
Archaeological Collagen Fingerprinting in the Neotropics; Protein Survival in 6000 Year Old Dwarf Deer Remains from Pedro González Island, Pearl Islands, Panama

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10.1 Introduction

Within the Neotropics ecozone, the tropical ecoregions of Central America host a unique collection of biodiversity that has been impacted greatly by human settlement. Due to major recent losses of diversity, studies that solely rely on the use of extant taxa provide a limited view of species change through human activity. Archaeological faunal remains can yield insights into this process of human-animal interactions in prehistory, but in many humid tropical regions these biological remains degrade relatively quickly rendering those that do survive more significant. This study looks at a Late Preceramic archaeological site occupied between 6190 and 5550 calibrated years before present (cal BP) (Table 10.1). This site is located on Pedro González Island, 8 km North of San José Island (Cooke and Jiménez 2009), Pearl Islands, Panama (Fig. 10.1), where the average temperature is $>30^{\circ}\text{C}$ throughout the year. Osseous remains were found during fieldwork undertaken between 2008 and 2010 at the pre-Columbian site of Playa Don Bernardo (hereafter

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M. Mondini et al. (eds.), *Zooarchaeology in the Neotropics*,

DOI 10.1007/978-3-319-57328-1_10

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Table 10.1 Radiocarbon dates from the Preceramic shell bearing midden at Playa Don Bernardo, Pedro González Island, Pearl Island archipelago, Panama

Cut	Level (10 cm)	Stratum	Macrostratum	Material	Laboratory number	Radiocarbon age BP	$\delta^{13}C$	Conventional age BP	Conventional age BC	Calibrated age (2 σ)	Calibrated age (1 σ)	Intercept
L-19	5		1	Marine bivalve (<i>Argopecten circularis</i>)	β -256752	4860 \pm 50	-0.6	5260 \pm 50	3310	5720–5560 ^a	5650–5580	5600
L-19	8		1	Carbonized palm fruit	β -256751	4900 \pm 40	-26	4880 \pm 40	2930	5660–5880	5640–5590	5600
L-19	12		1	Marine bivalve (<i>Argopecten circularis</i>)	β -243898	4980 \pm 40	0	5390 \pm 40	3440	5870–5650 ^a	5870–5650	5740
L-20	13		2	Charred plant matter	β -261219	5240 \pm 50	-29	5170 \pm 50	3220	6000–5890 and 5810–5760	5980–5970 and 5940–5900	5920
L-20	19		3	Charred plant matter	β -261218	5140 \pm 40	-26	5120 \pm 40	3170	5840–5750 and 5840–5750	5920–5890 and 5800–5770	5900
L-20	26		3	Charred plant matter	β -261217	5150 \pm 40	-27	5120 \pm 40	3170	5930–5850 and 5840–5750	5920–5890 and 5800–5770	5900
B*17	39	7	3	Charred dolphin (<i>Delphinus</i>) bone	β -304632	5350 \pm 40	-14	5540 \pm 40	3590	5990–5870 ^a	5950–5890	5910
B*17	41	7	3	Charred plant matter	β -278902	5330 \pm 40	-28	5280 \pm 40	3330	6190–5930	6180–6150 and 6120–5990	6000

Dates and calibrations (INTEL-04) by Beta Analytic Inc.

^aMarine calibration used

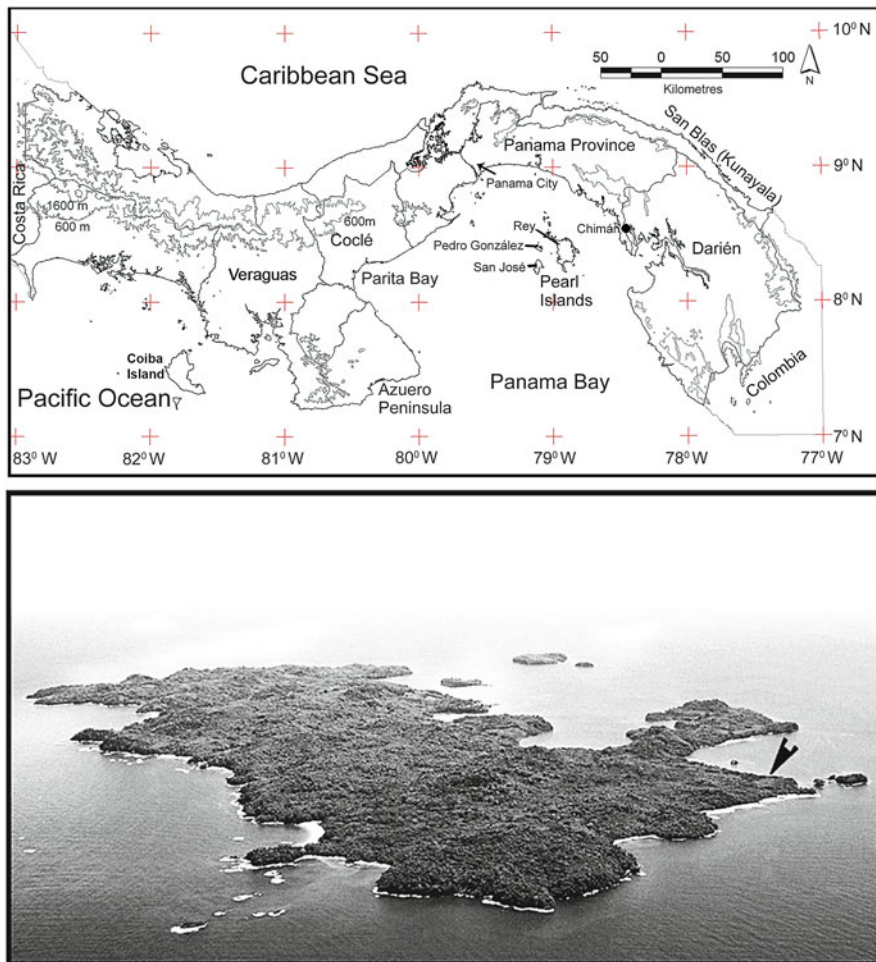


Fig. 10.1 Map of the Pearl Islands (*top*); Location of the Playa Don Bernardo (PDB) on Pedro González Island (*bottom, black arrow*; Photo by S. Redwood)

referred to as ‘PDB’), at which the remains of a very small cervid were the most abundant in the terrestrial vertebrate sample (Martínez-Polanco et al. 2015).

In the PDB midden, 2502 bone and antler specimens were assigned to Cervidae with a minimum number of individuals (MNI) of 22 (sum of MNI for each of three strata identified in each of three test pits) (Martínez-Polanco et al. 2015). The very high specimen/individual ratio across the site (114:1) is likely to be due, firstly, to the small size of the excavated test cuts, none of which uncovered complete cultural deposits, and, secondly, to the extreme fragmentation of the bones, which were heavily transformed by the actions of the pre-Columbian inhabitants while preparing the carcasses for food and tools (Fig. 10.2). A femur (TL: 132 mm) and a tibia



Fig. 10.2 Two deer antlers modified for use as tools, Playa Don Bernardo, Isla Pedro Gonzalez, Pearl Islands. *Top row*: Corte 1, Macrostratum IB (STRI cat.: 10–1979); *bottom row*: Corte 1, Macrostratum II (STRI cat.: 11–820)

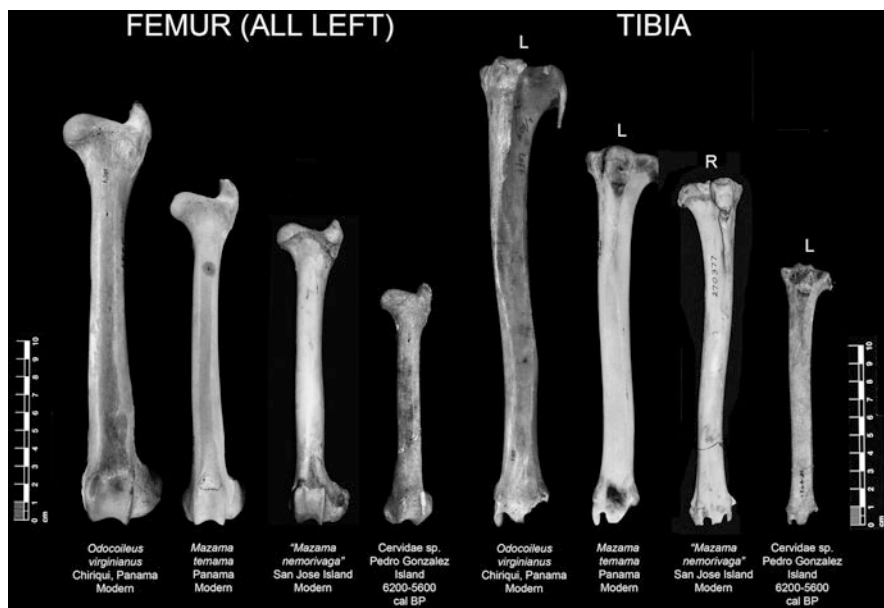


Fig. 10.3 Intact adult femur and tibia from the 6000 cal BP cervid population on Pedro González Island compared with those of modern white-tailed deer, Central American red brocket and a brocket species from San José Island, attributed to *Mazama nemorivaga*

(TL: 143 mm), both adult, are the only long bones that were recovered intact (Fig. 10.3).

10.1.1 Identifying the Source Species

Only three deer species currently occur in Central America outside Mexico. Two are still widespread in appropriate habitats where human population is low or hunting curtailed: the Central American red brocket (*Mazama temama* Kerr 1792) and the white-tailed deer (*Odocoileus virginianus* Zimmerman 1780) (Groves and Grubb 2011). The third extant species is restricted to San José Island in the Pearl Island archipelago in Panama Bay (Pacific) (Fig. 10.1). This small deer was first observed by biologists in the 1940s; Kellogg (1946) assigned it to *Mazama permira*, whereas Handley (1966) treated it as a subspecies of the South American gray brocket (*M. gouazoubira* Fischer 1814), a designation that has been followed by others (e.g., Wilson and Reeder 2005). However, Groves and Grubb (2011) retain the species name *Mazama permira*, in spite of their acknowledged unfamiliarity with specimens. In accordance with more recent phylogeographic data, Rossi et al. (2010) intuitively referred the San José Island population to the Amazonian brown brocket (*Mazama nemorivaga* Cuvier 1817), the remainder of whose distribution lies in northwest South America (Black-Decima et al. 2010). The Amazonian

brown brocket and the Amazonian gray brocket are considered by some to be parapatric species (Medellín et al. 1998; Rossi and Duarte 2008). White-tailed deer were introduced to Contadora and San José islands in the Pearl Island archipelago from the mainland after 1970, and thrive there under protection although it is not known how they interact with the native brocket deer on San José Island.

10.1.2 Archaeological Deer Bone Identification

Cervid archaeofaunal samples from pre-Columbian sites on the Pacific side of the Panamanian mainland, which span the period 8000–500 cal BP, appear to contain only white-tailed deer remains (Cooke et al. 2007, 2008). Previously, bone and tooth size rather than morphology have been used to distinguish between *O. virginianus* and *M. "americana"* (now *M. temama* Kerr 1792) at the only pre-Columbian site in Panama at which both taxa have been formally reported (Cerro Brujo, western Caribbean, 1350–1050 cal BP) (Linares and White 1980). Conversely, the presence of *M. "americana"* was rejected by Cooke and Jiménez in samples from several Panamanian pre-Columbian archaeological sites located in ancient wooded savannas and dry littoral zones of the Pacific watershed, in which deer bones were frequent to abundant. This was because none of the adult deer specimens fell within the size ranges of modern Central American red brocket deer (Cooke et al. 2007, 2008). Nor were the typical *Mazama* spike antlers retrieved. However, the tiny size of the PDB Preceramic cervid invalidates a size-based division between *Odocoileus* and *Mazama*, while the demonstrated polyphyly of genus *Mazama* (Duarte et al. 2008) and the need for its re-definition confound the issue even more.

10.1.3 Pedro González Island Archaeological Deer

The most logical inference under the current situation is that the tiny deer encountered by ostensibly the first human seafarers who arrived on Pedro González Island in the Pearl Island Archipelago of Panama Bay ca 6000 cal BP should represent the same phylogenetic lineage as that of the extant population on nearby San José Island, which is now arguably assumed to be an isolated population of the Amazonian brown brocket deer (*Mazama nemorivaga*) (Rossi and Duarte 2008; contra Reid) although being a dwarfed form of the white tailed deer cannot be ruled out due to on-going uncertainties concerning cervid inter-species relationships, which will only be resolved with additional genomic data. However, the extreme anthropogenic modification of the cervid bone sample in the PDB midden, as well as the unusually small size of the deer that produced the specimens, compromised the objective assignation of the remains below the Family level. For this reason, the taxon represented in the archaeological remains was originally reported as an unknown genus and species in Cervidae (Cooke and Jiménez 2009). Specimens of cervids and other mammalian taxa from PDB were evaluated for isotopic analysis

in 2012 (Ugan personal communication) but found poor collagen preservation (i.e., <1% yield), lowering the likelihood that quality ancient DNA (aDNA) could be extracted for analysis (Sosa et al. 2013); attempts at aDNA extraction have not proved successful. Therefore, in order to improve understanding of the Pedro González Island sub-fossil deer, we have resorted to protein-based sequence information since it, despite providing a more highly conserved source of molecular sequence information, is more likely to be available in this archaeological material.

10.2 Experimental

10.2.1 Materials for Collagen Analyses

Modern specimens of Central American red brocket deer (*M. temama*) from Panama and white-tailed deer (*O. virginianus*) from Panama and Illinois, USA, were obtained from the Smithsonian Tropical Institute's zooarchaeology skeleton reference collection. Modern specimens of deer identified by the collectors as Amazonian brown brocket (*M. gouazoubira*) were obtained from the von Humboldt Institute, Colombia but it should be noted that these identifications precede the split between *M. gouazoubira* and *M. nemorivaga* (Black-Decima et al. 2010; Rossi et al. 2010). Given their geographic distributions all of these individuals are likely to be the latter. Archaeological specimens for collagen fingerprint analysis from Panama comprised 22 deer bones (Cervidae) from the Preceramic midden at Playa Don Bernardo (PDB).

10.2.2 Morphological Analyses

All measurements taken on deer bones followed (von den Driesch 1976). They were compared with corresponding measurements from (1) the modern skeletons of *O. virginianus* and *M. temama*, (2) two individuals of the San José island *Mazama*, and (3) several bones of white-tailed deer from two Preceramic sites in Central Panama: Cerro Mangote (8000–5000 cal BP) and Zapotal (4300–3300 cal BP). Live weights were estimated on the basis of ten astragali following Purdue (1987) and the breadth of ten femur heads following Reitz and Wing (1999).

10.2.3 Collagen Extraction and Digestion

From each of the specimens sampled, small bone chips (~10 mg) were taken and collagen extracted and analysed following Buckley et al. (2011). In brief, samples were demineralised prior to collagen extraction using 1 mL 0.5 M HCl, overnight at 4 °C. The samples were centrifuged (13,000 × g, 5 min) and the supernatant was discarded. The remaining acid-insoluble pellet from each sample was resuspended

using 500 μL of 50 mM ABC (pH 7.4) and gelatinised at 70 °C for 3 h. Subsequent to gelatinisation, the sample was centrifuged (13,000 \times g, 15 min), precipitating the ungelatinised protein from the supernatant. The supernatant was then removed for tryptic digestion where 2 μL of 1 $\mu\text{g}/\mu\text{L}$ sequencing-grade trypsin solution was added to the supernatant and incubated at 37 °C for 18 h.

To produce peptide mass fingerprints (PMFs), C18 pipette tips were used to purify (desalt and concentrate the peptides) and fractionate the generated peptide mixture. The pipette tips were firstly equilibrated for sample binding, washing, and elution. This was done with two bed volumes of 50% ACN/0.1% TFA, followed by two bed volumes (100 μL) of 0.1% TFA. Post digestion, the samples were centrifuged (13,000 \times g, 15 min) and acidified to 0.1% TFA. The samples were then loaded onto the activated C18 pipette tips by aspirating and dispensing with 10 cycles. The pipette tips were then washed twice with 100 μL 0.1% TFA and a stepped gradient of increasing ACN concentration was applied to the tips to fractionate and elute the peptides (100 μL 10% ACN and 50% ACN with 0.1% TFA). The eluent was aspirated and dispensed ten times, dried using a centrifugal evaporator and re-suspended with 10 μL 0.1% TFA.

10.2.4 MALDI-ToF Mass Spectrometric Analysis

1 μL of the sample solution was spotted onto a Bruker ultraflex 384 target plate, mixed together with 1 μL of α -cyano-4-hydroxycinnamic acid matrix solution (1% in ACN/H₂O 1:1 v/v) and dried to air. Each of the collagen digest fractions were analysed by Matrix Assisted Laser Desorption Ionization Time of Flight Mass Spectrometry (MALDI-ToF-MS) in reflectron mode using a Bruker ultraflex II MALDI ToF/ToF mass spectrometer equipped with a Nd:YAG smart beam laser. MALDI-ToF peptide mass fingerprints were acquired over a mass range of m/z 700–3700 using 1000 laser acquisitions. Final mass spectra were externally calibrated against an adjacent spot containing five calibrant peptides. To confirm the homology of peptides between different species, tandem MS (MS/MS) was carried out on selected peptide markers (precursor ion selected with 500 laser acquisitions, up to 4500 laser acquisitions were used for the fragment ions, and argon was used as the collision gas).

10.3 Results

10.3.1 Measurements and Estimated Body Mass

The live weights of the PDB deer were estimated to be 5.77 ± 1.1 kg (range: 3.5–7 kg) and 7.7 ± 0.9 kg (range 6.9–9.4 kg) (Table 10.2). Epiphyseal fusion and tooth eruption sequences show that the PDB sample includes juveniles, sub-adults, adults and mature adults (>5 years). The variations in estimated body mass should therefore reflect age and sex differences among individuals.

Table 10.2 Body mass estimates for the ~6000 cal BP deer on Pedro González Island based on the breadth of the femur head and area of the astragalus

Skeletal element	Reference	N	Mean (kg)	SD	Min.	Max.
Femur	Reitz and Wing (1999)	8	7.70	0.86	6.85	9.38
Astragalus	Purdue (1987)	10	5.77	1.10	3.50	7.00

The above estimates align the size of this sub-fossil cervid with that of the two smallest deer in South America: northern pudu (*Pudu mephistophiles* de Winton 1896): 5–6 kg (Loyola et al. 2010), and Mérida brocket deer (*Mazama bricenii* Thomas 1908): 8–13 kg (Lizcano et al. 2010a). They are below the body mass range estimates for two other small brocket species: dwarf red brocket deer (*M. rufina* Pucheran 1951) (Lizcano et al. 2010b): 10–15 kg, and Brazilian dwarf red brocket deer (*M. nana* Hensel 1872): 10–15 kg (Veltrini-Abril et al. 2010). Three male specimens from a Brazilian population of the Amazonian brown brocket (*M. nemorivaga*) weighed between 14 and 15.5 kg (Viera-Rossi et al. 2010). Small populations in *Odocoileus virginianus* lineages are known on islands. They have estimated body masses above 20 kg, e.g., in the western Florida Keys (*O. virginianus clavium*): 20–35 kg (Miller et al. 2002); on Margarita Island, Venezuela (*O. v. margaritae* or *O. margaritae* (Osgood 1910; Molina and Molinari 1999; Molinari 2007)): 28–31 kg, and on Coiba Island, Panama (*O. v. rothschildi*) (Allen 1904), caveat Olson (2008): about 25 kg.

The neighbouring San José deer appear to be somewhat larger than the sub-fossil deer on Pedro González Island. One measure (total length of the tibia and femur) shows that these San José individuals are intermediate in size between Central American adult red brocket from Panama and the sub-fossil deer from Pedro González (Fig. 10.3, Table 10.3). On the other hand, body mass estimated from the femur head depth of specimen M-22177 was 10 kg whereas the astragalus area (Purdue 1987) of specimen M-27716 gave an estimated body mass of 9.2 kg. The same measures show a much lower body mass for the 6000 year Pedro González deer (5.8 ± 1.1 – 7.7 ± 0.9 kg) (Table 10.2). These size differences can be explained, firstly, by the fact that the land area of San José Island (44 km²) is three times that of Pedro González (14 km²) following Foster's rule and, secondly, by a plausible separation time of 9000–8300 uncalibrated radiocarbon years ago (Martín et al. 2016) between the two island deer populations. It is feasible also that the lack of appropriate mammalian and avian predators on the archipelago in Holocene pre-Columbian and modern times, e.g. felids, canids, mustelids and procyonids, also influenced size reduction in deer because of over-population and competition for restricted resources (Handley 1966; Wolverton et al. 2007; Angehr and Dean 2010; van der Geer et al. 2010; Martínez-Polanco et al. 2015). When deer become isolated on islands, changes occur in morphology, not only a gradual reduction in size, but also a tendency towards hypsodont teeth (high-cusped molars), more massive limbs, a reduction in the length of the autopodium relative to the body, and increases in the numbers of fusions in the foot bones (van der Geer et al. 2010). The PDB cervid samples were studied for these indicators and two cases of fusion

Table 10.3 Body mass estimates for the Pedro González sub-fossil deer

Taxon	Age	Locality	N	Mean	SD	Min.	Max.
Cervidae sp.	6200–5500 cal BP	Isla Pedro González	10	5.8	1.1	3.5	7.0
<i>O. virginianus</i>	8000–3000 cal BP	Central Pacific Panama	9	50.6	8.0	37	66.3
<i>O. virginianus</i>	Modern	Illinois and Chiriqui	2	40.0	4.1	36.9	45.1
<i>M. temama</i>	Modern	Central Panama	2	21.7	4.8	16.9	26.4
<i>M. nemorivaga</i>	Modern	Isla San José	1	9.2			

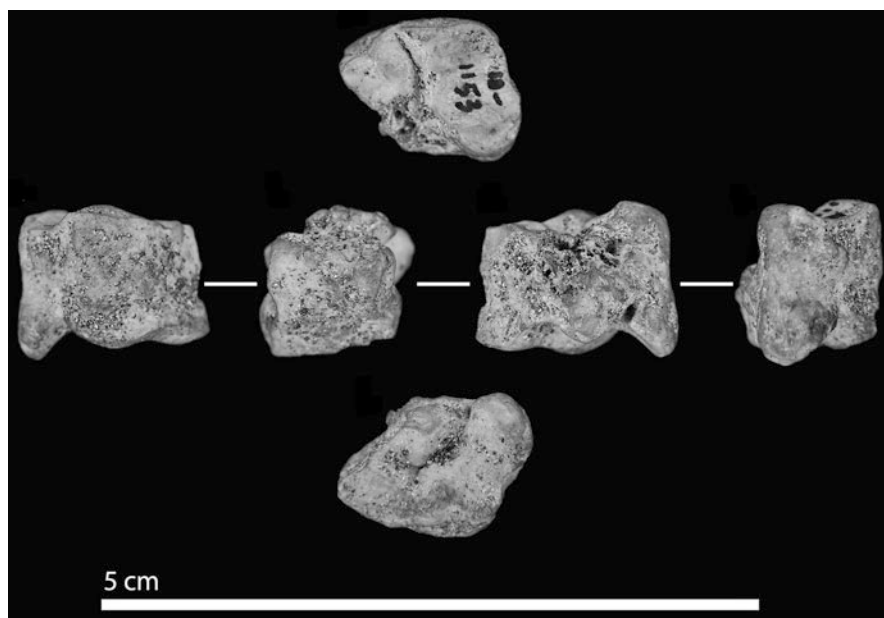


Fig. 10.4 Fused deer podials, Playa Don Bernardo, Isla Pedro González, Pearl Islands: right lunar and cuneiform from Corte 1, Macrostratum III (STRI cat.: 10-1153). *Centre left to right*: anterior, lateral, posterior and medial views with proximal view (*top*) and distal view (*bottom*). Photo by Raiza Segundo

in tarsals were observed: (1) a right cuboid fused with the internal cuneiform and (2) a lunar fused with the right cuneiform (Fig. 10.4).

10.3.2 Collagen Fingerprint Analyses

Collagen peptide fingerprints (e.g., Fig. 10.5) could be identified to separate most cervid taxa studied so far at the genus level (to the exception of *Dama* and *Cervus*) but could not be used to separate at the species level. Confounding this matter with

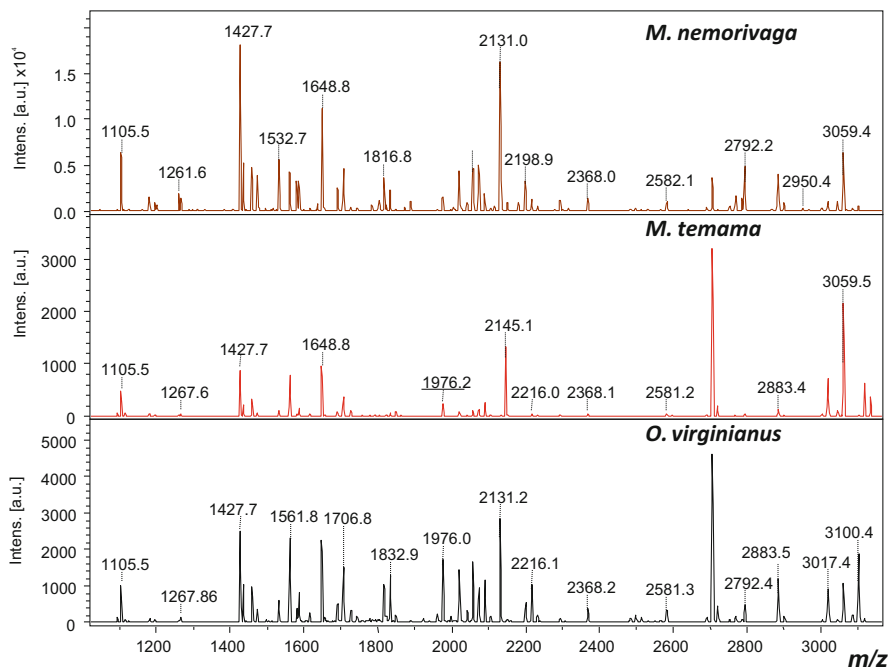


Fig. 10.5 MALDI-ToF-MS peptide mass fingerprints of collagen extracted from *Odocoileus virginianus*, *Mazama temama* and *M. nemorivaga* bone showing only 50% ACN fractions

the current taxa is the demonstrated polyphyly of genus *Mazama* (Duarte et al. 2008), where in this case the two *Mazama* species included in this study, *M. temama* from Panama had distinct markers from the species assumed intuitively by Duarte et al. (2008) to be *M. nemorivaga*, whereas the latter and *O. virginianus* surprisingly shared the same markers.

Collagen fingerprints from modern samples of *M. temama*, deer identified in collections in Argentina as *M. gouazoubira*¹ and *O. virginianus* (Fig. 10.5) revealed only one of the previously published peptide markers (Buckley et al. 2009; Buckley and Collins 2011; Buckley and Kansa 2011) as being variable: peptide D can be seen at m/z 2145.1 in red brocket deer (*M. temama*) but at m/z 2131 in white-tailed deer (*O. virginianus*) and the Amazonian brown brocket deer (*M. nemorivaga*¹; Fig. 10.5) and all other deer previously analysed (Buckley and Kansa 2011). No other homologous peptide markers could be readily identified that distinguish between these two groups. However, note that all of these South American deer appear to share a previously identified roe deer peptide marker (labelled G in

¹Note that our specimens were listed as *M. gouazoubira*, but sampled from a dataset in which these identifications pre-dated the taxonomic split between gray and brown brocket populations, hence given locality, supported by difference from Argentinian specimens, are assumed as *M. nemorivaga*.

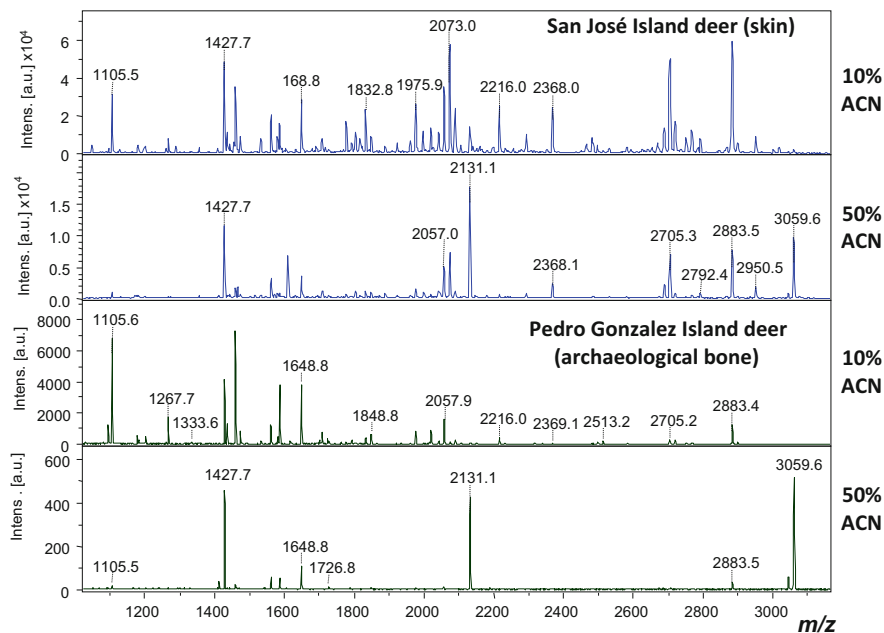


Fig. 10.6 MALDI-ToF-MS peptide mass fingerprints of collagen extracted from the modern San José Island deer skin and the archaeological Pedro González Island deer bone (showing both 10 and 50% ACN fractions)

Buckley et al. 2009) at m/z 3059.4. When compared to these three standards, all archaeological cervids from Playa Don Bernardo as well as the San José deer skin collagen, yielded identical collagen fingerprints with the Amazonian brown brocket and white-tailed deer (e.g., Fig. 10.6; peptide markers needed to separate them from other artiodactyls were C & G—see Buckley et al. 2009).

10.4 Discussion

Although the results of this work clearly indicate that the Pedro González Island deer most likely represent the same species as that present on the nearby San José Island, and that the Central American red brocket (*M. temama*) can be ruled out as an ancestral source, we cannot currently confirm on collagen fingerprints alone whether the ancestral source of these species the white-tailed deer (*O. virginianus*) or the Amazonian brown brocket (*M. nemorivaga*).

10.4.1 The Pearl Islands' Fauna

Although there are no native or introduced deer on Pedro González Island today, a proximal adult femur attributed to *O. virginianus* was found in a shallow (0.4 m) shell-bearing midden (PG-L-106), located on the same beach ridge as PDB (see location in Fig. 10.1) but associated stratigraphically with charred material dated to 1230 ± 40 BP (Martínez-Polanco et al. 2015). The femur was found to be similar in size to adult white-tailed deer femora from pre-Columbian middens in drier areas of Panama's Pacific lowlands and foothills (Cooke et al. 2007, 2008). This is the only cervid specimen that was identified in zooarchaeological samples from test excavations at ceramic sites on ten islands in the Pearl Island archipelago where human occupations between 2300 and 500 calibrated years ago were recorded (Martín et al. 2016). San José Island, the third largest in the Pearl Island archipelago (Panama Bay), is the only location where the Amazonian brown brocket (*M. nemorivaga*) has been reported in Central America. Since its discovery by biologists in the 1940s, this population has been moved from one taxon (*Mazama permira*) to another (*M. gouazoubira*) and yet another (*M. nemorivaga*), but only intuitively apparently following new data on speciation and geographic distribution among South American brocket deer (*Mazama*), but without elucidation of each name change and lacking consensus among specialists. It is apparent from Duarte et al.'s (2008) and Hassanin et al.'s (2012) revisions of South American deer phylogeny, that only molecular sequencing of the San José Island deer population in comparison to different mainland populations of the Central American red brocket (*M. temama*) can be used to resolve satisfactorily the phylogenetic history, not only of this small deer, ostensibly a relic species in disjunction with South American populations assumed to belong to the same lineage, but also of the extinct sub-fossil population on Pedro González Island, which we propose is an earlier form of the same taxon in spite of its separation from the San José Island population for 9000-8300 uncalibrated radiocarbon years (Martín et al. 2016).

10.4.2 Recent Molecular Phylogenies of *Mazama* and *Odocoileus*

However, the rapidly advancing molecular research into the phylogeny of New World deer has recently yielded conflicting results. Using cytochrome b sequence data, Pitra et al. (2004) showed white-tailed deer (*O. virginianus*) in a clade with North American mule deer (*O. hemionus*), and sister to *Mazama* sp., whereas Smith et al. (1986), using isozyme data, suggested that the red brocket (*M. americana*) was more closely related to South American forms of white-tailed deer (*O. virginianus*) than to the gray brocket (*M. gouazoubira*). A more recent analysis by Duarte et al. (2008) confirmed that the morphologically-cohesive genus *Mazama* does not form a monophyletic group, but represents separate radiations with high levels of molecular and cytogenetic divergence (Duarte and Merino 1997). Red and gray brockets form two clades with *Odocoileus* spp. nested between two red brocket sub-clades (Duarte et al. 2008). Hassanin et al. (2012) confirmed the existence of two clades

for brockets, one containing *M. gouazoubira* and *M. nemorivaga*, and the other, *M. americana* and *M. rufina*, which are themselves grouped together with *Odocoileus*. Several of these associations are in conflict with the results reported here.

Central American brockets were not included in these molecular phylogenetic studies. Since red brocket ("*M. americana*") karyotypes were demonstrated to be distinct between Central and South American populations, Central American red brockets were elevated to species level (*M. temama*) (Jorge and Benirschke 1977; Geist 1998), but they remain poorly studied. *Mazama temama* has not been given a Red List Category and Criterion by the IUCN due to defective knowledge about its distribution in relation to the re-defined South American red brocket (*M. americana*), whereas its persistence and continuity within its known range (Belize to the Panama/Colombia border) are not well documented (Bello et al. 2008). A popular Central American field guide emphasises the unsubstantial nature of the evidence for separating *M. temama* from *M. americana*, and retains the latter species name for *all* red brocket populations (Reid 2009).

10.4.3 Pedro González Island: Past and Present

Available data show that Playa Don Bernardo was abandoned half-way through the sixth millennium cal BP. On this and other islands in the Pearl Island archipelago, no evidence has yet been found for further human occupation until the middle of the third millennium cal BP. The Preceramic settlers of Pedro González hunted the small deer until they abandoned the site, but in diminishing numbers. No cervid bones of this size have been recorded in test excavations conducted at ten ceramic-using sites on five islands. Iguanas (*Iguana iguana*), cane rats (*Zygodontomys*), and spiny rat (*Proechimys*) are the most frequent terrestrial vertebrate taxa by rank order in a sample of only 147 taxonomically significant specimens (Cooke et al. 2016).

Whether it ultimately turns out to be an ultra-dwarfed white-tailed deer with a long history on the archipelago or more likely a lineage of brown brocket closest to *Mazama nemorivaga*, the preservation of the stocks of the San José island deer should be considered a conservation priority. Despite efforts by the owners of the island to protect the deer, poachers still enter clandestinely to hunt the deer and other mammals. The possibility of competition with introduced white-tailed deer and even hybridization is high. On the one hand, obtaining additional tissues for molecular sequencing would improve confidence in the deer's phylogenetic status. On the other hand, archaeological sites in whose middens lies a wealth of information about the Holocene distribution of deer and other vertebrates on the Pearl Island archipelago, are fast disappearing under the machinery of development projects.

10.4.4 The Pedro González Island Terrestrial Environment at 6.2–5.5 cal BP

No archaeobotanical data from sediment cores are available for reconstructing the vegetation on Pedro González Island or elsewhere in the Pearl Island archipelago at the time of first identifiable human occupation. The presence of ecologically healthy forested habitats during the initial part of the Preceramic occupation is strongly suggested by the high rank in the terrestrial vertebrate archaeofauna of green iguana (*Iguana iguana*) and agouti (*Dasyprocta*), as well as *Boa constrictor* and large (>1 m length) colubrid snakes, which, in the absence of mammalian carnivores, were the major predators on these taxa and continue to be so (Cooke et al. 2016). Six of the less frequent terrestrial vertebrate taxa in the PDB archaeofaunas also require heavily vegetated habitats although they are differentially tolerant of forest fragmentation and strong seasonal aridity: paca (*Cuniculus paca*), mud turtles (*Kinosternon leucostomum* and *K. scorpioides*), spiny rat (*Proechimys semispinosus*), rufous tree rat (*Diplomys labilis*) and a medium-sized monkey, probably a capuchin (*Cebus capucinus*). None of these six taxa are currently present on Pedro González Island whereas paca, monkeys and mud turtles have not been recorded anywhere on the Pearl Island archipelago. The presence of mud turtles, which were widely used for food by the pre-Columbian inhabitants of mainland Pacific central Panama (Cooke et al. 2007, 2008), suggests that island streams may have carried more water than today although *Kinosternon* spp. are known to aestivate in the moist mud of water-less streams (Morales-Verdeja and Vogt 1997; Berry and Iverson 2011).

Therefore the pre-human deer on Pedro González likely inhabited vegetation formations similar to the strongly seasonal ones that persist on the Pearl Islands. Small water courses may have contained more water than today. The Preceramic islanders were cultivating maize and other cultigens on the island (Cooke et al. 2016). Therefore they would have cut and burnt the vegetation to prepare fields. How extensive these activities were, however, and whether they would have enhanced the rapid decline of deer and other terrestrial vertebrates that is apparent in the archaeofaunal record by the end of the Preceramic occupation and thereafter, cannot yet be elucidated.

10.5 Conclusions and Future Research

The archaeofaunal deer remains from Playa Don Bernardo hold the key to achieving a far better understanding, not only of the relationship between a mid-Holocene and a modern form of deer possibly in the same lineage, but also of theoretical aspects concerning the effects of dwarfing and isolation on morphology and osteology in artiodactyls. Due to the low likelihood that aDNA extraction and sequencing from these archaeological remains would be feasible, we resorted to a relatively new technique of collagen fingerprinting to identify the pre-Columbian remains. Results indicated that all of the archaeological specimens sampled that yielded

collagen fingerprints do not derive from Panamanian *M. temama* (Central American brocket) but from a group of taxa that includes the white-tailed deer and distinctive Colombian populations of *Mazama* whose attribution to species remains in abeyance because of uncertainties regarding the genetics and distribution of *M. gouazoubira* and *M. nemorivaga*. Furthermore, analyses of collagen extracted from the San José deer also match the PDB fingerprint. Future work should try to improve the taxonomic resolution obtainable from protein sequencing methods, perhaps through the study of other non-collagenous proteins (Buckley and Wadsworth 2014) or potentially through screening archaeological samples for aDNA preservation via protein fingerprinting analyses.

Acknowledgements The Pearl Island project (2007–2010) was supported by funds from Panama’s Secretaría Nacional de Ciencia, Tecnología e Innovación (SENACYT), the National Geographic Society and the Smithsonian Tropical Research Institute’s education programs. Field logistics were coordinated by Reynaldo Tapia and Conrado Tapia. Juan J. Amado, then of the Pearl Island Development Company, and INGEMAR (Panama), generously provided funds for travel, subsistence, archaeology and student participation on Pedro González Island in 2009 and 2010. Test excavations at Playa Don Bernardo benefitted greatly from the assistance of Clara Arango, Eugenia Mellado, Marlene Klages, Yessi Ortiz, Ninel Pleitez, and Aureliano Valencia. Laboratory analyses by MB were supported by funding from the NERC and the Royal Society and carried out in The University of Manchester’s Faculty of Life Sciences Biomolecular Analysis Core Research Facility as well as the Manchester Institute of Biotechnology. Special thanks are due to several members of the Pedro González community who assisted in the excavations. This research would not have been possible without *Mazama* samples graciously provided by the Instituto de Investigación de Recursos Biológicos Alexander von Humboldt, Villa de Leyva, Colombia, thanks to the courtesy of Carlos Jaramillo and Claudia Alejandra Uribe Medina. Lastly, thanks are due also to Raiza and Roxana Segundo for assistance with images.

Author Contributions

MB designed and undertook the biochemical research. He planned and wrote the article with RGC. RGC designed and supervised the archaeological field work on the Pearl Island archipelago (2007–2010). FB and JGM directed excavations at the Playa Don Bernardo site and also analyzed material culture. AL participated in the Playa Don Bernardo excavations and assisted with the curation and preliminary analysis of the archaeofaunas. MJ undertook the initial analysis of all the vertebrate remains. MFM updated and expanded the analysis of the cervid remains and organized materials for photography.

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