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Genetic Variation in Archaeological *Rattus exulans* Remains from the Emily Bay Settlement Site, Norfolk Island

ELIZABETH MATISOO-SMITH[1], K. ANN HORSBURGH[1], JUDITH H. ROBINS[1] AND ATHOLL ANDERSON[2]

[1] Pacific Palaeoecological Research Laboratories, Centre for Archaeological Research, Department of Anthropology, University of Auckland, Private Bag 92019, Auckland, New Zealand
e.matisoo-smith@auckland.ac.nz

[2] Department of Archaeology & Natural History, Research School of Pacific and Asian Studies, Australian National University, Canberra ACT 0200, Australia
aja@coombs.anu.edu.au

ABSTRACT. Analyses of mitochondrial DNA variation in archaeological samples of *Rattus exulans* obtained during the 1997 excavations at Emily Bay, Norfolk Island suggest a high degree of variation in the prehistoric populations on the island. The ten samples sequenced produced five unique haplotypes. This result is consistent with a scenario of multiple introductions of the species to the island. There are clear affiliations with East Polynesian and New Zealand samples, however other lineages also appear to be present on Norfolk Island. Three haplotypes that had previously not been identified in tropical East Polynesia appear on Norfolk. One of these has also been identified in an archaeological sample from New Zealand. The other two haplotypes have yet to be identified elsewhere.

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It has been argued that patterns of genetic variation in Pacific populations of the Pacific rat, *Rattus exulans*, serve as a model for prehistoric human movement in the region. Specifically they have been valuable for identifying points of origin for voyages of exploration, colonization and later contact (Matisoo-Smith *et al*., 1998). More recently, analyses of degrees of genetic variation in archaeological and modern samples of *R. exulans* have been used to assess the degree of contact with and isolation of particular island groups within Polynesia (Matisoo-Smith *et al*., 1999). Both of these approaches are applied here to archaeological samples collected during the 1997 Emily Bay excavations on Norfolk Island (see Anderson, Smith and White, this vol.).

Materials and methods

A total of 33 *Rattus exulans* bone samples were provided for analysis. From this material, 13 samples were considered to be large enough and in good enough condition for ancient DNA extraction. We were able to obtain enough DNA from 10 of these samples to amplify, using the Polymerase Chain Reaction (PCR), and directly sequence a 175 base-pair (bp)

Table 1. Bone samples processed. MRL = mandibular alveolar length (MRL) as described in Matisoo-Smith and Allen (2001).

sample	trench	square, spit	skeletal element	weight (g)	MRL (mm) mandibles	maximum length femur (mm)	sequence obtained?
NIPP 574A	EB97:23	E6, spit3	mandible/L	0.09	6.63	—	N
NIPP 574B	EB97:23	E6, spit3	mandible/L	0.10	5.59	—	Y
NIPP 635A	EB97:23	F10, spit3	mandible/R	0.10	6.36	—	Y
NIPP 641	EB97:23	F10, spit7	mandible/L	0.10	6.56	—	Y
NIPP 740A	EB97:24	C3, spit3	mandible/L	0.13	5.89	—	Y
NIPP 575A	EB97:24	E6, spit4	femur/L	0.11	—	incomplete	Y
NIPP 635B	EB97:24	F10, spit3	femur/L	0.13	—	26.99	Y
NIPP 692	EB97:24	A2, spit1	femur/L	0.22	—	29.15	Y
NIPP 699	EB97:24	A4, spit2	femur/R	0.11	—	23.43	N
NIPP 740B	EB97:24	C3, spit3	femur/L	0.14	—	incomplete	Y
NIPP 739	EB97:24	C3, spit2	femur/R	0.12	—	incomplete	Y
NIPP 573	EB97:23	E6, spit2	femur/R	0.11	—	incomplete	N
NIPP 575B	EB97:23	E6, spit4	femur/L	0.13	—	24.37	Y

fragment of a hypervariable region of the mtDNA d-loop. The sample identification numbers, location, morphological measurements and other information are shown in Table 1. The Emily Bay settlement site has been interpreted as representing a single-phase occupation dating to the thirteenth to fifteenth centuries A.D. (see Anderson, Higham and Wallace, this vol.).

Extraction, amplification and sequencing methods are as described previously with all ancient DNA work carried out with strict precautions to avoid and identify contamination (Matisoo-Smith *et al*., 1999). PCR primers EGL 8 (L5'GGACATACCTGTGTTATCA 3') and EGL 9 (H5' CCCTGAAGTAAGAACCAGA 3') were used for amplification and sequencing, providing sequence data of approximately 175 base pairs for each sample (bases 15594–15765 in Gadaleta *et al*., 1989). Gene diversity (*h*) was calculated using Nei's (1987) equation, where x = haplotype frequency and n = number of haplotypes sampled:

$$h = (1 - \Sigma x^2)\, n / (n - 1)$$

All sequences were compared with those derived from *R. rattus*, *R. norvegicus* and *R. praetor* material and were confirmed as belonging to *R. exulans*. Distance analyses were performed using MEGA, version 1.01 (Kumar *et al*., 1993) and a phylogeny was constructed using the neighbor-joining method. One thousand bootstrap replicates were performed and values are shown on the phylogenetic tree in Fig. 1.

Results

Morphology and bone quality. In terms of maximum length (ML) for femora, and mandibular alveolar length (MRL), the material from Emily Bay overall appears relatively large, but fits well within the normal range for *Rattus exulans* from around the Pacific Islands (20.5–30.3 mm for ML and 4.79–7.3 mm for MRL). However, sample NIPP 692 approaches the maximum size recorded for archaeological *R. exulans* material (Matisoo-Smith and Allen, 2001). All material appeared to be well preserved, with most femora and mandibles weighing more than 0.1 g, as is typical of *R. exulans* material of good quality. None of the bones appeared to be burned or have any other distinguishing features.

Genetic variation. We had a particularly high success rate for amplification and sequencing of the material, with only three of 13 samples not providing DNA of sufficient quality for direct sequencing. From the 10 samples analysed, five unique sequences and six phylogenetically informative sites were identified, which produced a gene diversity value for Norfolk Island rats of 0.80. These five sequences were compared to all existing sequences for *Rattus exulans* (Matisoo-Smith *et al*., 1998 [GenBank accession numbers AF104120–104211] and unpublished data). The phylogenetically informative sites were identified and are shown

Table 2. Phylogenetically informative sites for 175 bp of *Rattus exulans* mtDNA sequence. Variable sites 1–6 refer to sites 255, 257, 272, 293, 317, and 332 in Matisoo-Smith (1996). East Polynesian consensus sequence is from Matisoo-Smith (1996). All NIPP samples are archaeological samples from Emily Bay, Norfolk Island; all AI samples are from archaeological sites in New Zealand (AI 536, 537, and 539 from the Washpool Midden site, and AI 552 from Paremata) and were provided by the Archaeozoology Laboratory, Museum of New Zealand. A, T, C, and G represent the bases adenine, thymine and cytosine and guanine—which make up DNA.

variable site	1	2	3	4	5	6
East Polynesian consensus	C	T	C	C	C	G
AI537	C	T	C	C	C	G
AI552	C	T	C	C	C	G
NIPP 574B	C	T	C	C	C	G
NIPP 641	C	T	C	C	C	G
NIPP 739	C	T	C	C	C	G
NIPP 740B	C	T	C	C	C	G
NIPP 575A	C	T	C	C	C	A
NIPP 575B	C	T	T	T	T	G
NIPP 635A	C	T	T	T	A	G
NIPP 635B	C	T	T	T	A	G
NIPP 692	C	T	T	T	A	G
NIPP 740A	A	C	T	T	A	G
AI536	A	C	T	T	A	G
AI539	C	T	T	C	A	G

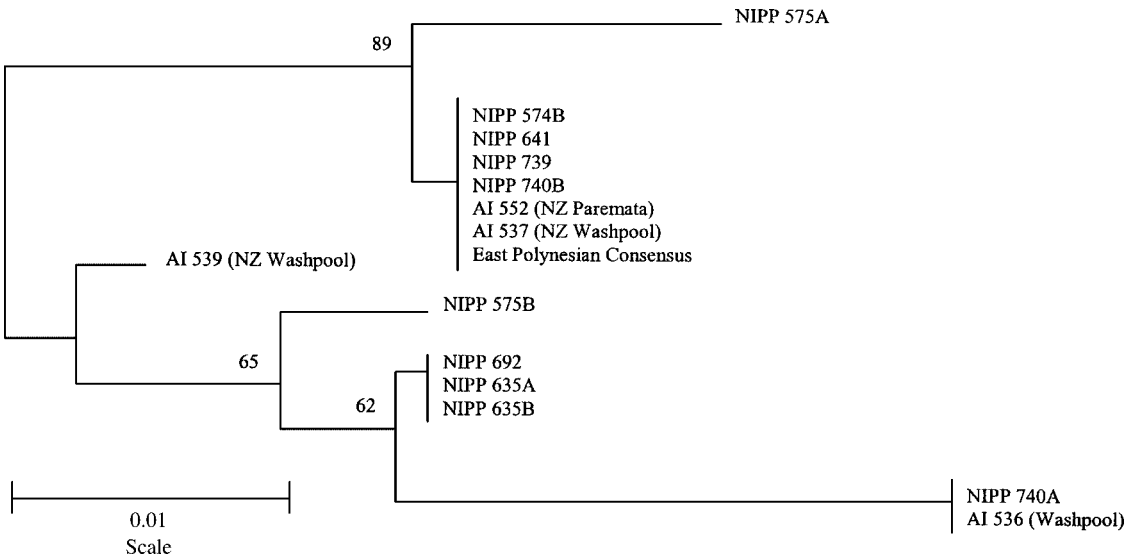

Figure 1. Neighbor Joining Tree for 175 bp of mtDNA d-loop sequence. NIPP samples from Emily Bay settlement site, Norfolk Island.

in Table 2. In addition to the Norfolk Island samples, sequences from archaeological samples from New Zealand, and an East Polynesian consensus sequence from modern *R. exulans* (from the Southern Cook Islands, the Society Islands, Raoul Island, the Marquesas Islands, the Hawaiian Islands and New Zealand) are also shown for comparison.

Four of the samples (NIPP 574B, 641, 739 and 740B) were identical to the East Polynesian sequence and to two archaeological samples from New Zealand, AI 552 and AI 537. Sample NIPP 575A was identical to these four samples with the exception of a single point mutation (a transition, G to A, identified as variable site 6 in Table 2). Sample NIPP 740A is unique amongst the Norfolk Island sequences and similar only to one other sample sequenced so far, an archaeological sample (AI 536) from the earliest layer from the Washpool Midden site (N168/22), located in Palliser Bay in the south of the North Island of New Zealand (Leach, 1979). These two samples differ from all others at variable sites 1 (C to A) and 2 (T to C) shown in Table 2. Three samples, NIPP 635A, 635B, and 692, were identical to one another, and unlike any East Polynesian samples, though they differ from a New Zealand sample (AI 539) at only one site (C to T at site 4, Table 2). Sample NIPP 575B is unique, but differs from the NIPP 635A, 635B and 692 by one point mutation (A to T at variable site 5, Table 2).

Discussion

Genetic variation as an indicator of contact. Irwin (1992) classes Norfolk Island with the Kermadecs in his discussion of prehistoric voyaging, suggesting that both may have served as stepping stone islands for voyages to and from New Zealand, and as such "they could show signs of multiple contacts, from New Zealand and elsewhere in East Polynesia" (1992: 111). The Kermadecs, lying between New Zealand and tropical East Polynesia, would probably have been contacted more frequently than Norfolk Island, but less frequently than the Chatham Islands which are particularly isolated and unlikely to have had regular prehistoric contact after initial occupation.

Tajima (1990) suggests that genetic diversity is likely to be high where migration rates are high and low where migration rates are low. Given this as an assumption, together with the commensal relationship between *Rattus exulans* and prehistoric Pacific Islanders, then the degree of genetic diversity in an island population of this rat could be an indicator of the degree of human contact with that island (Matisoo-Smith *et al*., 1998). It is, of course, also possible that genetic diversity varied within transported populations.

Gene diversity (*h*), as calculated by Nei (1987: 179), is an indicator of genetic diversity within species. If each haplotype scored is unique, then the maximum value of *h* equals (n+1)/n, where n is the number of haplotypes scored. An *h* value of 0 denotes a population with no haplotype variation. The Norfolk Island *h* value of 0.80 suggests a relatively variable *Rattus exulans* population. A phylogenetic analysis of these samples (Fig. 1) shows not only the variability, but the fact that these are quite divergent sequences, suggesting that they are not closely related.

Previously (Matisoo-Smith *et al*., 1999), gene diversity was calculated for an archaeological population of *R. exulans* from Chatham Island, and modern samples from New Zealand, and Raoul Island in the Kermadec group. As predicted, given suggestions of isolation, the Chatham Island value was relatively low, 0.54. In contrast, the New Zealand and Raoul values were much higher, with *h* equal to 0.985 and 0.90 respectively. For the 175 bp region of the genome analysed in this study, the Raoul Island value was the same, 0.90, with four unique sequences from seven samples. The Chatham Island samples, however, showed no variation in this region of the genome, and therefore had an *h* value of 0. The intermediate Norfolk Island value is consistent with a level of prehistoric contact between those of the Chathams and the Kermadecs.

Origins of Norfolk Island *Rattus exulans*. Four of the Norfolk Island *R. exulans* sequences (NIPP 574B, 641, 739 and 740B) were identical to the consensus sequence for East Polynesian extant *R. exulans*. This sequence was also

identified in two archaeological rat bones from the Washpool Midden and Paremata sites in New Zealand (samples AI 537 and 552). Sample NIPP 575A differs from this typical East Polynesian sequence by only one point mutation. These results are thoroughly consistent with other archaeological evidence suggesting East Polynesian influence in Norfolk Island and ties between Norfolk Island and New Zealand (Specht, 1984).

A third haplotype also suggests a connection between Norfolk Island and New Zealand—that haplotype shared between NIPP 740A and AI 536. This New Zealand sample (AI 536) is also from the Washpool Midden site (N168/22). It is associated with Level 1, lens B, which is part of the earliest phase of occupation of the site, dated to about A.D. 1180 (Leach, 1979). What is particularly interesting is that this sequence has not been identified elsewhere in East Polynesia, nor have the fourth haplotype, a sequence shared by three other Norfolk Island samples, NIPP 635A, 635B and 692, and the fifth haplotype (NIPP 575B) which differs from these three by a single point mutation.

While the common haplotype found between NIPP 740A and AI536 strongly suggests a Norfolk-New Zealand connection, it is impossible to say which direction this represents—from Norfolk Island to New Zealand or from New Zealand to Norfolk. It could, of course, merely represent a common source for both samples, and not necessarily a direct connection between the locations. As yet the source of this haplotype and the other two that are closely related has not been identified, despite analysis of more than 200 *R. exulans* sequences from East Polynesia, both archaeological and modern. It is possible that this sequence therefore represents either a very rare and/or extinct East Polynesian lineage, or a non-East Polynesian source for Norfolk Island and/or some New Zealand *R. exulans* populations. Alternatively, there may have been a highly variable source population for the Norfolk Island rats. We are currently collecting and analysing samples from West Polynesia (Tonga, Niue and Fiji) and from more westerly locations such as New Caledonia, Vanuatu, and sites in Near Oceania, in addition to studying archaeological samples from additional East Polynesian sites. Only through continued genetic analyses of *R. exulans* remains from a range of archaeological sites throughout the Pacific Ocean will we potentially identify the source of these mystery sequences.

In conclusion, results of analyses of genetic variation in *R. exulans* remains from Emily Bay, Norfolk Island are consistent with other archaeological evidence suggesting links between Norfolk, New Zealand and East Polynesia. However, several samples have mtDNA sequences that have not yet been identified in East Polynesian populations. This may suggest a link between Norfolk, New Zealand and a third region that we have not yet been able to identify.

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