Supporting Information

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SI Materials and Methods

Archaeological Specimens. Eighty ancient chicken bones from 12 archaeological sites, representing three major European locations (United Kingdom, Central Europe, and Greece) and two major time bins [(*i*) Late Iron Age La Tène C and D, Late Hellenistic/Early Roman to Roman, and (*ii*) medieval and postmedieval periods] were extracted for DNA (Table S1).

Although our sampled specimens are relatively modern and geographically distant relative to the domestication centers in southeast Asia, which date to as early as 6000 B.C. (1) (although, see the main text for a discussion on issues relating to *Gallus* finds in some early East Asian contexts), they represent among the earliest, if not the earliest, introductions to Europe. The first wave of introduction likely arrived around 900–700 B.C. in Greece and on the Iberian Peninsula (by Phoenician sea trade). The central European chicken was likely derived from the early Greek founder population, which was distributed throughout the northern Mediterranean, and from there introduced to Central Europe across the Alps around 600–400 B.C. Zooarchaeological evidence for the presence of chicken in Central Europe (Germany, Austria, and Switzerland) dates to the Hallstatt C–D period *ca*. 800–475 B.C. One major mechanism of introduction consisted of trade with ancient Greek settlement colonies in the coastal regions of the northwestern Mediterranean. During the following La Tène period (475–30/1 B.C.) *Gallus* bones remain rare in archaeological contexts, with frequencies increasing slightly during its later phases. After the Roman conquest, poultry-keeping became relatively rapidly and widely established in the northwestern Provinces of the Roman Empire, but not so in regions outside the area of influence of Rome, where it only became economically important in medieval times (2). We can therefore safely assume that these Late Iron Age/Early Roman birds descend from early *Gallus* introduced into Europe. For the broadly contemporaneous birds from Classical Greece, however, this is not necessarily the case, considering the fact that in the meantime "Greece" had been part of the Persian, Greek, and Roman Empire with trade connections to Mesopotamia and India (3). Southeast Europe may therefore have witnessed multiple introductions from the Near East.

Dating of Archaeological Specimens. The majority of the specimens analyzed in this study were dated using stratigraphic and/or contextual evidence (Fig. S1 and Table S1). Given the ease with which chicken bones can move between occupation phases, we primarily sampled faunal assemblages from sites with no overlaying building structures or archaeological strata. A single specimen from Altenburg, Germany (Ch38 in Table S1) presumed to date to Iron Age La Tène C/D contexts (280–15 B.C.), however, was the only ancient sample that possessed homozygous genotypes for both the sweep *TSHR* allele and *yellow skin* associated Gray Junglefowl (GJF) *BCDO2* (β-carotene dioxygenase 2) allele (two genotypes primarily associated with modern commercial breeds). In addition, although there was no subsequent occupation layer over the majority of the site, there is evidence of a medieval occupation at the outskirts of the site, and German troops potentially used the walls surrounding the Late Iron Age settlement during World War II. Given the genetic signals and the higher potential that this sample was intrusive, we directly dated this bone, the result of which showed that it dated to 150 ± 30 cal. B.P. (2 σ calibration; Beta Analytic, no. 356195).

We also directly radiocarbon dated a specimen from Kassope (Greece) where there was no evidence of subsequent occupation layers, and this sample produced a date of 2150–1990 cal. B.P. (2 σ calibration; Beta Analytic, no. 351382), thus confirming the contextual dating (Table S1). None of the other stratigraphically dated samples come from sites with intrusive or overlaying occupation layers, and the genetic results strongly suggest the archaeologically derived dates are accurate.

Description of Key Archaeological Sites. ***Kassope, Greece.*** Kassope, Greece was an ancient Greek city founded 350 B.C. and abandoned 30 B.C. (3). Friedl (4) presented an analysis of the fauna. The chicken remains studied here originate from house 3, occupied during the second and first century B.C.

Magdalensberg, Austria. Magdalensberg, Austria, was a trading settlement founded during the second third of the first century B.C. and inhabited until about A.D. 50 (5). The bird remains, including numerous chicken bones excavated at this site, have been identified by Dräger (6).

Altenburg-Rheinau, Germany. Altenburg-Rheinau, Germany was a Celtic oppidum occupied between *ca*. 150 and 15 B.C. (7). Excavations on the eastern peninsula during the 1970s produced the chicken remains, which have been analyzed by Moser (8).

Abodiacum/Epfach, Germany. Abodiacum/Epfach, Germany was a Roman military station and settlement, occupied with some interruptions from the first until the beginning of the fifth century A.D. (9). Chicken remains have been found throughout the sequence (10).

Quintana/Künzing, Germany. The *Gallus* finds originate from the Mithras sanctuary (*Mithraeum*) near the Roman settlement of Quintana/Künzing, Germany. This sanctuary was in use from the second century A.D. until the second half of the third century A.D. (11). The birds represent offerings to the God Mithras. Excavations produced an assemblage of >7,500 chicken remains (12).

Manching, Germany. Manching, Germany was a Celtic oppidum inhabited between *ca*. 300 B.C. until 50/30 B.C., probably the largest city north of the Alps during this period. After abandonment, some small-scale Roman occupation took place, probably in form of a *mansio* or perhaps *vicus*, lasting until the middle of the third century A.D. (13). The chicken remains form part of a huge faunal assemblage (>350,000 finds) constituting a most useful source of information about late La Tène animal husbandry in Central Europe (14).

United Kingdom: Arbeia, South Shields Roman Fort, England. Arbeia was a Roman fort built at the mouth of the River Tyne in the late second century A.D., which was converted into a supply base for food in the early second century A.D. After a large fire in the late third or early fourth century A.D., the fort was redesigned and rebuilt, and continued in use into the early fifth century A.D. (15).

The remaining chicken remains, from the north of England, which were used in this study, came from two archaeological sites in York (Spurriergate and St. Saviourgate) (16), located within the core of the medieval city; from a site in Beverley, East Riding of Yorkshire (17); and from South England and an excavation in East London (recovered from excavations in preparation for the construction of the Docklands Light Railways) (18).

The vertebrate material from Spurriergate was recovered from excavations in a former car park and beneath several 1960s buildings that were subsequently demolished before the archaeological

interventions. The chicken bones were retrieved mainly from fills of rubbish pits and dump layers associated with occupation of Anglian/Anglo-Scandinavian, medieval and postmedieval dates. The excavations at St. Saviourgate largely revealed pits containing refuse of a mixed nature, from primary butchery waste to household rubbish. The chicken bones were recovered from pit fills of late medieval date.

Chicken remains from Beverley, East Riding of Yorkshire, were recovered from excavations at the site of the former Picture Playhouse and Swimming Pool in the heart of the medieval town at the north side of Saturday Market. This had been a market area of Beverley since the 12th century, becoming known as the Corn Market by the 14th century and as Saturday Market by the 16th. The site was the location of a meat market by the 18th century, and quite probably much earlier, with an arcaded butchers' shambles built in 1753 and a fish shambles built behind the butchers' market in 1777. All of the contexts from which the fowl bones came were pit fills or ground raising deposits of medieval and postmedieval date (16, 17).

Ancient DNA Laboratories and Experimental Set-Up. DNA extractions and PCR amplifications were performed in a dedicated ancient DNA laboratory in the Department of Archaeology (Durham Evolution and Ancient DNA) at Durham University, United Kingdom. We followed strict laboratory procedures according commonly used guidelines (19, 20). All equipment and work surfaces were cleaned before and after each use with a dilute solution of bleach [5–10% (wt/vol) active sodium hypochlorite] followed by ddH_2O and ethanol [99% (vol/vol)]. Pipettes and plastic racks were UV-irradiated in a dedicated cross-linker (at <15 cm for at least 30 min at 254-nm wavelength) before and after use. Pre- and post-PCR laboratories are physically isolated; access to the pre-PCR laboratories is restricted to Ancient DNA laboratory users only and access is also prohibited if the laboratory user had entered post-PCR areas the same day. Ancient DNA laboratory users wear clean laboratory coats, double layer of gloves (nitrile and latex), and overshoes to avoid introducing contaminants from post-PCR areas.

Independent replication of 12 ancient specimens was performed in a dedicated ancient DNA laboratory at the Evolutionary Biology Centre, Uppsala University, Sweden. Laboratory work follows commonly used guidelines (19, 20). Laboratory users wear coveralls, facemasks, dedicated laboratory clogs, and double layers of gloves. The laboratory is equipped with positive air pressure and UV lamps and is routinely deep cleaned with bleach [1–5% (wt/vol) active sodium hypochlorite], ddH_2O, and dilute ethanol [70% (vol/vol)].

Ancient DNA Extraction. The ancient chicken bones were prepared for DNA extraction (one DNA extract per specimen) by removing an approximately 1-mm layer of outer bone surface by abrasion using a Dremel drill with clean, one-time use cut-off wheels (Dremel no. 409). A subsection of the bone was subsequently isolated and pulverized in a Micro-Dismembrator (Sartorious-Stedim Biotech), followed by collection in 15-mL Grainer tubes. Milling containers and grinding balls were subsequently suspended and cleaned in 1% virkon, and rinsed in absolute [99% (vol/vol)] ethanol.

Next, 50- to 100-mg bone powder/specimen was digested in 0.425M EDTA (pH 8), 0.05% SDS, 0.05M Tris·HCI and 400 μg Proteinase K, and incubated overnight on a rotator at 50 °C until fully dissolved. The DNA extraction master mix, excluding Proteinase K, was UV-irradiated at 254 nm for 1 h in a cross-linker before use. Once dissolved overnight, 2 mL of solution was concentrated in a Millipore Amicon Ultra-4 30 kDa molecular weight cut-off to a final volume of 100 μL. The concentrated DNA extract was purified using the QIAquick PCR Purification Kit (Qiagen) following manufacturers recommendations, except that the final elution step was performed twice (2 × 50 μL) to produce a final volume of 100 μL. One in five DNA extractions were blank negative controls containing only extraction buffer and Proteinase K.

During replication in Uppsala, the ancient specimens were UV-irradiated at 1 J/cm^2 per side (254-nm wave length) and 1 mm of the surface was removed before powderization. DNA was extracted as in Svensson et al. (21). Next, 40- to 80-mg bone powder was incubated in 1 mL of 0.5M EDTA, pH 8, 1 M Urea, and 100 μg Proteinase K for 22 h at 38 °C together with four negative, blank controls. An additional 100 μg Proteinase K was then added and the samples were incubated for 3 more hours at 55 °C. DNA was further extracted using Qiagen PCR Purification Kit and finally eluted in 100 μL Elution Buffer (Qiagen).

PCR Amplification. A 201-bp mitochondrial control region (CR) fragment (22, 23) and one SNP in each of two autosomal nuclear loci (*BCDO2* and thyroid-stimulating hormone receptor, *TSHR*) were targeted for PCR amplification (Table S6). PCR set-up was performed in a fume hood in a dedicated facility adjacent to the dedicated ancient DNA extraction facility. The PCR set-up facility is subject to positive air pressure that minimizes the risk of introducing contaminant DNA. One in eight PCR reactions were negative controls. In addition, one positive control (a modern GJF) was included for each round of PCR amplifications. To avoid contaminating the ancient DNA PCR reactions with modern GJF DNA, the modern positive control was stored in the dedicated PCR/post-PCR room and added to the reaction tubes once placed on the thermal cycler. PCRs were visualized on a 1–2% (wt/vol) agarose gel using GelRed and UV illumination. PCR products were purified using ExoSAP-IT (USB Affymetrix) and stored at −20 °C before sequencing.

Independent replication of CR sequences and the *TSHR* SNP was performed in Uppsala with slight modifications to the PCR protocol: addition of RSA (rabbit serum albumine) was used instead of BSA (bovine serum albumine) and Smart Taq (Naxo) was used instead of Taq Gold (see below). Apart from following the PCR cycling conditions described below, replicate PCRs for the CR sequence were also performed following Storey et al. (23).

Mitochondrial DNA CR (201 bp). PCRs were setup in 25-μL reactions using 1 U Taq GOLD (Applied Biosystems), 1× Gold buffer (Applied Biosystems), 2.5 mM $MgCl_2$, 0.5 μg/μL BSA, 200 μM of each dNTP, 0.4 μM of each forward and reverse primers, and 2–5 μL of aDNA extract. One in eight PCR reactions were negative blank controls. PCR cycling conditions were 95 °C for 5 min, 50 cycles of 94 °C for 30 s, 54 °C for 30 s, and 72 °C for 30 s, followed by 72 °C for 10 min.

BCDO2. PCRs were set up in 25-μL reactions using 1.0–1.25 U Taq GOLD (Applied Biosystems), 1× Gold buffer (Applied Biosystems), 2.5 mM $MgCl_2$, 0.5 μg/μL BSA, 1 M betaine, 200 μM of each dNTP, 0.4 μM of each primer, and 2–5 μL of ancient DNA extract. One in eight PCR reactions were negative blank controls. PCR cycling conditions were 95 °C for 5 min, 50 cycles of 94 °C for 30 s, 57 °C for 30 s, and 72 °C for 30 s, followed by 72 °C for 10 min.

TSHR. PCRs were set up in 25 μL reactions using 1.0–1.25 U Taq GOLD (Applied Biosystems), 1× Gold buffer (Applied Biosystems), 2.5 mM $MgCl_2$, 0.5 μg/μL BSA, 200 μM of each dNTP, 0.6 μM of the biotinylated forward primer, and 0.8 μM of the reverse primer, and 2–5 μL of aDNA extract. One in eight PCR reactions were negative blank controls. PCR cycling conditions were 95 °C for 5 min, 50 cycles of 94 °C for 30 s, 56 °C for 30 s, and 72 °C for 30 s, followed by 72 °C for 10 min.

DNA Sequencing, Genotyping, and Data Analysis. Sanger sequencing was performed on the Applied Biosystems 3730 DNA Analyzer at the DNA Sequencing Service at the School of Biological and Biomedical Sciences (Durham University). Trace files were manually inspected using 4Peaks (Mekentosj) or Geneious v.5.4 (24) and built into contigs by hand in Se-Al (http://tree.bio.ed.ac.uk/software/seal) or automatically in Geneious v.5.4 using the assembly function (default parameters). At least two (but usually three) independent PCR replicates/sequences (mitochondrial DNA, mtDNA) per sample were performed to ensure authenticity. Sporadic nonreplicable postmortem DNA template damage was observed in several sequences (C→T transitions). This type of damage often results from deamination through hydrolysis resulting in the conversion of cytosine bases to uracil (alternatively hydroxyuracil) or adenine to hypoxanthine. Uracil is read as thymine and hypoxanthine as guanine by DNA polymerases during PCR amplification and subsequently induces the common type 2 (C/T or A/G) damage (20, 25, 26).

Pyrosequencing was performed in-house at the Archaeology department in Durham using the PyroMark Q24 (Qiagen) following manufacturers guidelines, and using Qiagen Q24 sequencing reagent kits. Results, sequences, and genotypes were analyzed in the PyroMark Q24 software (Qiagen) using modified settings: accepted peak deviation and minimum peak heights were set to less strict to account for low signal intensity and slight deviations in peak heights (which, if observed, could be the result of Type-2 C→U deamination/error). Dispensation order was automatically generated using the PyroMark Q24 software (Qiagen) (Table S6).

To account for allelic dropout that is common in ancient DNA studies (21), each SNP/genotype was confirmed by repeated genotyping from two to eight independent PCRs (at least two independent replications for heterozygous specimens, but up to eight replications in for homozygous specimens and heterozygous specimens for which we repeatedly observed allelic dropout). The probability of falsely assigning a heterozygous individual as homozygous was calculated as follows: $P(\text{false homozygote}) = K \times (K/2)^{n-1}$, where n is the number of replicates and K is the observed number of allelic dropouts divided by the total number of genotypings of heterozygous individuals (21, 27).

Fisher's exact test, as implemented in R v.2.15.2 (28), was used to test for differences in allele (and mtDNA CR haplotype) frequencies between modern and ancient populations. In addition, binomial probability distributions (the *pdbinom, dbinom*, and *gbinom* functions in R v.2.15.2) were used to examine differences in allele frequencies between ancient and modern populations and to authenticate the ancient DNA results, assuming that putative contamination would reflect genotype frequencies in modern populations (Fig. S2 and Table S2).

In Uppsala, the TSHR SNP was genotyped on a PSQ 96MA (Biotage), using pyrosequencing technology and the SNP software and SNP reagent kit (Biotage). Sample preparation was performed according to Pyrosequencing instructions using 25 µL of PCR product.

Phylogenetic Reconstruction. An alignment of previously published mtDNA CR haplotypes (29, 30) was used as a reference to identify haplotypes (Table S4 depicts unique E-clade haplotypes). Modern reference and ancient DNA consensus sequences were aligned in Geneious v.5.4 (24) using MAFFT (31). Phylogentic analysis was performed using PhyML v.3.0 (32) as implemented in Geneious v.5.4 (24). The nucleotide substitution model was estimated in using MrModeltest 2.3 (33) and PAUP* 4.0 (34), as implemented in MrMtGui (35). The best-fit model using both adjusted likelihood ratio test statistics and Akaike Information Criterion was HKY+I+G. Nodal support values were estimated through bootstrapping. A *Gallus gallus bankiva* sequence (AB007718) was used as an outgroup.

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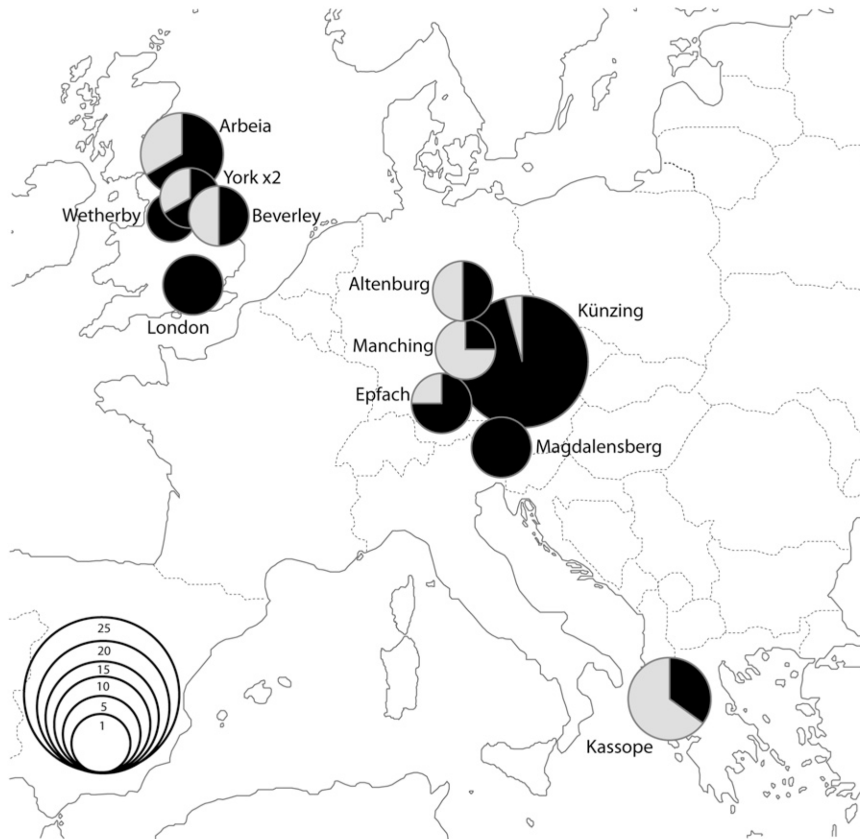

Fig. S1. A map depicting archaeological sites, the number of sampled specimens and DNA retrieval success rate (success indicated as at least one reproducible genotype/specimen) (Table S1). Black represents success, gray represents failure.

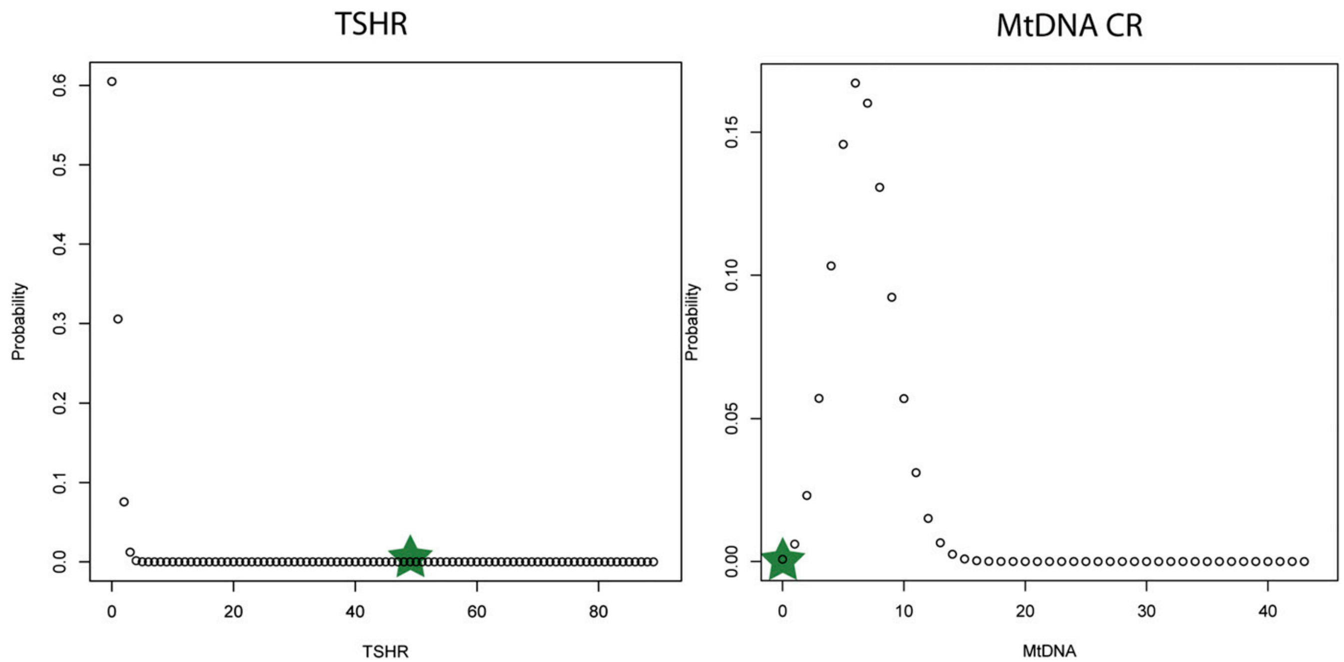

Fig. S2. A figure depicting the number of expected *TSHR* wild type alleles in a sample of 44 (corresponding to the biallelic genotypes obtained from the ancient European samples) given frequencies reported previously for modern chickens (1) (Table S2), and the expected number of non–E-clade mtDNA haplotypes in a sample of 43 (representing the ancient European chickens for which we have available mtDNA CR haplotypes) given the frequencies reported previously for modern European populations (~15%) (2–4) (Table S5). The green stars depict the empirically observed frequencies in the ancient European samples (Tables S2 and S5).

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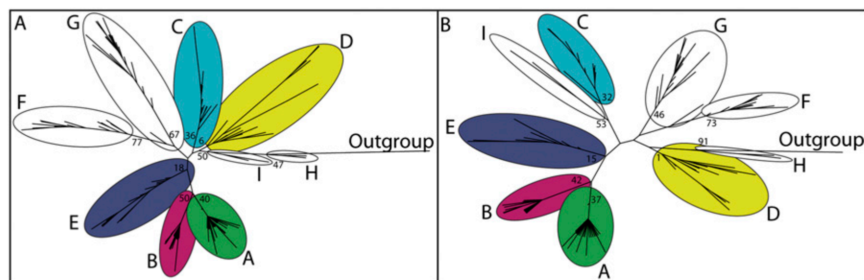

Fig. S3. (*A*) A maximum-likelihood tree constructed using the haplotypes reported by Liu et al. (30). Numbers indicate bootstrap support values. (*B*) A maximum-likelihood tree constructed using the 201-bp (23) mtDNA CR haplotypes of Liu et al. (30) amplified for the ancient European specimens (*SI Materials and Methods*, and Tables S4 and S6).

Table S1. Archaeological chicken bones investigated for this study

Sample	Reference ID	Country	Site	Cultural context	Dates	Contact	mtDNA (GenBank accession)	*TSHR*	*BCDO2*
Ch1	Nr D553771 ob 1513 A	Germany	Quintana/Künzing	Roman	Second–third century A.D.	J.P.	E* (KF753251)		
Ch2	Nr D553771 ob 1513 A	Germany	Quintana/Künzing	Roman	Second–third century A.D.	J.P.	E* (KF753252)		
Ch3†	Nr D53772 ob 1513B	Germany	Quintana/Künzing	Roman	Second–third century A.D.	J.P.	E* (KF753253)	W/W	GJF/RJF
Ch4†	Nr D53772 ob 1513B	Germany	Quintana/Künzing	Roman	Second–third century A.D.	J.P.	E* (KF753254)	S/S	RJF/RJF
Ch5	Nr D53830 ob 1513 A	Germany	Quintana/Künzing	Roman	Second–third century A.D.	J.P.	E* (KF753255)		
Ch6	Nr D53739 ob 1509	Germany	Quintana/Künzing	Roman	Second–third century A.D.	J.P.			
Ch7†	Nr D53760 ob 1513 A	Germany	Quintana/Künzing	Roman	Second–third century A.D.	J.P.	E* (KF753256)	S/S	
Ch8	Nr D53760 ob 1513 A	Germany	Quintana/Künzing	Roman	Second–third century A.D.	J.P.	E* (KF753257)		
Ch9	Nr D53760 ob 1513 A	Germany	Quintana/Künzing	Roman	Second–third century A.D.	J.P.	E* (KF753258)		
Ch10†	Nr D53771 ob 1513 A	Germany	Quintana/Künzing	Roman	Second–third century A.D.	J.P.	E* (KF753259)	W/W	RJF/RJF
Ch11	Nr D53772 ob 1513 B	Germany	Quintana/Künzing	Roman	Second–third century A.D.	J.P.	E* (KF753260)		
Ch12	Nr D53772 ob 1513 B	Germany	Quintana/Künzing	Roman	Second–third century A.D.	J.P.	E* (KF753261)	W/S	GJF/RJF
Ch13†	Nr D53772 ob 1513 B	Germany	Quintana/Künzing	Roman	Second–third century A.D.	J.P.	E* (KF753262)	W/S	RJF/RJF
Ch14	Nr D53772 ob 1513 B	Germany	Quintana/Künzing	Roman	Second–third century A.D.	J.P.	E* (KF753263)	W/W	
Ch15	Nr D53772 ob 1513 B	Germany	Quintana/Künzing	Roman	Second–third century A.D.	J.P.	E* (KF753264)	W/S	RJF/RJF
Ch16	Nr D53772 ob 1513 B	Germany	Quintana/Künzing	Roman	Second–third century A.D.	J.P.	E* (KF753265)	W/S	RJF/RJF
Ch17	Nr D53772 ob 1513 B	Germany	Quintana/Künzing	Roman	Second–third century A.D.	J.P.	E* (KF753266)	W/S	
Ch18	Nr D53884 ob 1509 B	Germany	Quintana/Künzing	Roman	Second–third century A.D.	J.P.	E* (KF753267)		
Ch19	Nr D53858 ob 1509	Germany	Quintana/Künzing	Roman	Second–third century A.D.	J.P.	E* (KF753268)	S/S	GJF/RJF
Ch20	Nr D53858 ob 1509	Germany	Quintana/Künzing	Roman	Second–third century A.D.	J.P.	E* (KF753269)	W/S	RJF/RJF
Ch21	Nr D53771 ob 1513 A	Germany	Quintana/Künzing	Roman	Second–third century A.D.	J.P.	E* (KF753270)	W/W	RJF/RJF
Ch22	Nr D53771 ob 1513 A	Germany	Quintana/Künzing	Roman	Second–third century A.D.	J.P.		W/S	
Ch23	Nr D53771 ob 1513 A	Germany	Quintana/Künzing	Roman	Second - third century A.D.	J.P.		W/W	
Ch24	Nr D53771 ob 1513 A	Germany	Quintana/Künzing	Roman	Second–third century A.D.	J.P.	E* (KF753271)	W/S	
Ch25	56/1121	Germany	Abodiacum, Epfach	Early Roman	First–fifth century A.D.	J.P.	E* (KF753272)	W/S	
Ch26	56/554	Germany	Abodiacum, Epfach	Early Roman	First–fifth century A.D.	J.P.		W/W	
Ch27†	56/1009	Germany	Abodiacum, Epfach	Early Roman	First–fifth century A.D.	J.P.	E* (KF753273)	W/S	RJF/RJF
Ch28	56/1121	Germany	Abodiacum, Epfach	Early Roman	First–fifth century A.D.	J.P.			
Ch29	?/367	Germany	Manching	Iron Age La Tène C and D	200–30 B.C.	J.P.			
Ch30	62/336	Germany	Manching	Iron Age La Tène C and D	200–30 B.C.	J.P.			
Ch31†	239	Germany	Manching	Iron Age La Tène C and D	200–30 B.C.	J.P.	E* (KF753274)	W/W	RJF/RJF
Ch32	395	Germany	Manching	Iron Age La Tène C and D	200–30 B.C.	J.P.			
Ch33†	55	Austria	Magdalensberg	Early Roman	100 B.C.–A.D.50	J.P.	E* (KF753275)	W/S	
Ch34	24	Austria	Magdalensberg	Early Roman	100 B.C.–A.D. 50	J.P.		W/S	
Ch35	3	Austria	Magdalensberg	Early Roman	100 B.C.–A.D.50	J.P.		S/S	
Ch36	38	Austria	Magdalensberg	Early Roman	100 B.C.– A.D.50	J.P.		W/S	RJF/RJF
Ch37	A140-431	Germany	Altenburg	Iron Age La Tène C and D	280–15 B.C.	J.P.		W/S	
Ch38‡	A115-406	Germany	Altenburg	Postindustrial	150 ± 30 cal. B.P.	J.P.	E* (KF753276)	S/S	GJF/GJF
Ch39	A147-439	Germany	Altenburg	Iron Age La Tène C and D	280–15 B.C.	J.P.			
Ch40	A46-202	Germany	Altenburg	Iron Age La Tène C and D	280–15 B.C.	J.P.		W/S	
RB367	8209/5589	England	Arbeia, South Shields	Roman	A.D. 120–400	A.C.			
RB368†	2413/5589	England	Arbeia, South Shields	Roman	A.D. 120–400	A.C.	E* (KF753277)	W/W	GJF/RJF
RB369	712/5589	England	Arbeia, South Shields	Roman	A.D. 120–400	A.C.	E* (KF753278)	W/W	RJF/RJF
RB370†	3522/89	England	Arbeia, South Shields	Roman	A.D. 120–400	A.C.	E* (KF753279)	W/W	RJF/RJF
RB371	702/5589	England	Arbeia, South Shields	Roman	A.D. 120–400	A.C.			
RB372	219/5589	England	Arbeia, South Shields	Roman	A.D. 120–400	A.C.	E* (KF753280)	W/S	

Table S1. Cont.

Sample	Reference ID	Country	Site	Cultural context	Dates	Contact	mtDNA (GenBank accession)	*TSHR*	*BCDO2*
RB373	2, (MFI87) context [156]	England	London, DLR	Roman, Flavian period	A.D. 69–96	F.G.		W/W	
RB374†	12, (MFI87) context [156]	England	London, DLR	Roman, Flavian period	A.D. 69–96	F.G.		W/S	GJF/RJF
RB375	5, (MFI87) context [156]	England	London, DLR	Roman, Flavian period	A.D. 69–96	F.G.		W/S	RJF/RJF
RB376	11, (MFI87) context [156]	England	London, DLR	Roman, Flavian period	A.D. 69–96	F.G.		W/S	RJF/RJF
RB378	SLW04 [169]	England	Scott Lane, Wetherby	Medieval	11th/12th century A.D.	D.D.	E* (KF753281)	W/W	
RB379	1995.434 [114]	England	St. Saviourgate, York	Medieval	14th/15th century A.D.	D.D.			
RB380†	1995.434 [115]	England	St. Saviourgate, York	Medieval	14th/15th century A.D.	D.D.		W/S	RJF/RJF
RB381	2000.584 [3029]	England	Spurriergate, York	Medieval	11th/12th century A.D.	D.D.	E6 (KF753287)	W/W	
RB382	2000.584 [7004]	England	Spurriergate, York	Medieval	10th/14th century A.D.	D.D.			
RB383	2000.584 [3080]	England	Spurriergate, York	Medieval	10th/11th century A.D.	D.D.		W/W	
RB384	2000.584 [3757]	England	Spurriergate, York	Medieval	12th/14th century A.D.	D.D.	E6 (KF753288)	S/S	
RB385	[1146]	England	Playhouse, Beverley	Postmedieval	16th-18th century A.D.	D.D.		W/S	
RB386	[1216]	England	Playhouse, Beverley	Medieval	12th-15th century A.D.	D.D.			
RB387	[1209]	England	Playhouse, Beverley	Medieval	12th-15th century A.D.	D.D.			
RB388	[1109]	England	Playhouse, Beverley	Postmedieval	16th-18th century A.D.	D.D.	E3 (KF753289)	W/S	
RA6	H3-Huhn-sca1	Greece	Kassope	Late Hellenistic/Early Roman	200–40 B.C.	J.P.	E* (KF753282)	S/S	RJF/RJF
RA7	H3-Huhn-sca2	Greece	Kassope	Late Hellenistic/Early Roman	200–40 B.C.	J.P.			
RA8	H3-Huhn-sca3	Greece	Kassope	Late Hellenistic/Early Roman	200–40 B.C.	J.P.			
RA9	H3-Huhn-sca4	Greece	Kassope	Late Hellenistic/Early Roman	200–40 B.C.	J.P.			
RA10	H3-Huhn-sca5	Greece	Kassope	Late Hellenistic/Early Roman	200–40 B.C.	J.P.	E* (KF753283)		
RA11	H3-Huhn-cor1	Greece	Kassope	Late Hellenistic/Early Roman	200–40 B.C.	J.P.			RJF/RJF
RA12	H3-Huhn-cor2	Greece	Kassope	Late Hellenistic/Early Roman	200–40 B.C.	J.P.			
RA13	H3-Huhn-cor3	Greece	Kassope	Late Hellenistic/Early Roman	200–40 B.C.	J.P.			
RA14	H3-Huhn-cor4	Greece	Kassope	Late Hellenistic/Early Roman	200–40 B.C.	J.P.			RJF/RJF
RA15	H3-Huhn-cor5	Greece	Kassope	Late Hellenistic/Early Roman	200–40 B.C.	J.P.			
RA16	H3-Huhn-cor6	Greece	Kassope	Late Hellenistic/Early Roman	200–40 B.C.	J.P.			
RA17	H3-Huhn-tmt1	Greece	Kassope	Late Hellenistic/Early Roman	200–40 B.C.	J.P.			
RA18	H3-Huhn-tmt2	Greece	Kassope	Late Hellenistic/Early Roman	200–40 B.C.	J.P.	E* (KF753284)	S/S	RJF/RJF
RA19	H3-Huhn-tmt3	Greece	Kassope	Late Hellenistic/Early Roman	200–40 B.C.	J.P.			
RA20	H3-Huhn-tmt4	Greece	Kassope	Late Hellenistic/Early Roman	200–40 B.C.	J.P.	E* (KF753285)		
RA21	H3-Huhn-tit1	Greece	Kassope	Late Hellenistic/Early Roman	200–40 B.C.	J.P.			
RA22§	H3-Huhn-tit2	Greece	Kassope	Late Hellenistic/Early Roman	200–40 B.C.	J.P.	E* (KF753286)	S/S	RJF/RJF
RA23	H3-Huhn-tit3	Greece	Kassope	Late Hellenistic/Early Roman	200–40 B.C.	J.P.			
RA24	H3-Huhn-tit4	Greece	Kassope	Late Hellenistic/Early Roman	200–40 B.C.	J.P.			
RA25	H3-Huhn-tit5	Greece	Kassope	Late Hellenistic/Early Roman	200–40 B.C.	J.P.			

E*= E1, E5, E12, E15 or E16 according to the haplotype nomenclature of Liu et al. (29). See further information in Table S4. Sources: A.C., Alex Croom, Tyne and Wear Archives and Museums, South Shields, United Kingdom; D.D., Deborah Jaques, Palaeoecology Research Services Ltd, Kingston upon Hull, United Kingdom; F.G., Francis Grew, Museum of London, London; J.P, Joris Peters. Genotype nomenclature: *TSHR*: S, sweep allele; W, wild-type allele; *BCDO2*: GJF, Gray Junglefowl allele (*yellow skin*); RJF, Red Junglefowl allele.

†Independently replicated in Uppsala for *TSHR* and the mtDNA CR.

‡Directly radiocarbon dated to 150 ± 30 cal. B.P. (2 σ calibration).

§Directly radiocarbon dated to 2150–1990 cal. B.P. (2 σ calibration).

Table S2. Genotype frequencies in ancient and modern populations

Populaton type	*TSHR*			*BCDO2* [SNP B in Eriksson et al. (1)]		
	W/W	W/S	S/S	GJF/GJF	GJF/RJF	RJF/RJF
Modern [Eriksson et al. (1) and Rubin et al., (2)]	0	7	264	73	1	36
Roman England	4	4	0	0	2	4
Medieval and postmedieval England	3	3	1	0	0	1
Iron Age and Roman Central Europe	7	15	4	0	3	10
Late Hellenistic/Early Roman Greece	0	0	3	0	0	5
Total (ancient)	14	22	8	0	5	20

1. Eriksson J, et al. (2008) Identification of the yellow skin gene reveals a hybrid origin of the domestic chicken. *PLoS Genet* 4(2):e1000010.
2. Rubin C-J, et al. (2010) Whole-genome resequencing reveals loci under selection during chicken domestication. *Nature* 464(7288):587–591.

Table S3. Hardy–Weinberg test on *TSHR* genotypes from Künzing

Genotypes	Observed	Expected
Homozygote reference	5	5.1
Heterozygote	8	7.9
Homozygote variant	3	3.1
Variant (sweep) allele frequency	0.44	

χ^2 = 0.004; χ^2 *P* value with 1 df. = 0.95.

Table S4. E-clade haplotypes from Liu et al. (30) and ancient European haplotypes observed in this study

	Nucleotide position																	
	22	44	199	222	224	243	246	249	256	261	281	330	342	344	355	372	391	446
Reference (A2)	T	C	T	A	T	T	C	A	T	C	A	C	A	A	T	A	C	C
E*	?	?	.	.	.	C	.	.	C	T	.	.	.	.	.	?	?	?
E1 (E*)	.	.	.	.	.	C	.	.	C	T	.	.	.	.	.	.	.	T
E2	.	.	.	.	.	C	.	.	C	.	.	T	.	.	.	.	.	T
E3	.	.	.	.	.	C	.	.	C	T	.	T	.	.	.	.	.	T
RB388	**?**	**?**	.	.	.	**C**	.	.	**C**	**T**	.	**T**	.	.	.	**?**	**?**	**?**
E4	.	.	.	.	.	.	.	.	C	T	.	.	.	.	.	.	.	T
E5 (E*)	.	.	.	.	.	C	.	.	C	T	.	.	.	.	.	.	.	.
E6	.	.	C	.	.	C	.	.	C	T	.	.	.	.	.	.	.	T
RB381	**?**	**?**	**C**	.	.	**C**	.	.	**C**	**T**	.	.	.	.	.	**?**	**?**	**?**
RB384	**?**	**?**	**C**	.	.	**C**	.	.	**C**	**T**	.	.	.	.	.	**?**	**?**	**?**
E7	.	.	C	.	.	C	.	.	.	T	.	.	.	.	.	.	.	T
E8	.	.	.	.	.	C	T	.	C	T	.	.	.	.	.	.	.	T
E9	.	.	.	.	.	C	.	.	C	T	.	.	G	.	.	.	.	T
E10	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	T
E11	.	.	.	G	.	C	.	.	C	T	.	T	.	.	.	.	.	T
E12 (E*)	.	.	.	.	.	C	.	.	C	T	.	.	.	.	.	.	T	T
E13	.	.	.	.	.	C	T	G	C	T	.	.	.	G	C	.	.	T
E14	G	T	.	.	.	C	T	.	C	T	.	.	.	.	.	.	.	T
E15 (E*)	.	.	.	.	.	C	.	.	C	T	.	.	.	.	.	.	A	T
E16 (E*)	.	.	.	.	.	C	.	.	C	T	.	.	.	.	.	G	.	T
E17	.	.	.	.	C	C	T	.	C	T	.	.	.	.	.	.	.	T
E18	.	.	.	G	.	C	.	G	C	T	G	.	.	.	C	.	.	T

The shaded region corresponds to the 201-bp sequence (23) amplified and sequenced for this study. Areas outside the shaded region correspond to the remaining 318-bp region presented by Liu et al. (30). E* = ancient 201-bp mtDNA haplotype (Table S1) and corresponds to several 519-bp E-clade haplotypes reported by Liu et al. (30). Bold haplotypes/specimens correspond to the 201-bp haplotypes amplified and sequenced from ancient European chickens in this study (Table S1). Variable nucleotide positions are highlighted in accordance to Table S1 (haplotype A2) from Liu et al. (30) (corresponding to nucleotide positions in, e.g., GenBank accession no. AY392407).

Table S5. MtDNA haplogroup frequencies in ancient and modern datasets

Source	Hg A	Hg B	Hg C	Hg D	Hg E
Dana et al. (1)	30	15	12	6	323
Liu et al. (2)	4	1	0	0	53
Revay et al. (3)	1	11	0	0	65
Total modern	35	27	12	6	441
Proportion modern	0.07	0.05	0.02	0.01	0.85
(Ancient) Storey et al. (4)	0	0	0	0	5
(Ancient) Present study	0	0	0	0	38
Ancient total	0	0	0	0	43
Proportion ancient	0.00	0.00	0.00	0.00	100.00

1. Dana N, et al. (2010) East Asian contributions to Dutch traditional and western commercial chickens inferred from mtDNA analysis. *Anim Genet* 42(2):125–133.
2. Liu Y-P, et al. (2006) Multiple maternal origins of chickens: Out of the Asian jungles. *Mol Phylogenet Evol* 38(1):12–19.
3. Revay T, Bodzsar N, Mobegi VE, Hanotte O, Hidas A (2010) Origin of Hungarian indigenous chicken breeds inferred from mitochondrial DNA D-loop sequences. *Anim Genet* 41(5): 548–550.
4. Storey AA, et al. (2012) Investigating the global dispersal of chickens in prehistory using ancient mitochondrial DNA signatures. *PLoS ONE* 7(7):e39171.

Table S6. PCR and sequencing primers for the mtDNA CR, *TSHR*, and *BCDO2*

Locus	Primer sequence (5′–3′)	Primer name	Source	Sequence to analyze
MtDNA CR	ACCCATTATATGTATACGGGCATTAA	GG144F	Storey et al. (23)	
MtDNA CR	CGAGCATAACCAAATGGGTTAGA	GG387R	Storey et al. (23)	
TSHR	CTTTCTTCTTGCCCTTTT	TSHR-F (biotin)	Present study	
TSHR	GATGCTGACTTTGCTGTA	TSHR-R	Present study	
TSHR	TGCTGTAGCTGCTGACTC	TSHR-S	Present study	C/TAACCAGTGG
BCDO2	ACTCTTGCATGGATCTGG	BCDO2-F (biotin)	Present study	
BCDO2	TGTGGTCTCAGAATTTGG	BCDO2-R	Present study	
BCDO2	TCAGAATTTGGGACG	BCDO2-S	Present study	C/TTGGCAATGC