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Ancient DNA of the Pacific rat (*Rattus exulans*) from Rapa Nui (Easter Island)

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Abstract

We report analysis of ancient mitochondrial DNA sequences from nine archaeological specimens (8 femura and 1 incissor) of *Rattus exulans* excavated from Anakena Beach Dune on Rapa Nui. Sequence of a 239-base-pair fragment of the hypervariable mitochondrial control region reveals a single mitochondrial DNA (mtDNA) sequence of all samples corresponding to the R9 haplotype prevalent in East Polynesia. This suggests a single or very limited introduction of *Rattus exulans* to the island. Rapa Nui, like other remote islands of Polynesia, remained effectively isolated following colonization.

Keywords: Ancient DNA; Island colonization; Polynesia; Rapa Nui; *Rattus exulans*

1. Introduction

Recent research now documents the relatively late colonization of Rapa Nui (Easter Island) at about AD 1200 [13]. This late colonization date accords well with results of recent field work [6], re-dating, and re-evaluation of East Polynesian chronologies (e.g., [1,3,4,23]). Given the forbidding distance and extreme windward position of the Rapa Nui, many scholars have posited that colonization occurred as a single [22], or limited number [8,9,16] of events originating in central eastern Polynesia. Proponents of South American [11] or other non-Polynesian [15] origins for Rapa Nui's early settlement have found little support.

The question of single, few, or multiple colonization events of Pacific Islands, particularly those most remote, is significant to evaluating alternative models for the region. Anderson [2:173] has re-evaluated assumptions drawn from modern experimental voyaging in Polynesia and shows that "colonization voyages were much more difficult and slower than envisioned by orthodox opinion." As a consequence, Anderson [2:173] argues, "colonization was coincident with discovery, and interaction between archipelagos was limited in extent." Such questioning of the neo-traditional perspective has implications for island chronologies and the potential for episodic discoveries and colonization that came with rapid bursts of dispersal in Polynesia. Anderson also suggests island colonization was propelled by rapid human population growth rates, opportunistic exploitation and quick depletion of faunal resources that drove migrations at low densities. Does the evidence for colonization and inter-island interactions for Rapa Nui fit the model Anderson [2] has outlined?

The number of colonization events and the degree to which Rapa Nui was in contact with its far-flung neighbors will be reflected in the DNA variation of its inhabitants. However, with the synchronic analysis of modern populations may come multiple interpretations of genetic data [5]. Analysis of ancient DNA from archaeological samples allows a temporal as well as spatial measure of variation. [12]. Using mitochondrial DNA (mtDNA) from archaeological human bone samples, Hagelberg et al. [10] was able to confirm the Polynesian origins of the Rapanui.

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Polynesian colonists routinely carried with them domestic animals—among them dog, pig, chicken, and rat—as elements of a portable economy. Thus analysis of these commensal animals' genetic variation acts as proxy for human variation and interaction history, while limiting the sometimes culturally sensitive and technical issues associated with ancient human DNA analysis. This commensal approach has been most successfully applied to the Pacific region with the Pacific rat (*Rattus exulans*) [17,19]. Chicken and rat are the only commensal animals known to have been brought to Rapa Nui by early colonists. *Rattus exulans* has been extirpated on Rapa Nui, but abundant remains are found archaeologically [14,16,24]. In this study, we analyze mtDNA from nine archaeological samples of Pacific rat excavated recently at the Anakena Beach Dune (Fig. 1).

2. Methods

The specimens of *Rattus exulans* we analyze and report in this paper come from collections made in the 2004 University of Hawai'i Rapa Nui field school excavations at the Anakena Beach Dune (Fig. 1). Details of these excavations will be published in a forthcoming paper [14], but here we provide information on their context and age.

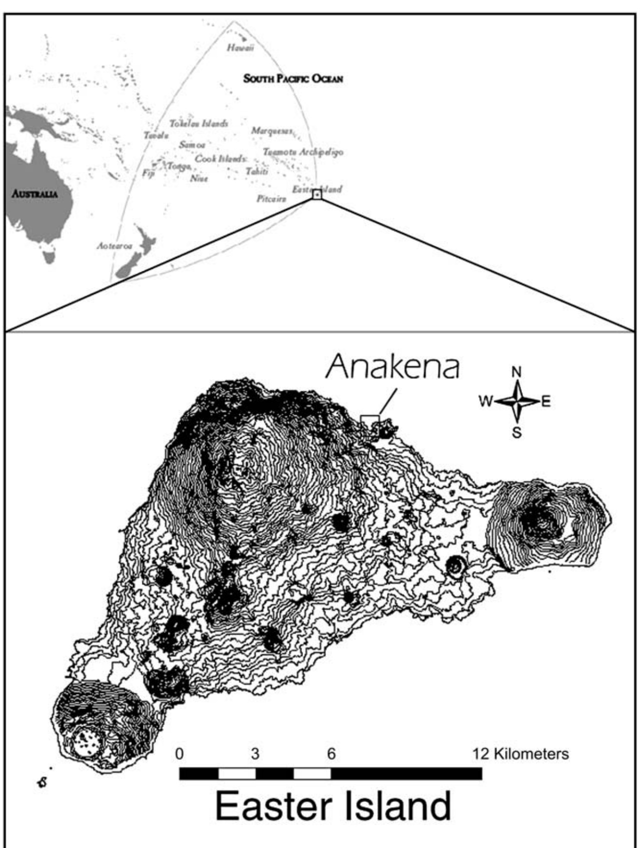

Fig. 1. Rapa Nui map showing Anakena.

In 2004 the University of Hawaii archaeological field school under the direction of Terry Hunt undertook excavations at Anakena. The goal was to acquire well preserved remains, including faunal materials, dating to the initial period of Polynesian settlement on Rapa Nui. An excavation unit of 4×2 m (long axis oriented 339°) was placed near the crest of the sand dune formation at ca. 25 m north of Ahu Nau Nau. This is an area near earlier (1991) excavations made by Steadman et al. [24]. This 8 m^2 area was designated "Anakena North Unit 1." Excavation proceeded by natural stratigraphic units with arbitrary levels dividing the strata for controlled collection of materials. All excavated sediment was sieved through 1/8-inch (3.18 mm) mesh. We recovered abundant well-preserved faunal remains (dominated by rat), artifacts, and other materials including wood charcoal in the deposits.

Excavations revealed a well-stratified sequence comprised of primarily calcareous sand deposited through aeolian action on the north facing beach dune (Fig. 2). The strata also included some terrigenous sediments deposited as colluvium (slope wash from the adjacent cinder cones of volcanic ash and developed soils). Layers I to V appear to be recent sand deposits separated by short periods of stability when thin organic horizons develop in situ (indicated by dark coloration). Layer V is aeolian sand, but has inter-bedded lenses of colluvium (poorly sorted clay with cobbles and boulders) from the slopes above. Layers VI to XI are comprised of primarily well-sorted aeolian sands. These layers are distinguished by thin horizons of organic material evident from dark colorations. The basal layer (XII) is an in situ weathered volcanic ash and primeval soil (paleosol). It has a clear and distinctive boundary with the sand layer above. Within 8–10 cm depth of Layer XI excavations revealed the tubular root molds associated with the extinct *Jubaea* palms (e.g., [21]). The upper boundary of Layer XI represents an ancient, stable surface with clear indications of soil formation and vegetation, including the giant, extinct *Jubaea* palm.

Fourteen archaeological samples (13 bone and 1 incisor tooth) (Fig. 3) were processed for DNA extraction at the University of Auckland ancient DNA facility using a modified silica/guanidinium thiocyanate technique as previously described [20]. Of these 14 samples, DNA was successfully extracted, amplified, and sequenced from nine on the first attempt (8 bones and 1 incisor tooth) (Table 1). Re-extraction was performed a second time on the five unsuccessfully extracted samples but still failed to yield quantifiable DNA. Sequence was obtained from the nine successfully amplified samples. This sequence consisted of a 239-base-pair fragment of the hypervariable mitochondrial control region from position 15355 to 15594 [7]. Primers used were: EGL-4L (5′ ccaccatcaacacc caaaag 3′) and EGL-7H (5′ tgataacacaggtatgtcc 3′).

3. Results

As shown by the radiocarbon chronology for Anakena North Unit 1 excavations, the deeper deposit appears to date to within a range of cal AD 1200–1400 (see Table 1). As

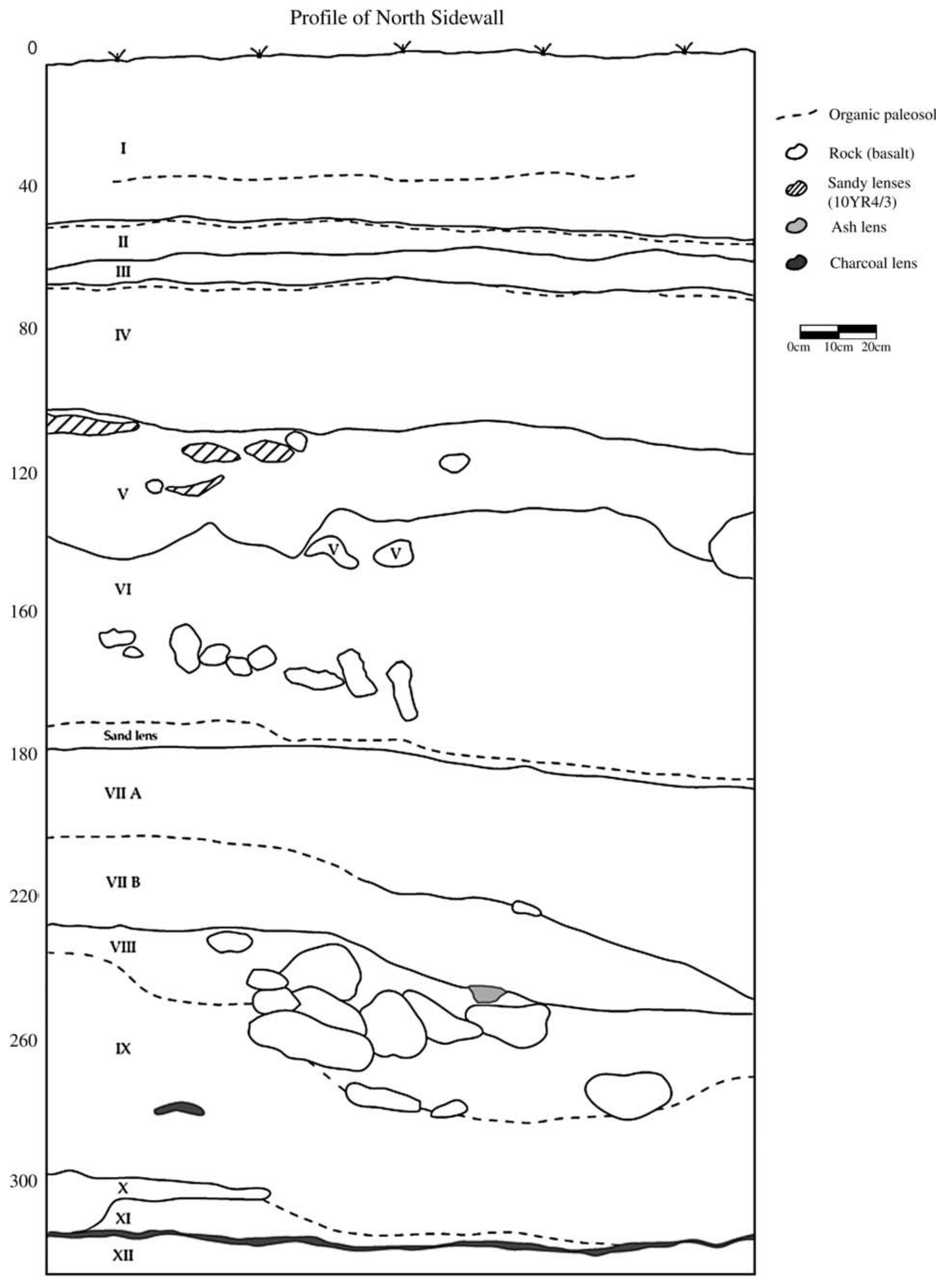

Fig. 2. Stratigraphy of Anakena North Unit I.

suggested by the stratigraphic features and composition, the deposits must have formed relatively quickly, as dates from the base (Layer XII) to Layer V overlap at one standard deviation. The actual duration represented in the lower, prehistoric deposits (Layers V–XII) is short, likely to fall within about 100 years. The evidence suggests that the lower layers at Anakena record the first colonization of Rapa Nui [13]. The most recent strata (I–III) appear to have formed recently and include historic and modern artifacts in their expected stratigraphic superposition.

The *Rattus exulans* specimens analyzed and reported in this paper come from a larger faunal assemblage of approximately 2383 Polynesian rat bones recovered in the 2004 excavations. The Polynesian rat ranks first (1) in abundance followed in rank order by (2) fish; (3) sea mammals; (4) bird; (5) medium mammal; (7) human; and (7) turtle. Counts and corresponding rank order are preliminary, as the analysis of faunal assemblages from the 2004 excavations is currently underway [14]. These rank order abundances are comparable to the early faunal assemblage reported from the Anakena Dune by Steadman et al. [24].

4. Conclusion and discussion

All *Rattus exulans* samples shown in Table 1 share an identical genetic sequence, which match the R9 haplotype

Fig. 3. Archaeological sample of *Rattus exulans* femur.

(Genbank accession number AY604228) described by Matisoo-Smith and Robins [17]. Matisoo-Smith and Robins [17] also found this R9 haplotype in all eight archaeological *Rattus exulans* samples analyzed from Te Niu on the west coast of Rapa Nui dated in the range of cal AD 1299–1443 [27]. These data reflect an island-wide coverage, as represented from northwest to northeast (at Anakena) and a temporal sequence from possible initial colonization in the 13th century AD to historic times.

Rattus exulans is not native to North or South America. The fact that *Rattus exulans* bone was found in what may be the earliest layers of human occupation strongly rejects any non-Polynesian origin for the inhabitants of Rapa Nui.

The absence of any variation in these Rapa Nui rat sequences suggests a single or limited introduction of the animal to the island and more extreme isolation than Matisoo-Smith et al. [18] have shown on the Chathams and Kermadecs, which both share an isolated geographic location yet show greater rat genetic variation. This extreme genetic isolation of Rapa Nui is greatly contrasted with the mtDNA record for larger, remote archipelagos such as Hawaii and New Zealand [19]. However, it our conclusions are based on rat, and not human DNA. Therefore, these data cannot rule out arrivals to Rapa Nui from colonists who did not bring Pacific rats with them. Nor can we rule out sustained migration to Rapa Nui by Polynesians bringing with them rats of a single genetic population.

The R9 haplotype is widespread throughout the Pacific, but is most concentrated in East Polynesia and notably is the only sequence identified in the archaeological samples ($n = 8$) from Mangareva Island in the Gambier Group. The R9 haplotype is one of five lineages identified in analyses of Marquesan rats [16]. While we cannot therefore identify a specific source population for the Rapa Nui rats, a single or at least a limited number of introductions to Rapa Nui of *Rattus exulans* from East Polynesia is consistent with multiple lines of evidence [9]. While long-distance inter-archipelagic interactions may have occurred with some regularity in other parts of remote Polynesia [18,26], based on the mtDNA evidence of the introduced Polynesian rat that we report here, Rapa Nui appears to have

Table 1
Rattus exulans haplotypes and details of their archaeological context at Anakena Dune, Rapa Nui

Layer	Sediment[a]	Color[b]	Notes	Radiocarbon age (cal. AD range, 2SD, 95% probability)[c]	*Rattus exulans* sample
I	Med. calc. sand	10 YR 7/3	Modern artifacts		
II	Med. calc. sand	10 YR 3/3	Recent historic artifacts		Left femur + left incisor
III	Med.-fine sand	10 YR 6/4	Recent historic artifacts		
IV	Med. calc. sand	10 YR 6/4	Prehistoric artifacts		
V	Clay-boulder coll. and med. calc. sand	10 YR 2/2–7.5 YR 4/6	Colluvial deposit ("mudslide"); prehistoric artifacts	Beta-196712: cal AD 1250–1410	2 right femura
VI	Med. calc. sand	10 YR 6/4	Structural features; prehistoric artifacts		
VII	Med. calc. sand	10 YR 76/4	Structural features; prehistoric artifacts; rapid deposition?		2 right femura
VIII	Sandy loam calc.	10 YR 3/4	Prehistoric artifacts	Beta-196713: cal AD 1270–1400; Beta-196711: cal AD 1280–1400	Right femur
IX	Med. calc. sand	10 YR 6/4	Prehistoric artifacts	Beta-196714: cal AD 1290–1430	
X	Med. calc. sand	7.5 YR 3/4	Prehistoric artifacts		Right femur
XI	Med. calc. sand	10 YR 6/4	Burn lens at base; prehistoric artifacts	Beta-196715: cal AD 1260–1430 and cal AD 1370–1380	
XII	Compact, weathered clay, in situ	10 YR 3/3	Prehistoric artifacts and charcoal upper 5 cm (approx); palm root molds below; primeval soil	Beta-196716: cal AD 1210–1320 and cal AD 1340–1390	2 right femura

[a] Med., medium; Calc., calcareous; Coll., colluvium.
[b] Moist Munsell soil color recorded in the field.
[c] Radiocarbon dates from wood charcoal; calibrations follow Stuiver et al. [25] (INTCAL 98 radiocarbon age calibration as provided by Beta Analytic). Fuller details of the radiocarbon dates and their implications for Rapa Nui chronology will be presented in a forthcoming paper [13].

remained relatively isolated following initial colonization. Such isolation has implications for the evolutionary history of one of Polynesia's most remote and remarkable islands.

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