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Cite this article: Tomiya S, Meachen JA. 2018 Postcranial diversity and recent ecomorphic impoverishment of North American gray wolves. *Biol. Lett.* **14**: 20170613. <http://dx.doi.org/10.1098/rsbl.2017.0613>

Received: 30 September 2017

Accepted: 30 November 2017

Subject Areas:

evolution, ecology, palaeontology

Keywords:

Canis lupus, postcrania, Quaternary

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Electronic supplementary material is available online at <https://dx.doi.org/10.6084/m9.figshare.c.3950767>.

Evolutionary biology

Postcranial diversity and recent ecomorphic impoverishment of North American gray wolves

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Recent advances in genomics and palaeontology have begun to unravel the complex evolutionary history of the gray wolf, *Canis lupus*. Still, much of their phenotypic variation across time and space remains to be documented. We examined the limb morphology of the fossil and modern North American gray wolves from the late Quaternary (<ca 70 ka) to better understand their postcranial diversity through time. We found that the late-Pleistocene gray wolves were characterized by short-leggedness on both sides of the Cordilleran–Laurentide ice sheets, and that this trait survived well into the Holocene despite the collapse of Pleistocene megafauna and disappearance of the ‘Beringian wolf’ from Alaska. By contrast, extant populations in the Midwestern USA and northwestern North America are distinguished by their elongate limbs with long distal segments, which appear to have evolved during the Holocene possibly in response to a new level or type of prey depletion. One of the consequences of recent extirpation of the Plains (*Canis lupus nubilus*) and Mexican wolves (*C. l. baileyi*) from much of the USA is an unprecedented loss of postcranial diversity through removal of short-legged forms. Conservation of these wolves is thus critical to restoration of the ecophenotypic diversity and evolutionary potential of gray wolves in North America.

1. Background

Ancestors of modern North American gray wolves (*Canis lupus*) migrated from Eurasia in the late Pleistocene (ca 70–23 ka) and gave rise to at least two genetically and morphologically distinct groups—one represented by extant populations and the other by the extinct Beringian wolf [1,2]. The Beringian morph of the gray wolf was first described from Alaska, and is distinguished from its extant relatives by a unique mitochondrial haplogroup and robust skulls that suggest greater bone consumption and predation on large megafauna [2,3]. Although these groups can be distinguished by skull morphology [2,4], their postcranial differences have remained unexplored, leading to confusion (occasionally also with the extinct dire wolf, *Canis dirus*) and uncertainty of their presence at fossil sites. Similarly, much of the morphological research on extant gray wolves has, to date, focused on their skulls because of the traditional emphasis on skull characters in taxonomy and wide interest in feeding ecology (e.g. [5,6]).

Postcranial skeletons are strongly linked to locomotor behaviours and, hence, ecology of carnivores including canids [7–11]. For example, elongation of distal limb segments associated with digitigrady is common in cursorial species, whose geographic distributions in turn are constrained by climatic and vegetational conditions [8–11]. Thus, spatiotemporal patterns of postcranial diversity may provide insights into a species’ evolutionary history

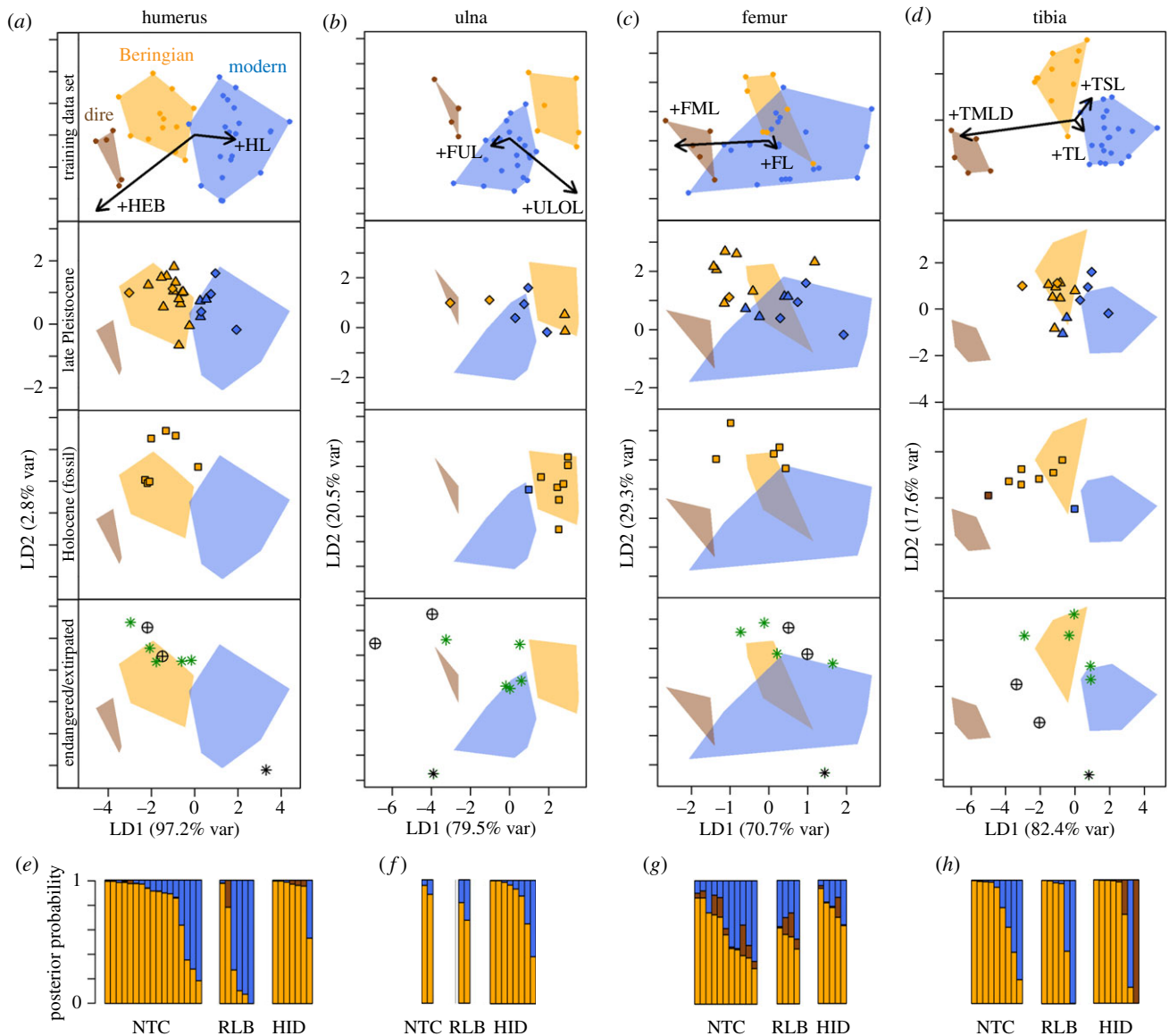


Figure 1. Morphometric differentiation of wolf samples along the first two discriminant axes (LD1 and LD2) for humerus (a), ulna (b), femur (c), and tibia (d). See table 1 for variables. Top row: training data set (dire wolf, brown; Alaskan Beringian wolf, orange; modern gray wolf, blue) and vectors representing proportional variable loadings (see table 1). Rows 2–3: fossil gray wolf samples from late-Pleistocene natural trap cave (NTC, triangles) and Rancho La Brea (RLB, diamonds), and Holocene of Idaho (HID, squares); fill colours indicate group assignments by classification functions. Row 4: Mexican wolf (*Canis lupus baileyi*; crossed circles) and recent-historical specimens collected before 1900 in areas from which wolves were subsequently extirpated (primarily Plains wolf, *C. l. nubilus*; stars). Bar charts (e–h) show posterior probabilities of group assignments for individual specimens and correspond to elements in a–d. See table 1 for variables.

encompassing its response to environmental changes. Toward this goal, we conducted quantitative comparison of appendicular elements of fossil and modern North American gray wolves, including specimens belonging to recently extirpated populations.

2. Methods

We measured 272 specimens with appendicular elements ([9,12]; electronic supplementary material, table S1) consisting of the dire wolf from the late Pleistocene of Rancho La Brea, California [13], and six samples of the gray wolf from: (1) late Pleistocene of Alaska [1,2]; (2) late Pleistocene of Natural Trap Cave, Wyoming [4]; (3) late Pleistocene of Rancho La Brea, California [13]; (4) Holocene of Middle Butte Cave (less than 7.6 ka) and Moonshiner Cave (greater than 3 ka), Idaho [14]; (5) modern populations in Alaska, Canada and the Midwestern USA, likely representing *Canis lupus occidentalis* and the ‘Great Lakes wolf’ [15,16]; (6) now-extirpated populations in western and

Midwestern USA, collected before 1900 and mostly, if not entirely, belonging to the Plains wolf (*C. l. nubilus*); and (7) the Mexican wolf (*C. l. baileyi*) in Arizona. An initial screening of the dataset indicated that samples (6) and (7) would constitute multivariate outliers if combined with the northern modern gray wolf sample (5), hence they were analysed separately. We were unable to fully control for the combined effects of age and sex on skeletal dimensions; however, the key patterns as described below are sufficiently clear that our conclusions are robust to potential sampling bias.

Linear discriminant analyses were performed to identify morphometric distinctions among the groups with established identities (dire wolf, Alaskan Beringian wolf and modern gray wolf). We selected classification functions that best differentiated these groups based on jackknife cross-validation and used them to evaluate the characteristics of the remaining fossil samples. Because the fossil samples consisted of isolated elements with uncertain associations, individual elements were analysed separately. Models with different groupings of samples were compared to identify morphometrically cohesive clusters, and those with the

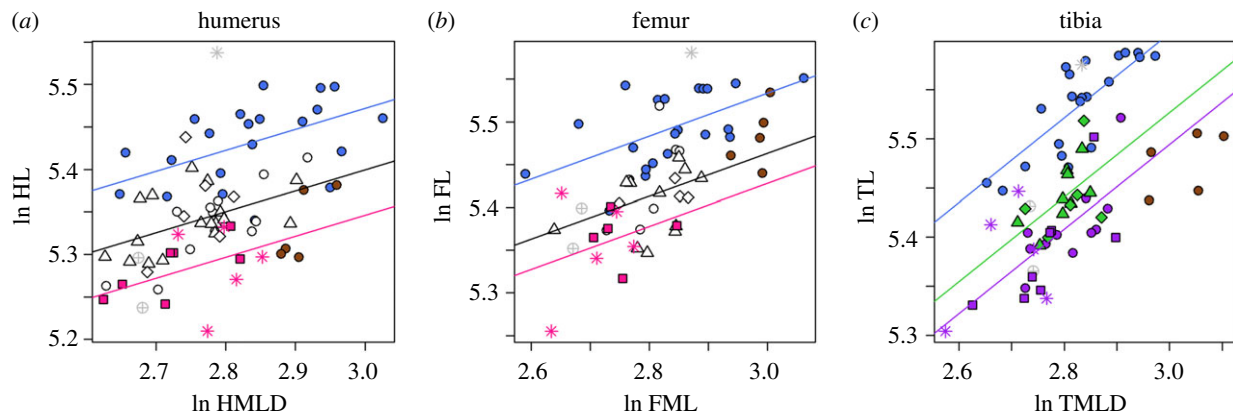


Figure 2. Regression lines from ANCOVA of humeral (a), femoral (b) and tibial (c) lengths, with midshaft widths as covariates. Symbols as in figure 1. See table 1 for variables. Colours and lines correspond to groups of samples for best-supported models (electronic supplementary material, table S4): Pleistocene (white/black), non-modern Holocene (including recent historical; pink), Natural Trap Cave + Rancho La Brea (green), and Alaskan Beringian + non-modern Holocene (purple). The dire wolf, Mexican wolf, and USNM A977 (grey star) were excluded from analysis but plotted for comparison. HMLD, humerus midshaft width.

Table 1. Linear discriminant analysis result. Variable abbreviations (see electronic supplementary material, table S1): FL, femur length; FML, femur midshaft width; FUL, ulnar length excluding olecranon process; HEB, humerus distal width; HL, humerus length; MT3L, metatarsal III length; RL, radius length; TL, tibia length; TMLD, tibia midshaft width; TSL, tibial cnemial crest length; ULOL, ulnar olecranon process length. *Linear discriminant functions based on FEB (femur distal width) and FL produced identical classification success rates to those listed here.

element	max. no. variables	best-performing set of functions [proportion of trace]	classification success rate (jackknife cross-validation)			
			modern gray wolf	Alaskan Beringian morph	dire wolf	mean across groups
humerus	4	LD1 [0.972] = $-0.454 \text{ HEB} + 0.187 \text{ HL}$ LD2 [0.028] = $0.239 \text{ HEB} - 0.014 \text{ HL}$	0.952	0.917	1.000	0.956
ulna	2	LD1 [0.795] = $-0.093 \text{ FUL} + 0.342 \text{ ULOL}$ LD2 [0.205] = $-0.029 \text{ FUL} - 0.217 \text{ ULOL}$	0.905	0.833	1.000	0.913
femur	3	LD1* [0.707] = $-0.807 \text{ FML} + 0.065 \text{ FL}$ LD2* [0.293] = $-0.059 \text{ FML} - 0.088 \text{ FL}$	0.905	0.429	0.800	0.711
tibia	3	LD1 [0.824] = $-1.321 \text{ TMLD} + 0.105 \text{ TL} + 0.191 \text{ TSL}$ LD2 [0.176] = $-0.135 \text{ TMLD} - 0.092 \text{ TL} + 0.191 \text{ TSL}$	1.000	0.909	1.000	0.970
radius	1	LD1 [1.000] = 0.112 RL	0.905	0.941	0.000	0.615
metatarsal III	1	LD1 [1.000] = 0.242 MT3L	0.950	0.000	1.000	0.650

highest support as measured by the sample size-adjusted Akaike's information criterion were selected for subsequent analyses [17]. Statistical significance of across-group differences was assessed by multivariate analysis of variance (MANOVA). We then performed analyses of covariance (ANCOVA) to examine differences in limb-element lengths while controlling for variations in midshaft widths, which we regarded as body-mass proxies [18]. Additionally, we selected inter-elemental ratios that reflect carnivore habitat and ecology (e.g. large metatarsal III/femur ratios characterize cursorial species) [7–10], and compared their values between: (1) the modern and recent-historical (pre-1900) gray wolf samples using ANOVA; and (2) the fossil samples and bootstrapped pseudo-replicates of the modern gray wolf sample using randomization test, thus simulating dissociation of skeletal elements. Additional methodological details are provided in electronic supplementary material, text S1.

3. Results

The tibiae, humeri and ulnae of the dire wolf, Alaskan Beringian wolf and modern North American gray wolf (excluding

outliers and extirpated populations) are clearly distinguishable (figure 1; electronic supplementary material, figure S2; table 1). Within the gray wolf, the modern sample differs from the Alaskan Beringian morph principally in having more elongate elements. Femoral dimensions are highly variable within the modern gray wolf sample and overlap substantially with those of the Alaskan Beringian morph. The univariate discriminant models for the metatarsal III and radius performed poorly, so we did not attempt classification of these elements in samples outside the training dataset.

Classification functions assigned a large majority of the late-Pleistocene fossils from Wyoming and southern California to the Beringian morph, although several specimens were located outside the observed morphological ranges for both the Beringian morph and the modern gray wolf sample (figure 1). Likewise, the fossils from the Holocene of Idaho showed close similarities with the Alaskan Beringian wolf, more so than the late-Pleistocene gray wolves from southern California. Depending on the element, multivariate linear models with two to three groups of samples

had the highest support values; these models consistently indicated a division between the modern gray wolf and other gray wolf samples (electronic supplementary material, table S2). With all of the best-supported models, MANOVA detected significant differences in multivariate means between all pairs of groups, indicating geographic or temporal variations (e.g. Pleistocene versus non-modern Holocene; electronic supplementary material, table S3).

The highly endangered Mexican wolf and pre-1900 specimens of the Plains wolf (from regions where they subsequently became extirpated) generally fell outside the morphological ranges of the restricted modern gray wolf sample, and instead plotted either near the Alaskan Beringian wolf or in their own unique regions in the discriminant space (figure 1). One anomalous individual, represented by USNM A977 (supposedly collected in Montana in 1850), appears to be an outlier in the direction of the modern gray wolf sample but is characterized by even longer limb elements (figure 2, electronic supplementary material, figure S2).

The best-supported ANCOVA models identified three groups of gray-wolf samples and highlighted the distinctiveness of the modern gray wolf sample (figure 2; table 2; electronic supplementary material, table S4). Their limb elements were significantly longer than those of other gray-wolf groups by 7–13% for humerus, 7–11% for femur and 8%–12% for tibia after adjusting for body size. The observed levels of differentiation approach those achieved in a laboratory population of mice that were artificially selected for elongate limbs and demonstrated significant increases in stance duration and stride length relative to the control population [19]. The Mexican wolf and the largely extirpated Plains wolf more closely resembled the late-Pleistocene and Holocene fossil gray wolves in their short-leggedness. ANOVA and randomization tests showed the brachial index, crural index and intermembral index to be generally indistinguishable among the gray wolf samples (electronic supplementary material, figure S3, tables S5 and S6); however, in the two cases where significant differences were detected, they both indicated relative elongation of distal limb segments (radius over humerus, metatarsal over femur and tibia over femur) in the modern gray wolf sample. Consistently with this pattern, the metatarsal III–femur ratio was significantly larger in the modern gray wolves compared to the recent-historical and the Pleistocene Natural Trap Cave samples.

4. Discussion

The extirpation of gray wolves from much of the contiguous USA erased a large swath of their genetic and—as demonstrated here—morphological diversity in North America [1,20]. Our analysis of fossil and modern gray wolves revealed a previously unappreciated dimension of their diversity history. The postcranial distinctions among the dire wolf, the Alaskan Beringian wolf and the modern gray wolf parallel the divergence of their skull morphology [2,4]. Likewise, the overall similarities of samples from Wyoming and eastern Beringia lend additional support for the presence of the Beringian morph south of the Laurentide–Cordilleran ice sheets in the late Pleistocene, which was recently reported based on analysis of mandibular shape [4]. Our results highlight persistence of short-legged morphotypes well into the Holocene—a phenomenon that was apparently masked in a previous study using a smaller dataset with a different grouping of fossil and modern samples [21].

Table 2. ANCOVA statistics. Sample abbreviations: AKB, Alaskan Beringian morph; HID, Idaho Holocene; MG, modern gray wolf; NTC, Natural Trap Cave; RHG, recent-historical gray wolf (primarily *C. l. nubilus*); RLB, Rancho La Brea gray wolf. See table 1 and figure 2 for variables. Grouping of samples follow models with highest support (electronic supplementary material, table S4). p_{adj} , p -values for unplanned pairwise comparisons adjusted with Holm's sequential Bonferroni procedure (separately for each element).

Y	covariate	groups compared	d.f.	adjusted group means ($\bar{Y}_{adj}$)	slope	intercept	t	p_{adj}
In HL	In HMLD	MG, [AKB + NTC + RLB]	64	5.418 (MG)	0.247	4.731 (MG)	6.288	<0.001
		[AKB + NTC + RLB], [HID + RHG]		5.347 ([AKB + NTC + RLB])		4.659 ([AKB + NTC + RLB])	4.215	<0.001
				5.293 ([HID + RHG])		4.605 ([HID + RHG])		
In FL	In FML	MG, [AKB + NTC + RLB]	49	5.484 (MG)	0.250	4.782 (MG)	5.663	<0.001
		[AKB + NTC + RLB], [HID + RHG]		5.415 ([AKB + NTC + RLB])		4.712 ([AKB + NTC + RLB])	2.196	0.016
				5.379 ([HID + RHG])		4.677 ([HID + RHG])		
In TL	In TMLD	MG, [NTC + RLB]	56	5.521 (MG)	0.430	4.204 (MG)	7.064	<0.001
		[NTC + RLB], [AKB + HID + RHG]		5.441 ([NTC + RLB])		4.317 ([NTC + RLB])	3.061	0.002
				5.407 ([AKB + HID + RHG])		4.237 ([AKB + HID + RHG])		

In retrospect, the gray wolves are notable in the Quaternary history of the North American fauna not only for their survival across the Pleistocene–Holocene transition but also in their retention of short-leggedness through that period, because megafaunal extinctions could have prompted strong selection for increased cursoriality. What, then, prompted the apparently recent emergence of long-legged gray wolves with more pronounced elongation of distal limb segments? We hypothesize that a new level or type of prey depletion necessitated a novel prey-capture behaviour or increase in foraging distances and led to selection for increased limb lengths. Interspecific comparative studies of carnivorans suggest positive relationships between limb lengths on one hand and hunting behaviour or home range size on the other [22,23], while territory size in modern *Canis lupus* is correlated with the available prey biomass at local and regional scales [24]. A direct role of climate is difficult to envision given the broad geographic distribution of short-leggedness across a glacial–interglacial transition.

Within this broad picture, we recognize regional variations in postcranial morphology among the late-Pleistocene gray wolves. The moderate differentiation of eastern-Beringian and Wyoming populations echoes a similar case within the contemporaneous stilt-legged horse, which exhibits a pattern of geographic differentiation in metatarsal dimensions across the same regions [25]. These variations may reflect environmental conditions or genetic divergence after the closure of the Yukon corridor at *ca* 23 ka [1]. We anticipate that additional research on the morphoclines, when combined with palaeoenvironmental and genetic data, will enable us to test these hypotheses.

The Mexican wolf—a highly endangered species that is phylogenetically positioned outside all other extant North

American gray wolves [1]—and pre-1900 specimens of the Plains wolf from the Midwestern and western USA retain the short-leggedness seen in late-Pleistocene wolves. Together, they reveal a rich diversity of forms that occupied the continent until recently. The modern impoverishment of postcranial diversity in North American gray wolves is apparently unprecedented in their history. Protection of the threatened *C. l. baileyi* and the severely diminished *C. l. nubilus* is an essential step toward restoring the ecophenotypic as well as genetic diversity of the species and, with it, its evolutionary potential.

Data accessibility. Primary data are available from Dryad Digital Repository [12].

Authors' contributions. J.A.M. conceived of the study; S.T. and J.A.M. designed the study, collected and analysed data, and drafted the manuscript. Both authors gave final approval for publication and agree to be held accountable for the work performed therein.

Competing interests. We have no competing interests.

Funding. This study was supported by Division of Earth Sciences (NSF EAR/SGP 1425059).

Acknowledgements. We thank Eileen Westwig, Alana Gishlick and Judy Galkin (American Museum of Natural History), William Stanley and Adam Ferguson (Field Museum), Aisling Farrell and Gary Takeuchi (Tar Pits Museum), Mary Thompson (Idaho Museum of Natural History), Mark Omura (Museum of Comparative Zoology), Maria Eifler, Kelsey Cloyd, Megan Sims, David Burnham and Desui Miao (University of Kansas Museum of Natural History), Laura Vietti (University of Wyoming Geological Museum), Linda Gordon (US National Museum of Natural History), Kathy Molina (UCLA) and Kristof Zyskowski (Yale Peabody Museum) for access to, and assistance in, collections under their care; Robert Wayne and Blaire Van Valkenburgh (UCLA) and Alan Cooper (University of Adelaide) for discussion; and editors and two anonymous reviewers for helpful comments on earlier versions of this paper. This is UCMP publication no. 2086.

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