1.5
Bison antiquus	Length	1.44	0.04	1.42
	Width	1.72	0.005	1.42
	Depth	1.86	0.002	1.42
Equus occidentalis	Length	2.15	<0.001	1.38
	Width	1.11	0.30	1.38
	Depth	3.95	<0.001	1.38
Camelops hesternus	Length	0.57	0.037	1.58
	Width	1.39	0.11	1.58
	Depth	1.31	0.17	1.58

Coefficients of Variation (CV)

As shown in Table 1, most of the coefficients of variation (20 out of 27) are higher for intermembranous bones than for their corresponding measurements of the endochondral bones. CVs from the samples of *Panthera atrox* are less than 10 with the exception of the maximum width of the astragalus, which has a CV of 11.95. However, the CVs of the endochondral bones (astragalus) are generally higher than those of the intermembranous bones (patella), in contrast to our expectations. *Smilodon fatalis* bones have CVs within the range of 5-13. The CVs of the endochondral bones of this species are all less than 10. *Smilodon fatalis* patellae CVs are also under 10. However, all three dimensions measurements of the carpal sesamoids in this species demonstrate CVs greater than 10. In addition, the CVs of both intermembranous bones (patella and sesamoids) are consistently higher than those of the astragalus, as expected in our hypothesis.

In both species of ground sloths (*Paramylodon harlani* and *Nothrotheriops shastensis*), all CVs are in the range of 3-10. The highest coefficient of variation in the order Xenarthra is the maximum depth of the outer sesamoid of digit 3 of the *Paramylodon harlani* with a value of 9.71. However, the CVs of the endochondral bones are in the same range as those of the intermembranous bones and not consistently lower. The sample size for *Nothrotheriops shastensis* is much smaller, but similar trends are observed. Mean, standard deviation, and coefficient of variation were not calculated for the *Nothrotheriops shastensis* patellae as only a few specimens were available.

In *Camelops hesternus*, the CVs were unusually high, with three values above 10 (Table 1). In contrast to our predictions, two out of three CVs

TABLE 3. Results of t-tests assuming equal variances.

T-tests Assuming Equal Variances				
Species	**Measurement**	**t Stat**	**t critical values**	**P value**
Paramylodon harlani	Length	-5.92	1.65-1.97	<0.001
Smilodon fatalis	Depth	37.18	1.64-1.96	<0.001
Equus occidentalis	Width	25.11	1.65-1.97	<0.001
Panthera atrox	Depth	18.07	1.65-1.97	<0.001
Camelops hesternus	Length	10.40	1.66-1.98	<0.001
Camelops hesternus	Width	-1.82	1.66-1.98	0.07
Camelops hesternus	Depth	0.42	1.66-1.98	0.33

TABLE 4. Results of t-tests assuming unequal variances.

T-tests Assuming Unequal Variances				
Species	**Measurement**	**t Stat**	**t-critical values**	**P-value**
Paramylodon harlani	Width	6.87	1.65-1.98	<0.001
	Depth	-2.93	1.65-1.98	0.002
Smilodon fatalis	Length	8.73	1.65-1.96	<0.001
	Width	-14.48	1.65-1.96	<0.001
Panthera atrox	Length	1.37	1.65-1.97	0.08
	Width	-1.07	1.65-1.97	0.14
Equus occidentalis	Length	21.79	1.65-1.97	<0.001
	Depth	-21.93	1.65-1.97	<0.001
Bison antiquus	Length	-12.84	1.65-1.97	<0.001
	Width	23.06	1.65-1.97	<0.001
	Depth	-1.49	1.65-1.97	0.068

(length and depth) of the astragalus were consistently higher than those of the patella. In *Bison antiquus*, the CVs of the astragalus were slightly lower than those of the patella, consistent with our initial hypothesis. The same is true of *Equus occidentalis* (Table 1).

Summarizing the CV data, 20 out of 27 of the comparisons of the CV of an intermembranous bone to the endochondral astragalus yield a higher CV for the intermembranous bone, in agreement with our prediction. Many of our taxa (*Equus occidentalis, Bison antiquus*, and *Smilodon fatalis*) show the predicted trend of greater CVs in intermembranous bones than in endochondral bones. The data from *Paramylodon harlani* are equivocal, with overlapping values of CVs for intermembranous and endochondral bones. But two of the taxa (*Camelops hesternus* and *Panthera atrox*) seem to have higher CVs in their endochondral bones (astragalus) than in their intermembranous bones.

DISCUSSION

Results for each taxon are summarized below and in Table 7.

Paramylodon harlani

Coefficients of variation yielded the initial estimates in determining the degree of variability between the intermembranous and endochondral

TABLE 5. ANOVA f-values and f-critical values for samples meeting ANOVA requirements.

ANOVA Species	Measurement	F	F-critical
Paramylodon harlani	Length	35.02	3.91
Equus occidentalis	Width	630.53	3.88
Smilodon fatalis	Depth	1382.78	3.85
Panthera atrox	Depth	510.41	3.91
Camelops hesternus	Length	108.13	3.93
Camelops hesternus	Width	3.32	3.93
Camelops hesternus	Depth	0.18	3.93

bones of the species in this study. The majority of observations CVs are between 4 and 10, and 5 and 6 are the average values. Values lower than 4 usually mean that the sample size was not sufficient to show the variability. Table 1 shows that the CVs of the *Paramylodon harlani* and *Nothrotheriops shastensis* all fall under 10, which initially indicated that neither the intermembranous bones nor endochondral bones are highly variable. There is no consistent pattern of astragalus CVs being lower than patellar CVs, as would be predicted by our hypothesis. This is true even for the small inner and outer sesamoid bones, which might be expected to be the most variable in the skeleton (as they are in examples given below).

F-tests performed on *Paramylodon harlani* determined that variances between astragali and patellae widths and depths were unequal. The t-tests performed on *Paramylodon harlani*, as shown in Tables 3 and 4, indicate that the means are all statistically different because the t-stat values fall outside of the range of t-critical values. Therefore, the null hypothesis that the means are equal is rejected. All p-values in *Paramylodon harlani* t-tests also fell below the 0.05 significance level; this result strongly rejects the null hypothesis. ANOVA results (as shown in Table 5) give f-values greater than f-critical values; this also rejects the null hypothesis of equal means and suggests a statistically significant difference. All results except the width dimensions indicate that the intermembranous bones are statistically different than the endochondral bones of this species. However, Levene's test (Table 6) indicated that none of the dimensions of the patellae were significantly more variable than those of the astragali.

Smilodon Fatalis

CVs for the comparison of astragali and patellae of the *Smilodon fatalis* are all under 10, but the sesamoids have CVs of 11.71 and 12.11 (Table 1). All dimensions of measurement of the patellae and sesamoids (the intermembranous bones) had CVs greater than CVs of measurements of corresponding astragali, which is consistent with our hypothesis. T-test results reject the null hypothesis of equal means because all values of the t-stat fall outside of the range of t-critical values (Tables 3 and 4). P-values also all fall below the 0.05 confidence interval; this strongly rejects the null hypothesis. In addition, because ANOVA yielded an f-value larger than the f-critical value in the comparison of astragalus to patella depth, the difference is considered statistically significant (Table 5). The same was true using Levene's test (Table 6). All results indicate that the endochondral bones are statistically different than the intermembranous bones in this species. In the case of *Smilodon fatalis,* intermembranous bones are consistently and significantly more variable than endochondral bones.

Panthera atrox

The CVs of *Panthera atrox* generated the most surprising results. CVs of patella length and depth were *lower* than those of astragalus length and depth. This implies that the patellae of this species are *less variable* than the astragali (Table 1). T-statistic values were outside of the range of t-critical value, which indicates that the means are statistically different, and the null hypothesis is rejected. Only the p-values of comparison of astragalus to patella depth falls below the significance level of 0.05, which strongly rejects the null hypothesis of equal means. However, p-values for comparison of astragalus to patella length and width are above the 0.05 significance level; this signifies that the results do not strongly reject the null hypothesis and therefore, may be due to a random sampling error. ANOVA results displayed an f-value larger than the f-critical value in the comparison of maximum depth. This indicates that the results are statistically significant, but contradict the prediction of our hypothesis. Levene's test, however, showed that none of these dimensions of astragali were statistically different from the corresponding dimensions of the patellae (Table 6).

Bison antiquus

The CVs of all dimensions of the patellae of *Bison antiquus* are higher than those of the astragalus, which agrees with our hypothesis (Table 1). In

TABLE 6. ANOVA of data transformed by the modified Levene's method.

Species	Measurement	F	F-critical	P-value	Patella signif. more variable?
Bison antiquus	Depth	4.894	3.897	0.029	Yes
(n = 87)	Width	0.458	3.897	0.499	No
	Length	11.657	3.897	0.001	Yes
Equus occidentalis	Depth	79.034	3.887	0.001	Yes
(n = 102)	Width	0.800	3.887	0.372	No
	Length	4.158	3.887	0.043	Yes
Camelops hesternus	Depth	0.004	3.974	0.949	No
(n = 37)	Width	1.925	3.974	0.169	No
	Length	0.165	3.974	0.686	No
Paramylodon harlani	Depth	0.091	3.929	0.762	No
(n = 55)	Width	0.040	3.929	0.841	No
	Length	0.268	3.929	0.606	No
Smilodon fatalis	Depth	26.648	3.854	0.001	Yes
(n = 366)	Width	29.412	3.854	0.001	Yes
	Length	13397.410	3.854	0.000	Yes
Panthera atrox	Depth	0.705	3.916	0.403	No
(n = 64)	Width	2.859	3.916	0.093	No
	Length	1.895	3.916	0.171	No

the f-tests (Table 2), the f values were always greater than the f-critical value, so the variances are not equal. In the t-tests assuming unequal variances, all three t values fell outside the critical range, so the means are statistically different. Thus, all three dimensions are consistent with our hypothesis and statistically significant. Using the modified Levene test (Table 6), the widths of patellae are not significantly more variable than those of the astragali, but the length and depth are significantly more variable.

Equus occidentalis

The CV values of the horses are higher for the patella than for the astragalus in two dimensions (length and depth), consistent with our hypothesis of greater intermembranous bone variability, but not for the width measurements. The variances also show the same trend (Table 1). In the f-test (Table 2), two dimensions of the horse bones (length and depth) have unequal variances, but the third (width) has equal variances. The t-test (Tables 3 and 4) shows that these differences are significant, as does the ANOVA (Table 5). Thus, the intermembranous bones of the horse are significantly more variable than the endochondral bones in two dimensions (length and depth), but not so in the third dimension (width). The modified Levene test (Table 6) gives the same result.

Camelops hesternus

As mentioned above, the CVs of the patellae of *C. hesternus* are *lower* than those of the astragali for the length and depth measurements, but not for the width (Table 1). Using the f-test, the variances of all three dimensions were statistically equal, and the t-test (Table 3) shows that the means are also significantly different because they fall outside the range of critical values. In the ANOVA analysis (Table 5), the length dimension was significantly different, but not the width or depth dimensions. However, the modified Levene test (Table 6) shows that none of the dimensions of the patellae are significantly more variable than those of astragali. Thus, *C. hesternus* gives mixed results, with some that are consistent with our hypothesis, and others that are not.

Table 7. Summary of comparisons indicating where intermembranous bones (patellae) are more variable than the corresponding astragali. Statistically significant results are indicated by bold face.

Taxon	Dimension	T-test	ANOVA	Levene test	CV
Bison antiquus	Length	**Yes**		**Yes**	Yes
	Width	**Yes**		No	Yes
	Depth	**Yes**		**Yes**	Yes
Equus occidentalis	Length	**Yes**		**Yes**	Yes
	Width	**No**	**No**	No	No
	Depth	**Yes**		**Yes**	Yes
Camelops hesternus	Length	**No**	**No**	No	No
	Width	**Yes**	Yes	No	Yes
	Depth	**No**	No	No	No
Paramylodon harlani	Length	**Yes**	**Yes**	No	Yes
	Width	**No**		No	No
	Depth	**Yes**		No	Yes
Smilodon fatalis	Length	**Yes**		**Yes**	Yes
	Width	**Yes**		**Yes**	Yes
	Depth	**Yes**	**Yes**	**Yes**	No
Panthera atrox	Length	Yes		No	Yes
	Width	No		No	No
	Depth	**No**	**No**	No	No

CONCLUSIONS

Comparison and statistical analysis of measurements of about 1800 fossilized bones of Pleistocene mammals show mixed results. In many cases (e.g., nearly all the measurements of *Smilodon fatalis, Bison antiquus, Equus occidentalis*, and some dimensions of *Panthera atrox, Paramylodon harlani*, and *Camelops hesternus*), there is significantly higher variability in intermembranous bones than in endochondral bones. In others (one dimension of *Panthera atrox,* two of *Paramylodon harlani,* and *Camelops hesternus* astragali and patellae, and some the sesamoids of *Paramylodon harlani*), the trend is reversed or equivocal. In general (in the CV data set, 21 out of 27 dimensions of all the taxa combined), most of these findings agree with the hypothesis that intermembranous bones are more variable than endochondral bones because they are not as tightly constrained by articular surfaces with other bones, joints, or tendons. The results of these analyses suggest that movement and growth play less of an important role in constraining the growth of intermembranous bones than previously suggested in anatomical literature.

ACKNOWLEDGMENTS

We thank J. Sadd, M. Lacy, S. Meiri, D. Polly, and D. Cope for advice regarding the statistical portion of this project. We thank G. Gage for her help with measuring *Camelops* astragali and A. Sutyagina for helping with measurements of the felids. We thank C. Shaw and J. Harris for access to the Page Museum collections, and X. Wang and L. Barnes for access to the San Josecito Cave specimens stored in the Natural History Museum of Los Angeles County. We thank A. Clifford for his suggestions, and B.L. Beatty, D. Cope, M. Lacy, S. Meiri, D. Polly, and an anonymous reviewer for helpful comments on the manuscript. Prothero is grateful to the late Howard Levene, who taught him biostatistics at Columbia University in the late 1970s and provided a link to Dobzhansky and other great scientists of the twentieth century.

REFERENCES

Arroyo-Cabrales, J., and Polaco, O.J. 2003. Caves and the Pleistocene vertebrate paleontology of Mexico, pp. 273-291. In Schubert, B.W., Mead, J.I., and Graham, R.W. (eds.), *Ice Age Cave Faunas of North America*. Indiana University Press. Bloomington.

Bland, Y.S., and Ashhurst, D.E. 1997. Fetal and post-natal development of the patella, patellar tendon and suprapatella in the rabbit; changes in the distribution of the fibrillar collagens. *Journal of Anatomy,* 190:327-342.

Bongers, E., van Kampen, A., van Bokhoven, H., and Knoers, N. 2005. Human syndromes with congenital patellar anomalies and the underlying gene effect. *Clinical Genetics,* 68(4):302-319.

Currey, J.D. 2006. *Bones: Structure and Mechanics*. Princeton University Press, Princeton, New Jersey.

Dayan, T., Wool, D., and Simberlofff. D. 2002. Varation and covariation of skulls and teeth: modern carnivores and the interpretation of fossil mammals. *Paleobiology* 28:508-526.

De Iuliis, G., and Cartelle, C. 1999. A new giant megatheriine ground sloth (Mammalia: Xenarthra: Megatheriidae) from the late Blancan to early Irvingtonian of Florida. *Zoological Journal of the Linnaean Society,* 127:495-515.

Goldberg, I., and Nathan, H. 2004. Anatomy and pathology of sesamoid bones. *International Orthopaedics,* 1:141-147.

Hall, B.K. 2005. *Bones and Cartilage: Developmental Skeletal Biology*. Academic Press, London.

Kurtén, B. 1953. On variation and population dynamics of fossil and recent mammal populations. *Acta Zoologica Fennica,* 76:1-122.

Lande, R. 1977. On comparing coefficients of variation. *Systematic Zoology* 26:214-217.

Levene, H. 1960, Robust tests for equality of variances. In Olkin, I., Ghurye, G., Hoeffding, W., Madow, W.G., and Mann, H.B. (eds.), *Contributions to Probability and Statistics*: Stanford University Press, Stanford, California, p. 278-292.

Lewontin, R.C. 1966. On the measurement of relative variability. *Systematic Zoology,* 15:141-142.

Marcus, L.F., and Berger, R. 1984. The significance of radiocarbon dates for Rancho La Brea.. In Martin, P.S., and Klein, R.G. (eds.), *Quaternary Extinctions: A Prehistoric Revolution*: University of Chicago Press, Chicago, pp. 159-188.

McDonald, J.N. 1981. *North American Bison: Their Classification and Evolution*. University of California Press, Berkeley, California.

Meiri, S., Dayan, T., and Simberloff, D. 2005. Variability and correlations in carnivore crania and dentition. *Functional Ecology,* 19:337-343.

Merriam, J.C., and Stock, C. 1932. The Felidae of Rancho La Brea. *Carnegie Institute of Washington Publications,* 422.

Plavcan, J.M., and Cope, D.A. 2001. Metric variation and species recognition in the fossil record. *Evolutionary Anthropology,* 10:204-222.

Polly, P.D. 1998. Variability in mammalian dentitions: size-related bias in the coefficient of variation. *Biological Journal of the Linnaean Society*, 64:83-99.

Prothero, D.R. 2005. *The Evolution of North American Rhinoceroses*. Cambridge University Press, Cambridge.

Prothero, D.R., and Raymond, K.R. 2008. Variation and sexual size dimorphism in Pleistocene ground sloths (Xenarthra). *New Mexico Museum of Natural History and Science Bulletin,* 44:331-334.

Prothero, D.R., Raymond, K.R., Syverson, V., and Molina S. 2009. Stasis in late Pleistocene birds and mammals from La Brea tar pits over the last glacial-interglacial cycle. *Cincinnati Museum Center Scientific Contributions,* 3:291-292.

Sarin, V.K., Erickson, G.M., Giori, N.G., Bergman, A.G., and Carter, D.R. 1999. Coincident development of sesamoid bones and clues to their evolution. *Anatomical Record,* 257:174-180.

Schultz, B.B. 1985. Levene's test for relative variation. *Systematic Zoology,* 34:449-456.

Simpson, G.G., Roe, A., and Lewontin, R.C. 1960. *Quantitative Zoology*. Harcourt Brace and World, New York.

Sokal, R.R. and Rohlf, F.J. 1994. *Biometry* (3^{nd} ed.). W.H. Freeman, New York.

Sokal, R.R., and Rohlf, F.J. 2009. *Introduction to Biostatistics* (2^{nd} ed.). Dover Press, New York.

Stock, C. 1925. Cenozoic gravigrade edentates of western North America, with special reference to the Pleistocene Megalonychidae and Mylodontidae of Rancho La Brea. *Carnegie Institute of Washington Publications* 331.

Stock, C,. and Harris, J.M. 1992. Rancho La Brea: A record of Pleistocene Life in California. *Natural History Museum of Los Angeles County, Science Series,* 37:1-113.

Trinkaus, E. 2000. Human patellar articular proportions: Recent and Pleistocene patterns. *Journal of Anatomy,* 196:473-483.

Trinkaus, E., and Rhoads, M.L. 1999. Neanderthal knees: power lifters of the Pleistocene? *Journal of Human Evolution,* 73:833-859.

Vickaryous, M.K., and Olson, W. 2007. Sesamoids and ossicles in the appendicular skeleton, pp. 323-341. In Hall. B.K. (ed.), *Fins and Limbs: Evolution, Development and Transformation*. University of Chicago Press, Chicago.

Walmsley, R. 1940. The development of the patella. *Journal of Anatomy* 74(3):360-368.

Ward, C.V., Ruff, C.B., Walker, A., Teaford, M.F., Rose, M.D., and Nengo, I.O. 1995. Functional morphology of *Proconsul* patellas from Rusinga Island, Kenya, with implications for other Miocene-Pliocene catarrhines. *Journal of Human Evolution,* 29:1-19.

Webb, S.D. 1965. The osteology of *Camelops. Bulletin of the Los Angeles County Museum, Science Series,* 1:1-54.

Yablokov, A.V. 1974. *Variability in Mammals*. Amerind Press, New Delhi.