

Supplementary Information for Loog et al. (2017) “Inferring allele frequency trajectories from ancient DNA indicates that selection on a chicken gene coincided with changes in medieval husbandry practices”

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Wet Laboratory Procedures and Protocols

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 to commonly used guidelines (Cooper and Poinar 2000; Gilbert et al. 2005). 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 ddH₂O 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.

Ancient DNA Extraction

The ancient chicken bones were prepared for DNA extraction by removing an approximately 1-mm layer of outer bone surface by abrasion using a Dremel drill with clean, single-use cut-off wheels (Dremel no. 409). A subsection of the bone was subsequently isolated and pulverized in a Micro-Dismembrator (Sartorius-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·HCl and 400 µg Proteinase K, and incubated overnight on a rotator at 50 °C until fully dissolved. 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. Approximately 1 in 10 DNA extractions were blank negative controls containing only extraction buffer and Proteinase K.

PCR Amplification. 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. Approximately 1 in 11 PCR reactions were negative controls. BCDO2: PCRs were set up in 25-µL reactions using 1.0–1.25 U Taq GOLD (Applied Biosystems), 1× Gold buffer (Applied Bio- systems), 2.5 mM MgCl₂, 0.5

$\mu\text{g}/\mu\text{L}$ BSA, 1 M betaine, 200 μM of each dNTP, 0.4 μM of each primer, and 2–5 μL of ancient DNA extract. 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 Bio- systems), 2.5 mM MgCl_2 , 0.5 $\mu\text{g}/\mu\text{L}$ 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. 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. 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.

DNA Genotyping

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 S8).

To account for allelic dropout that is common in ancient DNA studies (Svensson et al. 2007), 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 drop-out). 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 (Gagneux et al. 1997; Svensson et al. 2007)

Archeological background for newly genotyped samples

The 14 new samples come from three different archaeological sites located in Morocco and Turkey.

Mogador

Four samples (RB579; RB585; RB585; RB587) come from the site of Île de Mogador in Morocco. The site has three main occupational periods: The first corresponds to a Phoenician inhabitation between the 7th and 5th century BC. The second major occupation phase dates to the Roman period and lasted from the 1st till the 3rd century CE. The chicken samples newly genotyped for this study come from a third period, corresponding to a modern re-occupation, which started in the 17th/18th century and lasted into the 20th century CE. The chicken samples, newly genotyped for this study, are dated to be not older than 50 years (Table S7). General information about the site together with a detailed study of its fauna is available in Becker et al. (2012).

Doliche/Dülük Baba Tepes

Three samples (RB593; RB594; RB595) come from a hill-top site of Doliche/Dülük Baba Tepesi near modern Gaziantep in Southern Anatolia, Turkey. The site, famous for being the place where the cult of Jupiter Dolichenus originated, was occupied from the 6th century BC until the 13th century AD. In modern times (until recently) the site witnessed civilian and military use. The chicken samples, newly genotyped for this study, come from modern refuse overlying antique occupation and are dated to be not older than 50 years (Table S7). Archaeological background of the site and a detailed report containing information about the faunal assemblage can be found in Pöllath and Peters (2011).

Korucutepe/Elazığ

Seven samples (RB598; RB599; RB600; RB601; RB602; RA26; RA27) come from the site on Korucutepe/Elâzığ located in Eastern Anatolia, Turkey. Site occupation started in the second half of the 4th millennium BC and continued during the Bronze and early Iron Ages. Around 800 BC the site was abandoned until its resettlement by Seljuqs some two thousand years later (between 1200 and 1400 AD). After this time period occupation ceased at the site. Samples genotyped for this study have been dated to be around 700 years old (Table S7), thus coinciding with the Seljuk occupation. General information about the site can be found in Boessneck and von den Driesch (1975).

Supplementary Figures

Supplementary Figure 1

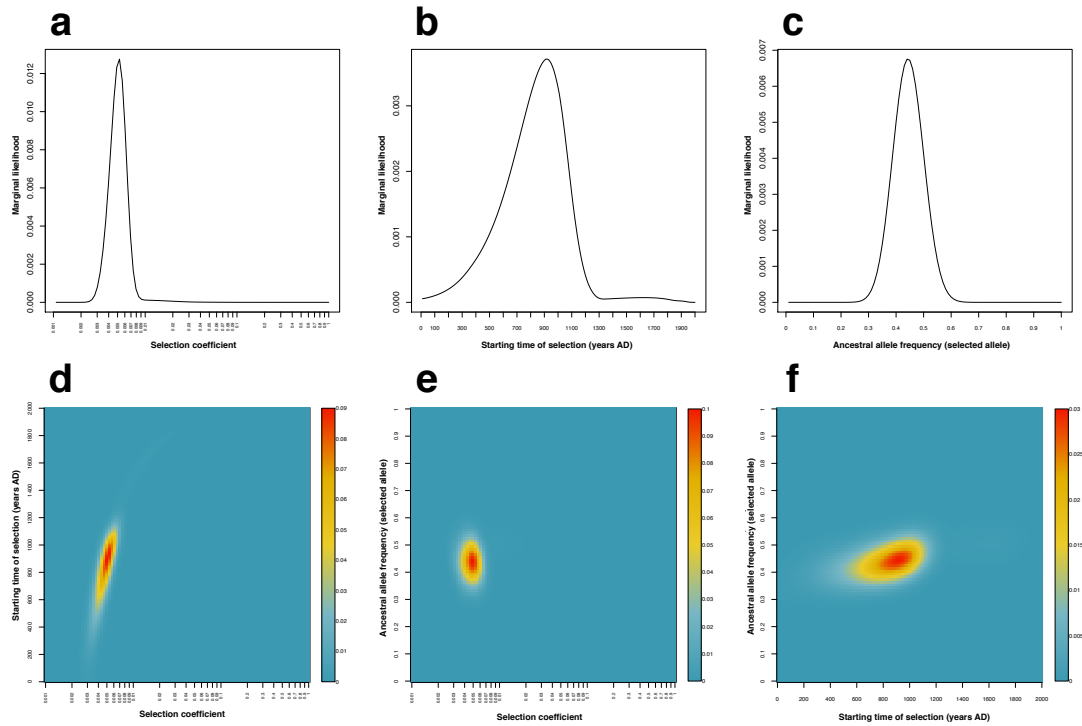


Figure S1. Marginal posterior distribution of parameters for the TSHR locus: selection coefficient (a), starting time of selection (b) and ancestral allele frequency (c). Panels d-f show joint marginal posterior distribution of pairs of parameters.

Supplementary Figure 2

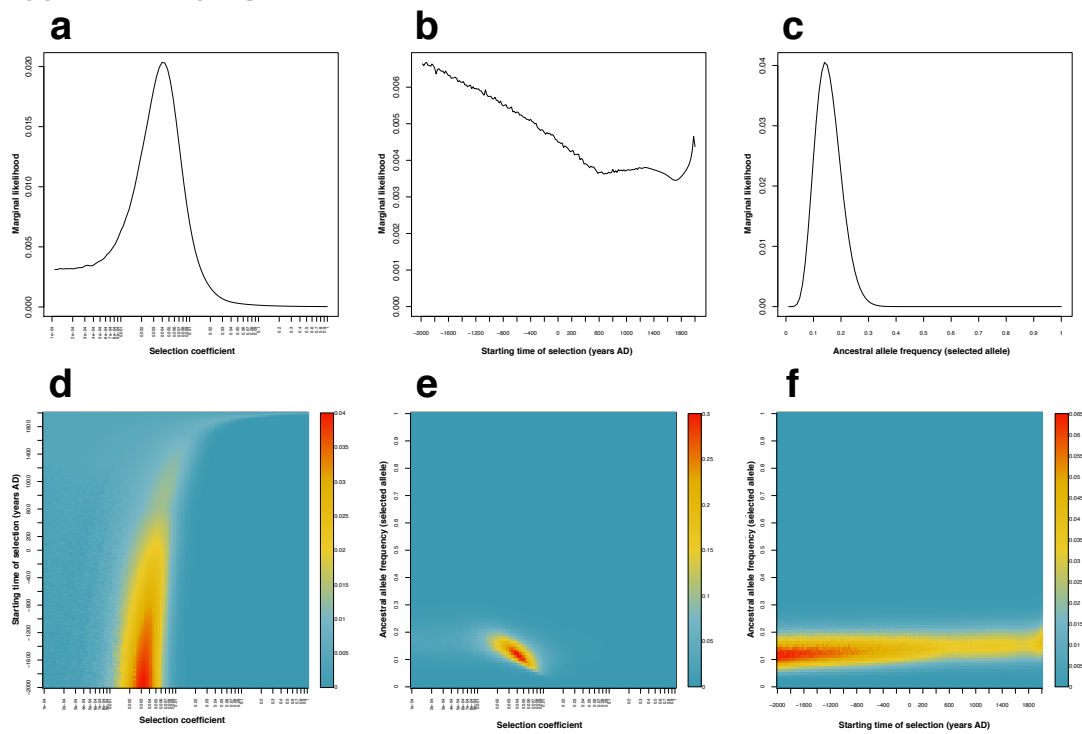


Figure S2. Marginal posterior distribution of parameters for the BCD02 locus: selection coefficient (a), starting time of selection (b) and ancestral allele frequency (c). Panels d-f show the joint marginal posterior distribution of pairs of parameters.

Supplementary Figure 3

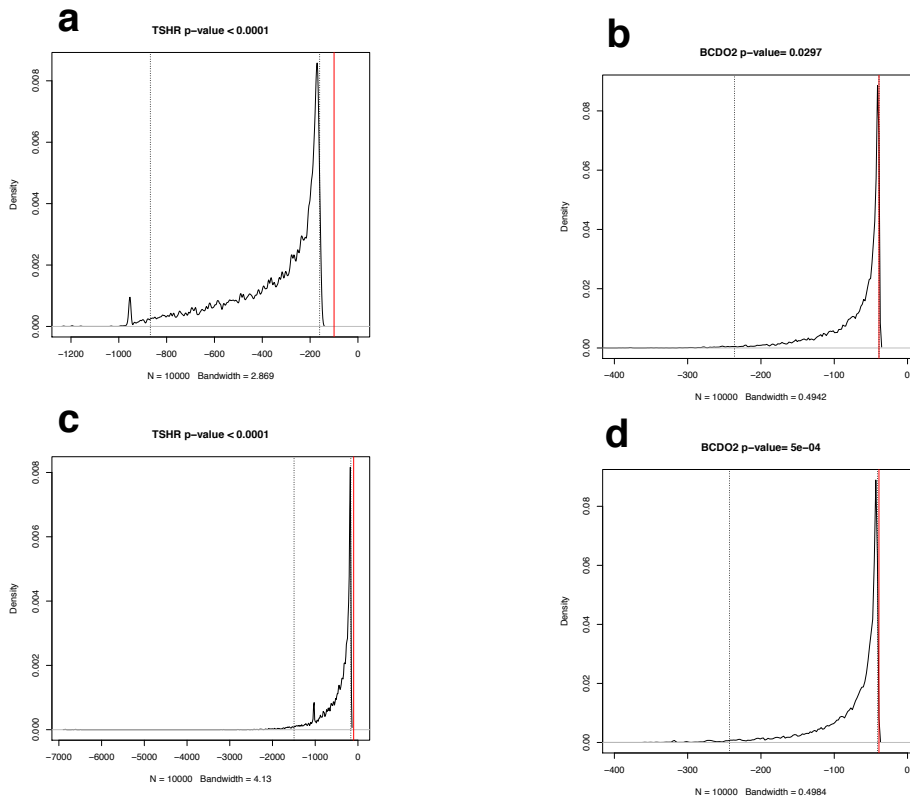


Figure S3. Distribution of data likelihoods for the TSHR locus from 10,000 simulations of random genetic drift with (a) and without (c) gene flow from imported Asian jungle fowl. Panels b and d show the corresponding results for the BCDO2 locus.

Supplementary Figure 4

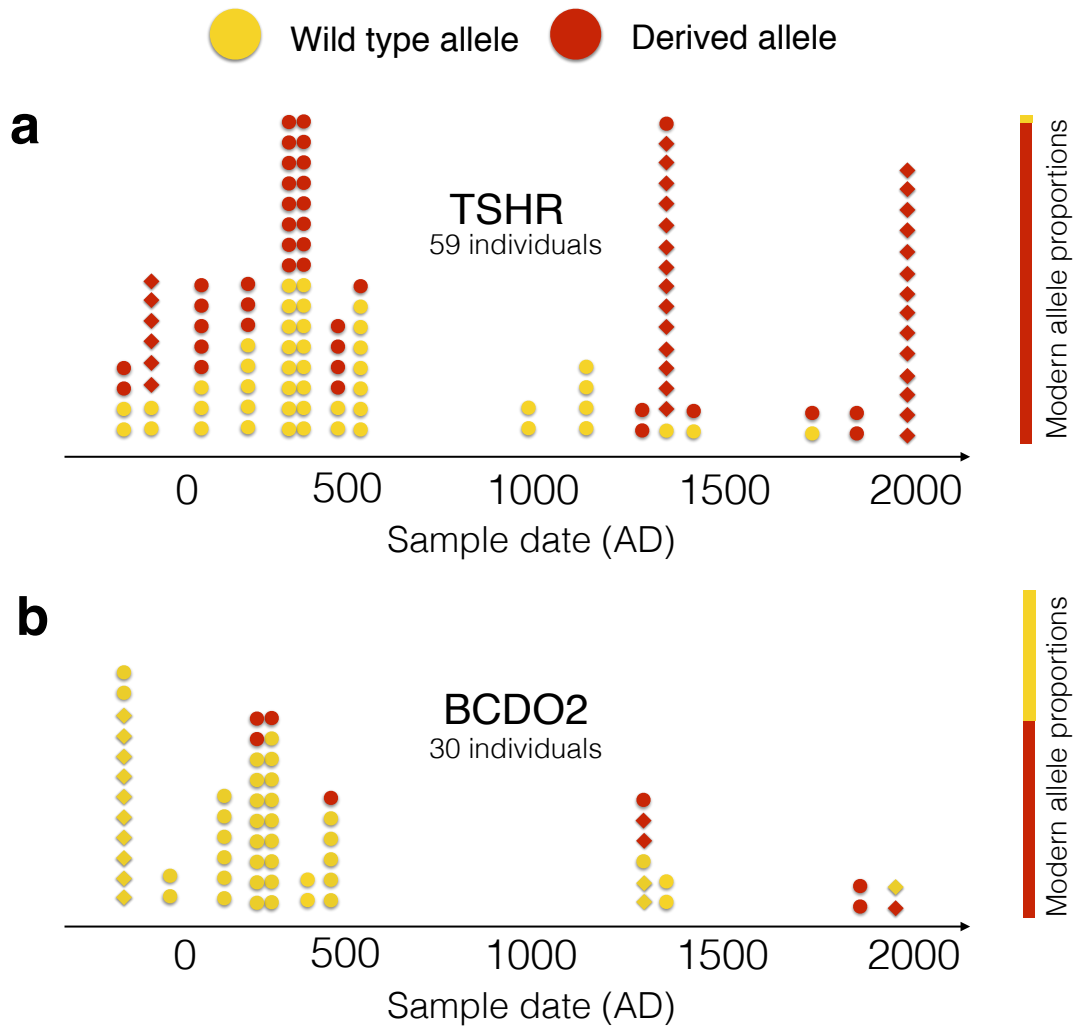


Figure S4: Observed allele counts through time for the TSHR locus **(a)** and the BCDO2 locus **(b)**. Wild type alleles in ancient samples are represented by yellow dots and derived alleles by red dots. Modern allele proportions are shown as solid bars to the right of each panel. Circles represent ancient samples from North-Western Europe and diamonds represent ancient samples from the Mediterranean region.

Supplementary Figure 5

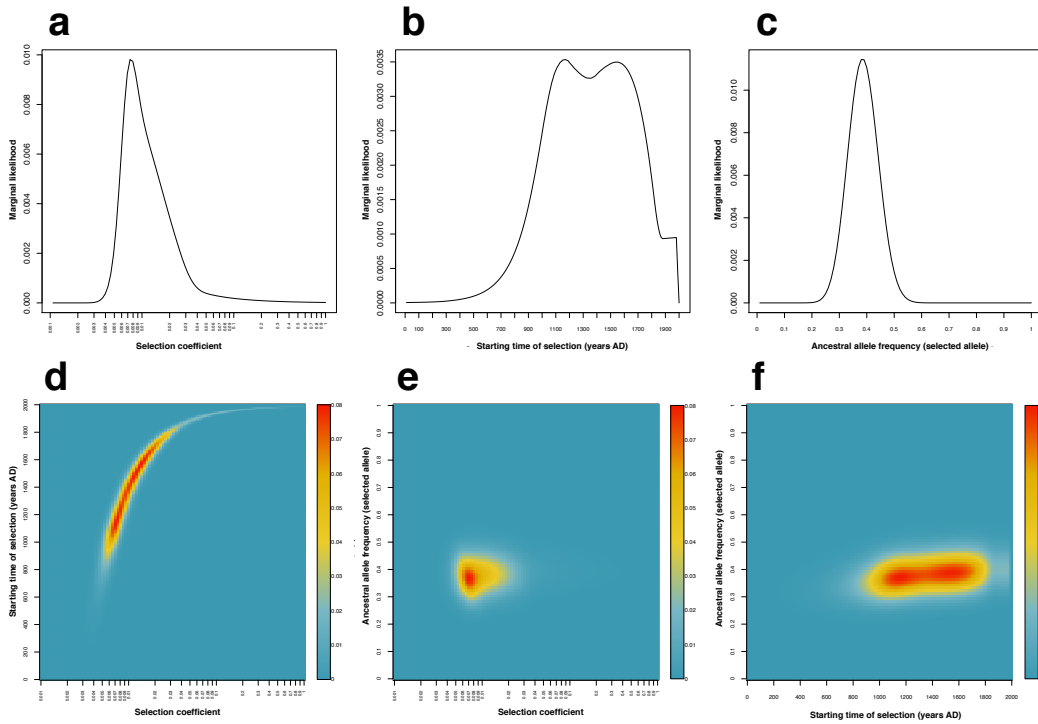


Figure S5. Marginal posterior distribution of parameters for the TSHR locus in the North-Western Europe subset of the data: selection coefficient (**a**), starting time of selection (**b**) and ancestral allele frequency (**c**). Panels **d-f** show joint marginal posterior distribution of pairs of parameters.

Supplementary Figure 6

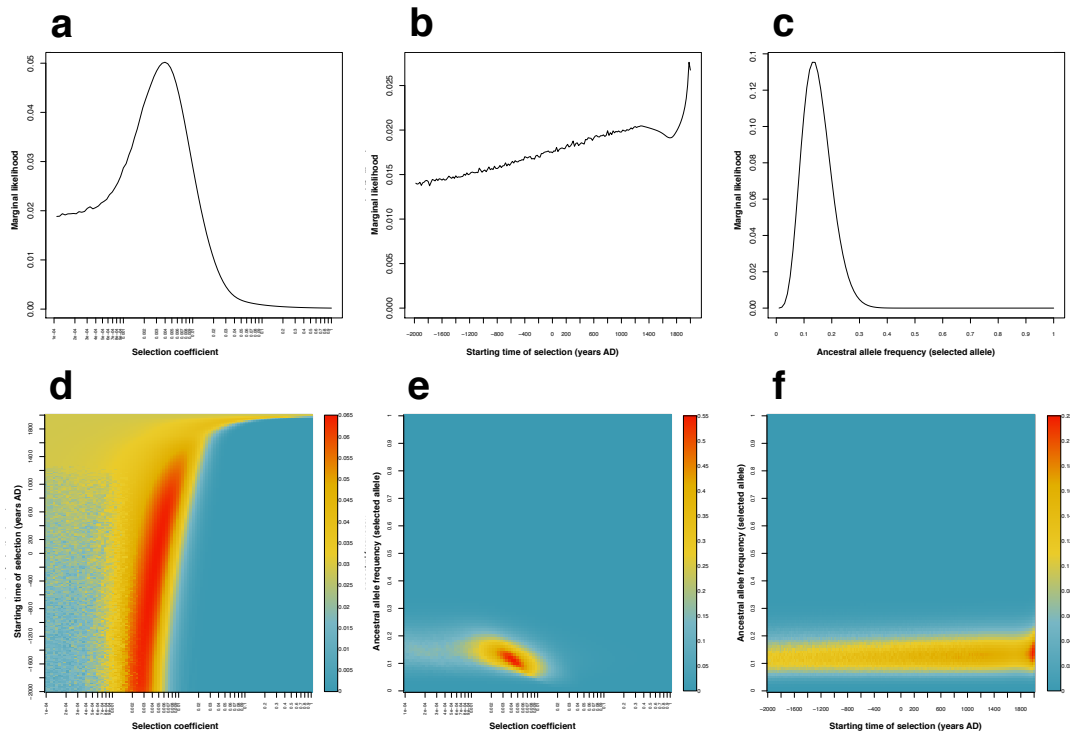


Figure S6. Marginal posterior distribution of parameters for the BCD02 locus in the North-Western Europe subset of the data: selection coefficient (a), starting time of selection (b) and ancestral allele frequency (c). Panels d-f show the joint marginal posterior distribution of pairs of parameters.

Supplementary Figure 7

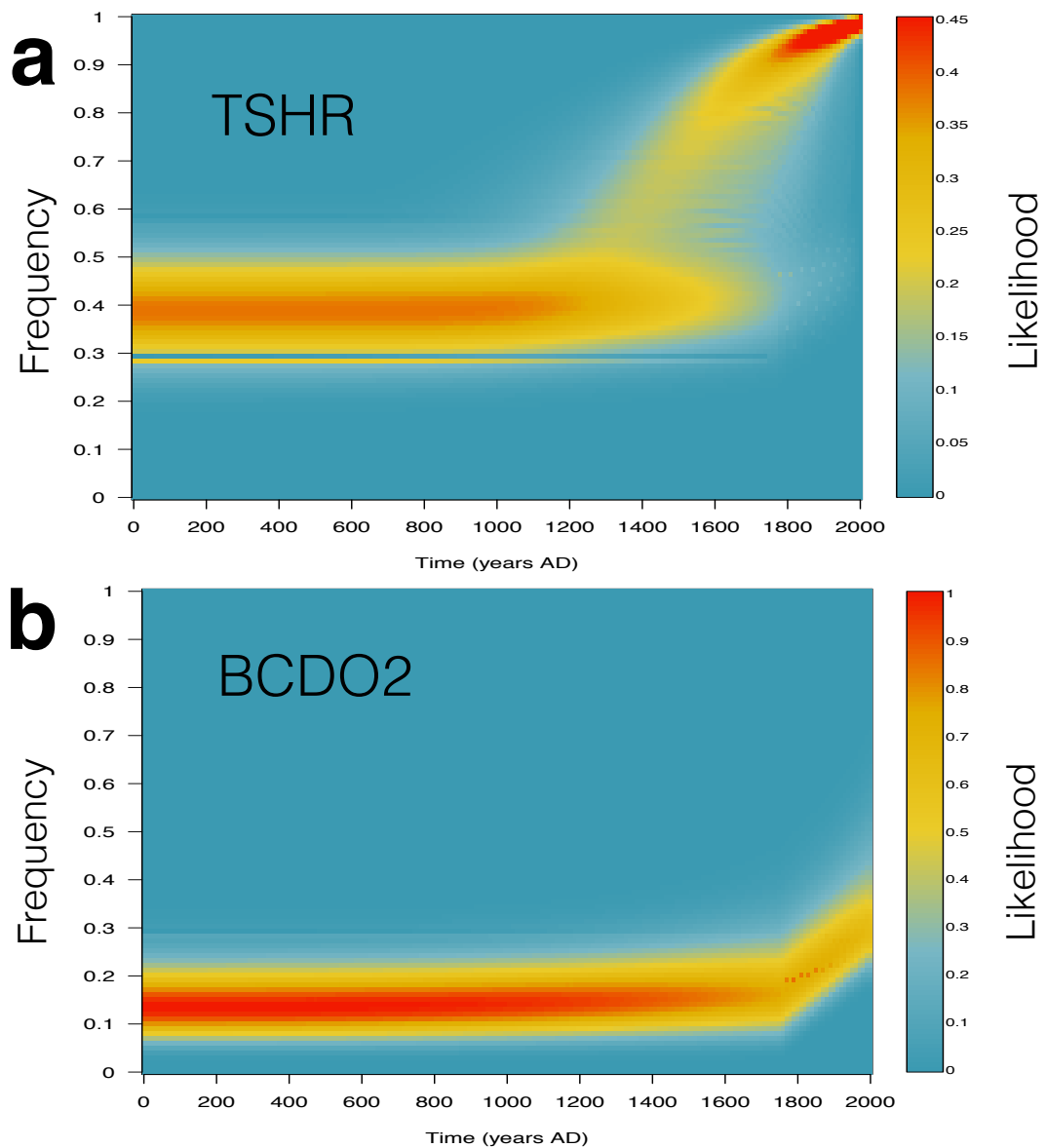


Figure S7: Posterior distribution of the derived allele frequency as a function of time for the TSHR locus (a) and the BCDO2 locus (b) in the North Western Europe subset of the data. The likelihoods are color coded (see color bar) and are shown relative to the maximum likelihood in the plot.

Supplementary Figure 8

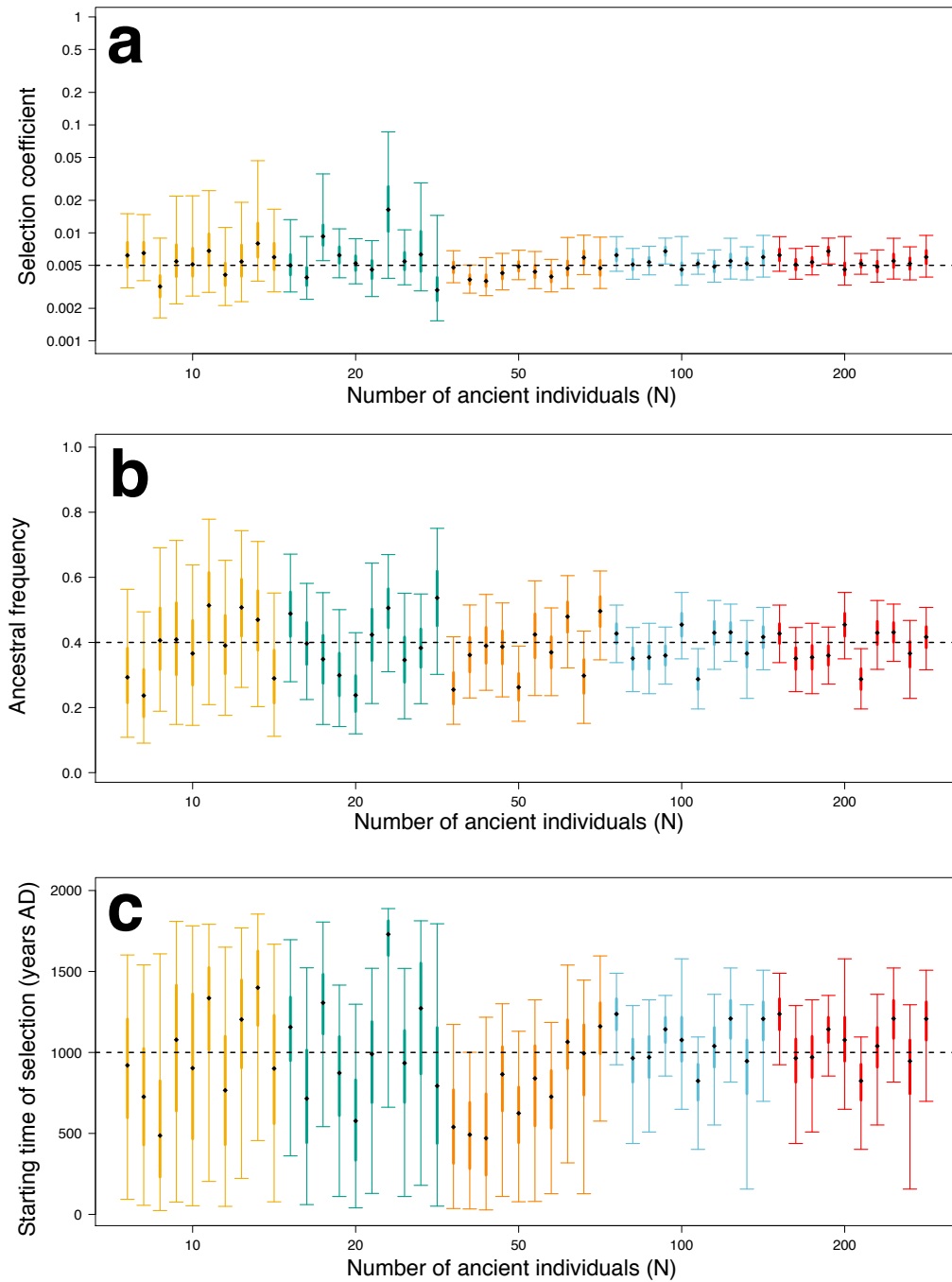


Figure S8: Effect of sample size on the parameter estimates in simulated data. Marginal estimated parameter distributions for selection coefficient (**a**), ancestral derived allele frequency (**b**) and starting time of selection (**c**, in years AD). For each simulated dataset, we show the median (black dot), inter-quartile range (thick line) and symmetric 95% confidence intervals (thin line). Data sets are grouped according to number of simulated ancient individuals (N) and coloured by size: $N=10$ (yellow), $N=20$ (green), $N=50$ (orange), $N=100$ (blue) and $N=200$ (red).

Supplementary Tables

Supplementary Table 1a

| Period (century AD) | Total number of chicken remains | Total number animal remains | Proportion of chicken remains | 95% Confidence Intervals | |
|---------------------|---------------------------------|-----------------------------|-------------------------------|--------------------------|------------|
| 5th to 9th | 8029 | 215655 | 0.037230762 | 0.04137207 | 0.03308946 |
| 9th to 11th | 3856 | 89374 | 0.043144539 | 0.04955772 | 0.03673136 |
| 11th to 12th | 5237 | 71435 | 0.073311402 | 0.0803708 | 0.06625201 |
| 12th to 14th | 6228 | 73729 | 0.084471511 | 0.09137825 | 0.07756478 |

Supplementary Table 1b

| Period (century AD) | Total number of chicken remains | Total number animal remains | Proportion of chicken remains | 95% Confidence Intervals | |
|---------------------|---------------------------------|-----------------------------|-------------------------------|--------------------------|------------|
| 7th to 9th | 45 | 5364 | 0.008389262 | -0.01825982 | 0.03503835 |
| 9th to 11th | 866 | 42353 | 0.020447194 | 0.01102118 | 0.0298732 |
| 11th to 12th | 1456 | 44713 | 0.032563237 | 0.02344627 | 0.04168021 |
| 12th to 14th | 6754 | 191586 | 0.035253098 | 0.03085484 | 0.03965136 |

Table S1. Relative frequency of domestic chicken remains (by number of identified specimens) from 184 English archaeological faunal assemblages (Table S1a) and 104 German archaeological faunal assemblages (Table S1b).

Supplementary Table 2

| ID | Country | Genotype | From (Years AD) | To (Years AD) | Dating Method | Reference |
|-------|---------|----------|-----------------|---------------|---------------|-------------------|
| Ch37 | Germany | GA | -280 | -15 | Context | Flink et al. 2014 |
| Ch40 | Germany | GA | -280 | -15 | Context | Flink et al. 2014 |
| RA6 | Greece | AA | -200 | -40 | Context | Flink et al. 2014 |
| RA18 | Greece | AA | -200 | -40 | Context | Flink et al. 2014 |
| RA22 | Greece | AA | -200 | -40 | Direct | Flink et al. 2014 |
| Ch31 | Germany | GG | -200 | -30 | Context | Flink et al. 2014 |
| Ch33 | Austria | GA | -100 | 50 | Context | Flink et al. 2014 |
| Ch34 | Austria | GA | -100 | 50 | Context | Flink et al. 2014 |
| Ch35 | Austria | AA | -100 | 50 | Context | Flink et al. 2014 |
| Ch36 | Austria | GA | -100 | 50 | Context | Flink et al. 2014 |
| RB373 | England | GG | 69 | 96 | Context | Flink et al. 2014 |
| RB374 | England | GA | 69 | 96 | Context | Flink et al. 2014 |
| RB375 | England | GA | 69 | 96 | Context | Flink et al. 2014 |
| RB376 | England | GA | 69 | 96 | Context | Flink et al. 2014 |
| Ch3 | Germany | GG | 100 | 300 | Context | Flink et al. 2014 |
| Ch4 | Germany | AA | 100 | 300 | Context | Flink et al. 2014 |
| Ch7 | Germany | AA | 100 | 300 | Context | Flink et al. 2014 |
| Ch10 | Germany | GG | 100 | 300 | Context | Flink et al. 2014 |
| Ch12 | Germany | GA | 100 | 300 | Context | Flink et al. 2014 |
| Ch13 | Germany | GA | 100 | 300 | Context | Flink et al. 2014 |
| Ch14 | Germany | GG | 100 | 300 | Context | Flink et al. 2014 |
| Ch15 | Germany | GA | 100 | 300 | Context | Flink et al. 2014 |

| | | | | | | |
|-------|---------|----|------|------|---------|-------------------|
| Ch16 | Germany | GA | 100 | 300 | Context | Flink et al. 2014 |
| Ch17 | Germany | GA | 100 | 300 | Context | Flink et al. 2014 |
| Ch19 | Germany | AA | 100 | 300 | Context | Flink et al. 2014 |
| Ch20 | Germany | GA | 100 | 300 | Context | Flink et al. 2014 |
| Ch21 | Germany | GG | 100 | 300 | Context | Flink et al. 2014 |
| Ch22 | Germany | GA | 100 | 300 | Context | Flink et al. 2014 |
| Ch23 | Germany | GG | 100 | 300 | Context | Flink et al. 2014 |
| Ch24 | Germany | GA | 100 | 300 | Context | Flink et al. 2014 |
| Ch25 | Germany | GA | 0 | 500 | Context | Flink et al. 2014 |
| Ch26 | Germany | GG | 0 | 500 | Context | Flink et al. 2014 |
| Ch27 | Germany | GA | 0 | 500 | Context | Flink et al. 2014 |
| RB368 | England | GG | 120 | 400 | Context | Flink et al. 2014 |
| RB369 | England | GG | 120 | 400 | Context | Flink et al. 2014 |
| RB370 | England | GG | 120 | 400 | Context | Flink et al. 2014 |
| RB372 | England | GA | 120 | 400 | Context | Flink et al. 2014 |
| RB383 | England | GG | 900 | 1100 | Context | Flink et al. 2014 |
| RB378 | England | GG | 1000 | 1200 | Context | Flink et al. 2014 |
| RB381 | England | GG | 1000 | 1200 | Context | Flink et al. 2014 |
| RB384 | England | AA | 1100 | 1400 | Context | Flink et al. 2014 |
| RB388 | England | GA | 1100 | 1500 | Context | Flink et al. 2014 |
| RB599 | Turkey | AA | 1272 | 1334 | Direct | This study |
| RB600 | Turkey | AA | 1275 | 1337 | Context | This study |
| RB601 | Turkey | AA | 1275 | 1337 | Context | This study |
| RB602 | Turkey | AA | 1275 | 1337 | Context | This study |
| RA26 | Turkey | AA | 1275 | 1337 | Context | This study |
| RA27 | Turkey | AA | 1275 | 1337 | Context | This study |
| RB598 | Turkey | AA | 1278 | 1340 | Direct | This study |
| RB380 | England | GA | 1300 | 1500 | Context | Flink et al. 2014 |
| RB385 | England | GA | 1500 | 1800 | Context | Flink et al. 2014 |
| Ch38 | Germany | AA | 1820 | 1880 | Direct | Flink et al. 2014 |
| RB579 | Morocco | AA | 1950 | 2000 | Direct | This study |
| RB585 | Morocco | AA | 1950 | 2000 | Context | This study |
| RB586 | Morocco | AA | 1950 | 2000 | Direct | This study |
| RB587 | Morocco | AA | 1950 | 2000 | Context | This study |
| RB593 | Turkey | AA | 1950 | 2000 | Context | This study |
| RB594 | Turkey | AA | 1950 | 2000 | Context | This study |
| RB595 | Turkey | AA | 1950 | 2000 | Context | This study |

Table S2. List of ancient samples used in the analysis of the TSHR locus (position 43,250,347 on chromosome 5). G is the ancestral allele and A is the derived allele.

Supplementary Table 3

| ID | Country | Genotype | From (Years AD) | To (Years AD) | Dating Method | Reference |
|-----------|----------------|-----------------|----------------------------|--------------------------|--------------------------|-------------------|
| RA6 | Greece | AA | -200 | -40 | Context | Flink et al. 2014 |
| RA11 | Greece | AA | -200 | -40 | Context | Flink et al. 2014 |
| RA14 | Greece | AA | -200 | -40 | Context | Flink et al. 2014 |
| RA18 | Greece | AA | -200 | -40 | Context | Flink et al. 2014 |
| RA22 | Greece | AA | -200 | -40 | Context | Flink et al. 2014 |
| Ch31 | Germany | AA | -200 | -30 | Context | Flink et al. 2014 |
| Ch36 | Austria | AA | -100 | 50 | Context | Flink et al. 2014 |
| RB374 | England | GA | 69 | 96 | Context | Flink et al. 2014 |
| RB375 | England | AA | 69 | 96 | Context | Flink et al. 2014 |
| RB376 | England | AA | 69 | 96 | Context | Flink et al. 2014 |
| Ch3 | Germany | GA | 100 | 300 | Context | Flink et al. 2014 |
| Ch4 | Germany | AA | 100 | 300 | Context | Flink et al. 2014 |
| Ch10 | Germany | AA | 100 | 300 | Context | Flink et al. 2014 |
| Ch12 | Germany | GA | 100 | 300 | Context | Flink et al. 2014 |
| Ch13 | Germany | AA | 100 | 300 | Context | Flink et al. 2014 |
| Ch15 | Germany | AA | 100 | 300 | Context | Flink et al. 2014 |
| Ch16 | Germany | AA | 100 | 300 | Context | Flink et al. 2014 |
| Ch19 | Germany | GA | 100 | 300 | Context | Flink et al. 2014 |
| Ch20 | Germany | AA | 100 | 300 | Context | Flink et al. 2014 |
| Ch21 | Germany | AA | 100 | 300 | Context | Flink et al. 2014 |
| Ch27 | Germany | AA | 0 | 500 | Context | Flink et al. 2014 |
| RB368 | England | GA | 120 | 400 | Context | Flink et al. 2014 |
| RB369 | England | AA | 120 | 400 | Context | Flink et al. 2014 |
| RB370 | England | AA | 120 | 400 | Context | Flink et al. 2014 |
| RA26 | Turkey | AA | 1275 | 1337 | Context | This study |
| RA27 | Turkey | GG | 1275 | 1337 | Context | This study |
| RB598 | Turkey | GA | 1278 | 1340 | Context | This study |
| RB380 | England | AA | 1300 | 1500 | Context | Flink et al. 2014 |
| Ch38 | Germany | GG | 1820 | 1880 | Context | Flink et al. 2014 |
| RB579 | Morocco | GA | 1950 | 2000 | Context | This study |

Table S3. List of ancient samples used in the analysis of the BCDO2 locus (position 6,273,428 on chromosome 24). A is the ancestral allele and G is the derived allele.

Supplementary Table 4

| Breed | Origin | Samples in pool | Frequency |
|---------------------------------|----------------|-----------------|------------------------------|
| White Leghorn Line 13 (WL-A) | Sweden | 11 | 1 |
| Rhode Island Red | Netherlands | 10 | 1 |
| Rhode Island Red (RIR) | France | 8 | 1 |
| Jaärhöns | Norway | 8 | 1 |
| Poltava clay | Ukraine | 8 | 1 |
| Dorking | UK | 3 | 0.67 |
| Broiler Dam Line B (CB-2) | France | 9 | 1 |
| Broiler Sire Line B | France | 10 | 1 |
| Bourbonnaise | France | 8 | 1 |
| Coucou de Rennes | France | 7 | 1 |
| Czech Golden Pencilled | Czech Republic | 5 | 1 |
| Finnish Landrace | Finland | 5 | 1 |
| Friesian fowl | Netherlands | 5 | 1 |
| Houdan | France | 5 | 1 |
| Marans | France | 8 | 1 |
| Owl-bearded | Netherlands | 8 | 1 |
| Red Villafranguina | Spain | 5 | 1 |
| Transsylvanian Naked Neck | Hungary | 5 | 1 |
| Westfa_lischer Totleger | Germany | 3 | 0.67 |
| Yurlov crower | Russia | 4 | 1 |
| Icelandic | Iceland | 12 | 0.92 |
| Inhibition of Gold | France | 3 | 1 |
| Coucou du Vercors | France | 5 | 1 |
| SASSO | France | 12 | 1 |
| Total nr of individuals: | | 167 | Total frequency: 0.98 |

Table S4. Frequency of the TSHR derived allele (position 43,250,347 on chromosome 5) in modern European breeds. (Data from (Rubin et al. 2010))

Supplementary Table 5

| Breed | Area of breed origin | Sampling country | Skin colour |
|----------------------------|----------------------|------------------|-------------|
| Bedouin | Middle east | Israel | White |
| Owl-bearded (Uilenbaarder) | Netherlands | Netherlands | White |
| Friesian fowl | Netherlands | Netherlands | White |
| Bresse noire | France | France | White |
| Houdan | France | France | White |
| Marans | France | France | White |
| Dorking | England | England | White |
| Finnish Landrace | Finland | Finland | White |
| Sicilienne Buttercup | Italia | Italia | White |
| Yurlov crower, Russia | Russia | Russia | White |
| Westfaeliche Totleger | Germany | Germany | White |
| Sundheimer | Germany | Germany | Yellow |
| Old Scand. Ref. Pop | Scandinavia | n/a | Yellow |
| Green-legged Partridge | Poland | Poland | Yellow |
| Orlov | Russia | Russia | Yellow |
| Ukrainian bearded | Ukraine | Ukraine | Yellow |
| Poltava clay | Ukraine | Ukraine | Yellow |
| Yurlov crower, Ukraine | Russia | Russia | Yellow |

Table S5. List of modern European breeds included for BCDO2 derived allele frequency calculation. (Data from AVIANDIV (<https://aviandiv.tzv.fal.de>) database)

Supplementary Table 6

| Breed | Origin | Breed phenotype | Sample size | Frequency of the derived allele in breeds |
|------------------------|----------------|-----------------|-------------|---|
| Friesian Fowl | Netherlands | white skin | 6 | 0 |
| Padova | Italia | white skin | 4 | 0 |
| Westfälischer Totleger | Germany | white skin | 3 | 0 |
| Houdan | France | white skin | 5 | 0 |
| Dorking | England | white skin | 4 | 0 |
| Red Villafrantina | Spain | white skin | 5 | 0.1 |
| Czech Golden Pencilled | Czech Republic | white skin | 5 | 0 |
| Total: | | | 32 | Total frequency: 0.015625 |

Table S6. Frequency of BCDO2 derived allele (position 6,273,428 on chromosome 24) in modern European breeds. (Data from (Eriksson et al. 2008))

Supplementary Table 7

| Sample_ID | Reference | Lab_code | Radiocarbon_Age(BP) |
|-----------|------------------|-------------|---------------------|
| RB599 | This study | OxA-X-2504 | 754 ± 27 |
| RB598 | This study | OxA-27436 | 738 ± 24 |
| Ch38 | Flink et al.2014 | Beta-356195 | 80 ± 30 |
| RB579 | This study | OxA-27588 | 1.12172 ± 0.00631 |
| RB586 | This study | OxA-27435 | 1.28372 ± 0.00326 |
| RA22 | Flink et al.2014 | Beta-351382 | 2030 ± 30 |

Table S7. Radiocarbon ages (BP) for directly dated samples used in the analyses.

Supplementary Table 8

| Locus | Primer sequence (5'–3') | Primer name | Source | Sequence to analyze |
|-------|-------------------------|------------------|-------------------|---------------------|
| TSHR | CTTTCTTCTGCCCTTTT | TSHR-F (biotin) | Flink et al. 2014 | |
| TSHR | GATGCTGACTTTGCTGTA | TSHR-R | Flink et al. 2014 | |
| TSHR | TGCTGTAGCTGCTGACTC | TSHR-S | Flink et al. 2014 | C/TAACCAGTGG |
| BCDO2 | ACTCTTGCATGGATCTGG | BCDO2-F (biotin) | Flink et al. 2014 | |
| BCDO2 | TGTGGTCTCAGAATTTGG | BCDO2-R | Flink et al. 2014 | |
| BCDO2 | TCAGAATTTGGGACG | BCDO2-S | Flink et al. 2014 | C/TTGGCAATGC |

Table S8. Polymerase Chain Reaction and sequencing primers used for TSHR and BCDO2

Supplementary Table 9a

| BCDO2 | s | f.anc | TSHR | s | t.sel.start | f.anc |
|----------------|-------------|-------|-----------------|-------------|-------------|-------|
| Cl.lower (95%) | 0.000131826 | 0.07 | Cl.lower(0.025) | 0.003019952 | 290 | 0.34 |
| Cl.upper (95%) | 0.01584893 | 0.25 | Cl.upper(0.975) | 0.00691831 | 1210 | 0.54 |
| Max | 0.003630781 | 0.14 | Max | 0.004897788 | 920 | 0.44 |

Supplementary Table 9b

| BCDO2 | s | f.anc | TSHR | s | t.sel.start | f.anc |
|----------------|-------------|-------|-----------------|-------------|-------------|-------|
| Cl.lower (95%) | 0.000120226 | 0.06 | Cl.lower(0.025) | 0.004570882 | 750 | 0.28 |
| Cl.upper(95%) | 0.02089296 | 0.25 | Cl.upper(0.975) | 0.05495409 | 1900 | 0.49 |
| Max | 0.003630781 | 0.13 | Max | 0.00691831 | 1170 | 0.38 |

Table S9. Marginal likelihoods for the selection coefficient (s); ancestral allele frequency (f.anc) and starting time of selection (t.sel.start) estimated for the TSHR and BCDO2 loci. Table 9a contains the estimates and the 95% confidence intervals (CIs) from the analysis with the full dataset. Table 9b contains the estimates and the confidence intervals (CIs, calculated at the 0.025 and 0.975 quintiles))from the analysis with the subset of full dataset containing samples from North Western Europe Only.

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