

Radiocarbon and DNA evidence for a pre-Columbian introduction of Polynesian chickens to Chile

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Communicated by Patrick V. Kirch, University of California, Berkeley, CA, May 1, 2007 (received for review February 10, 2007)

Two issues long debated among Pacific and American prehistorians are (i) whether there was a pre-Columbian introduction of chicken (*Gallus gallus*) to the Americas and (ii) whether Polynesian contact with South America might be identified archaeologically, through the recovery of remains of unquestionable Polynesian origin. We present a radiocarbon date and an ancient DNA sequence from a single chicken bone recovered from the archaeological site of El Arenal-1, on the Arauco Peninsula, Chile. These results not only provide firm evidence for the pre-Columbian introduction of chickens to the Americas, but strongly suggest that it was a Polynesian introduction.

ancient DNA | *Gallus gallus* | Polynesia

Argument about the origins and date of introduction of the domestic fowl or chicken (*Gallus gallus*) to the Americas has raged for over 30 years. Despite claims that it might be native to the region (1), it has never been recovered or reported from paleontological, Paleo-Indian, or, until now, prehistoric archaeological contexts in the Americas. A Portuguese or Spanish introduction to the east coast of South America around AD 1500 has been suggested (2), but when Pizarro reached Peru in 1532, he found that chickens were already an integral part of Incan economy and culture, suggesting at least some history of chickens in the region. Consequently, there have been numerous suggestions of a pre-European chicken introduction to the west coast of South America (3–5), in which both Asian and Polynesian contacts have been proposed (1, 4, 6). Here, we provide the first unequivocal evidence for a pre-European introduction of chickens to South America and indicate, through ancient DNA evidence, that the likely source of that introduction was Polynesia. This evidence has implications for debates about ancient Polynesian voyaging capabilities as well as those addressing prehistoric population interactions and exchange. This study also presents the first published ancient DNA sequences for chickens providing valuable data for researchers concerned with the loss of genetic variation in modern domestic stocks (7).

The Indo-Pacific origins of the Polynesians are linked to Southeast Asia through the Austronesian expansion and particularly to the Lapita culture that first appears in the Pacific ≈3300 years before present (B.P.). Lapita colonists moved rapidly through eastern Melanesia to Samoa and Tonga by ≈2900 B.P. Beginning ≈1500–1000 B.P., the settlement of East Polynesia began, probably from Samoa, with colonization of Hawai'i by 1000 B.P., Easter Island by 800 B.P. and New Zealand by 700 B.P. Polynesians introduced dogs, pigs, rats and chickens to many of the islands they settled (8). Chicken remains first appear in Vanuatu and Tonga between 3000 and 2800 B.P., where they are associated with Lapita sites (9, 10), in Niue (11) from ≈2000 B.P. and in early occupation layers throughout most of East Polynesia (8).

Some prehistoric contact between the Americas and Polynesia is evident from the presence of South American sweet potato (*Ipomoea batatas*) in pre-European archaeological sites in Polynesia (6, 12, 13), most notably from Mangaia, Cook Islands, where it is dated indirectly to ≈AD 1000 (13). Linguistic and archaeological evidence suggest that the bottle gourd (*Lagenaria siceraria*), also from the Americas, was present in Eastern Polynesia before AD 1200 (14, 15). Voyaging from Polynesia to the Americas has been proposed (16), and debated (17) recently in relation to linguistic and archaeological evidence for the occurrence of some watercraft, namely sewn plank canoes, and fishhook forms found in southern California which resemble Polynesian types. Sewn plank canoes have also been documented in Chile by ethnographers (18) and claims have been made suggesting artifactual and linguistic evidence for Polynesian influence in the Mapuche region of south central Chile (3). Computer simulations suggest that voyaging eastward from Polynesia in the southern hemisphere where the mid-latitude westerlies are more accessible, is a more likely prospect than a northern route to the Americas (19). These southern hemisphere voyages would have brought landfalls in the central and southern regions of Chile and could have introduced the Polynesian chicken to South America. However, no securely dated pre-Columbian chicken remains or unequivocal archaeological evidence for Polynesian contact with the Americas has been reported until now.

The archaeological site of El Arenal-1 (see Fig. 1), first excavated in 2002, is three kilometers inland on the southern side of the Arauco Peninsula, south central Chile (lat 37°22'15"S, long 73°36'45"W). Analyses of pottery and other artifacts show that the site belongs to the El Vergel Cultural Complex of horticulturalist communities dating to the period between AD 1000 and 1500 (20). Three thermoluminescence dates on ceramics from the site place the occupation between AD 700 and 1390 (D.Q. and L. Contreras, unpublished data). Most importantly, excavators recovered 50 chicken bones from the site, representing a minimum of five individual birds. These remains provide

Author contributions: A.A.S., J.M.R., and E.A.M.-S. designed research; A.A.S., L.H., and E.A.M.-S. performed research; J.M.R., D.Q., D.V.B., D.J.A., R.W., A.J.A., T.L.H., and J.S.A. contributed new reagents/analytic tools; A.A.S. and E.A.M.-S. analyzed data; and A.A.S., J.M.R., D.Q., D.V.B., D.J.A., R.W., A.J.A., T.L.H., J.S.A., L.H., and E.A.M.-S. wrote the paper.

The authors declare no conflict of interest.

Data deposition: The sequences reported in this paper have been deposited in the GenBank database (accession nos. EF535236–EF535249).

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This article contains supporting information online at www.pnas.org/cgi/content/full/0703993104/DC1.

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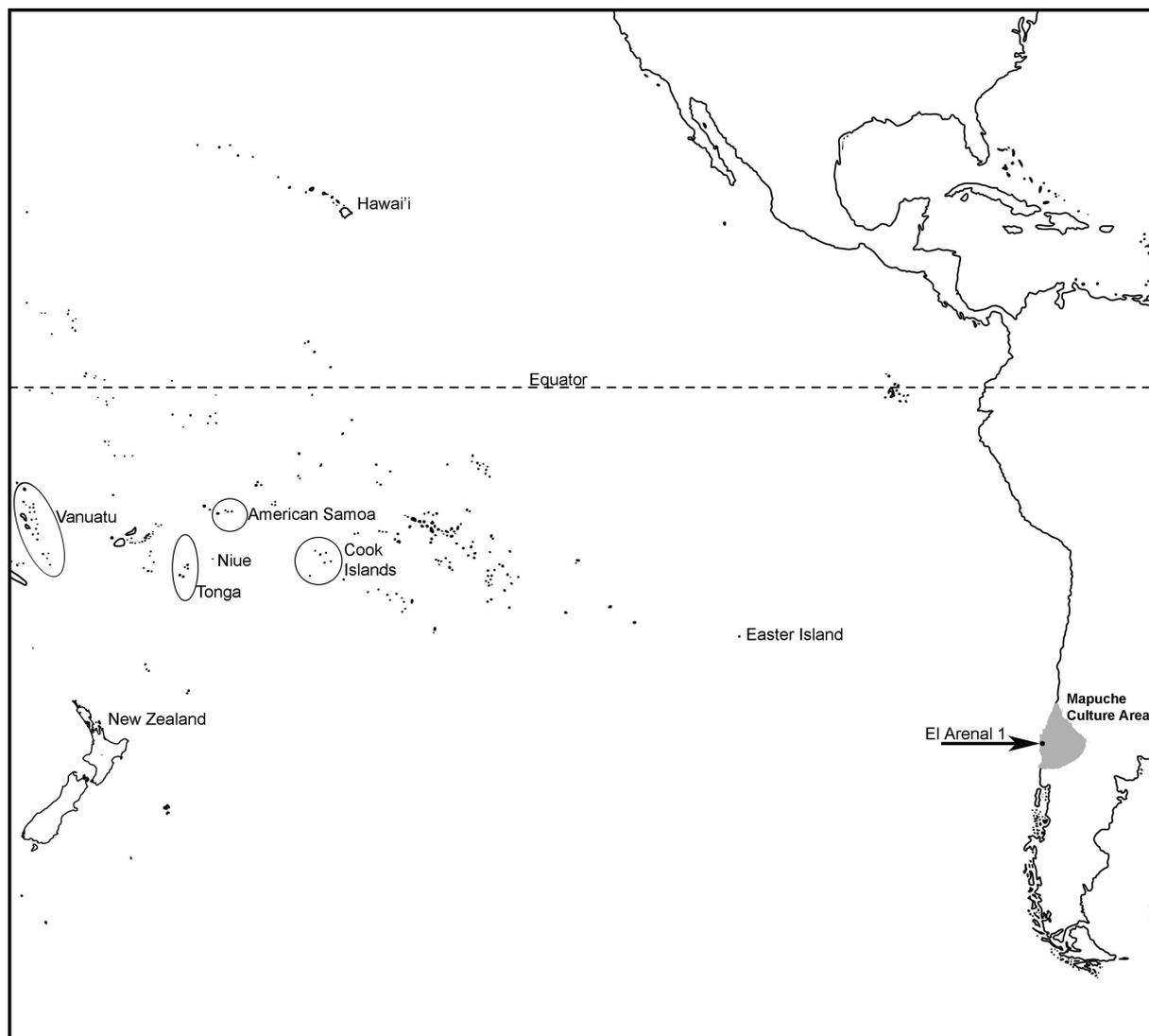


Fig. 1. Map of the Pacific showing locations mentioned in the text.

the earliest evidence for the presence of chicken in the Americas. Direct dating and ancient DNA analyses of these remains were therefore essential for identifying the origins and pre-Columbian provenience of chickens in Chile. These were compared with ancient mtDNA obtained from chicken bones from archaeological sites in Polynesia and with modern Araucana chicken materials.

Results and Discussion

The radiocarbon date obtained for the El Arenal chicken bone was 622 ± 35 B.P., resulting in a calibrated age range of AD 1321–1407 with two intercepts. At two sigma (AD 1304–1424) the sample still lies within the pre-Columbian era, and thus provides directly dated evidence for pre-Columbian chicken in South America. Chicken first occurs in Pacific archaeological sites $\approx 3,000$ years ago in the Reef/Santa Cruz (22) and shortly thereafter in Vanuatu (9), but people did not reach Central and East Polynesia for another 1,500–2,000 years. The direct dating of chicken bones from the El Arenal-1 site in Chile falls within the expected range of dates, 600–800 B.P., for colonization in the easternmost islands of Polynesia, including Rapa, Pitcairn, and Easter Island (23). As a result of this temporal affinity, ancient DNA analyses were warranted to understand the relationship

between these South American chickens and ancient Oceanic populations and to identify the likely origins of the El Arenal bone.

A total of 37 chicken bones obtained from prehistoric archaeological sites dating from between 2900 and 500 years B.P. from five Polynesian archipelagos were obtained for use in our study. Positive PCR amplification and DNA sequence was obtained for 12 of the 37 ancient samples attempted, resulting in a success rate of 32%, which is consistent with the degraded nature of biomolecules in ancient remains (24). The variable sites identified in the archaeological chicken bones, modern Araucana chicken feathers and modern chicken sequences obtained from GenBank are shown in Table 1. In the most variable area of the D-loop, the common SNPs (25) are observed. In ancient material, these include an additional four sites that identify two haplogroups in prehistoric chickens. In total, SNPs were most common between sites 199 and 339. Therefore, the most interesting section of the D-loop was <150 bp long creating an ideal target length for ancient DNA studies.

The El Arenal bone produced an identical sequence to chicken bones from two prehistoric archaeological sites in the Pacific: Mele Havea in Tonga, from upper plainware layers dating to between 2000 and 1550 B.P., which is significantly earlier than El

Table 1. Variable sites identified in ancient and modern chicken samples

Sample no.	Variable site location									GenBank accession no.
	196	214	222	225	278	293	303	331	339	
Reference sequence	T	T	T	C	A	C	T	G	A	NC001323
CHLARA001	T	C	C	C	A	C	T	G	A	EF535241
Ton_HB	T	C	C	C	A	C	T	G	A	EF535237
AMSFTF001	T	C	C	C	A	C	T	G	A	EF535240
Ton_TD	C	C	C	C	A	C	T	G	A	EF535236
NIUPKI009	C	C	C	C	A	C	T	G	A	EF535239
HWIKUA001	C	C	C	C	A	C	T	G	A	EF535238
PAQANA011	Y*	C	C	C	A	C	T	G	A	EF535246
PAQANA004	T	T	C	C	G	T	C	G	G	EF535242
PAQANA006	T	T	C	C	G	T	C	G	G	EF535243
PAQANA009	T	T	C	C	G	T	C	G	G	EF535244
PAQANA010	T	T	C	C	G	T	C	G	G	EF535245
PAQHAN001	T	T	C	C	G	T	C	C	G	EF535247
Araucana feather 1	T	C	C	T	A	C	T	G	A	EF535248
Araucana feather 2	T	T	T	C	A	C	T	G	A	EF535249
Miyake Lombok 1	T	T	C	C	G	C	C	G	G	AB009437, AB009438
Miyake Lombok 2	T	T	C	C	G	C	C	G	A	AB009436
Miyake Vietnam 1	C	T	C	C	A	C	T	G	A	AB009435
Miyake Vietnam 2	T	T	C	C	G	C	T	G	A	AB009434
Miyake Thailand 1	T	T	C	C	G	C	C	G	A	AB009432
Miyake Thailand 2	T	T	C	C	G	C	C	G	G	AB009441
Miyake Philippines	T	T	C	C	G	C	C	G	A	AB009433
Liu Yunnan	T	T	C	C	G	C	T	G	A	AF512163, AF392339

Numbering based on Desjardins and Morais (42).

*Y, ambiguous site that can be either a C or a T.

Arenal-1; and Fatu-ma-Futi in American Samoa, which dates to about the same period as El Arenal-1. All ancient West Polynesian samples, early samples from Anakena, Easter Island and Kualoa, Hawai'i, and the El Arenal sample share a single unique point mutation (a T to C transition) at site 214. One of the modern Araucana feather samples also shares this unique mutation. Three other SNPs (all transitions) at sites 278, 303, and 339 are shared by these West Polynesian, early Anakena and Hawai'i, and the Chilean bone samples and sequences reported from modern chickens in Southeast Asia, specifically samples from the Yunnan region of China and Vietnam (see Table 1 for GenBank accession numbers). Interestingly, samples from archaeological layers dating to later periods at Anakena and from another later prehistoric period Easter Island site, Hanga Hahaione, did not share these three SNPs. These sequences appear to be more closely related to those of chickens from Island Southeast Asia, specifically from Lombok, the Philippines, and Thailand (see Table 1). This suggests that there were two mitochondrial lineages present in prehistoric Polynesian chicken populations. This result is consistent with ancient DNA analyses of Polynesian dogs that suggest two distinct lineages originating from similar geographic locations: mainland and island Southeast Asia (26). The presence of the Polynesian sequence in a contemporary Chilean Araucana chicken also suggests that some modern populations of this breed may be at least partially derived from this ancient Polynesian source. Only further research on mtDNA variation in other American chickens, both modern and archaeological, and particularly those associated with early Spanish and Portuguese settlements, will provide data on the overall impact of Polynesian chickens to the American chicken gene pool.

Voyaging and Settlement of the Pacific. Since Heyerdahl's Kon Tiki expeditions and resulting works (27), public attention has been focused on the possibility of American origins for Polynesians and/or later prehistoric contact between the Americas and

Polynesia. Archaeological, linguistic, and human genetic data have shown that Polynesian origins are clearly in the region of island Southeast Asia and Melanesia rather than in the Americas (8). In the past 10 years, mounting evidence has indicated at least some interaction between Polynesians and the indigenous peoples of South America. Computer simulations (19) and experimental sailings (28) have shown that Polynesian voyaging was indeed purposeful. The presence of the sweet potato in Mangaia (13) and possibly also the American haplotypes present in Polynesian bottle gourds (15) demonstrate that some level of contact occurred between Polynesia and South America. Despite the similarities of a few artifact types and the presence of sewn plank canoes (3, 18), no conclusive evidence existed for the arrival or presence of Polynesians in South America.

Debates About the Origin of the Chicken in South America. Several hypotheses about how the chicken reached the Americas have been presented in the past. The possibility of natural dispersal has been explored and repudiated (29), and most scholars believe the chicken was introduced to the New World by Spanish or Portuguese explorers when they arrived on the east coast around AD 1500 (30). However, the presence of chickens in Peru when Pizarro arrived in 1532 and its integration in Incan culture would require not only a very rapid dispersal across the continent, but the almost immediate incorporation of chickens into the economy, a highly unlikely combination of events. Recognition of this has led to several suggestions for the presence of pre-Columbian chicken on the west coast. Those supporting what has been referred to as the hyperdiffusionist perspective assert that chickens were brought to South America as part of a cultural complex directly from mainland or island Southeast Asia (4, 31), but no conclusive archaeological evidence has yet come to light to support this. An alternative theory presented is that chickens were introduced from Polynesia (1, 5, 32). The evidence presented here supports the latter hypothesis, and the similarities in ancient DNA patterns across wide geographic distances and

Table 2. Archaeological context for chicken bones analyzed

Sample name	Site and archipelago	Context	Assoc. radiocarbon age (reported dates)	Ref.
CHLARA001	El Arenal-1, Chile	El Vergel complex	Cal AD 1304–1424 [†] (note directly dated bone)	21
TON_HB	Mele Havea, Tonga	Plainware	2000–1550 [†] cal B.P.	D.V.B., unpublished data
ASMFTF001	Fatu-ma-Futi, American Samoa	A-ceramic period or ‘Dark Ages’	1000–500 B.P.	42
TON_TD	Ha’ateiho, Tonga	Plainware	2000–1550 [†] cal B.P.	D.V.B., unpublished data
NIUPKI009	Paluki, Niue	Colonization Period	Cal AD 360–540*	11
HWIKUA001	Kualoa, Hawai’i	Prehistoric Hawai’ian	Cal AD 1040–1280 [†]	43
PAQANA011	Anakena, Easter Island	Early, settlement phase	Cal AD 1270–1400 [†]	23
PAQANA004	Anakena, Easter Island	Early, settlement phase	Cal AD 1260–1430 [†] and cal AD 1370–1380 [†] ; cal AD 1210–1320 [†] and cal AD 1340–1390 [†]	23
PAQANA006	Anakena, Easter Island	Early, settlement phase	Cal AD 1280–1430 [†]	23
PAQANA009	Anakena, Easter Island	Early, settlement phase	Cal AD 1290–1430 [†]	23
PAQANA010	Anakena, Easter Island	Early, settlement phase	Between cal AD 1280–1400 [†] and cal AD 1250–1410 [†]	23
PAQHAN001	Hanga Hahave, Easter Island	Classic, Ahu-Moai Period Crematoria	Prehistoric	44

*Date reported at one sigma.

[†]Date reported at two sigma.

perhaps 1,500 years suggest that the pre-Columbian chicken remains from El Arenal-1 are descended from Polynesian stock.

Commensal Models. Commensal models are now widely applied in the Pacific to understand migration and interactions in prehistory. Through the examination of the ancient DNA of fauna and flora that was purposefully transported into the Pacific, archaeologists are able to add another dimension to evaluate the direction and spread of Lapita and Polynesian peoples. One interesting finding of commensal studies is that some domesticates were introduced to the Pacific more than once. Data for Pacific rats (*Rattus exulans*) shows at least two introductions of the species into Oceania (33), studies of Pacific dogs (*Canis familiaris*) have revealed two mitochondrial lineages of canines to Remote Oceania (26), and studies of the bottle gourd have shown influences from both Asia and the Americas in the distribution of modern plants in the Pacific (15). This study has revealed not only a Polynesian origin for pre-Columbian chickens at El Arenal-1 in Chile, but the existence of two chicken mitochondrial lineages in the Pacific indicating the possibility of two introductions of these domesticates to the region. The idea of multiple introductions of chickens to the Pacific was originally proposed by Carter (5) based on linguistic grounds and by Crawford (32) based on morphology. The analysis of further archaeological chicken samples from across Oceania will help to elucidate the specific timing of introductions and geographic origins of these stocks.

Ancient DNA of the Chicken and Issues of Conservation. In addition to their value for tracking prehistoric migrations and providing evidence for the origins of South American chickens, there are several other implications of the ancient mtDNA data presented here. The first is incorporation into studies of the origin(s) of chicken domestication (34, 35). The second is in the evaluation of the variability of the chicken mitochondrial genome (7). It has been shown that the mtDNA of modern chickens is lacking in variability. The populations examined in this study have been subject to repeated bottlenecks through the movement of starter stocks to new islands, and this material shows little variability in the most mutable sites (25) over more than 1,000 years. This suggests that mtDNA is not highly variable in the chicken and that concerns about the genetic conservation of the species may

be well founded (36). We have also shown that an ancient Polynesian haplotype persists in modern populations of Chilean chickens and that in the 600 years or more since the introduction of European chicken these sequences have deviated little from their ancient Pacific ancestors. Selective breeding for particular phenotypic traits associated with the Araucana breed may have by chance preserved the Polynesian haplotype, keeping it from being swamped by later European-introduced chicken lineages.

Conclusion

This article presents well dated and securely provenienced evidence of a pre-Columbian chicken introduction to the Americas. We are not suggesting that the El Arenal-1 site represents the exact location of introduction or that the related date corresponds to the first or only introduction of chicken to South America. The date corresponds well with current archaeological evidence for the eastward expansion of the Polynesians. Most importantly, the current results demonstrate that chickens with a Polynesian genetic signature reached the south central coast of Chile before European contact with the Americas. Further analyses of additional samples from East Polynesia and South America may allow us to narrow down the source population and timing of introduction of chickens to the Americas. In addition, further archaeological research to examine possible points and timing of contact(s) along the coast and on the coastal islands of South America is clearly warranted.

Methods

Research on the origins of Pacific chickens has been undertaken in the Department of Anthropology at the University of Auckland as part of a larger program focusing on the use of mtDNA variation in commensal animals as a proxy for tracing human migrations through the Pacific (33, 37). We obtained one of the El Arenal-1 chicken bones and identified the need for both a direct radiocarbon date and at least two independent DNA analyses from the bone. Therefore, the bone was broken into three pieces under sterile conditions in the ancient DNA laboratory in the Department of Anthropology at the University of Auckland. The first piece was submitted to the Rafter Radiocarbon Laboratory at the Institute of Geological and Nuclear Sciences (Wellington, New Zealand) for radiocarbon dating. The two remaining pieces of the El Arenal bone were processed

independently in two separate dedicated ancient DNA laboratories at the University of Auckland and Massey University.

Dating. Based on visual inspection the bone appeared to be well preserved. Microscopic particles of dirt were removed with a brush and scalpel before demineralization in an acid wash. The extracted gelatin fraction (NZ 26115) was dated and returned a radiocarbon age of 622 ± 35 B.P. ($d13 = -21\text{‰}$). The sample was calibrated by using the Calib software package (Ver. 5.0.1) (21); and the southern hemisphere atmospheric data (38).

DNA Analyses. The genetics of the fowl are now well understood with the complete nuclear genome sequenced in 2004 (39). Analyses of the mitochondrial genome (7) have shown that there is little variation in chickens, with studies of SNPs (25) revealing only 11 variable sites in the hypervariable D-loop region. Five of the eleven SNPs occur between site numbers 217 and 261 (40). We therefore targeted this region for polymerase chain reaction (PCR) amplification of our archaeological chicken samples.

DNA was extracted, and ≈ 400 bp of mitochondrial DNA (bases 144–556) was PCR amplified by using two overlapping primer sets. All sequencing was conducted by the Allan Wilson Centre Genome Service at Massey University. Each amplicon was sequenced in both directions, and the raw sequences were aligned by using the Sequencher software package [GeneCodes Corp., Ann Arbor, MI; see [supporting information \(SI\) Methods](#) for descriptions of methods].

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In addition to the El Arenal sample, we also processed 11 chicken bones recovered from a range of pre-European archaeological sites in Tonga, American Samoa; Niue, Hawai'i; and Easter Island. The age ranges and cultural affinities for these archaeological samples appear in Table 2. It has been suggested that the unique type of chicken known as the Araucana, which has no tail and lays blue eggs, is descended from pre-European stock bred by the Mapuche (formerly called Araucanos) people of Southern Chile (41). To assess the possible Polynesian origin of the blue-egg breed we also extracted and amplified the DNA from feathers belonging to two modern individuals identified as possible Araucana chickens.

We thank Dr. Frédérique Valentin (CNRS, Paris), who codirected the excavations on Tutuila with D.J.A.; Kelly Esh (University of Hawai'i), who sorted the Anakena chickens for use in this study; Gonzalo Figueroa García-Huidobro for providing Araucana chicken feathers; Karolyn Buhning Rabanal for translations from Spanish; Peter Quinn for drawing the map; Judith Robins (University of Auckland), Dr. Thegn Ladefoged (University of Auckland), and Prof. David Lambert (Massey University) and as always Prof. Roger Green (University of Auckland) for assistance and support; and two anonymous reviewers for suggestions on the manuscript. Finally, the authors also acknowledge Dr. George F. Carter who, despite heavy criticism, maintained that Polynesians introduced the first chickens to South America. We wish he were alive to see this result. Funding for laboratory work and a scholarship to A.A.S. were provided by the Allan Wilson Centre for Molecular Ecology and Evolution. Additional funds for laboratory work were provided by the Royal Society of New Zealand Skinner Fund.