

# **Rancho La Brea Weasels as a Special Case of Response to Pleistocene Disturbance**

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## ***ABSTRACT***

The climatic transition and megafaunal extinctions at the end of the Pleistocene epoch approximately 11,000 years ago presumably affected non-megafaunal survivors, including mesocarnivores: small to medium-sized mammalian carnivores. Here, we investigate how these perturbations may have impacted weasels (*Mustela*), a common member of modern North American ecosystems. Using linear and two-dimensional geometric morphometrics, we compared fossil specimens from the Pleistocene- to Holocene-age Rancho La Brea (RLB) asphalt seeps in Los Angeles, California, to the three extant species of *Mustela* currently inhabiting the region. We determined the species at Rancho La Brea to be *Mustela frenata*, the long-tailed weasel. We found that body size in Los Angeles *M. frenata* today did not change significantly from before the Last Glacial Maximum to the present; however, other ecomorphological shifts are apparent. Based on analysis of the cranial skeleton, weasels today display greater capacity for omnivory than their fossil counterparts, and this was accompanied by less massive limbs and more proximal mechanical advantage in the postcranial skeleton. The ecomorphological response of Los Angeles weasels to the end-Pleistocene climatic warming and megafaunal extinctions was likely governed primarily by climatic effects and low-level trophic interactions. Top-down trophic cascade effects (i.e. character release) did not appear to greatly influence weasel ecomorphology in the late Pleistocene. These ecomorphological shifts invite inquiry into how large-scale Pleistocene disturbances set the stage for extant North American carnivore communities dominated by animals of smaller size.

## **1. INTRODUCTION**

### **1.1 General Introduction**

The field of conservation biology is becoming increasingly concerned with understanding species' responses to environmental and ecological disturbance on a systematic level (1). In this era of anthropogenic climate change, one of the best defenses against biodiversity loss and subsequent ecosystem collapse is understanding the biological response of organisms to disturbance. The end of the last Ice Age and the transition from Pleistocene to Holocene open a unique window through which to look at the responses of ecosystems and organisms to large-scale extinctions and climate change. From the Last Glacial Maximum (LGM) ~25-20 kya to the present, the earth experienced a period of dramatic warming that contributed to the Pleistocene Megafaunal Extinctions (PME) (2). setting the stage for modern ecosystems and the ongoing anthropogenic warming and extinction crisis (3). The Pleistocene Megafaunal Extinctions are also the oldest human-influenced extinction events (2), and there is evidence that human mammoth-hunting may have contributed to the global climate changes that preceded the PME (4). Recent work has found evidence of human culture in northern Mexico that implies a

pre-LGM dispersal in Northern Mexico (5), creating the potentiality that humans were contributing to climatic and extinction dynamics in Los Angeles and the rest of the American Southwest before the LGM. These characteristics of this period in earth's history make it a useful study system for informing how the current period of anthropogenic warming and extinction might affect extant ecosystems.

Mesocarnivores -small to medium sized members of the mammalian order Carnivora- include some of the most common inhabitants of terrestrial ecosystems including raccoons, foxes, coyotes, and skunks. They occupy scavenging and hunting roles, have adapted to a wide range of habits including fossoriality (badgers), semi-aquaticism (otters), and arboreality (martens) (6), and they are known to have high adaptability to disturbance (7,8). To be able to predict how a mesocarnivore species will respond to an ecological disturbance, it is necessary to understand which traits respond to extrinsic change and how those traits facilitate the organism's role in its ecosystem.

Mesopredator release -the proliferation of and/or behavior changes of mesopredators in response to the removal of apex predators (9,10)- has been observed on all continents except Antarctica (9–12). However, release is not always the default for mesopredators in a large-predator removal event (LPRE). LPREs sometimes lead to other trajectories for mesopredators, and mesopredator responses to LPREs need not be uniform within an ecosystem (9).

The skeletal morphology of many carnivorans correlates uniquely with their habitat and climate (6,13). Many species of Mustelidae, for example, possess shortened legs relative to their body length than most carnivorans (ex. weasels, *Mustela spp.*). The relationship between Mustelid skeletal morphology and habitat and climate is known to deviate from established patterns within Carnivora; North American martens (*Martes spp.*) have been shown to deviate from Allen's Rule and exhibit geographic morphological variation related to locomotor adaptations to vegetation density (13). Weasels and other Mustelids are known to possess high levels of geographic variation and sexual dimorphism relative to other carnivorans (14).

In this paper, we explore hypotheses of ecomorphological variation in fossil mustelids at RLB based on abiotic factors -change driven by climate and habitat change- and biotic factors -mesopredator release, character displacement, and other trophic interactions.

## 1.2 Study System and Goals

With the exception of Australia and Antarctica, *Mustela spp.* are found on every continent, and their range in the Americas spans coast to coast from the Great Lakes in the north to the northern tip of South America (15). Within this range, they are found in temperate and tropical areas, preferring fields and lightly wooded areas as opposed to deserts and dense forests (16). Weasels have a long, slender body and short legs; their tail can be up to 50% of their body length (16). They exhibit high levels of geographic size variation and sexual dimorphism (13). The body mass range of weasels can range from 30 g at the smallest (the least weasel, *Mustela*

*nivalis*) to 1.5 kg at the largest (European polecat, *M. putorius*) (17). Their dental formula is I3/3, C1/1, P4/3, M1/2 (16). Weasels are highly carnivorous, consuming rodents and lagomorphs primarily (18,19); females prefer smaller rodents, into whose burrows they are small enough to fit, while males eat larger mammals that smaller females can not bring down as easily (16). Weasels will also eat lizards and birds, and they supplement their diet with fruits and berries in the summer (16). Weasels are diurnal but are more active at night (16). Their predators consist of owls, coyotes, and rattlesnakes (16), all of which have been found in the fossil record and analyzed in detail at Rancho La Brea (20).

Rancho La Brea is a well-known lagerstätte in Los Angeles, California, that has yielded over 5 million late Quaternary fossils known for their exceptional preservation (21). The “tar pits” there are a series of asphaltic deposits consisting of a matrix of sands, silts, gravels, and clays containing the fossilized remains of animals and plants (21). From ~55 kya until 200 ya, Miocene-aged hydrocarbons migrating upwards in the soil resulted in intermittent surficial liquid asphalt seeps that preserved biological tissues -mainly bone collagen and plant cellulose- from plants and animals that became entrapped in the seeps (20). The activity of the Rancho La Brea tar seeps spans from well before the Last Glacial Maximum (LGM) to well after the Pleistocene Megafaunal Extinctions, opening an exceptionally preserved window into the ecology and climate into two of the most extreme environmental periods in earth’s recent history. Analysis of the RLB collections has historically focused on larger fauna with less attention given to lower trophic levels and smaller fauna (20,22). Thus, our holistic understanding of ecosystem dynamics at RLB during the Pleistocene is lacking, despite a wealth of plant and small animal specimens in the RLB collections waiting to be identified and analyzed.

In this paper, we analyze ecomorphological shifts in Los Angeles and Western North American weasels from before the Last Glacial Maximum to the present. Using both linear morphometrics and geometric morphometrics we address the following questions: *i) which species of Mustela were present at Rancho La Brea?, ii) what was the pattern of change -if any- in Los Angeles weasels from before the LGM ~25-20 kya to after the Pleistocene Megafaunal Extinctions ~11 kya to the present?, and iii) to what degree were the environmental and trophic selection pressures acting on RLB Mustelids during the end-Pleistocene shared throughout the American Southwest?*

## **2. MATERIALS AND METHODS**

### **2.1 Materials**

Our fossil material for this study comprised 92 craniodental specimens (either a skull or a dentary or isolated teeth from either) and 52 individual postcranial elements from the Hancock, Pit 91, and Project 23 Collections at La Brea Tar Pits and Museum in Los Angeles, California, USA. These fossils were preserved in 17 separate asphaltic deposits and are estimated to be between 55,000 to potentially 200 years old, based on predominantly large-mammal dates found in association with these fossils.

Our extant comparative material consisted of 71 specimens containing both a postcranial skeleton and a skull/dentary from the Mammalogy collection at the Los Angeles County Museum of Natural History (LACM). These extant specimens represented 2 genera and 3 species (*Mustela frenata*, *M. erminea*, *Neovison vison*). We restricted our extant sampling to specimens from California and Nevada to reduce the effects of high geographic variation in *M. frenata* (14) when comparing extant specimens to our fossil specimens (which are all from LA County before it was LA County). When possible, only extant specimens from LA County were used in the linear and geometric morphometric comparisons to focus our analysis on tracking weasel ecology in the Los Angeles area through time (see 2.4 Statistical Analysis).

## **2.2 Time Intervals:**

When per-element sample sizes were adequate, we sorted fossil specimens into four time intervals: pre-Last Glacial Maximum (preLGM; ~35 to ~25 kya), mid-LGM (~25 to ~20 kya), post-glacial pre-extinction (PostLGMpreExt; ~16 to ~12 kya), and Holocene post-extinction (HoloPostExt; ~9,000 to ~500 ya). Though Rancho La Brea also actively entrapped fauna during the peak mid-LGM interval, *Mustela* was largely not preserved in these deposits. Time intervals for fossil specimens were assigned based on the median age of the deposit from which they were excavated (21,23–30). All median deposit dates were calculated using published radiocarbon dates from Rancho La Brea. If a given deposit spanned more than one of the above-defined time intervals (e.g. Deposit “No Data”), then we excluded that deposit’s specimens from our analysis as the chronological uncertainty was too great.

When sorting into the three time intervals reduced per-element sample sizes to statistically unusable levels, we pooled RLB specimens into a single “fossil” time category for comparison with extant (“modern”) specimens.

## **2.3 Linear Morphometrics:**

Linear measurements were taken with Mitutoyo Absolute Digimatic (Model No. CDN-8” CX) calipers. Forty raw measurements (25 craniodental, 15 postcranial) were taken from which 29 indices (16 from craniodental measurements, 13 from postcranial measurements) were computed; craniodental indices followed Friscia et al (2013) (31) (Table 1), and postcranial indices were taken from Samuels et al (2013) (6) (Table 2). Tables 3 and 4 describe the raw measurements used to compute the indices. Postcranial indices that were composites of multiple measurements on two or more bones were excluded, as our fossil postcranial specimens were all isolated elements. Our proxy for body mass (BM) was the length of the lower first molar, following Van Valkenburgh and Press (1990) (32). Van Valkenburgh and Press (1990) (32) give the equation  $\log(BM) = 3.48 * \log(m1L) - 3.04$  as a predictor for body mass in mustelids and reports an r-value of .93 for this equation (32). Data was excluded based on poor preservation (taphonomy), presence of bone disease, juvenile or sub-adult age class as observed

by incomplete epiphyseal fusion and incomplete eruption of dentition. For more information on data exclusion, see Supplementary Information.

Index	Description
RBL*	Relative blade length of lower first molar (m1 carnassial), measured as the ratio of trigonid length to total anteroposterior length of m1
RLGA*	Relative lower grinding area, measured as the square root of the summed areas of the m1 talonid and m2 (if present) divided by the length of the m1 trigonid. Area was estimated as the product of maximum width and length of the talonid of m1 and m2, respectively
RUGA*	Relative upper grinding area, measured as the square root of the summed areas of M1 and M2 (if present) divided by the anteroposterior length of P4 (upper carnassial). Area was estimated by the product of width and length of M1 and M2, respectively
M1BS	m1 blade size relative to dentary length, measured as the length of the trigonid of m1 (carnassial) divided by dentary length. Dentary length was measured as the distance between the posterior margin of the mandibular condyle and the anterior margin of the canine
M2S	m2 size relative to dentary length, measured as the square root of m2 area (if present) divided by dentary length. Tooth area measured as in RLGA and dentary length (measured as in M1BS). If no m2 was present in the taxon, M2S was recorded as zero
IXP4	Second moment area of the dentary at the interdental gap between the third and fourth lower premolars relative to dentary length. Moment area is used as an estimate of resistance of the dentary to bending. Second moment of area was calculated using the formula $I_x = (pDxDy^3)/64$ , where Dx is maximum dentary width and Dy is maximum dentary height at the p3–p4 interdental gap. $I_x$ relative to dentary length was then estimated as the fourth root of $I_x$ divided by dentary length (measured as in M1BS)
IXM2	Estimate of resistance of dentary to bending, as measured by the second moment of area at the interdental gap between the first and second molars (or posterior to the first molar if no second molar was present). Measured as IXP4, except maximum dentary width and height were taken at the m1–m2 interdental gap (or posterior to m1 if m2 was not present in the taxon)
MAT*	Mechanical advantage of the temporalis muscle, measured as the distance from the mandibular condyle to the apex of the coronoid process divided by dentary length (measured as in M1BS)
MAM*	Mechanical advantage of the masseter muscle, measured as the distance from the mandibular condyle to the ventral border of the mandibular angle divided by dentary length (measured as in M1BS)
C1	Relative size of the upper canine, measured by the square root of the basal area of C1 divided by square root of the size of the lower first molar (carnassial). C1 area was calculated as the product of length and width of the tooth. m1 size was used as a proxy for body size and was calculated as the product of length and width of the tooth
P4P*	Relative size of the protocone of the upper fourth premolar (carnassial), measured as the ratio of maximum width of P4 divided by maximum length of P4
UM21	Square root of upper second molar area (if present) divided by square root of upper first molar area. Areas estimated as RUGA. If no M2 was present in a taxon, UM21 was recorded as zero

P4S	Lower fourth premolar shape, measured as maximum width of p4 divided by its maximum length
PMZ	Relative total length of premolars, measured as the sum of the maximum lengths of p2, p3, p4 divided by dentary length (measured as in M1BS)
P4Z	Relative length of fourth lower premolar, measured as the maximum length of p4 divided by dentary length (measured as in M1BS)

Table 1. Craniodental indices used in this study. Descriptions from Friscia et al (2013) (31). \* indicates indices used in Time Interval Analysis.

Index	Description
SMI*	Deltpectoral crest length divided by humerus length (DPCL/HL). Indicates mechanical advantage of the deltoid and pectoral muscles acting across the shoulder joint.
BI	Radius length divided by humerus length (RL/HL). Indicates relative proportions of proximal and distal elements of the forelimb.
HRI*	Mediolateral diameter of humerus divided by humerus length (HMLD/HL). Indicates robustness of the humerus and its ability to resist bending and shearing stresses.
HEI*	Epicondylar breadth of humerus divided by humerus length (HEB/HL). Indicates relative area available for the origins of the forearm flexors, pronators, and supinators.
OLI	Olecranon process length divided by functional length of the ulna (ULOL/FUL). Indicates relative mechanical advantage of the triceps brachii and dorsoepitrochlearis muscles used in elbow extension. This is identical to the index of fossorial ability used by Hildebrand (1985a,b) (33,34).
URI	Mediolateral diameter of ulna divided by functional length of the ulna (UMLD/FUL). Indicates robustness of the ulna and its ability to resist bending and shearing stresses, and relative area available for the origin and insertion of forearm and manus flexors, pronators, and supinators.
CI	Tibia length divided by femur length (TL/FL). Indicates relative proportions of proximal and distal elements of the hind limb.
FRI	Anteroposterior diameter of femur divided by femur length (FAPD/FL). Indicates robustness of the femur and its ability to resist bending and shearing stresses (AP diameter is used due to transverse expansion of the femora in some semiaquatic mammals).
GI*	Length of distal extension of the greater trochanter of the femur divided by femur length (FGT/FL). Indicates relative mechanical advantage of the gluteal muscles used in retraction of the femur.
FEI	Epicondylar breadth of femur divided by femur length (FEB/FL). Indicates relative area available for the origins of the gastrocnemius and soleus muscles used in extension of the knee and plantar-flexion of the pes.

TRI	Mediolateral diameter of tibia divided by tibia length (TMLD/TL). Indicates robustness of the tibia and its ability to resist bending and shearing stresses.
TSI	Length of distal extension of the tibial tuberosity (spine) divided by tibia length (TSL/TL). Indicates relative mechanical advantage of the hamstrings and biceps femoris muscles acting across the knee and hip joints.
IM	Lengths of the humerus and radius divided by lengths of the femur and tibia $[(HL + RL) / (FL + TL)]$ . Indicates the length of the forelimbs relative to the hind limbs.

Table 2. Postcranial indices used in this study. Descriptions from Samuels et al (2013) (6).

Measurement	Description
CL	Maximum anteroposterior length of upper canine
CW	Maximum mediolateral width of upper canine
P4L*	Maximum anteroposterior length of upper fourth premolar
P4W	Maximum mediolateral width of upper fourth premolar
M1L*	Maximum anteroposterior length of upper first molar
M1W	Maximum mediolateral width of upper first molar
M2L	Maximum anteroposterior length of upper second molar
M2W	Maximum mediolateral width of upper second molar
p2L	Maximum anteroposterior length of lower second premolar
p3L	Maximum anteroposterior length of lower third premolar
p4L	Maximum anteroposterior length of lower fourth premolar
p4W	Maximum mediolateral width of lower fourth premolar
m1TrL*	Anteroposterior length of trigonid blade on lower first molar
m1L*	Maximum anteroposterior length of lower first molar
m1W*	Maximum mediolateral width of lower first molar
m1TaW	Mediolateral width of talonid basin on lower first molar
m2L	Maximum anteroposterior length of lower second molar
m2W	Maximum mediolateral width of lower second molar

JL	Length of lower jaw as measured by the distance from the posterior margin of the condyloid process to the anterior margin of the lower canine socket
MAT	Moment arm of the temporalis as measured by the distance from the dorsal margin of the coronoid process to the posterior margin of the condyloid process
MAM	Moment arm of the masseter as measured by the distance from the posterior margin of the condyloid process to the dorsoposterior margin of the angular process
p3p4JD	Depth of jaw as measured by the distance from the junction of /p3 and /p4 to the bottom of the jaw along the line perpendicular to the line of /m1 along the jaw
p3p4JW	Mediolateral width of jaw at junction of /p3 and /p4 as measured by the distance from the buccal margin of the jaw to the lingual margin along the plane perpendicular to the plane defined by /m1 along the jaw
m1m2JD	Depth of jaw as measured by the distance from the junction of /m1 and /m2 to the bottom of the jaw along the line perpendicular to the line of /m1 along the jaw
m1m2JW	Mediolateral width of jaw at junction of /m1 and /m2 as measured by the distance from the buccal margin of the jaw to the lingual margin along the plane perpendicular to the plane defined by /m1 along the jaw

Table 3. Descriptions of craniodental measurements used in this study. \* indicates measurements included in Time Bin Analysis.

Measurement	Description
DPCL*	Length of deltopectoral crest
HL*	Length of humerus
HEB*	Epicondylar breadth of humerus
RL	Length of radius
OPL	Length of olecranon process
FUL	Functional length of ulna
UMLD*	Mediolateral diameter of ulna
TL	Length of tibia
FL*	Length of femur
FAPD	Anteroposterior diameter of femur
GTL	Length of greater trochanter



FEB	Epicondylar breadth of femur
TMLD	Mediolateral diameter of tibia
TTL	Length of distal extension of tibial tuberosity

Table 4. Descriptions of postcranial measurements used in this study.

## 2.4 Geometric Morphometrics:

To complement the linear morphometrics and quantify cranial shape differences holistically, we employed two-dimensional geometric morphometrics on cranial elements (skulls and dentaries). Photos were taken with a Fujifilm X-T10 digital camera equipped with a 35 mm lens. A ruler was placed in all photos to provide a scale bar. Seventeen landmarks were placed on the palate after Loy et al. (2004) (Table 5, Fig 1) (14); sixteen were placed on the dentary after Romaniuk (2018) (Table 6, Fig 2) (35). LM1 on the dentaries (Table 6) was excluded due to lack of preservation in the fossil specimens. Landmarks were recorded in Fiji (ImageJ) (36), and configurations were superimposed through Generalized Procrustes Analysis (37). All photos were taken by author M. Balisi, and all images were digitized by author J. Feingold. Best practices for geometric morphometrics were followed throughout photography and data digitization (38).

Landmark	Description
LM1*	Posterior margin of the most distal socket in the incisor row
LM2*	Anterior margin of canine socket
LM3*	Posterior margin of canine socket
LM4*	Anterior margin of P3 socket
LM5*	Posterior margin of P3 socket
LM6*	Anterior margin of buccal lobe of P4
LM7*	Anterior margin of lingual lobe of P4
LM8*	Posterior margin of P4
LM9*	Lingual margin of M1
LM10*	Posterior margin of buccal lobe of M1
LM11	Buccal margin of M1

LM12	Vertex between posterior margins of the lingual and buccal lobes of M1
LM13	Posterior margin of lingual lobe of M1
LM14	Anterior margin of lingual lobe of M1
LM15	Vertex between anterior margins of the lingual and buccal lobes of M1
LM16	Anterior margin of buccal lobe of M1
LM17	Vertex between lingual and buccal lobes of P4

Table 5. Descriptions of palatal landmarks; \*- indicates landmarks used for symmetry testing in the fossil specimens. All 17 were used for symmetry testing of the modern specimens.

Landmark	Description
LM1*	Anterior margin of canine socket
LM2	Posterior margin of canine socket
LM3	Anterior margin of m1
LM4	Vertex between anterior and posterior lobes of M1
LM5	Posterior margin of m1
LM6	Dorsal margin of coronoid process
LM7	Vertex between dorsal margin of coronoid process and posterior margin of condyloid process
LM8	Buccal tip of condyloid process
LM9	Vertex between condyloid process and ventral margin of angular process
LM10	Ventro-posterior margin of angular process
LM11	vertex between bottom of jaw and LM12
LM12	Point along the dorsal margin of the jaw with the same x-coordinate as LM5
LM13	Point along the dorsal margin of the jaw with the same x-coordinate as LM4
LM14	Point along the dorsal margin of the jaw with the same x-coordinate as LM3
LM15	Point along the dorsal margin of the jaw with the same x-coordinate as LM2
LM16	Anterior margin of masseteric fossa

Table 4. Descriptions of dentary landmarks. \* indicates landmarks excluded from analysis based on preservation.

Because specimens—especially fossils—can be fragmentary, we digitized cranial landmarks on only one side. To determine that taphonomic distortion did not preclude such an analysis, we tested symmetry in the crania using  $n=6$  fossil specimens for which all landmarks were present on both sides. These images were digitized on one side, flipped, and re-digitized. Symmetry was tested between the left and right sides with a pairwise morphological disparity test (function `morphol.dispar()`) in the `geomorph` package in R (39,40). Many specimens in both the RLB and LACM collections are fragmentary, and establishing symmetry in the data set greatly expanded the sample size for geometric morphometric analysis of cranial specimens. For more information on symmetry testing, landmark selection, and data exclusion, see Supplementary Information. Following the establishment of symmetry, landmarks on the palate were placed on the left side of the specimen when possible; if the specimen was broken on the left side, the right side was digitized instead and the photo was flipped prior to comparison with left-side specimens. Because fossil dentaries were often disarticulated, we were not able to test them for symmetry in the same way as the crania. Instead we chose to digitize specimens of only one side to avoid pseudoreplication. Left-sided specimens were chosen because there were more left-sided than right-sided specimens in the RLB collections.

## 2.5 Statistical Analysis:

### 2.5.1 Ecomorphological Variation in Extant Sample:

To quantify levels of sexual dimorphism in extant *Mustela* as context for potential sexual dimorphism in fossil *Mustela*, we compared cranial and postcranial linear morphometrics of known extant male and female individuals using non-parametric Mann-Whitney U tests. Similarly, to quantify morphological variation by geography (county) and subspecies, we compared subsets of the extant cranial and postcranial linear morphometric dataset using non-parametric Kruskal-Wallis tests.  $p < .05$  was treated as significant, and  $.05 < p < .10$  was treated as marginally significant.

### 2.5.2 Comparison Between Extant and Fossil Samples: Linear Morphometrics:

To determine broad ecomorphological differences in the Los Angeles weasel population through time, we compared extant LA County and fossil RLB specimens across all raw measurements and indices using non-parametric Mann-Whitney U tests. Because fragmentary fossils meant that not all specimens preserved all linear metrics of interest, this broad analysis maximized the number of linear metrics compared.  $p < .05$  was treated as significant, and  $.05 < p < .10$  was treated as marginally significant.

To refine the above fossil-versus-extant analysis, we selected the best-represented and most informative raw measurements and indices (Table 1,2,3,4) to test further for differences

across time intervals (see Time Intervals). To perform a space-for-time substitution analysis (41), these Time Intervals were then compared to extant specimens from LA County and other counties in California and Nevada from which we had adequate sample size. *P*-values were computed with Kruskal-Wallis non-parametric tests. Post-hoc tests were performed to examine the relationship between pairs of counties, fossil time intervals, and both. Because low sample sizes caused our distributions and variances to be non-uniform, *p*-values were computed with Games-Howell post-hoc tests.  $p < .05$  was treated as significant, and  $.05 < p < .10$  was treated as marginally significant.

### 2.5.3 Comparison Between Extant and Fossil Samples: Geometric Morphometrics:

Because of the lower number of usable specimens in the geometric morphometrics analysis compared to the linear morphometric analysis, the geometric morphometric analysis was not a Time Interval Analysis and instead distinguished only between fossil and extant groups. Only extant specimens from Los Angeles County were used in this analysis. *P*-values were calculated via a pairwise morphological disparity test in the geomorph package (39) in R (40).

## 3. RESULTS

### 3.1 Species Identification and Body Mass Estimate of Fossil *Mustela*:

The three extant species of *Mustela* in Los Angeles County—*Mustela erminea*, *M. frenata*, and *Neovison vison*—visibly differ in size (Fig 3), showing low overlap in the distributions of mass estimates ( $p = 3.80 \times 10^{-7}$ ). Mass estimations for RLB *Mustela* fell within the range of extant *Mustela frenata* (Fig 3), suggesting that this is the only species that was present at RLB. Post-hoc tests revealed significant differences between RLB fossils compared to *M. erminea* and *N. vison*, but not between RLB fossils and extant *M. frenata*. Because we excluded juvenile and sub-adult specimens (see Supplementary Information), we are confident that none of the fossil specimens are juvenile *N. vison* that fall within the range of adult *M. frenata*.

Based on the length of the lower first molar, we estimated RLB *M. frenata* to have a median body size of ~.4 kg (median absolute deviation ~.06 kg) (Fig 3).

### 3.3 Tracking Change Through Time in Los Angeles:

#### 3.3.1 Linear Morphometrics (Time Interval Analysis):

For the craniodental measurements and indices, the three RLB fossil time intervals were singled out for comparison with each other and with extant LA County *M. frenata*. Using the length of the lower first molar (m1L) as a body mass proxy, no significant differences in weasel body size across the RLB Time Intervals and extant LA County ( $p = .401$ ) (Fig 4). Post-hoc tests also failed to reveal significant differences between any two time intervals. This conclusion is supported in the geometric morphometrics by a lack of significance between fossil and extant centroid sizes in both the dentaries ( $p = .863$ ) and skulls ( $p = .452$ ).

However, other craniodental measurements and indices shifted through time in LA weasels. Indices of dental grinding area differed significantly (relative lower grinding area, RLGA;  $p = 1.58 * 10^{-3}$ ) or marginally (relative upper grinding area, RUGA;  $p = .081$ ) across time intervals (Fig 5), generally increasing from past to present. Post-hoc tests revealed significant differences between pre-LGM and extant LA County weasels for both indices of dental grinding area. The pattern in these indices is mirrored in the raw measurements, as the anteroposterior length of the upper first molar also significantly increased through time ( $p = 4.47 * 10^{-3}$ ). As well, the mechanical advantage of the masseter muscle (MAM, used in chewing-grinding motion) marginally differed across time intervals ( $p = .064$ ) (Fig 5), decreasing from pre-LGM to after the end-Pleistocene extinctions, and increasing post-extinction to a present value higher than before the LGM. Though no significant differences were found between groups for the mechanical advantage of the temporalis muscle (MAT, used in shearing and carnivorous feeding) (Fig 5), post-hoc tests revealed significant differences in MAT in LA weasels from after the LGM to now, with extant LA weasels displaying greater mechanical advantage of the temporalis muscle than LA weasels before the LGM ( $p = .01$ ), indicating a higher bite force.

As well, analysis of postcranial elements suggests changes in limb morphology, though low sample sizes hampered interpretation for many raw measurements and indices. Because the earliest two Time Intervals (pre-LGM and PostLGMpreExt) had uninformatively low representation, analysis of postcranial elements was restricted to a “fossil vs extant” comparison. Humeral robustness (index HRI; Table 2) differed significantly through time ( $p = 9.99 * 10^{-4}$ ), with extant Los Angeles weasels displaying less robust humeri (Fig 6). This was accompanied by less robust ulnae in extant specimens, with significant differences ( $p = .034$ ) found in the mediolateral diameter of the ulna (measurement UMLD; Table 4) (Fig 7). Mechanical advantage of the deltoid and pectoral muscles (index SMI; Table 2) differed marginally significantly ( $p = .083$ ) through time, with extant LA weasels displaying proportionally stronger shoulders (Fig 6). This was supported by marginally significant differences ( $p = .072$ ) found in the length of the deltopectoral crest (measurement DPCL; Table 4) (Fig 7). The relative attachment area of the forearm flexors, supinators, and pronators (index HEI; Table 2) increased significantly ( $p = 6.99 * 10^{-3}$ ) from fossil to extant weasels (Fig 6). Marginally significant differences ( $p = .066$ ) were found in the mechanical advantage of the gluteal muscles (index GI; Table 2), with extant weasels displaying higher mechanical advantage in the retraction of the femur (Fig 6). Though statistically significant differences were not observed between fossil and extant in the length of the tibia (measurement TL, Table 4;  $p = .825$ ) and the mediolateral diameter of the tibia (measurement TMLD, Table 4;  $p = .370$ ), visual inspection of their respective box plots suggests that extant Los Angeles weasels may have elongated and less robust tibiae (Fig 7).

### 3.3.2 Geometric Morphometrics

The smaller sample sizes in the geometric morphometric analysis necessitated restricting the comparison between extant and pooled RLB fossil specimens. To complement the linear morphometric analysis in tracking change in the LA weasel population through time, only extant specimens from LA County were used in this analysis.

Figure 8 shows the principal-component morphospace comprising the extant and fossil skulls, and a separation between fossil and extant specimens is apparent. Grid deformation plots along PC1 and PC2 show that specimens with higher scores on PC1 have anteroposteriorly shorter and mediolaterally wider M1s, mediolaterally wider carnassials, and a broader muzzle (Fig 9). Individuals with higher scores on PC2 have P3s that are angled more obliquely with respect to the anteroposterior axis (Fig 9). Extant specimens tend to group more positively for PC1 and more negatively for PC2 compared to the fossil specimens. Marginally significant differences were found between the fossil and extant groups (pairwise morphological disparity test  $p = .088$ ), persisting when allometric effects were considered (pairwise morphological disparity test  $p = .051$ ). Altogether, these results illustrate that extant specimens have shorter and wider M1s, wider carnassials, a broader muzzle, and more obliquely angled P3s than the fossils.

Figure 10 shows the principal-component morphospace comprising the extant and fossil dentaries. Again, a separation between fossil and extant specimens is apparent, though the groups are not as distinct as for the skulls. Grid deformation plots along PC1 and PC2 show that specimens with higher scores on PC1 have a coronoid process that is sloped more gradually on its anterior ascent and more steeply on its posterior descent, along with a masseteric fossa that extends more anteriorly (Fig 11). Specimens with higher scores on PC2 exhibit greater jaw depth along the tooth row (Fig 11). Extant specimens may tend to group more positively on PC1 and PC2 than the fossil specimens (Fig 10), appearing to possess less robust jaws, a flattened coronoid process, and a larger masseteric fossa. However, statistically significant differences were not found between the fossil and extant groups ( $p = .377$ ), and the lack of significant differences persisted when allometric effects were considered ( $p = .919$ ).

### **3.4 Space For Time Substitution:**

Pre-LGM LA *M. frenata* displayed significantly lower grinding area on m1 than weasels from most extant counties, including Los Angeles County (Fig 13).

Modoc County *M. frenata* consistently differed from other extant CA and NV counties and the fossil RLB time intervals in the measurements and indices tested. Post-hoc tests revealed Modoc weasels to be significantly smaller than weasels from all RLB time intervals and all extant counties from which sample size was high enough to obtain a p-value (Fig 12). Accordingly, linear measurements of the dentition were consistently lower in Modoc County weasels than in other extant counties and RLB time intervals. Though the sample size for extant Ventura County weasels was only 2, Ventura weasels displayed consistently higher relative

lengths of the trigonid blade on m1 and a greater lower grinding area than other extant CA and NV counties.

#### **4. DISCUSSION**

##### **4.1 Fossil Identification:**

There will always be the possibility that some or all of our fossil *M. frenata* are actually extant *M. frenata*, individuals who wandered into the seeps and died since the tar seeps resurfaced in the last ~100 years. Work by Brown et al. (2017) (42) has shown that the asphalt seep environment can cause carcasses to decay at a faster rate than normal; after 40 days of submersion ligaments were destroyed, and after 70 the bones were no longer in their relative anatomical position, suggesting that animals that fell into the seeps even within the last few years could be mistaken for fossils. Because ages of fossil specimens were inferred from fossils found in association with our specimens, we do not have direct dates to confirm or deny this phenomenon. Studies for the future plan to use newer, less destructive radiocarbon methods dating to get tighter and more confident age ranges on our fossil specimens.

##### **4.3 Tracking Change Through Time in Los Angeles:**

The lack of significant differences in body size across time in Los Angeles does not support the “top-down” mesopredator release hypothesis of change through time in LA *M. frenata*. One of the hallmarks of trophic ascension is an increase in body size, generally reflecting a shift to eating larger prey (10). Altogether, the range of median body masses in extant CA and NV counties is greater than the range of body masses of *M. frenata* through time in Los Angeles County. This relative stability in body size may suggest that competition-based interactions were not as primary a driver of body size variation in LA weasels through time as they are a driver of geographical body size variation in extant CA and NV weasels.

The patterns of variation observed in the craniodental measurements and indices in the Time Interval Analysis suggest that as the climate warmed from after the LGM to the Present, weasels began to exhibit a more omnivorous phenotype characterized by greater grinding ability. Every measure of grinding ability included in the Time Interval Analysis showed increased from after the PME to the present (Fig 5). This may reflect an increasing reliance on plant material to supplement the diet in response to ecological disturbance. Though long-tailed weasels are known to be obligate carnivores (43), omnivory in varying degrees is exhibited in the genus *Mustela* and their close relatives (44,45). The observed increase in grinding ability may indicate that weasels in the Los Angeles area began to supplement their diets with plant matter in response to an extreme ecological disturbance (the PME) and continued this trend to the present. Other mesocarnivore taxa from RLB have demonstrated similarly increased consumption of plant matter after PME (22,46).

The factors that most greatly limited the informativeness of the Time Interval Analysis were small sample sizes and a lack of direct dates. More often for the postcranial

measurements/indices and more often for the earlier Time Intervals (PreLGM and PostLGMpreExt), sample sizes of Time Intervals were too small to be maximally informative, sometimes consisting of 2 or fewer specimens. Additionally, the lack of direct dates on our fossil specimens makes continuous trends hard to observe. As more fossil specimens are added from ongoing excavations at RLB and direct dates are calculated on fossil specimens using less destructive dating methods, the informativeness of the Time Interval Analysis will increase and our picture of trends in the ecomorphology of LA weasels from preLGM to now will be more complete.

Overall, the results of the geometric morphometric analysis support the conclusion that Los Angeles weasels have been evolving a more omnivorous phenotype since the LGM. Extant weasels tended to have shorter and wider M1s (maximizing grinding surface area while also increasing robusticity compared to a longer, thinner tooth) and wider carnassials (possibly reflecting an expansion of the grinding surface of the carnassial) (Fig 8). Extant weasels may also have wider muzzles than they did in the past, suggesting a heavier reliance on rabbits and other prey larger than themselves. Extant Los Angeles weasels may have had larger masseteric fossae (indicative of higher grinding ability), along with less robust jaws and less pronounced coronoid processes (indicative of reduced mechanical advantage of the temporalis) (Fig 9). The lack of statistical significance between the fossil and extant dentary groups may be a result of small sample sizes for both fossil and extant dentaries. As more fossil specimens are uncovered from the Project 23 deposits at RLB, the geometric morphometric analysis can begin to split the data into the same Time Intervals that were used in the linear morphometric analysis.

Changes in the postcranial skeleton of Los Angeles weasels through time may represent responses to changing climate and habitat in the region. Research on martens (genus *Martes*) -a primarily arboreal mustelid mesocarnivore of similar size to *Mustela spp.*- has shown that individuals from areas with less dense undergrowth have more robust limbs and epiphyses, reflecting a decrease in arboreal tendencies as the ground becomes less obstructed (13). Observed patterns of morphological change in the postcranial skeleton (i.e. less robust limbs and higher mechanical advantage of proximal limb muscles) may reflect an increase in cursorial tendencies (47). Insect habitat and climate proxies show a trend of increasing temperature and aridity at RLB beginning after the LGM, and habitat in the area was consistently chaparral and grassland (30). Though there was a lack of Southern California woodland insect fauna in the fossil record at RLB, the decreasing ground vegetation cover after the LGM may have resulted in weasels exhibiting a more cursorial phenotype, similar to the behavioral mechanism by which ground vegetation cover influences the limb morphology of *Martes spp.* (13).

Weasel postcranial morphology may also have been influenced by small herbivore community dynamics after the LGM. The absence of large grazers may have led to increased abundance of lagomorphs, which can be up to 25% of the diet of weasels (18). Weasels have been shown to consume more lagomorphs in response to increased lagomorph abundance (18), and they are known to switch to larger prey when abundances of small rodents fall too low (19).



Though direct work has not been done on baseline community shifts across the LGM and the PME at RLB, recent work has documented increasing relative abundance of *Sylvilagus sp.* and decreasing relative abundance of small rodents of genera *Neotoma* and *Microtus* at RLB from 50-35 kya (48), and this trend may have continued through the end of the Pleistocene. An increase in lagomorph abundance may also explain the observed widening of the muzzle and higher bite force and the observed increase in shoulder and gluteal muscle mass in extant LA weasels. Rabbits are much larger than weasels (up to 10x their size), and weasels subdue them by wrapping their fore and hind limbs around the rabbit from the back to pin it down. Then, they clamp on to the back of the neck with the mouth and hold tightly until the rabbit is dead (19). A wider muzzle, higher bite force, and higher mechanical advantage of the proximal limb muscles would likely confer an advantage in performing this activity.

Altogether, morphological change in Los Angeles weasels from after the LGM to now was driven primarily by climatic factors and low-level trophic interactions. A greater capacity for omnivory in the crania of extant LA weasels may reflect instability in the food supply of meat from year to year following the post-LGM climate warming and later the PME. Other cranial and postcranial skeletal changes in LA weasels may reflect changing climate and habitat and shifting abundances of small herbivores. Museum collections -both fossil and extant- tend to be biased towards cranial elements relative to postcranial elements, partially due to taphonomy and partially due to field collecting bias. Small sample sizes of both fossil and extant postcranial elements forced us to restrict analysis of postcranial elements to a pure comparison between fossil and modern specimens, reducing our ability to gather information on continuous trends in postcranial ecomorphological change in LA weasels from the LGM to now.

#### **4.4 Space for Time Analysis:**

The observation that pre-LGM *M. frenata* at RLB had significantly lower grinding area on m1 than most sampled counties (including LA County), may suggest that the environmental pressures present at RLB after the LGM that caused LA weasels to begin exhibiting a greater capacity for omnivory were shared throughout the American Southwest. Pollen records from Lake Elsinore in Riverside County, CA -one of the counties sampled for our analysis- show similarity to RLB in post-LGM climate trends. Though Lake Elsinore shows a period of extreme aridity during the LGM followed by more mesic conditions that was not detected in more coastal environments, *Quercus* woodland and chaparral were consistently present at Lake Elsinore after the LGM, and aridity gradually increased after the Younger Dryas, similar to RLB at the time. Similarly significant differences between pre-LGM RLB weasels and extant CA and NV weasels were not found for measures of grinding ability other than the relative grinding area on m1, making conclusions hard to draw. Space for Time Analysis was only performed on craniodental measurements and indices due to sample size of fossil and extant postcranial specimens, further reducing our ability to conclusively generalize or degeneralize the environmental selection pressures present at RLB after the LGM to the rest of the American Southwest.

#### 4.5 Conclusions and Future Directions:

Overall, weasel response to the ecological disturbances caused by the interconnected post-LGM climatic warming and the PME was likely driven primarily by abiotic factors in the ecosystem and bottom-up trophic interactions. Evidence of top-down or intra-guild trophic effects on weasel ecomorphology was not clearly present, contrary to expectations. Weasels do not clearly demonstrate trophic ascension by way of the mesopredator release hypothesis (there were no changes in body size from past to present). Changes in skeletal morphology through time were small compared to the range of variation between extant CA and NV counties, potentially suggesting that competition-driven character displacement was not a primary driver of morphological change in Los Angeles weasels in the late Pleistocene and early Holocene. The continuous increase in grinding ability through time may reflect an increasing reliance on plant material as rising temperatures and -eventually- the PME created ecological instability. Weasel postcranial morphological variation from before the PME to the present most directly reflects climatic and habitat-based patterns of Mustelid morphological variation. An increased reliance on lagomorph prey is supported by analysis of both cranial and postcranial anatomy. This variation may represent phenotypic plasticity across temporal species ranges or adaptation of populations to changing environmental and trophic selective pressures. Los Angeles weasels differed from other mesopredators in their pattern of response to the end-Pleistocene climatic warming and megafaunal extinctions (22,46), demonstrating that the effects of these disturbances were not uniform across surviving taxa. It is still unclear whether the selection pressures acting on Los Angeles *M. frenata* during the end-Pleistocene were acting on *M. frenata* throughout the American Southwest. As sample sizes are increased for both fossil and extant specimens -especially in the postcranial skeleton-, direct dates are produced, and stable isotope and other analyses are incorporated, our picture of Los Angeles weasel response to end-Pleistocene environmental and ecological disturbance will become clearer, and our understanding of how this response reflects environmental and ecological change throughout the American Southwest during the end-Pleistocene will begin to take more shape.