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Long-term reciprocal gene flow in wild and domestic geese reveals complex domestication history

Marja E. Heikkinen[a,b,1], Minna Ruokonen[a,2], Thomas A. White[a,c], Michelle M. Alexander[d], İslam Gündüz[e], Keith M. Dobney[f,g,h], Jouni Aspi[a], Jeremy B. Searle[b,3], Tanja Pyhäjärvi[a,i,3]

[a] Department of Ecology and Genetics, PO Box 3000, Fi-90014 University of Oulu, Finland

[b] Department of Ecology and Evolutionary Biology, Cornell University, Ithaca, New York 14853, USA

[c] CMPG, University of Bern, Baltzerstrasse 6, 3012 Bern, Switzerland

[d] University of York, BioArCh, Environment Building, Wentworth Way, Heslington, York, YO10 5NG

[e] Department of Biology, Faculty of Arts and Sciences, University of Ondokuz Mayis, Samsun, Turkey

[f] Department of Archaeology, Classics and Egyptology, University of Liverpool, 12–14 Abercromby Square, Liverpool L69 7WZ, UK

[g] Department of Archaeology, University of Aberdeen, St Mary's, Elphinstone Road, Aberdeen, AB24 3UF, UK

[h] Department of Archaeology, Simon Fraser University, Burnaby, B.C. V5A 1S6, 778-782-419, Canada

[i] Biocenter Oulu, PO Box 5000, Fi-90014 University of Oulu, Finland

[1] To whom correspondence may be addressed. Email: marja.e.heikkinen@oulu.fi

[2] Deceased.

[3] Contributed equally to this work.

ORCID:

MEH, 0000-0002-7051-5654; MMA, 0000-0001-8000-3639; İG, 0000-0002-6436-5397; KMD, 0000-0001-9036-4681; JA, 0000-0002-2451-3201; JBS, 0000-0001-7710-5204; TP, 0000-0001-6958-5172

Abstract

Hybridization has frequently been observed between wild and domestic species and can substantially impact genetic diversity of both counterparts. Geese show some of the highest levels of interspecific hybridization across all bird orders, and two of the goose species in genus *Anser* have been domesticated providing excellent opportunity for joint study of domestication and hybridization. Until now, knowledge on the details of the goose domestication process has come from archaeological findings and historical writings supplemented with few studies based on mitochondrial DNA. Here, we used genome-wide markers to make the first genome-based inference of the timing of European goose domestication. We also analyzed the impact of hybridization on the genome-wide genetic variation in current populations of the European domestic goose and its wild progenitor: the greylag goose (*Anser anser*). Our dataset consisted of 58 wild greylags sampled around Eurasia and 75 domestic geese representing 14 breeds genotyped for 33,527 single nucleotide polymorphisms. Demographic reconstruction and clustering analysis suggested that divergence between wild and domestic geese around 5,300 generations ago was followed by long-term genetic exchange, and that greylag populations have 3.2–58.0% admixture proportions with domestic geese, with distinct geographic patterns. Surprisingly, many modern European breeds share considerable (> 10%) ancestry with Chinese domestic geese that is derived from the swan goose *Anser cygnoid*. We show that domestication process can progress despite continued and pervasive gene flow from the wild form.

Keywords: *Anser anser*, domestication, domestic goose, genotyping-by-sequencing, hybridization, population genomics

Significance Statement

Reproductive isolation between conspecific wild and domestic populations is a cornerstone of the domestication process, yet gene flow between such wild and domestic populations has been frequently documented. European domestic geese and their wild progenitor (greylags) co-occur and can hybridize and we show that they represent a particularly persuasive case where wild and domestic populations are not isolated gene pools. Our study makes a first genome-based estimate of goose domestication, which up to now has mostly relied on archaeological findings and historical writings. We show ongoing gene flow between greylags and European domestic geese following domestication, but we also observe a surprisingly large contribution of Chinese domestic geese (a separate species) to the genetic make-up of European domestic geese.

1. Introduction

Reproductive isolation is a defining feature of speciation and yet hybridization between species is an important general phenomenon in evolution (1, 2). Among birds, the Anseriformes (ducks, geese and swans) show particularly pervasive hybridization, 41.6% to > 60% of species hybridizing with each other (3, 4). Domestication generates differentiated gene pools and reproductive isolation between domestics and their wild progenitor, but hybridization between domestic and wild forms has been well demonstrated in both plants (1, 5) and animals (6, 7). The impacts include genetic and trait enrichment of domestics, for instance, in chicken the acquisition of a yellow skin phenotype is a result of past mating between red junglefowl and grey junglefowl (8). In geese, the high tendency for hybridization among wild species is also shown among wild and domestic forms (9, 10), creating an exciting opportunity to study the complex dynamics of hybridization and domestication.

The domestic geese of the world (European and Chinese forms) are derived from two different wild species: the greylag (*Anser anser*) and the swan goose (*Anser cygnoid*) (11, 12). *A. anser* and *A. cygnoid* shared a common ancestor about 3.4 Mya (13), but are still able to hybridize (4), and some domestic breeds are reportedly hybrid (14). The greylag has been divided into the western, nominate subspecies *A. a. anser* (Linnaeus, 1758) with a European breeding range and the eastern subspecies *A. a. rubrirostris* (Swinhoe, 1871) breeding further east, although the subspecific boundary is not well defined, and mitochondrial DNA has not been found to distinguish them (10). Of these subspecies, *rubrirostris* is larger and lighter colored than *anser* (15) and has a pink bill and cold pink legs in contrast to the orange bill and flesh-colored legs of *anser*, the bill color used as primary evidence in favor of the original domestication of *rubrirostris* (16). As with all domesticates, domestic geese varieties are morphologically more diverse than their wild counterparts, particularly in plumage and body size (14).

The current knowledge about goose domestication relies largely on ancient texts and archaeological evidence. Questions about where and when domestication took place, the genetic changes associated with it and the later history of domestic geese, however, remain largely unresolved (10). There are depictions from the New Kingdom of Egypt that suggest geese were already fully domesticated by the 18th Dynasty (1450-1341 BCE). The earliest reliable reference to domestic geese in western Eurasia is Homer's Odyssey (first half of 8th century BCE) and geese were certainly well-established poultry by Roman times (17).

Genetic diversity in the mitochondrial DNA (mtDNA) of greylag and European domestic geese showed reduced diversity in the domestics (10) which may result from an early domestication bottleneck or, alternatively, later breed formation. There is a particular mitochondrial haplogroup common in the domestics (10), and archaeological domestic goose bones from the High Medieval (11th-13th century CE) of Russia belonged to that haplogroup (18).

123 MtDNA relationships between extant Chinese and European domestic goose breeds confirm that the
124 former, excluding one breed, have swan goose ancestry, whereas European domestic goose and the
125 Chinese Yili breed have greylag ancestry (12, 19, 20). However, Chinese mtDNA haplotypes may
126 occasionally occur in European domestics, and vice versa (10, 19).
127 Genomic diversity data across populations is powerful in hybridization inference, and has been
128 applied, for instance, to New World cattle, which along with their taurine ancestry were shown to have a
129 greater proportion of indicine ancestry than previously assumed (21). Interpretation is still challenging and
130 it is best to infer jointly the genetic impact of initial domestication and subsequent hybridization of wild and
131 domestic populations, as the latter can obscure domestic-wild genetic relationships and may also give a
132 false impression of the number of times a species has been domesticated (22–24).
133 Here we investigate goose domestication history using genome-wide single nucleotide polymorphism
134 (SNP) data from thousands of loci, obtained by genotyping-by-sequencing (GBS). We used 56 and 50
135 samples of greylag and domestic geese from a previous mtDNA study (10), together with 2 new Turkish
136 greylag and 25 new domestic specimens. We studied the interplay between domestication and
137 hybridization by addressing the following questions: i) what is the extent of genetic differentiation amongst
138 wild and domestic geese? ii) what is the approximate time of domestication? and iii) what is the role of
139 intra- and interspecific hybridization in goose domestication history and the current genetic makeup?

140 **2. Materials and methods**

141 a) Sampling

142 The greylag wild-collected samples derive widely from Eurasia (Fig. 1, *SI Appendix*, Table S1). The European
143 domestic goose samples represented 14 different breeds (*SI Appendix*, Table S1) and individuals that did
144 not belong to any recognized breed or were presumed to be crosses between European and Chinese
145 domestic geese. Some specimens were reported to be Chinese domestic geese. The domestic samples were
146 obtained from local breeders in Denmark, Sweden and the UK and those from Turkey were collected
147 directly by the authors.

148 b) DNA extraction and GBS library construction

149 GBS (25) libraries were constructed at the Cornell Biotechnology Resource Center (BRC) following DNA
150 extraction with the DNeasy Blood and Tissue Kit (QIAGEN) with RNase treatment. Each individual DNA
151 sample and an adaptor with a unique barcode were combined in a 96-well plate along with a common
152 adaptor. Samples were treated with the EcoT-22I (ATGCAT) restriction enzyme to create fragmented DNA.
153 Barcoded adapters and common adapters with matching sticky ends were ligated to each sample with T4
154 DNA ligase. The samples were pooled and purified with a QIAquick PCR Purification Kit (QIAGEN). PCR

155 amplification of the library used primers complementary to barcoded and common adapters with products
156 purified as above, and the samples were 100 bp SE-sequenced with Illumina HiSeq 2000/2500 at the BRC.

157 c) GBS pipeline and SNP calling

158 Raw sequence reads were run through the Command Line Interface of the Tassel 5 GBS v2 Discovery and
159 Production pipelines (26). Details about the pipelines and SNP calling are in the *SI Appendix*. Good quality
160 reads were recorded as tags and aligned to the *A. cygnoid domesticus* GenBank assembly
161 (AnsCyg_PRJNA183603_v1.0 GCF_000971095.1) (27) using the Burrows-Wheeler Aligner with default
162 settings (28). After running the raw data through the pipelines, 69,865 SNPs were obtained.
163 The SNPs were subjected to additional filtering using VCFtools (29). We removed indels, loci with
164 more than two alleles and invariant loci. However, invariant loci were retained for phylogenetics, informing
165 about greylag-swan goose divergence. After preliminary analyses loci with observed heterozygosity over
166 0.75 were removed as potential paralogs. Individuals with more than 20% missing data across loci were
167 removed. The final dataset consisted of 33,527 biallelic SNPs and 133 individuals (58 wild and 75 domestic).

168 d) Population structure analyses

169 Population clustering and structure was analyzed with STRUCTURE 2.3.4 (30) and Principal Component
170 Analysis (PCA) (31). For the whole dataset, STRUCTURE was run with 1000 burn-in steps followed by 10 000
171 iterations of MCMC for data collection for K = 1-10 allowing admixture with five replicates of each run to
172 reach convergence. For the STRUCTURE analyses done separately on greylags and European domestic
173 geese, see *SI Appendix*. An admixture model with correlated allele frequencies among populations (32) was
174 used in all STRUCTURE analyses and the iterations were automated with StrAuto 1.0 (33). We applied both
175 likelihood of K and ΔK (34) of successive K values to determine the optimal number of clusters, using
176 STRUCTURE HARVESTER (35). CLUMPP 1.1.2 (36) was used to align the assignments from different
177 replicates of K and DISTRUCT 1.1 (37) for visualization. A PCA was performed with the prcomp function in R
178 (38) and the significance of eigenvalues determined based on the Tracy-Widom distribution (22, 31).
179 A neighbor-joining tree was constructed for phylogenetic analysis, with pairwise distance between
180 individuals obtained with the R package ape (39) based in 40,191 loci. The *A. cygnoid* reference genome
181 and the invariant sites that differed from it were included in the tree construction.

182 e) Tests for admixture and simulations of demographic history

183 The history of admixture was tested with a 3-Population test f_3(C; A, B) implemented in AdmixTools 4.1
184 (40). This method offers a formal test to explain observed patterns of admixture in a target population
185 without an outgroup. For identification of admixture between Chinese and European domestics, Grey and
186 White Chinese were combined to represent the Chinese, and the Landes breed that had minimum

187 indication of admixture in STRUCTURE was chosen to represent the European domestic source population.
188 See also *SI Appendix* for further information.

189 Different models of demographic history were tested with fastsimcoal2 ver 2.6 (41). We excluded all
190 SNPs that had missing data within the whole data set and executed the analyses with a site frequency
191 spectrum (SFS) based on 9,212 SNPs. As there are no estimates of the genetic diversity per base pair for
192 greylags, we estimated the proportions of variable and monomorphic sites in the data. From the BAM file
193 with –depth option in SAMtools 1.7 (42), we estimated 9,801,382 bp covered with GBS tags. We then
194 mimicked the filtering steps done for the biallelic SNPs to reduce the total number of sites in equivalent
195 proportions. We removed the same number of sites that corresponded to the number of SNPs that were
196 removed because they were indels, had more than 2 alleles or had heterozygosity over 0.75. Since some of
197 the SNPs were removed from this analysis due to missing data in some individuals, we removed an equal
198 proportion of sites from the total number of sites as well. The final SFS had 1,681,316 sites.

199 To infer the demographic history, we chose a subset of individuals from both wild-collected greylags
200 and domestic geese to represent the genetic variation in both groups. Therefore, 11 greylags with > 90.8%
201 of greylag ancestry and 15 domestic geese with > 91.4% of European domestic goose ancestry were
202 selected for the analysis. The mutation rate for the simulations was 1.38×10^{-7} (43). The parameter
203 estimation for each model tested involved 100,000 simulations and 40 conditional maximization (ECM)
204 cycles. The parameters for each model were estimated with 100 independent runs to obtain the global
205 maximum. The models tested were i) simple divergence of two populations with no migration, ii)
206 divergence of two populations with migration and iii) divergence of two populations with two different
207 migration matrices (Fig. 2). The best model was selected based on Akaike's weight of evidence as in (41).
208 For parametric bootstrapping 100 SFS were simulated with the parameter estimates obtained from the real
209 SFS, followed by maximum likelihood estimation with 50 independent runs for each bootstrap SFS. The 95%
210 confidence intervals were obtained from the bootstrap data for each estimated parameter.

211 f) The estimation of genetic diversity

212 Genetic diversity and pairwise F_{ST} values were investigated with the *hierfstat* R package (44). Expected
213 heterozygosity (H_E) was calculated for each locus and population and averaged across loci. Difference in
214 average H_E between greylags and European domestics was tested with a two-sample t-test with the Welch
215 correction for non-homogeneity of variance (45). For comparing the genetic diversity among wild and
216 domestics, only pure greylag populations (< 10% admixture with domestic geese) and pure European
217 domestic geese (< 10% admixture with Chinese domestic geese) were used to avoid hybridization effects on
218 the estimates. The admixture proportions were obtained from STRUCTURE.

219 The variance components across loci for hierarchical F-statistics for pure greylags and pure European
220 domestics were estimated using locus-by-locus analysis of molecular variance (AMOVA) implemented in
221 Arlequin 3.5.2.1 (46). The significance was tested with 16 000 permutations.

222 ***3.* Results**

223 a) Population structure

224 There was clear divergence between greylags and both European and Chinese domestic geese according to
225 STRUCTURE and PCA (Fig. 3a-b). At $K = 2$, populations/breeds are clustered based on their status (wild or
226 domestic) and, at $K = 3$, domestic geese are further separated into European and Chinese. At $K = 4$, the
227 fourth cluster is admixed within greylag populations but none of the individuals are unanimously assigned
228 to that cluster. The likelihood was highest for $K = 3$. These results were supported by PCA as the first two
229 PCs out of 14 significant PCs ($p < 0.05$) were enough to separate the three groups (wild, European domestic,
230 Chinese domestic) from each other (Fig. 3a). Overall the greylag populations showed 3.2% - 23.5%
231 admixture proportions with European domestic geese when $K = 3$. In contrast, not all European domestic
232 geese showed admixture with greylags and the admixture percentages ranged from 0.0 to 8.4%. At $K = 3$
233 many European domestic goose breeds showed mixed ancestry with Chinese domestic geese (0.0-27.1%).
234 The neighbor-joining tree repeated the major patterns observed with STRUCTURE and PCA revealing
235 a star shaped phylogeny and confirming that the domestic and greylag geese largely form different clades
236 (*SI Appendix*, Fig. S1). Surprisingly, the Chinese domestic geese were closer to European domestic geese
237 and greylags, than to the swan goose reference genome. In addition, one greylag from Turkey was more
238 closely related to the Chinese domestic geese than other greylags, also indicated by admixture proportions
239 from STRUCTURE. Further, two Crested Faroese individuals and four domestics from the USA (2 unknown
240 and 2 Toulouse crosses) were closer to Chinese than European domestic geese. These six individuals also
241 showed high proportions of admixture with Chinese domestics in the STRUCTURE analysis.
242 Some further population structure was observed within both greylags and domestic geese, when
243 analyzed separately with STRUCTURE and PCA. Geographically, greylags differentiated by subspecies (*SI*
244 *Appendix*, Fig S2-S3). STRUCTURE indicated little differentiation among European domestic geese, but the
245 PCA revealed separation between the European breeds and the Turkish domestic geese (*SI Appendix*, Fig
246 S4-S5).

247 b) Admixture and the time of domestication

248 Although, STRUCTURE implied considerable mixed ancestry from multiple genetic clusters for Dutch and
249 Turkish greylags, the f_3 analysis did not confirm admixture for these populations. The two Turkish greylag

250 samples came from the same area as our NW-Turkish domestic population, which among Turkish domestic
251 geese showed highest admixture with greylags (2.2%), but admixture was not confirmed with the f_3 test.
252 The f_3 analysis confirmed admixture of domestic geese in line with STRUCTURE results. Most notably,
253 the African breed is a hybrid between European and Chinese domestic geese (Z-score -6.399), unexpected
254 as this breed has been assumed to have originated solely from swan goose. The European-Chinese hybrid
255 status of the Kholmogor and Steinbacher breeds was also confirmed (Z-scores of -8.933 and -5.349,
256 respectively). The Kholmogor breed also fell halfway between European and domestic geese both in
257 STRUCTURE and PCA, whereas the Steinbacher was genetically closer to European domestic geese in the
258 PCA. However, the Diepholzer breed, which reportedly is also a hybrid, was not confirmed as such in our
259 analysis. Other domestic breeds/groups with admixture status in STRUCTURE were also confirmed to have
260 a European-Chinese admixture when a Z-score threshold of -3 (roughly corresponding to $p < 0.01$) was
261 used: Sebastopol, Toulouse cross, Domestic NY, Embden, Tufted Roman (Fig. 3c, *SI Appendix*, Table S2). The
262 Crested Faroese breed gave indication of admixture based on STRUCTURE analysis and the f_3 test supported
263 this (Z-score of -2.228, $p < 0.05$). Surprisingly, the Northern Turkish domestic population was not admixed
264 with Chinese domestic geese in STRUCTURE but f_3 analysis gave a contrasting signal (Z-score -2.459, $p <$
265 0.05).
266 The demographic model that best fit our data suggested divergence of greylag and domestic geese
267 with a recent migration rate change (Table 1, *SI Appendix*, Table S3). The model suggested divergence
268 around 5319 generations ago (95% confidence intervals (CI): 1538-8225) with asymmetric but close to
269 equal migration rates from greylags to domestic geese following divergence. About 159 (79-462)
270 generations ago, the migration matrix changed, suggesting higher gene flow from domestic geese to
271 greylags towards modern times. Given an estimated generation time for these geese of about 3 years, this
272 suggests divergence about 14 000 BCE and gene flow shift about 480 years ago.

273 c) Genetic diversity

274 An AMOVA was used to partition genetic diversity among greylag vs. domestic (group level), and among
275 populations (greylag) and among breeds (domestics), and within population levels (Table 2). The fixation
276 index between greylag and domestic geese was 0.158 and there was also significant differentiation among
277 greylag populations/domestic breeds. The average pairwise F_{ST} between greylag populations and domestic
278 breeds was 0.197, among greylag populations 0.088 and among domestic breeds 0.174 (*SI Appendix*, Table
279 S4).
280 The genetic diversity measured as average H_E was higher in pure greylags (0.146) than pure European
281 domestic geese (0.096) (Welch's t-test, degrees of freedom (df) = 10.594, $p = 3.91\times10^{-5}$). The difference in
282 average H_E remained when non-pure greylag and non-pure European domestics were included in the
283 comparison (0.156 vs. 0.107; Welch's t-test, df = 19.28, $p = 0.000418$) (*SI Appendix*, Table S1). The

284 Steinbacher and African breeds (*SI Appendix*, Table S1) were excluded from both estimates because the
285 Steinbacher is known to be a European-Chinese cross and the African has swan goose ancestry. The
286 average H_E ranged from 0.140 (Denmark) to 0.150 (Kazakhstan) in pure greylags and from 0.047 (Landes) to
287 0.123 (Domestic N-Turkey) in pure European domestics. The average H_E was higher in greylag populations
288 that showed high admixture proportions with domestic geese, Netherlands (0.157, 13.8% European
289 domestic, 2.2% Chinese domestic) and Turkey (0.236, 23.5% European domestic, 34.5% Chinese domestic),
290 but not significantly so (Welch's t-test, df = 1.002, p = 0.421, average H_E: pure greylags 0.146 vs non-pure
291 greylags 0.196). The average H_E was also higher in European domestics that showed high admixture
292 proportions with Chinese domestics, i.e. Crested Faroese (0.117, 17% Chinese domestic), Sebastopol
293 (0.133, 11.1% Chinese domestic), Domestic NY (0.143, 78.6% European domestic, 21.4% Chinese domestic)
294 and Toulouse cross (0.151, 72.9% European domestic, 27.1% Chinese domestic), and the difference was
295 also statistically significant (Welch's t-test, df = 9.0991, p = 0.0039, average H_E: pure European domestics
296 0.096 vs non-pure European domestics 0.136).

297 **4. Discussion**

298 We studied the dynamics of domestication and hybridization in grey (*Anser*) geese using genome-wide SNP
299 data. The results demonstrated genetic divergence between Eurasian wild greylag and European domestic
300 geese with long-term genetic exchange between them. We also inferred temporal changes in the direction
301 of gene flow. The degree of hybridization between greylag and domestic geese also varied geographically.
302 Surprisingly, several domestic goose breeds also showed a substantial genetic contribution of Chinese
303 domestic geese. We also provide insights about the origin and the timing of goose domestication.

304 a) Genetic diversity and differentiation of greylag and European domestic geese

305 Domestic species often show reduced genetic diversity compared to their wild ancestor, attributable to
306 genetic drift during population bottlenecks of initial domestication, combined with subsequent artificial
307 selection associated with breed formation (47). Domestic geese appear to follow the same trend. We found
308 European domestic geese to have lower H_E than wild greylags.

309 European domestic geese are genetically distinct from their wild progenitor but no more so than for
310 other domestic birds. The average pairwise F_{ST} values between greylag populations and domestic goose
311 breeds were lower than between red junglefowl and domestic chicken populations (48), and domestic
312 geese are less distinctive than domestic pigeons (49). Among domestic geese, the Turkish are particularly
313 interesting. From mtDNA, the Turkish domestic geese stand out as the most genetically variable group (10),
314 and although this is less evident from GBS, among the pure European domestic geese the Northern Turkish
315 showed the highest average H_E. The f_3 analysis indicates a history of admixture with Chinese domestics for
316 this population, which may explain its high genetic diversity.

317 We found a genetic separation between European and Near Eastern populations of greylags that
318 aligned with the western and eastern subspecies (*A. a. anser* and *A. a. rubrirostris*) (50), a distinction which
319 could not be made based on mtDNA (10). Hybridization between the western and eastern subspecies is
320 suggested from admixture in Dutch and Danish greylags in STRUCTURE. There is historical evidence for the
321 introduction of *rubrirostris* to Belgium in 1954 and to Netherlands in 1960s (9, 51); thus, *rubrirostris* genes
322 may have originated from the recently introduced gene pool spreading to Denmark.

323 b) When and where were geese domesticated?

324 Traditional views on goose domestication claim it first occurred in the eastern Mediterranean (possibly
325 Egypt) around the 3rd Millennium BCE (17, 52). Domestication of chicken and perhaps pigeon took place
326 earlier, but domestication of duck later, at least in Europe (23). Demographic modelling suggests that the
327 wild greylag and related domestic lineages split approximately 5,300 generations ago placing domestication
328 origins at 14 000 BCE assuming a 3-year generation time (15). This estimated genetic divergence time is,
329 admittedly, considerably earlier than any evidence for animal domestication except dog. It is important to
330 note that the estimated divergence times have large confidence intervals and merely indicates the split
331 between the ancestors of contemporary wild and domestic lineages. It is most likely that our demographic
332 modelling reflects the early divergence of different lineages of greylags, only one of which contributed to
333 later domestication. The subsequent reduction or even disappearance of that wild lineage means that,
334 despite wide geographical sampling, the possible modern wild population(s) of the greylag progenitor to
335 domestic geese was not sampled in this study and must, therefore, be sought by ancient DNA approaches.
336 Given that genetic diversity would be expected to be highest in the 'domestication center' and
337 reduce with increasing distance from there, the high mtDNA diversity of Turkish domestic geese means the
338 eastern Mediterranean remains a strong candidate for the origin of goose domestication, and our nuclear
339 data accord with that. However, as we have shown, hybridization between wild and domestic geese can
340 also generate high genetic diversity both within and outside the original domestication location.

341 c) The role of intra- and interspecific hybridization in goose domestication history

342 i. Evidence of current hybridization

343 Domestic animals and their wild relatives are often observed to interbreed, and this is also true for geese.
344 Both field observations and mtDNA results (9, 10) suggested some current hybridization between domestic
345 and greylag geese. Genome-wide analysis covering multiple greylag populations and domestic breeds
346 revealed a considerable impact of hybridization on genetic diversity of both wild and domestic geese.
347 Hybridization is particularly prevalent in certain geographical regions. Dutch and especially Turkish
348 wild greylag samples had more shared genetic affiliation with domestics than Scandinavian and Finnish
349 greylag populations (Fig. 3b). Some regions may offer more hybridization opportunities, e.g. climate may

350 allow greylags to be sedentary year-round and be favorable for keeping domestic geese. The Netherlands,
351 for instance, lies on the Atlantic flyway offering breeding, staging and wintering areas for greylags (53, 54).
352 Since pair-bonding of geese generally occurs on wintering grounds (55), hubs for migrating geese such as
353 the Netherlands may permit population mingling. Nevertheless, the f_3 test did not support a simple history
354 of admixture for the Netherlands. Patterson et al. (40) have stated that population-specific drift may mask
355 the signal of admixture in such analyses, leading to a non-negative f_3. The f_3 model is relatively simple, with
356 only two sources, and may not catch the signal of admixture in the Dutch greylag population because of the
357 previous contribution of *rubrirostris*, which was not included in the model.

358 Based on ringing data most greylag populations in Scandinavia follow the Atlantic flyway - some of
359 the geese wintering in the Netherlands and others in southwest Spain. However, Finnish greylags favor the
360 Central European flyway and winter in North Africa, with a minority of Finnish greylags using the Atlantic
361 Flyway (53, 54). The Finnish populations of greylag showed the lowest admixture proportions with
362 domestic geese (S-Finland 3.2% domestic goose, N-Finland 3.3% domestic geese) among greylag
363 populations. Rearing geese is not a popular practice in Finland and they constituted less than 5% of poultry
364 kept in Finland in 2014 (56). The Norwegian populations showed only slightly higher admixture proportions
365 with domestic geese, although domestic mtDNA haplotype ANS19 was detected from a wild greylag
366 collected in Finnmark, Norway (57). This haplotype is a partial sequence of the D5 haplotype identified by
367 Heikkinen et al. (10), and identical to that found in White Roman domestic geese (58).

368 Inferring the hybridization patterns in the Turkish greylags is more complicated, as Turkish greylags
369 indicate hybridization with both Chinese and European domestics. Both greylags sampled in Turkey showed
370 considerable admixture with domestic geese. One of them appeared genetically as a hybrid of European
371 and Chinese domestic goose with only a small proportion of greylag ancestry, whereas the other one was a
372 more equal mix of European domestic goose and greylag supplemented by a considerable Chinese
373 domestic goose ancestry. There is some indication of hybridization between greylags and domestic geese
374 within that area as the domestic geese sampled from the same area showed some admixture with greylags,
375 but this was not confirmed with f_3 analysis. These results may reflect a local practice of keeping captive
376 greylags within a flock of domestic geese. Another possibility is that the Turkish greylags have hybridized
377 with some unsampled distinct greylag population and simply appear genetically like domestic geese due to
378 lack of representation of the unsampled wild population. The greylag population breeding and wintering in
379 the Black Sea region is not well monitored (59).

380 ii. Long-term hybridization

381 Domestication can be seen as an analogy of speciation where an animal population transforms to an
382 ecotype that is adapted to the human niche (23) and at later stages of domestication is perpetuated with
383 reproductive isolation in the form of selection managed by humans (60). However, this reproductive

isolation may not be complete (7). While the genetic divergence of the greylag and its domestic descendant is evident, our results suggest extensive long-term genetic exchange between them. In addition, the demographic modelling suggests that the gene flow patterns have changed over time.

Initially, gene flow was greater from greylag to domestic geese. Possibly early goose herders supplemented the domestic gene pool with wild-caught greylags. Alternatively, wild ganders may have interbred with domestic geese and contributed to the domestic gene pool. It is unlikely that the early stages of goose domestication were rigorously managed, allowing matings outside the domestic gene pool. Both would explain the observed higher gene flow from greylag to domestic geese in the early stages of domestication. It is in the farmers' interest to keep the domestic geese and wild geese reproductively isolated to keep control over the traits that are being selected, but artificial selection of traits would have become possible only after the domestic gene pool had been established. After that, it may occasionally be beneficial to restock the flock to maintain enough genetic diversity. The natural tendency for imprinting in geese facilitates this practice. Later, the balance of gene flow shifted towards greylags. This likely occurred after goose-keeping became well-established in the Medieval period (17). The rise in number of domestic geese may have allowed an increase in domestic goose escapees resulting in increased gene flow from domestic geese back to greylags.

Furthermore, not only have domestic geese admixed with wild greylags but also European and Chinese domestic geese have hybridized. Hybridization with ancestral species or closely related species is frequent in domestic species, e.g., the genetic makeup of chicken derives from multiple different species of *Gallus* (8). Similarly, the genetic make-up of domestic geese seems to derive from two closely related species. This hybridization with Chinese domestic geese may have introduced some traits not present in greylags to European domestic geese and vice versa.

Authors' contributions.

MEH conceived the study, contributed to data collection, analyzed the data and drafted the manuscript. MR acquired the funding, conceived the study and contributed to data collection. TAW contributed to data collection and participated to data analysis. MMA and İG contributed to data collection. KMD, JA, JBS and TP conceived the study and contributed to writing and interpretation of data, with TP also participating in data analysis. All authors, excluding MR, reviewed, improved and approved the manuscript.

Competing interests.

The authors declare no conflict of interest.

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References

1. Arnold ML (2004) Natural hybridization and the evolution of domesticated, pest and disease organisms. *Mol Ecol* 13(5):997–1007.
2. Abbott R, et al. (2013) Hybridization and speciation. *J Evol Biol* 26(2):229–246.
3. Grant PR, Grant BR (1992) Hybridization of bird species. *Science* 256(5054):193–197.
4. Ottenburghs J, van Hooft P, van Wieren SE, Ydenberg RC, Prins HHT (2016) Hybridization in geese: a review. *Front Zool* 13(1):20.
5. Janzen GM, Wang L, Hufford MB (2019) The extent of adaptive wild introgression in crops. *New Phytol* 221(3):1279–1288.
6. Godinho R, et al. (2011) Genetic evidence for multiple events of hybridization between wolves and domestic dogs in the Iberian Peninsula. *Mol Ecol* 20(24):5154–5166.
7. Frantz LAF, et al. (2015) Evidence of long-term gene flow and selection during domestication from analyses of Eurasian wild and domestic pig genomes. *Nat Genet* 47(10):1141–1148.
8. Eriksson J, et al. (2008) Identification of the yellow skin gene reveals a hybrid origin of the domestic chicken. *PLoS Genet* 4(2):e1000010.
9. Kuijken E, Devos K (1996) The status of the greylag goose *Anser anser* in Flanders, Belgium. *Wetl Int Goose Spec Gr Bull* (8):26–28.
10. Heikkinen ME, et al. (2015) Relationship between wild greylag and European domestic geese based on mitochondrial DNA. *Anim Genet* 46(5):485–497.

11. Delacour J (1954) *The waterfowl of the world : Vol. 1, The magpie goose, whistling ducks, swans and geese, sheldgeese and shelducks* (Country Life, London). 2nd Ed.

12. Shi X-W, Wang J-W, Zeng F-T, Qiu X-P (2006) Mitochondrial DNA cleavage patterns distinguish independent origin of Chinese domestic geese and Western domestic geese. *Biochem Genet* 44(5–6):237–245.

13. Ottenburghs J, et al. (2016) A tree of geese: a phylogenomic perspective on the evolutionary history of True Geese. *Mol Phylogenet Evol* 101:303–313.

14. Buckland R, Guy G eds. (2002) Goose production. *FAO Animal Production and Health Paper - 154* (Food and Agriculture Organization of the United Nations, Rome).

15. Cramp S, Simmons KEL eds. (1977) *Handbook of the birds of Europe, the Middle East, and North Africa: The birds of the Western Palearctic. Vol. 1: Ostrich-Ducks.* (Oxford University Press, New York).

16. Kear J (1990) *Man and wildfowl* (T & AD Poyser Ltd, London).

17. Albarella U (2005) Alternate fortunes? The role of domestic ducks and geese from Roman to Medieval times in Britain. *Documenta Archaeobiologiae 3. Feathers, Grit and Symbolism; Birds and Humans in the Ancient Old and New Worlds.*, eds Grupe G, Peters J (Rahden: Verlag Marie Leidorf), pp 249–258.

18. Honka J, et al. (2018) Over a thousand years of evolutionary history of domestic geese from Russian archaeological sites, analysed using ancient DNA. *Genes* 9(7):367.

19. Sun J, et al. (2013) Matrilineal genetic structure of domestic geese. *J Poult Sci* 51(2):130–137.

20. Ren T, Liang S, Zhao A, He K (2016) Analysis of the complete mitochondrial genome of the Zhedong White goose and characterization of NUMTs: reveal domestication history of goose in China and Euro. *Gene* 577(1):75–81.

21. McTavish EJ, Decker JE, Schnabel RD, Taylor JF, Hillis DM (2013) New World cattle show ancestry from multiple independent domestication events. *Proc Natl Acad Sci USA* 110(15):E1398–E1406.

22. van Heerwaarden J, et al. (2011) Genetic signals of origin, spread, and introgression in a large sample of maize landraces. *Proc Natl Acad Sci USA* 108(3):1088–1092.

23. Larson G, Fuller DQ (2014) The evolution of animal domestication. *Annu Rev Ecol Evol Syst* 45(1):115–136.

24. Marshall FB, Dobney K, Denham T, Capriles JM (2014) Evaluating the roles of directed breeding and gene flow in animal domestication. *Proc Natl Acad Sci USA* 111(17):6153–6158.

475 25. Elshire RJ, et al. (2011) A robust, simple genotyping-by-sequencing (GBS) approach for high diversity
476 species. *PLoS One* 6(5):e19379.

477 26. Glaubitz JC, et al. (2014) TASSEL-GBS: a high capacity genotyping by sequencing analysis pipeline.
478 *PLoS One* 9(2):e90346.

479 27. Lu L, et al. (2015) The goose genome sequence leads to insights into the evolution of waterfowl and
480 susceptibility to fatty liver. *Genome Biol* 16(1):89.

481 28. Li H, Durbin R (2009) Fast and accurate short read alignment with Burrows-Wheeler transform.
482 *Bioinformatics* 25(14):1754–1760.

483 29. Danecek P, et al. (2011) The variant call format and VCFtools. *Bioinformatics* 27(15):2156–2158.

484 30. Pritchard JK, Stephens M, Donnelly P (2000) Inference of population structure using multilocus
485 genotype data. *Genetics* 155(2):945–959.

486 31. Patterson N, Price AL, Reich D (2006) Population structure and eigenanalysis. *PLoS Genet* 2(12):e190.

487 32. Falush D, Stephens M, Pritchard JK (2003) Inference of population structure using multilocus
488 genotype data: linked loci and correlated allele frequencies. *Genetics* 164(4):1567–1587.

489 33. Chhatre VE, Emerson KJ (2017) StrAuto: automation and parallelization of STRUCTURE analysis. *BMC*
490 *Bioinformatics* 18(1):192.

491 34. Evanno G, Regnaut S, Goudet J (2005) Detecting the number of clusters of individuals using the
492 software structure: a simulation study. *Mol Ecol* 14(8):2611–2620.

493 35. Earl DA, VonHoldt BM (2012) STRUCTURE HARVESTER: a website and program for visualizing
494 STRUCTURE output and implementing the Evanno method. *Conserv Genet Resour* 4(2):359–361.

495 36. Jakobsson M, Rosenberg NA (2007) CLUMPP: a cluster matching and permutation program for
496 dealing with label switching and multimodality in analysis of population structure. *Bioinformatics*
497 23(14):1801–1806.

498 37. Rosenberg NA (2003) distruct: a program for the graphical display of population structure. *Mol Ecol*
499 *Notes* 4(1):137–138.

500 38. R Core Team (2017) R: A Language and Environment for Statistical Computing. Available at:
501 https://www.r-project.org/.

502 39. Paradis E, Claude J, Strimmer K (2004) APE: analyses of phylogenetics and evolution in R language.
503 *Bioinformatics* 20(2):289–290.

504 40. Patterson N, et al. (2012) Ancient admixture in human history. *Genetics* 192(3):1065–1093.

505 41. Excoffier L, Dupanloup I, Huerta-Sánchez E, Sousa VC, Foll M (2013) Robust demographic inference

506 from genomic and SNP data. *PLoS Genet* 9(10):e1003905.

507 42. Li H, et al. (2009) The sequence alignment/map format and SAMtools. *Bioinformatics* 25(16):2078–
508 2079.

509 43. Pujolar JM, Dalén L, Olsen RA, Hansen MM, Madsen J (2018) First de novo whole genome
510 sequencing and assembly of the pink-footed goose. *Genomics* 110(2):75–79.

511 44. Goudet J (2005) hierfstat, a package for R to compute and test hierarchical F-statistics. *Mol Ecol*
512 *Notes* 5(1):184–186.

513 45. Welch BL (1938) The significance of the difference between two means when the population
514 variances are unequal. *Biometrika* 29(3/4):350.

515 46. Excoffier L, Lischer HEL (2010) Arlequin suite ver 3.5: a new series of programs to perform
516 population genetics analyses under Linux and Windows. *Mol Ecol Resour* 10(3):564–567.

517 47. Moyers BT, Morrell PL, McKay JK (2018) Genetic costs of domestication and improvement. *J Hered*
518 109(2):103–116.

519 48. Kanginakudru S, Metta M, Jakati R, Nagaraju J (2008) Genetic evidence from Indian red jungle fowl
520 corroborates multiple domestication of modern day chicken. *BMC Evol Biol* 8(1):174.

521 49. Stringham SA, et al. (2012) Divergence, convergence, and the ancestry of feral populations in the
522 domestic rock pigeon. *Curr Biol* 22(4):302–308.

523 50. Scott DA, Rose PM (1996) *Atlas of Anatidae populations in Africa and Western Eurasia* (Wetlands
524 International Publication No 41, Wageningen).

525 51. Rooth J (1971) The occurrence of the greylag goose *Anser anser* in the western part of its
526 distribution area. *Ardea* 59(1–2):17–27.

527 52. Zeuner FE (1963) *A History of Domesticated Animals* (Harper & Row, Publishers, New York and
528 Evanston).

529 53. Madsen J, Cracknell G, Fox T eds. (1999) *Goose populations of the Western Palearctic. A review of*
530 *status and distribution.* (Wetlands International Publication No. 48, Wageningen, Ronde).

531 54. Andersson Å, Follestad A, Nilsson L, Persson H (2001) Migration patterns of Nordic greylag geese
532 *Anser anser*. *Ornis Svecica* 11(1):19–58.

533 55. Rohwer FC, Anderson MG (1988) Female-biased philopatry, monogamy, and the timing of pair
534 formation in migratory waterfowl. *Current Ornithology*, ed Johnston RF (Springer US, Boston, MA),
535 pp 187–221.

536 56. Official Statistics of Finland (OSF): Number of livestock [e-publication]. Helsinki: Natural Resources

Institute Finland [referred: 17.12.2016]. Access method: http://www.stat.fi/til/klm/index_en.html (2016).

57. Pellegrino I, Cucco M, Follestad A, Boos M (2015) Lack of genetic structure in greylag goose (*Anser anser*) populations along the European Atlantic flyway. *PeerJ* 3:e1161.

58. Wang CM, et al. (2010) The origin of the White Roman goose. *Biochem Genet* 48(11–12):938–943.

59. Fox AD, et al. (2010) Current estimates of goose population sizes in western Europe, a gap analysis and an assessment of trends. *Ornis Svecica* 20(3–4):115–127.

60. Zeder MA (2012) The Domestication of Animals. *J Anthropol Res* 68(2):161–190.

61. BirdLife International and Handbook of the Birds of the World (2016) 2016. *Anser anser*. The IUCN Red List of Threatened Species. Version 2018-1.

553 **Table 1.** The parameter values that maximize likelihood estimates (MLE) of the preferred demographic
554 model for goose domestication history (see text) with their 95% confidence intervals (CI) and the search
555 range for the parameter values.

Model	Number of parameters	Parameter	MLE	95% CI	Search range
Divergence with 2 migration matrices	9	ANCSIZE	1112	291.95 - 9030.75	100 - 100000
		T_1	5319	1538.80 - 8225.45	100 - 20000
		$M1_{WD}$	$5.35x10^{-4}$	$2.96x10^{-4}$ - $6.75x10^{-4}$	$1x10^{-5}$ - 20
		$M1_{DW}$	$4.25x10^{-4}$	$1.73x10^{-4}$ - $6.03x10^{-4}$	$1x10^{-5}$ - 20
		T_2	159	79.65 - 462.95	50 - 10000
		$M2_{WD}$	$6.69x10^{-4}$	$4.57x10^{-4}$ - $9.69x10^{-4}$	$1x10^{-5}$ - 20
		$M2_{DW}$	$1.72x10^{-3}$	$1.33x10^{-3}$ - $2.15x10^{-3}$	$1x10^{-5}$ - 20
		N_{DOM}	959	879.95 - 1073.25	50 - 100000
		N_{WILD}	2504	2341.60 - 2695.55	50 - 100000

ANCSIZE, effective population size of ancestral population; T_1, time of divergence in generations; N_{DOM}, effective population size for domestic geese; N_{WILD}, effective population size for greylags; T_2, estimate of time in generations when the migration matrix switched; $M1_{WD}$ migration rate from wild to domestic following T_1; $M1_{DW}$ migration rate from domestic to wild following T_1; $M2_{WD}$ migration rate from wild to domestic following T_2; $M2_{DW}$ migration rate from domestic to wild following T_2.

556

557 **Table 2.** Hierarchical analysis of molecular variance (AMOVA) of greylags and their domestic descendants,
558 considering pure populations of greylags (first group) and pure breeds of European domestic geese (second
559 group).

Source of variation	Sum of squares	Variance components	Percentage variation	Fixation indices
Among groups	47565.119	431.4291	15.8	$F_{CT} = 0.158^{**}$
Among populations and breeds within groups	82960.489	302.51404	11.1	$F_{SC} = 0.131^{**}$
Within populations and breeds	345889.821	2003.45893	73.2	$F_{ST} = 0.268^{**}$
Total	476415.429	2737.40207		

** $p < 0.001$

560

561

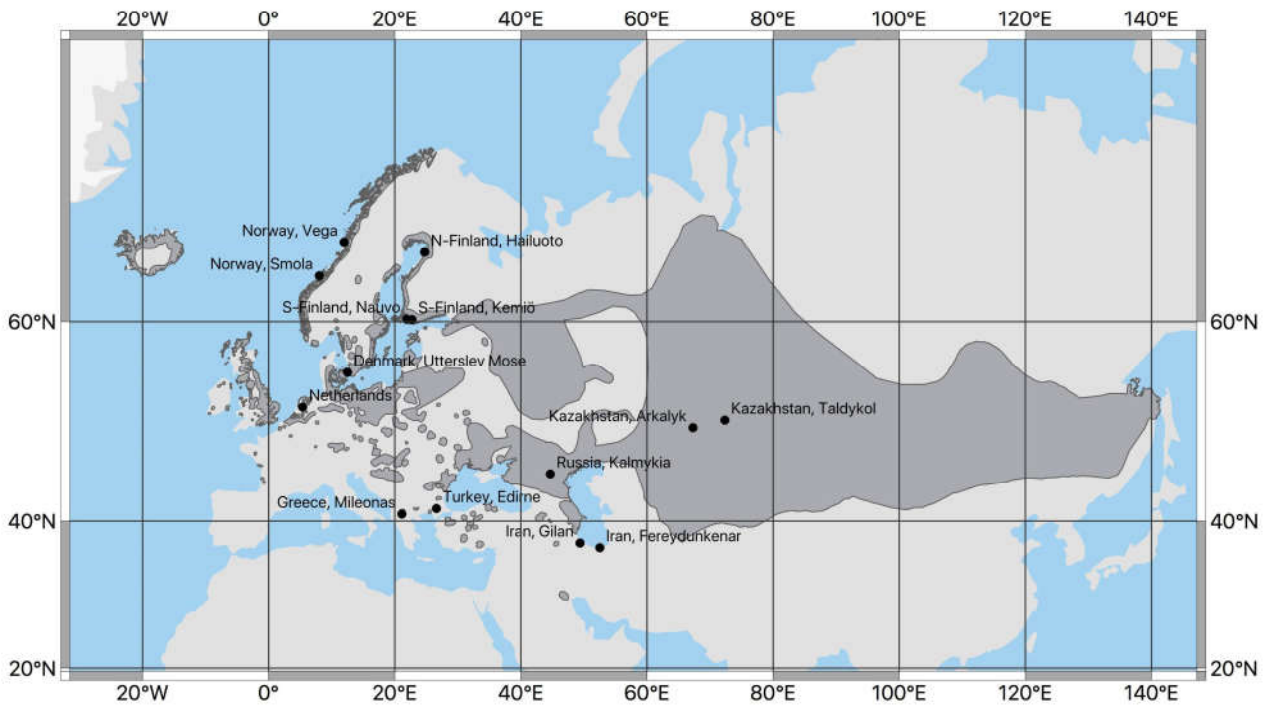


Fig. 1. Map showing the sampling sites for wild greylags used in this study. The breeding area of the species is shown on darker grey. The sampling sites in Kazakhstan were combined for analyses (one sample per location) and the sampling sites in Southern Finland included combined samples from the geographically close sites of Västanfjärd, Nauvo (shown) and Kemiö (shown). The Iranian samples were collected during the wintering season. Map modified from IUCN (61).

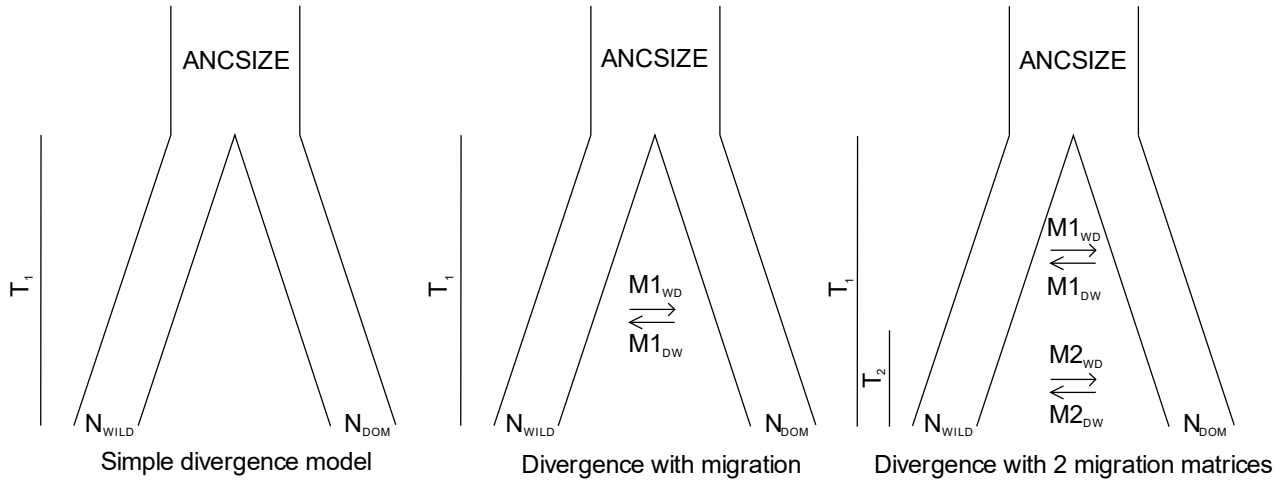


Fig. 2. Demographic histories of goose domestication as tested with fastsimcoal2.

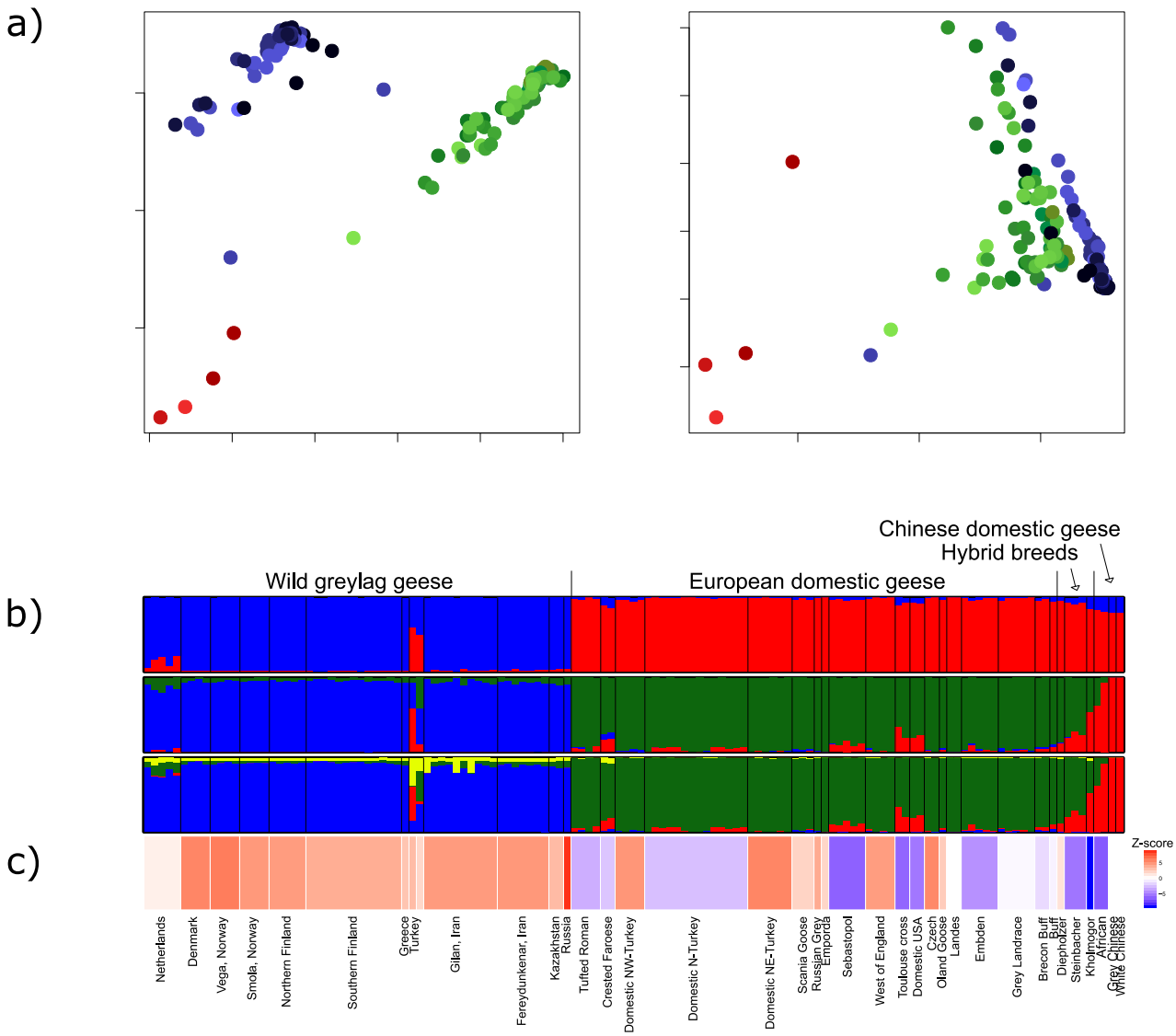


Fig. 3. The genetic divergence and hybridization patterns in greylag and domestic geese. Population status and names labelled as in *SI Appendix*, Table S1. The colors in a) and b) are associated to different groups as follows: greylags (blue), European domestics (green) and Chinese domestics (red). a) The first three principal components summarizing the genetic variation in geese (percentage explained by each PC is shown). b) STRUCTURE assignment plots for $K = 2$, $K = 3$, and $K = 4$. Each vertical line represents one individual with K number of colors indicating proportion of ancestry from the inferred clusters. c) Plot relating to the f_3 (*SI Appendix*, Table S2) values obtained for each population. The more negative the f_3, the more significant is Z-score in favor of admixture. The f_3 values were not calculated for Landes and the Chinese geese, as they were used as source populations, thus they were given an f_3 value of 0.

Supplementary Information for

Long-term reciprocal gene flow in wild and domestic geese reveals complex domestication history

Marja E. Heikkinen, Minna Ruokonen, Thomas A. White, Michelle M. Alexander, İslam Gündüz, Keith M. Dobney, Jouni Aspi, Jeremy B. Searle, Tanja Pyhäjärvi

Marja E. Heikkinen
Email: marja.e.heikkinen@oulu.fi

This PDF file includes:

Supplementary text
Figures S1 to S5
Tables S1 to S3
Legend for Table S4
SI References

Other supplementary materials for this manuscript include the following:

Table S4

Extended methods.

GBS pipeline and SNP calling

Raw sequence reads from Illumina were run through the Command Line Interface of the Tassel 5 GBS v2 Discovery and Production pipelines (1) on the Taito supercluster maintained by the CSC - IT Center for Science in Finland. The GBSSeqToTagDBPlugin was run with the default setting except for the minimum quality score set to 20. This plugin identifies good quality reads with the barcode and cut site from the raw sequence data and trims off barcodes and truncates sequences if another cut site is found in the sequence. The reads that were pulled from the raw data were trimmed to 64 bp (base pairs) but if a second cut site was found in the read, the sequence was truncated and kept if the remaining sequence was longer than 20 bp. The good quality reads are recorded as tags and along with individuals in which they appear, the tags are stored in the local database. Given the parameters used, the length of each good quality tag ranged from 20 to 64 bp and each position in the sequence had a minimum quality score of 20 from Illumina sequencing. After this, the tags were aligned to the *A. cygnoid domesticus* GenBank assembly (AnsCyg_PRJNA183603_v1.0 GCF_000971095.1) (2) using the Burrows-Wheeler Aligner with default settings (3). Then, the SAMToGBSdbPlugin was run with default settings to determine the potential positions of tags in the reference genome and the position information was recorded to the local database. Altogether 285,760 tags were mapped on the reference genome and 66,163 tags were left unmapped. In the next step the DiscoverySNPCallerPluginV2 was used to align the tags positioned in the same physical location with each other and called single nucleotide differences between aligned tags as SNPs. The SNP position and allele data were stored in the local database. The DiscoverySNPCallerPluginV2 was run with the default settings with the following change: the proportion of individuals with the genotype in the locus, the minimum locus coverage, was set to 0.8. Finally, the ProductionSNPCallerPluginV2 was used to convert the data from the local database to VCF format. The Tassel-GBS pipeline does not filter for sequencing depth per se because it is optimised for large numbers of markers in a large sample of individuals at the expense of sequencing depth (optimization of the pipeline is done for sequencing depth of 0.5 -3 x) (1). Therefore, the mean sequencing depths in our data ranged between 1.2 – 1310 x for each SNP averaged across individuals. The low coverage in some of the SNPs was compensated by the fact that the minimum locus coverage was set to 0.8 meaning that each SNP was genotyped in at least 80% of the samples. After running the raw data through Discovery and Production pipelines, the resulting number of SNPs was 69,865.

After that, the SNPs were subjected to additional filtering using VCFtools (4). We removed indels, loci with more than two alleles and invariant loci that differed from the reference. However, these invariant sites were retained in the phylogenetic tree construction as they were informative about the divergence

from the swan goose. After preliminary analyses we also removed loci with observed heterozygosity over 0.75, because they were potential paralogs mapping to same reference locus. We applied a filter that removed individuals that showed more than 20% missing data across loci. After these filtering steps, we had a dataset that consisted of 33,527 biallelic SNPs and 133 individuals that were successfully genotyped for at least 80% of the loci of which 58 were wild greylags and 75 were domestic geese.

Population structure analyses

Population clustering and structure at the individual level was analyzed with STRUCTURE 2.3.4 (5) and Principal Component Analysis (PCA) (6, 7) for the whole dataset and within greylags and domestic geese. The Bayesian STRUCTURE approach aims to find an optimal number of clusters (*K*) from a given dataset without prior population/group information by assuming that loci are in linkage equilibrium and each population is in Hardy-Weinberg equilibrium. The clustering of populations is done by considering the individual genotypes and estimating the allele frequencies in populations. For the whole dataset, STRUCTURE was run with 1,000 burn-in steps followed by 10,000 iterations of MCMC for data collection for *K* = 1-10 allowing admixture with five replicates of each run; this appeared to be enough to reach convergence. For the STRUCTURE analyses done separately on greylags and European domestic geese we increased the number of burn-in steps to 10,000 and number of MCMC to 50,000 and set the *K* to 1-7. As with the whole dataset, each run was repeated five times. An admixture model with correlated allele frequencies among populations (8) was used in all the STRUCTURE analyses. The iterations for STRUCTURE analyses were automated with the script StrAuto 1.0 (9). We used both likelihood of *K* and Δ*K* (10) of successive *K* values to determine the optimal number of clusters, as implemented in STRUCTURE HARVESTER (11). CLUMPP 1.1.2 (12) was used to align the assignments from different replicates of *K* and the results were used as an input for visualisation with the program DISTRUCT 1.1 (13). A PCA was performed with prcomp function in R (14) and the significance of the eigenvalues was determined based on the Tracy-Widom distribution (7, 15).

In order to visualize the genetic differences and distance between the reference genome and our data, a neighbor-joining tree was constructed. The tree estimation was performed based on a pairwise distance matrix computed between individuals with the R package ape (16). The *A. cygnoid* reference genome was included in the construction of the neighbor-joining tree and the invariant sites that differed from the reference genome within our data set were also included, thus the tree was constructed with 40,191 loci.

Tests for admixture and simulations of demographic history

The history of admixture was tested with 3-Population test f_3(C; A, B) implemented in AdmixTools 4.1 (17). This method offers a formal test of admixture that can be used to explain the observed patterns of

admixture in a target population and does not require an outgroup. The f_3 test allows separation of ancient polymorphisms from the effects of true admixture, which may be confounded in STRUCTURE. The admixture model is simple, with two source populations contributing to single target population. For identification of admixture between Chinese and European domestics, Grey and White Chinese were combined to represent the Chinese domestic source population and Landes breed that had minimum indication of admixture in STRUCTURE was chosen to represent the European domestic geese source population. The results did not change considerably when other pure breeds were used as a European source (data not shown). Combinations of domestic geese populations and greylag populations were also tested as source populations to see if any of them yielded negative Z-score values supporting history admixture in any of the greylag and domestic goose populations (data not shown).

Different models of demographic history were tested with fastsimcoal2 ver 2.6 (18). Only sites without missing data were used for demographic analyses. We excluded all the SNPs that had missing data within the whole data set and executed the analyses with a site frequency spectrum (SFS) that contained 9,212 polymorphic SNPs. The model estimation utilising the SFS also requires information on the number of monomorphic sites. As there are no estimates of the genetic diversity per base pair for greylags, we made a rough estimation of the proportions of variable and monomorphic sites in our data. The number of bases covered by the GBS tags was calculated from BAM file with –depth option available in SAMtools 1.7 (19). No threshold value was used for this, all the sites that were covered with the tags were recorded, regardless of their sequencing depth or quality. This resulted in 9,801,382 bases covered with tags. After this, we mimicked the filtering steps done for the biallelic SNPs to reduce the total number of sites in equivalent proportions. We removed the same number of sites that corresponded to the number of SNPs that were removed because they were indels, had more than 2 alleles or had heterozygosity over 0.75. Since a proportion of the SNPs were removed from this analysis due to missing data in some of the individuals, we removed an equal proportion of sites from the total number of sites as well. Therefore, the SFS was constructed with 1,681,316 sites in total.

For inferring the demographic history, we chose a subset of individuals from both wild greylags and domestic geese to represent the genetic variation in both groups. This selection was done based on their admixture coefficients from the STRUCTURE analysis so that each wild and domestic population, excluding those that were clearly of a hybrid origin, were represented by an individual with the least amount of admixture from other groups. Therefore, 11 greylags with > 90.8% of greylag ancestry and 15 domestic geese with > 91.4% of European domestic goose ancestry, were selected for the analysis. By doing this, we wanted to minimise the effect of recent admixture on the estimation of divergence time of these two groups, essentially to get the most accurate estimate of the domestication time available. In order to simulate possible population histories, the parameter estimation for each model involved 100,000

simulations and 40 conditional maximization (ECM) cycles. The parameters for each model were estimated with 100 independent runs to obtain the global maximum. The models tested were i) simple divergence of two populations with no migration, ii) divergence of two populations with migration and lastly, iii) divergence of two populations with two different migration matrices (Fig. 2). The best model to represent our data was selected based on Akaike's weight of evidence as in (18). For parametric bootstrapping 100 SFS were simulated with the parameter estimates obtained from the real SFS, followed by maximum likelihood estimation with 50 independent runs for each bootstrap SFS. The 95% confidence intervals were obtained from the bootstrap data for each estimated parameter.

The estimation of genetic diversity

Genetic diversity and pairwise F_{ST} values were investigated using the *hierfstat* R package (20). Expected heterozygosity (H_E) was calculated for each locus and population and averaged across loci. Difference in average H_E between greylags and European domestics was tested with a two-sample t-test with the Welch correction for non-homogeneity of variance (21). For comparing the genetic diversity among wild and domestics, only pure greylag populations (<10% admixture with domestic geese) and pure European domestic geese (<10% admixture with Chinese domestic geese) were used to avoid hybridization effects on the estimates. The admixture proportions were obtained from STRUCTURE analysis detailed above. Therefore, the greylag populations in the Netherlands and Turkey as well as the domestic populations Diepholzer, Crested Faroese, Sebastopol, Toulouse cross, Domestic NY, Buff, Steinbacher and Kholmogor were excluded from the group estimates.

The variance components across loci for hierarchical F statistics for pure greylags and pure European domestics were estimated using hierarchical locus-by-locus analysis of molecular variance (AMOVA, (22)) implemented in Arlequin 3.5.2.1 (23). The significance was tested with 16000 permutations.

Extended results

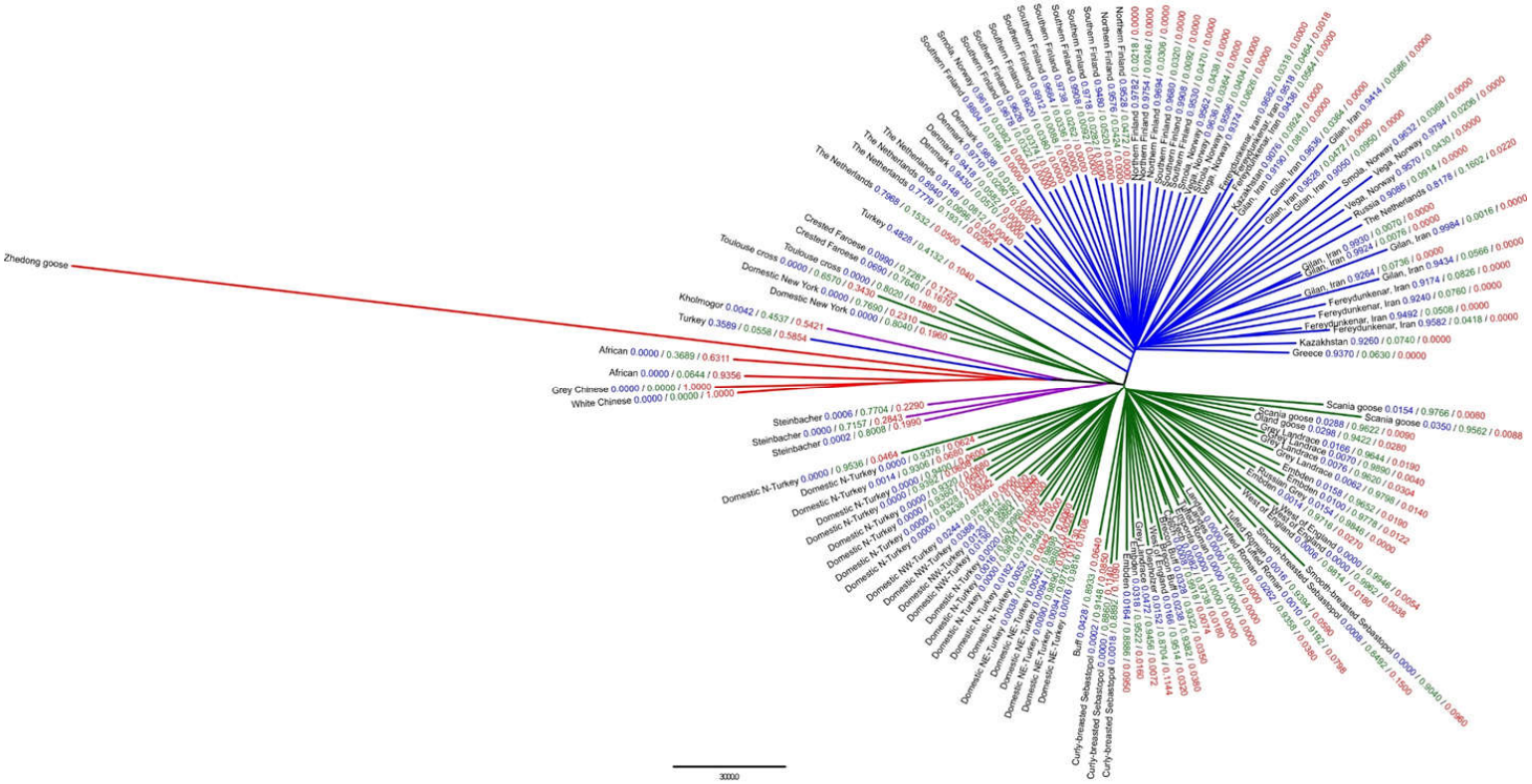
Genetic structure of greylags

Among the wild greylags, the Evanno and likelihood methods indicated that the most likely number of clusters in STRUCTURE was four (Fig. S2). Since the Turkish greylags showed the highest admixture with the domestic geese in the STRUCTURE analysis of the whole data, the STRUCTURE analysis on greylags was executed also without the Turkish greylags but K = 4 was deemed best in both analyses. The only difference between the analyses was that without the Turkish greylags, the Danish and Dutch (along with the eastern greylags) showed admixture with a cluster that was not so prominent in the analysis that included the Turkish greylags. The results suggested that there is some population structure, especially within the Iranian populations, but the major separation appears to be between eastern and western greylags. This was also visible in the PCA analysis where there was a tendency to separate the Western European

populations from the eastern greylag populations of Iran, Kazakhstan and Russia (Fig. S3). We found three significant PCs ($p < 0.05$) of which the first PC explained 7.2% of the variation, the second 4.3% and the third 4.1% (Fig. S3). In the neighbor-joining tree, eastern and western greylags mostly fall into separate clades (Fig. S1). Most Dutch and all Danish greylags formed their own clades whereas all Finnish and most of Norwegian greylags were in the same clade (Fig. S1). The Finnish and Norwegian greylags were in different branches of the tree with one individual from Norway being closely related to greylags from SW-Finland. Five samples from an island of Hailuoto in northern Finland formed a subclade that was separate from other Finnish samples that were collected in SW-Finland. The eastern greylags formed a clade with some subclades consistent with the geographical origin of the individuals, but three Norwegian individuals and one Dutch individual also fell into this clade.

Genetic structure of domestic geese

Based on STRUCTURE analysis conducted solely on domestic geese, the Evanno and likelihood methods indicated that there are two clusters within domestic geese ($K = 2$), approximately representing the European and Chinese domestic geese (Fig. S4). No subsequent split of breeds to different clusters was observed with higher values of K. Nearly all European domestic goose populations showed admixture with Chinese domestic geese. The separation between European and Chinese domestic geese was also confirmed by PCA where the first PC separated the Chinese and European domestic geese (Fig. S5). Moreover, within the European domestic geese, the Turkish domestic geese and mostly purebred European domestic geese formed their own groups (Fig. S5). This was also seen in the neighbor-joining tree (Fig. S1). According to Food and Agriculture Organization of the United Nations (FAO), the Diepholzer, Steinbacher and Kholmogor breeds are crosses of the two domestic goose types and they all showed admixture proportions with both types of domestic geese, Diepholzer (88.8% European, 11.2% Chinese), Steinbacher (76.6% European, 23.4% Chinese) and Kholmogor (45.8% European, 54.2% Chinese). In the PCA, Diepholzer and Steinbacher fell into the variation within European domestic goose breeds but Kholmogor was halfway between the European and Chinese domestic geese. Even though the STRUCTURE and PCA did not split breeds into separate genetic clusters, there was a trend of individuals of the same breed forming a clade in the neighbor-joining tree (Fig. S1). The Turkish domestics also formed subclades with individuals from nearby areas with some exceptions.


Zhedong goose
African 0.0000 / 0.3689 / 0.6311
African 0.0000 / 0.0644 / 0.9356
Grey Chinese 0.0000 / 0.0000 / 1.0000
White Chinese 0.0000 / 0.0000 / 1.0000
Turkey 0.3589 / 0.0558 / 0.5854
Kholmogor 0.0042 / 0.4537 / 0.5421
Domestic New York 0.0000 / 0.7690 / 0.2310
Domestic New York 0.0000 / 0.8040 / 0.1960
Domestic New York 0.0000 / 0.6570 / 0.3430
Toulouse cross 0.0000 / 0.8020 / 0.1980
Toulouse cross 0.0000 / 0.7440 / 0.2560
Toulouse cross 0.0000 / 0.7280 / 0.2720
Crested Farcese 0.0990 / 0.7287 / 0.1722
Crested Farcese 0.0990 / 0.7440 / 0.1570
Turkey 0.4828 / 0.4132 / 0.1040
The Netherlands 0.7969 / 0.1532 / 0.0500
The Netherlands 0.7779 / 0.1931 / 0.0290
The Netherlands 0.8940 / 0.0860 / 0.0200
The Netherlands 0.8148 / 0.0852 / 0.0000
Denmark 0.8418 / 0.0582 / 0.0000
Denmark 0.8710 / 0.0290 / 0.0000
Denmark 0.9689 / 0.0311 / 0.0000
Southern Finland 0.9854 / 0.0146 / 0.0000
Southern Finland 0.9912 / 0.0088 / 0.0000
Southern Finland 0.9622 / 0.0378 / 0.0000
Southern Finland 0.9678 / 0.0322 / 0.0000
Southern Finland 0.9818 / 0.0182 / 0.0000
Southern Finland 0.9884 / 0.0116 / 0.0000
Smola, Norway 0.9818 / 0.0182 / 0.0000
Southern Finland 0.9884 / 0.0116 / 0.0000
Southern Finland 0.9739 / 0.0261 / 0.0000
Southern Finland 0.9781 / 0.0219 / 0.0000
Southern Finland 0.9781 / 0.0219 / 0.0000
Southern Finland 0.9781 / 0.0219 / 0.0000
Northern Finland 0.9493 / 0.0507 / 0.0000
Northern Finland 0.9576 / 0.0424 / 0.0000
Northern Finland 0.9528 / 0.0472 / 0.0000
Northern Finland 0.9727 / 0.0273 / 0.0000
Northern Finland 0.9782 / 0.0218 / 0.0000
Northern Finland 0.9754 / 0.0246 / 0.0000
Northern Finland 0.9694 / 0.0306 / 0.0000
Southern Finland 0.9680 / 0.0320 / 0.0000
Southern Finland 0.9968 / 0.0032 / 0.0000
Southern Finland 0.9530 / 0.0470 / 0.0000
Vega, Norway 0.9652 / 0.0348 / 0.0000
Vega, Norway 0.9561 / 0.0439 / 0.0000
Vega, Norway 0.9596 / 0.0404 / 0.0000
Vega, Norway 0.9374 / 0.0626 / 0.0000
Fereydunkenar, Iran 0.9682 / 0.0318 / 0.0000
Fereydunkenar, Iran 0.9818 / 0.0182 / 0.0000
Kazakhstan 0.9876 / 0.0124 / 0.0000
Gilan, Iran 0.9536 / 0.0464 / 0.0000
Gilan, Iran 0.9190 / 0.0810 / 0.0000
Gilan, Iran 0.9528 / 0.0472 / 0.0000
Gilan, Iran 0.9636 / 0.0364 / 0.0000
Gilan, Iran 0.9414 / 0.0586 / 0.0000
Gilan, Iran 0.9950 / 0.0050 / 0.0000
Smola, Norway 0.9632 / 0.0368 / 0.0000
Vega, Norway 0.9794 / 0.0206 / 0.0000
Vega, Norway 0.9570 / 0.0430 / 0.0000
Russia 0.9086 / 0.0914 / 0.0000
The Netherlands 0.8178 / 0.1602 / 0.0220
Gilan, Iran 0.9930 / 0.0070 / 0.0000
Gilan, Iran 0.9924 / 0.0076 / 0.0000
Gilan, Iran 0.9984 / 0.0016 / 0.0000
Gilan, Iran 0.9264 / 0.0736 / 0.0000
Gilan, Iran 0.9434 / 0.0566 / 0.0000
Gilan, Iran 0.9174 / 0.0826 / 0.0000
Fereydunkenar, Iran 0.9240 / 0.0760 / 0.0000
Fereydunkenar, Iran 0.9492 / 0.0508 / 0.0000
Fereydunkenar, Iran 0.9582 / 0.0418 / 0.0000
Kazakhstan 0.9260 / 0.0740 / 0.0000
Greece 0.9370 / 0.0630 / 0.0000
Scania goose 0.0154 / 0.9766 / 0.0080
Scania goose 0.0350 / 0.9562 / 0.0088
Gland goose 0.0288 / 0.9622 / 0.0090
Grey Landrace 0.0298 / 0.9422 / 0.0280
Grey Landrace 0.0166 / 0.9644 / 0.0190
Grey Landrace 0.0070 / 0.9890 / 0.0040
Grey Landrace 0.0076 / 0.9620 / 0.0304
Embden 0.0062 / 0.9798 / 0.0140
Embden 0.0158 / 0.9652 / 0.0190
Russian Goose 0.0100 / 0.9778 / 0.0122
Emden 0.0154 / 0.9846 / 0.0000
Emden 0.0016 / 0.9984 / 0.0000
West of England 0.0000 / 0.9946 / 0.0054
West of England 0.0016 / 0.9984 / 0.0000
West of England 0.0000 / 0.9962 / 0.0038
Tufted Pomeranian 0.0016 / 0.9984 / 0.0000
Tufted Pomeranian 0.0000 / 0.9984 / 0.0016
Smooth-breasted Sebastopol 0.0000 / 0.9840 / 0.0160
Smooth-breasted Sebastopol 0.0000 / 0.9492 / 0.1500
Smooth-breasted Sebastopol 0.0000 / 0.9410 / 0.0960
Smooth-breasted Sebastopol 0.0000 / 0.9492 / 0.0508

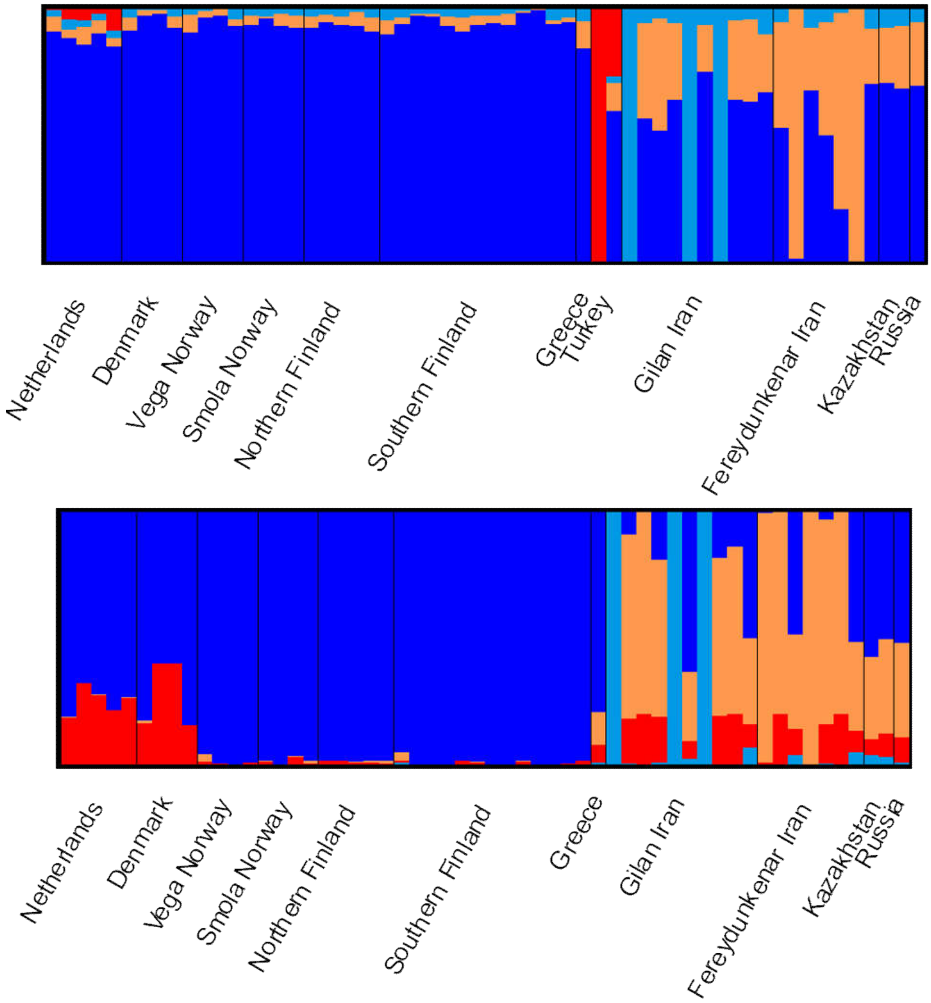


Fig. S2. STRUCTURE assignment plots for greylags when *K* = 4. Each vertical line represents one individual with different colors indicating proportion of ancestry from the inferred clusters. The analysis was done with (top) and without (bottom) Turkish greylags because the two Turkish greylags were highly admixed with domestic geese according to STRUCTURE analysis done for the whole data and we wanted to see how that affected the results.

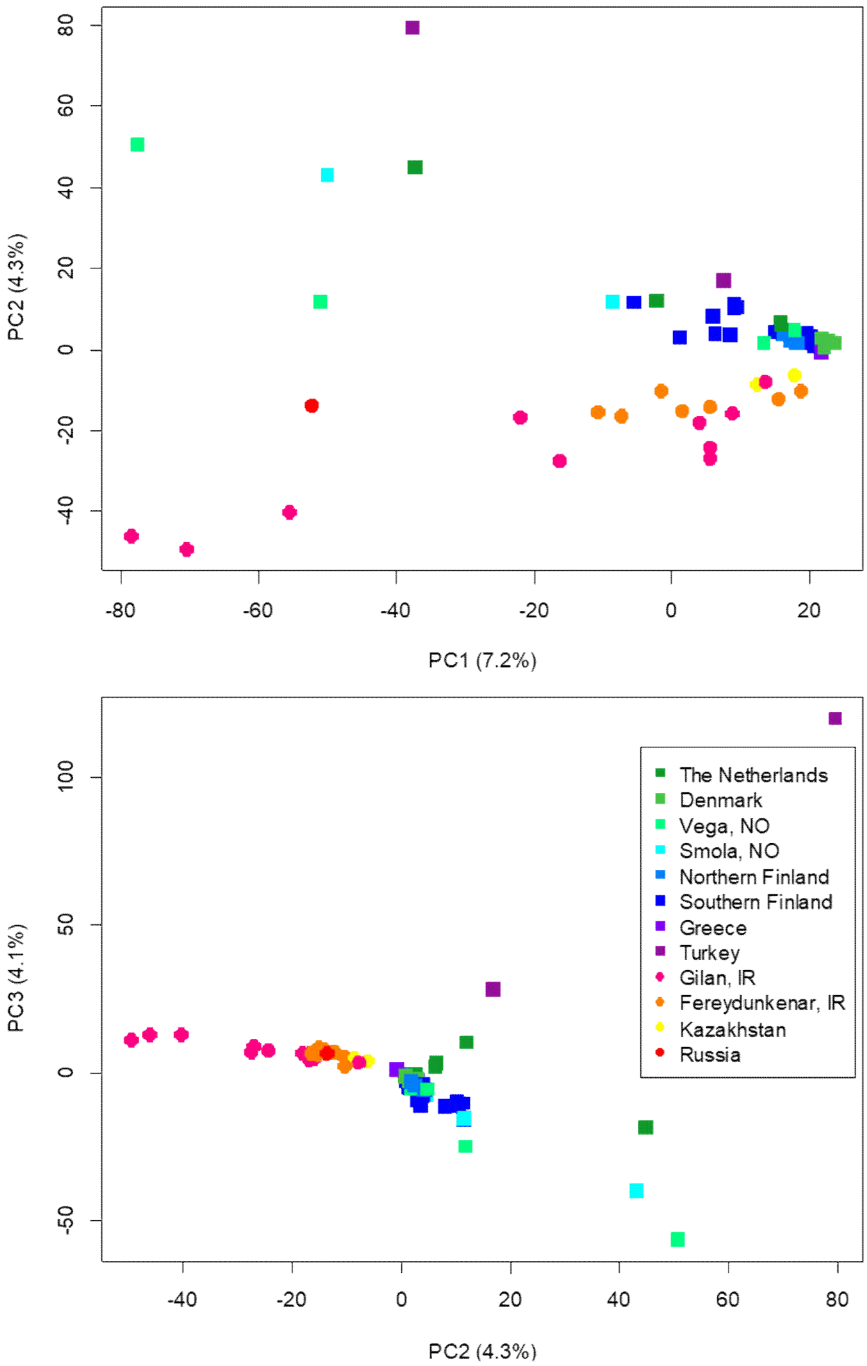


Fig. S3. The first three principal components summarizing the genetic variation within wild greylags. European populations are shown with shades of green and blue and purple square symbols while the Asian populations are depicted by yellow, orange and red round symbols. The percentages explained by each PC are shown on the X and Y axes.

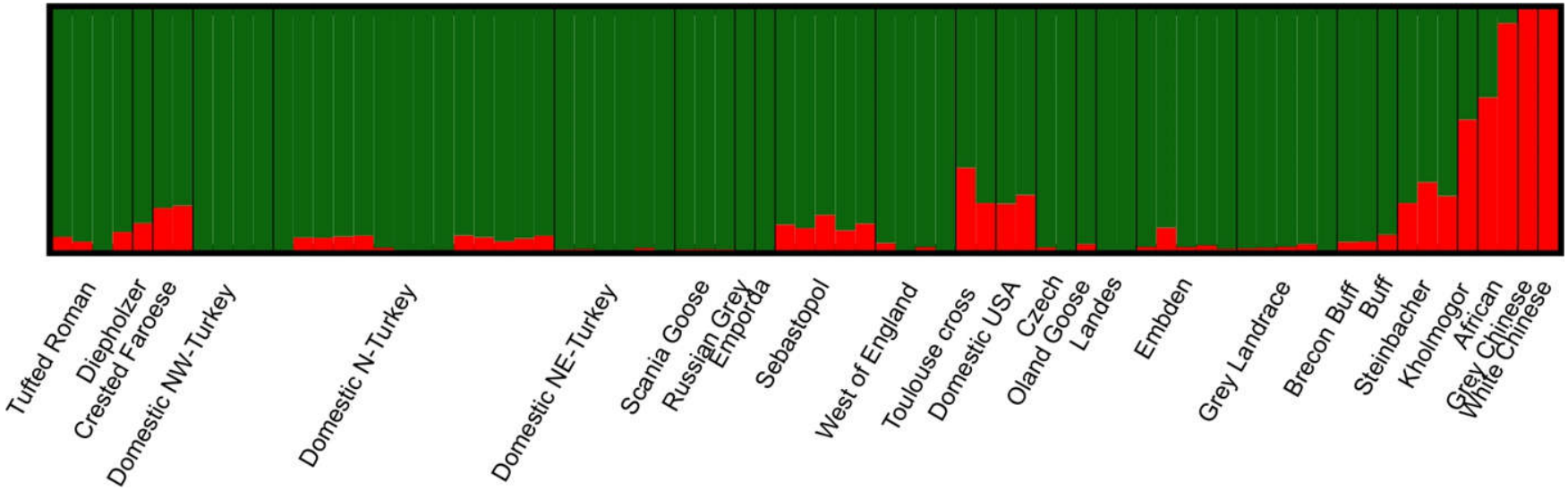


Fig. S4. STRUCTURE assignment plot for domestic geese when *K* = 2. Each vertical line represents one individual with different colors indicating proportion of ancestry from the inferred clusters where green indicates proportion of European domestic goose ancestry and red indicates proportion of Chinese domestic goose ancestry in each individual.

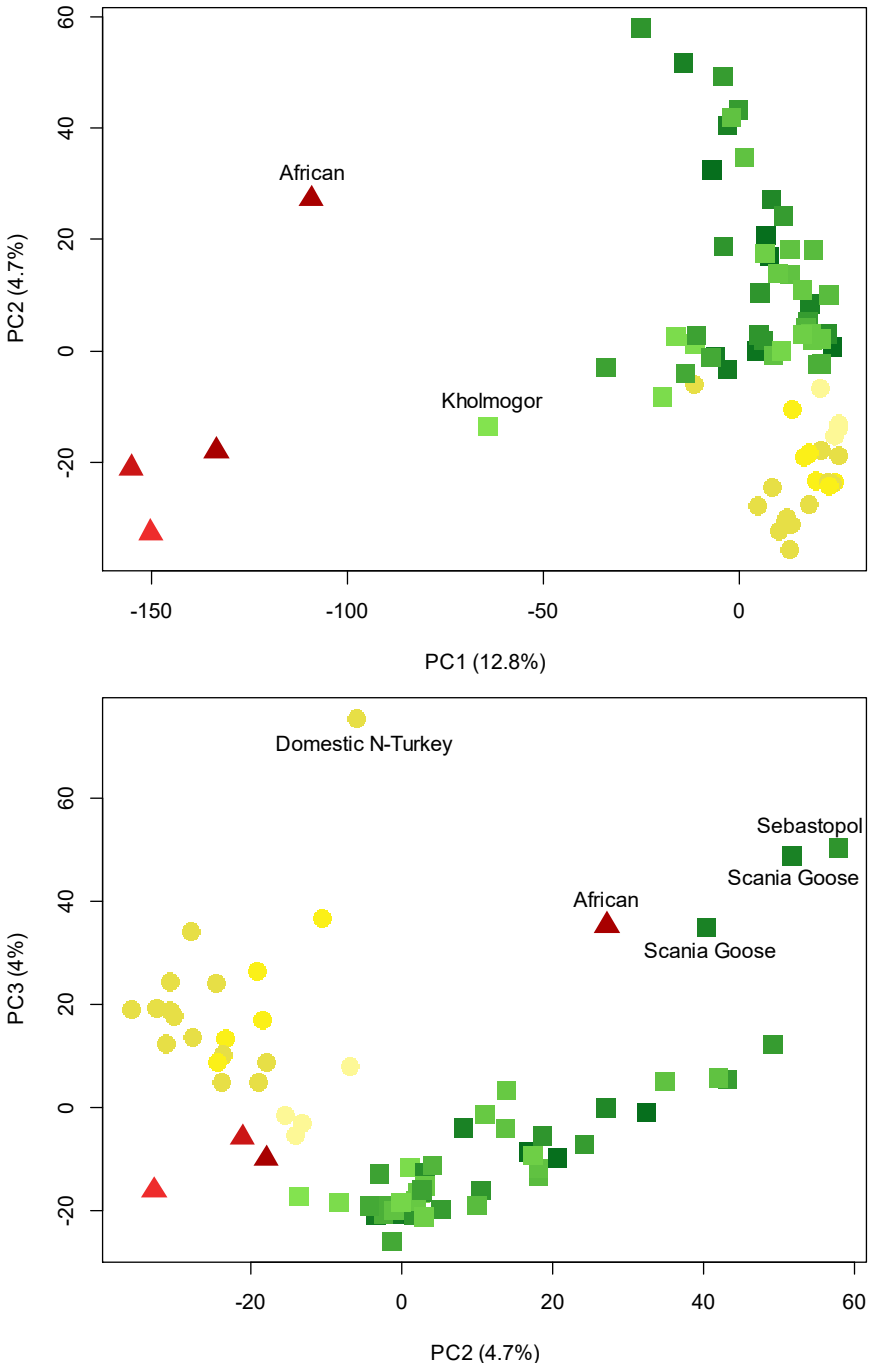


Fig. S5. The first three principal components summarizing genetic variation within the domestic geese only. The European breeds are square symbols with different shades of green, Turkish domestics are round symbols with different shades of yellow and Chinese breeds are triangular symbols with different shades of red. The percentages explained by each PC are shown on the X and Y axes.

Table S1. Diversity estimates in different greylag populations and breeds of domestic geese. The hybrid status of Diepholzer, Kholmogor and Steinbacher is based on Appendix 1 in Buckland & Guy (2002).

Population	Status	Sample size	Expected heterozygosity
Netherlands	Wild Greylag	5	0.157
Denmark	Wild Greylag	4	0.140
Vega, Norway	Wild Greylag	4	0.146
Smola, Norway	Wild Greylag	4	0.148
Northern Finland	Wild Greylag	5	0.147
Southern Finland	Wild Greylag	13	0.149
Greece	Wild Greylag	1	NA
Turkey	Wild Greylag	2	0.236
Gilan, Iran	Wild Greylag	10	0.145
Fereydunkenar, Iran	Wild Greylag	7	0.142
Kazakhstan	Wild Greylag	2	0.150
Russia	Wild Greylag	1	NA
Brecon Buff	European Domestic	2	0.110
Buff	European Domestic	1	NA
Crested Faroese	European Domestic	2	0.117
Czech	European Domestic	2	0.058
Domestic Northern Turkey	European Domestic	14	0.123
Domestic Northeast Turkey	European Domestic	6	0.094
Domestic Northwest Turkey	European Domestic	4	0.092
Domestic NY	European Domestic	2	0.143
Embden	European Domestic	5	0.120
Emporda	European Domestic	1	NA
Grey Landrace	European Domestic	5	0.107
Landes	European Domestic	2	0.047
Oland Goose	European Domestic	1	NA
Russian Grey	European Domestic	1	NA
Scania Goose	European Domestic	3	0.099
Sebastopol	European Domestic	5	0.133
Toulouse cross	European Domestic	2	0.151
Tufted Roman	European Domestic	4	0.113
West of England	European Domestic	4	0.093
Diepholzer	European x Chinese Domestic	1	NA
Steinbacher	European x Chinese Domestic	3	0.152
Kholmogor	European x Chinese Domestic	1	NA
African	Chinese Domestic	2	0.224
Grey Chinese	Chinese Domestic	1	NA
White Chinese	Chinese Domestic	1	NA

Table S2. The Patterson's 3-Population test statistics obtained to test the history of admixture in different greylag populations and breeds of domestic geese.

Source1	Source2	Target	f_3	Standard error	Z-score
Landes	Chinese	Netherlands	0.0098	0.0135	0.727
Landes	Chinese	Denmark	0.0775	0.0141	5.501
Landes	Chinese	Vega, Norway	0.0848	0.0144	5.875
Landes	Chinese	Smola, Norway	0.0575	0.0123	4.686
Landes	Chinese	Northern Finland	0.0598	0.0132	4.550
Landes	Chinese	Southern Finland	0.0389	0.0103	3.768
Landes	Chinese	Greece	0.0706	0.0260	2.715
Landes	Chinese	Turkey1	0.1902	0.0612	3.106
Landes	Chinese	Turkey2	0.1395	0.0758	1.840
Landes	Chinese	Gilan, Iran	0.0621	0.0135	4.603
Landes	Chinese	Fereydunkenar, Iran	0.0696	0.0147	4.749
Landes	Chinese	Kazakhstan	0.0426	0.0135	3.148
Landes	Chinese	Russia	0.3555	0.0425	8.366
Landes	Chinese	Tufted Roman	-0.0543	0.0175	-3.103*
Landes	Chinese	Crested Faroese	-0.0620	0.0278	-2.228**
Landes	Chinese	Domestic NW-Turkey	0.0942	0.0195	4.818
Landes	Chinese	Domestic N-Turkey	-0.0383	0.0156	-2.459**
Landes	Chinese	Domestic NE-Turkey	0.1133	0.0205	5.523
Landes	Chinese	Scania Goose	0.0578	0.0287	2.012
Landes	Chinese	Russian Grey	0.6133	0.1574	3.897
Landes	Chinese	Emporda	0.1028	0.0539	1.909
Landes	Chinese	Sebastopol	-0.1107	0.0182	-6.089*
Landes	Chinese	West of England	0.1028	0.0228	4.500
Landes	Chinese	Toulouse cross	-0.1240	0.0209	-5.921*
Landes	Chinese	Domestic NY	-0.1190	0.0227	-5.242*
Landes	Chinese	Czech	0.2749	0.0511	5.376
Landes	Chinese	Oland Goose	0.3046	0.1280	2.380
Landes	Chinese	Embden	-0.0693	0.0164	-4.223*
Landes	Chinese	Grey Landrace	-0.0041	0.0167	-0.248
Landes	Chinese	Brecon Buff	-0.0357	0.0257	-1.392
Landes	Chinese	Buff	-0.0207	0.0416	-0.497
Landes	Chinese	Diepholzer	0.0732	0.0565	1.296
Landes	Chinese	Steinbacher	-0.1149	0.0215	-5.349*
Landes	Chinese	Kholmogor	-0.1672	0.0187	-8.933*
Landes	Chinese	African	-0.1267	0.0198	-6.399*

* $p < 0.0.1$, ** $p < 0.05$

Table S3. Model selection results and parameter estimates for different demographic models that were tested (see text). Confidence intervals for the best model are shown below the bottom line.

Model	Number of parameters	log L	AIC	ΔAIC	AIC_W	ANCSIZE	N_{DOM}	N_{WILD}	T_1	T_2	$M1_{WD}$	$M1_{DW}$	$M2_{WD}$	$M2_{DW}$
Divergence with 2 migration matrices	9	-29766.4	59550.7	0	0.99999999	1112	959	2504	5319	159	$5.35x10^{-4}$	$4.25x10^{-4}$	$6.69x10^{-4}$	$1.72x10^{-3}$
Divergence with migration	6	-29787.7	59587.44	36.736	1.0541E-08	756	1056	2304	5952		$7.70x10^{-4}$	$8.86x10^{-4}$		
Divergence without migration	4	-29919.8	59847.53	296.83	3.5009E-65	7312	1047	2576	226					
						291.95-9030.75	879.95-1073.25	2341.60-2695.55	1538.80-8225.45	79.65-462.95	$2.96x10^{-4}$-$6.75x10^{-4}$	$1.73x10^{-7}$-$6.03x10^{-4}$	$4.57x10^{-4}$-$9.69x10^{-4}$	$1.33x10^{-3}$-$2.15x10^{-3}$

ANCSIZE: effective population size of ancestral population, N_{DOM}: effective population size for domestic geese, N_{WILD}: effective population size for greylags, T_1: time of divergence in generations, T_2: estimate of time in generations when the migration matrix switched, $M1_{WD}$: migration rate from wild to domestic following T1, $M1_{DW}$: migration rate from domestic to wild following T1, $M2_{WD}$: migration rate from wild to domestic following T2, $M2_{DW}$: migration rate from domestic to wild following T2

Table S4. (separate file) Pairwise F_{ST} values for each population analysed in this study.

References

1. Glaubitz JC, et al. (2014) TASSEL-GBS: a high capacity genotyping by sequencing analysis pipeline. *PLoS One* 9(2):e90346.
2. Lu L, et al. (2015) The goose genome sequence leads to insights into the evolution of waterfowl and susceptibility to fatty liver. *Genome Biol* 16(1):89.
3. Li H, Durbin R (2009) Fast and accurate short read alignment with Burrows-Wheeler transform. *Bioinformatics* 25(14):1754–1760.
4. Danecek P, et al. (2011) The variant call format and VCFtools. *Bioinformatics* 27(15):2156–2158.
5. Pritchard JK, Stephens M, Donnelly P (2000) Inference of population structure using multilocus genotype data. *Genetics* 155(2):945–959.
6. Menozzi P, Piazza A, Cavalli-Sforza L (1978) Synthetic maps of human gene frequencies in Europeans. *Science* 201(4358):786–792.
7. Patterson N, Price AL, Reich D (2006) Population structure and eigenanalysis. *PLoS Genet* 2(12):e190.
8. Falush D, Stephens M, Pritchard JK (2003) Inference of population structure using multilocus genotype data: linked loci and correlated allele frequencies. *Genetics* 164(4):1567–1587.
9. Chhatre VE, Emerson KJ (2017) StrAuto: automation and parallelization of STRUCTURE analysis. *BMC Bioinformatics* 18(1):192.
10. Evanno G, Regnaut S, Goudet J (2005) Detecting the number of clusters of individuals using the software structure: a simulation study. *Mol Ecol* 14(8):2611–2620.
11. Earl DA, VonHoldt BM (2012) STRUCTURE HARVESTER: a website and program for visualizing STRUCTURE output and implementing the Evanno method. *Conserv Genet Resour* 4(2):359–361.
12. Jakobsson M, Rosenberg NA (2007) CLUMPP: a cluster matching and permutation program for dealing with label switching and multimodality in analysis of population structure. *Bioinformatics* 23(14):1801–1806.
13. Rosenberg NA (2003) distruct: a program for the graphical display of population structure. *Mol Ecol Notes* 4(1):137–138.
14. R Core Team (2017) R: A Language and Environment for Statistical Computing. Available at: https://www.r-project.org/.
15. van Heerwaarden J, et al. (2011) Genetic signals of origin, spread, and introgression in a large sample of maize landraces. *Proc Natl Acad Sci USA* 108(3):1088–1092.
16. Paradis E, Claude J, Strimmer K (2004) APE: analyses of phylogenetics and evolution in R language. *Bioinformatics* 20(2):289–290.
17. Patterson N, et al. (2012) Ancient admixture in human history. *Genetics* 192(3):1065–1093.
18. Excoffier L, Dupanloup I, Huerta-Sánchez E, Sousa VC, Foll M (2013) Robust demographic inference from genomic and SNP data. *PLoS Genet* 9(10):e1003905.
19. Li H, et al. (2009) The sequence alignment/map format and SAMtools. *Bioinformatics* 25(16):2078–2079.
20. Goudet J (2005) hierfstat, a package for R to compute and test hierarchical F-statistics. *Mol Ecol Notes* 5(1):184–186.
21. Welch BL (1938) The significance of the difference between two means when the population variances are unequal. *Biometrika* 29(3/4):350.
22. Excoffier L, Smouse PE, Quattro JM (1992) Analysis of molecular variance inferred from metric distances among DNA haplotypes: application to human mitochondrial DNA restriction data. *Genetics* 131(2):479–491.
23. Excoffier L, Lischer HEL (2010) Arlequin suite ver 3.5: a new series of programs to perform population genetics analyses under Linux and Windows. *Mol Ecol Resour* 10(3):564–567.