The wild species genome ancestry of domestic chickens

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Short title: Chicken genome ancestry

Abstract

Hybridization and/or introgression play a key role in the evolutionary history of animal species. It is commonly observed in several orders in wild birds. The domestic chicken *Gallus gallus domesticus* is the commonest livestock species exploited for the benefit of mankind. The origin of its diversity remains unsettled. Here, we report a genome-wide analyses for signatures of introgression within domestic village chicken. We first established the genome-wide phylogeny and divergence time across the genus *Gallus*, showing the sister relationships between Grey junglefowl *G. sonneratii* and Ceylon junglefowl *G. lafayettii* and that the Green junglefowl is the first diverging lineage within the genus *Gallus*. Then, by analysing the whole-genome sequences of geographically diverse chicken populations, we reveal extensive bidirectional introgression between Grey junglefowl and domestic chicken and to a much less extent with Ceylon junglefowl. A single case of Green junglefowl *G. varius* introgression was identified. These introgressed regions include biological functions related to the control of gene expression. Our results show that while the Red junglefowl is the main ancestral species, introgressive hybridization episodes have impacted the genome and contributed to the diversity of domestic chicken, although likely at different level across its geographic range.

Keywords: *Chicken introgression, genetic diversity, chicken domestication, chicken migration, livestock, divergence time, Gallus species, interspecies hybridization, Galliformes, Speciation, Evolution, ABBA BABA*

Introduction

Despite the importance of domestic chicken *Gallus gallus domesticus* to human societies with more than 65 billion birds raised annually to produce meat by the commercial sector [1] and more than 80 million metric tons of egg produced annually for global human consumption, the origin and history of the genetic diversity of this major domesticate is only partly known. The Red junglefowl is the recognized maternal ancestor of domestic chicken [2, 3], with evidence from mitochondrial DNA (mtDNA) supporting multiple domestication centres [4] and the likely maternal contribution of several of its subspecies with the exception of *G. g. bankiva* (a subspecies with a geographic distribution restricted to Java, Bali and Sumatra).

However, the genus *Gallus* comprises three others wild species which may have contributed to the genetic background of domestic chicken. In South Asia, the Grey junglefowl *G. sonneratii* is found in Southwest India and the Ceylon junglefowl *G. lafayettii* in Sri Lanka. In South-East Asia, the Green junglefowl *G. varius* is endemic to Java and neighbouring islands [5] (Fig. 1*A*). Hybridization between the Red and the Grey junglefowls in their sympatric zones on the Indian subcontinent has been documented [5]. In captivity, hybridization between different *Gallus* species has been reported [6, 7], with Morejohn (1968) successfully producing F1 Red junglefowl x Grey junglefowl fertile hybrids in subsequent backcrossing with both species. Red junglefowl/domestic chicken mtDNA has been found in captive Grey junglefowls [8, 9] and the yellow skin phenotype is likely the result of the introgression of a Grey junglefowl chromosomal fragment into domestic chicken [10]. Captive F1 hybrids between female domestic chicken and male Green junglefowl, prized for their plumage colour and distinct voice, are common in Indonesia where they are known as Bekisar [5]. More generally, inter-species hybridization and introgression is an evolutionary process that plays a major role in the genetic history of species and their adaptation [11]. It may occur in the wild, when species live in sympatry, or in captivity following human intervention. While unravelling how

it happens and detecting its signatures at the genome level is central to our understanding the speciation process, inter-species hybridizations are commonly practiced in agricultural plants and livestock for improving productivity [12] with hybridization also known to occur between domestic and wild species in several taxa [13]. Hybridization and introgression are relatively common in wild birds, including in Galliformes [6, 14-17]. For example, the genetic integrity of the rock partridge *Alectoris graeca* is being threatened in its natural habitat through hybridization with the introduced red-legged partridge *A. rufa* [18], and the presence of Japanese quail alleles in the wild migratory common quail *Coturnix coturnix* reveals hybridization between domestic quail and the wild relative [19]. Additionally, mtDNA and nuclear microsatellite analysis indicate gene flow between Silver Pheasant *Lophura nycthemera* and Kalij Pheasant *L. leucomelanos* [20]. Infertile F1 hybrids between the common Pheasant *Phasianus colchicus* and domestic chicken have also been reported in captivity [21].

Here, we report whole genomes analysis of indigenous domestic village chickens from Ethiopia, Saudi Arabia, and Sri Lanka, together with domestic breeds from Indonesia and China, European fancy chickens and the four wild junglefowl species to infer the genetic contributions of different *Gallus sp.* to the domestic chicken genome. Our results show for the first time the presence of introgressed alleles in domestic chicken from the three non-red junglefowls species (Grey, Ceylon and Green). We also observed extensive introgression from domestic chicken/Red junglefowl into Grey junglefowl, some introgression from domestic chicken into Ceylon junglefowl but no introgression from domestic chicken to Green junglefowl. While our findings support the Red junglefowl as the primary ancestor of domestic chicken worldwide, they also indicate that the genome diversity of domestic chicken population was subsequently reshaped and enhanced following introgression from other *Gallus* species.

Results

Sampling, genetic structure and diversity

We analysed 87 whole genome sequences from domestic chickens (n = 53), Red junglefowls (Red (n = 6) and Javanese red (n = 3)), Grey junglefowl (n = 3), Ceylon junglefowl (n = 8), and Green junglefowl (n = 12)) and common Pheasant (n = 2)). Our dataset was made up of newly-sequenced genomes at an average depth of 30X, together with publicly available sequence data, which ranged from 8X to 14X. Across all the 87 genomes, a total of 91,053,192 autosomal single nucleotide polymorphisms (SNPs) were called with more than 50% of the polymorphisms found in common Pheasant (Supplementary Table S1). Summary statistics for read mapping and genotyping are provided in Supplementary Table S1.

To understand the genetic structure and diversity of the four *Gallus* species, we ran principal component (PC) and admixture analyses based on the autosomal SNPs filtered from linkage disequilibrium. PC1 clearly separated the Green junglefowl from the other *Gallus sp*, while PC2 distinguished Red, Grey and Ceylon junglefowls as well as slightly the Javanese red junglefowl subspecies from the other Red junglefowl (Fig. 1*B*), with the Grey and Ceylon junglefowls being positioned closer to each other than to any other junglefowls. The admixture analysis recapitulates these findings, providing some evidence for shared ancestry between the Red and Grey junglefowls at $K = 3$, but at the optimal $K = 5$ the ancestry of each junglefowl species is distinct (Fig. 1*C*).

132 **Detecting the true *Gallus* species phylogeny**

133 We constructed a neighbour-joining tree and a NeighorNet network using autosomal sequences
134 of 860,377 SNPs filtered to sites separated by at least 1 kb from the total 91 Million SNPs and
135 a maximum likelihood tree on 1,849,580 exon SNPs extracted from the entire autosomal
136 whole-genome SNPs. The trees were rooted with the common Pheasant as an outgroup (Fig.
137 2*A* and 2*B*; Supplementary Fig. S1*A*). Our results show that Grey and Ceylon junglefowls are
138 sister species and form a clade that is sister to the clade of Javanese red junglefowl, Red
139 junglefowl, and domestic chicken, with the latter two being paraphyletic. Green junglefowl is
140 outside of this clade, making it the most divergent junglefowl species. We also observe the
141 same relationships for the Z chromosome as well as for the mitochondrial (mt) genome (Fig.
142 2*C* and 2*D*). However, the latter show that the Grey junglefowl samples in this study do carry
143 a domestic/red junglefowl mitochondrial haplotype. All the trees show the Javanese red
144 junglefowl lineage at the base of the domestic/red junglefowl lineages.

145 Next, we investigated the extent to which other topologies are represented in the autosomal
146 genome using topology weighting by iterative sampling of sub-trees (*Twisst*) [22] based on
147 windows of 50 fixed SNPs [22]. To limit the number of topologies, the analysis was performed
148 twice using either the Red junglefowl or the Javanese red junglefowl along with the Grey,
149 Ceylon and Green junglefowls and the common Pheasant (outgroup). *Twisst* estimates the
150 relative frequency of occurrence (i.e. the weighting) of each of the 15 possible topologies for
151 these five taxa for each window and across the genome.

152 The most highly weighted topology genome-wide (T12), accounting for ~ 20% of the genome,
153 supports the autosomal species genome phylogeny: (((((Red or Javanese red junglefowl), (Grey
154 junglefowl, Ceylon junglefowl)), Green junglefowl), common Pheasant) (Fig. 3), while the
155 second highest topology (T9, ~ 18%) rather places the green junglefowl at the basal of the
156 monophyly Grey and Ceylon junglefowls: ((((Grey junglefowl, Ceylon junglefowl), Green
157 junglefowl), Red or Javanese red junglefowl), common Pheasant). There are also weightings
158 for other topologies. In particular, topologies 3 (2.9%), 10 (7.7%) and 15 (4.2%) show sister
159 relationships between the Red junglefowl and the Grey junglefowl; topologies 6 (2.2%) and 11
160 (6%) show sister relationships between the Ceylon junglefowl and the Red junglefowl and
161 topologies 1 (3.2%), 4 (3.1%) and 13 (9.7%) show sister relationships between the Green
162 junglefowl and the Red junglefowl.

163 The result of TreeMix shows similar trends in phylogenetic relationships (as above) but it
164 indicates multiple histories of admixture, namely from Red junglefowl to Grey junglefowl,
165 Ceylon junglefowl to Red junglefowl, and from the root of the monophyly Grey and Ceylon
166 junglefowls to Green junglefowl (Supplementary Fig. S1*B*), with the latter being consistent
167 with the observation on topology 9 in Fig. 3*A*.

168 **Species divergence time from autosomal genome**

169 To estimate the divergence time between lineages, we first inferred the average coalescence
170 times (CT) from the autosomes, which represents the sum of the accumulated divergence since
171 the split and the standing divergence among the average pair of individuals that was present in
172 the ancestral population at the time of the split. To estimate the split times, we subtracted the
173 estimated nucleotide diversity in each ancestral population from the CT (see materials and
174 methods for details). Among the junglefowls, the divergence times span a few million years.

175 Namely, ~ 1.1 MYA (Million Years Ago), between the Red and Javanese red junglefowls, ~
176 1.7 MYA between the Ceylon and Grey junglefowls, 2.6 to 2.8 MYA between the
177 Red/Javanese Red and Grey/Ceylon junglefowls, ~ 4 MYA between Green and the other
178 junglefowls species, while the junglefowls and common Pheasant lineages diverge ~ 21 MYA
179 (see Table 1 for details of all the pairwise divergence calculations). These split times agree
180 with autosomal and Z chromosome species trees relationships (Fig. 2). Using the same
181 approach, we estimate 8,093 (CI: 7,014 - 8,768) years for the accumulated divergence time
182 (domestication) between the domestic chicken and Red junglefowl (Table 1).

183 **Genome-wide tests for introgression between junglefowls and domestic chicken**

184 Having established a general pattern for the evolutionary history and relations of the
185 junglefowls, we next assess the presence of shared alleles between the domestic chicken and
186 the junglefowl species. We used *D*-statistics [23, 24] to test for a genome-wide excess of shared
187 alleles between the domestic chicken and each of non-red junglefowl species, relative to the
188 Red junglefowl. *D* is significantly greater than zero with strong Z-scores in all three cases
189 (Table 2), implying possible introgression between domestic chicken and the Grey, Ceylon and
190 Green junglefowls. However, because the Grey and Ceylon junglefowls are sister species,
191 introgression from just one of these species into domestic chicken could produce significantly
192 positive *D* values in both tests. Accordingly, the estimated admixture proportion (*f*) is similar
193 in both cases, ~ 12% and ~ 14% for Grey and Ceylon junglefowls, respectively. The estimated
194 admixture proportions are lower for the Z chromosome, ~ 6% with the Grey junglefowl and
195 ~10% for Ceylon junglefowl. The estimated admixture proportions between the domestic
196 chicken and the Green junglefowl are ~ 9% for the autosomes and ~ 7% for the Z chromosome.

197 **Genome scans for introgressed regions**

198 To identify specific loci harbouring introgressed variation, we calculated f_d [25] which estimate
199 local admixture proportion within a defined 100 kb windows size. This window size was
200 chosen because it is much greater than the expected size of tracts of shared ancestry from
201 incomplete lineage sorting (ILS) between these species. Given their estimated divergence time
202 and a recombination rate of 3×10^{-8}, tracts of shared variation across the species that resulted
203 from ILS would be expected to be very small, on the order of ~ 8 bp (95% CI: 7 – 10 bp) on
204 average (see methods). Next, we separated the domestic chicken into three groups based on
205 their geographic origin and in relation to the geographic location of the junglefowl species: (*i*)
206 Ethiopian and Saudi Arabian domestic chicken at the West of the Grey and wild Red
207 junglefowl geographic distributions (*ii*) Sri Lankan chicken inhabiting the same island as the
208 Ceylon junglefowl, and (*iii*) South-East and East Asian chickens, which include two breeds
209 (Kedu Hitam and Sumatra) from the Indonesian Islands, a geographic area where the Red and
210 the Green junglefowl are found, and Langshan, a breed sampled in the UK but originally from
211 China.

212 For introgression between domestic chicken and Grey junglefowl, we first selected the three
213 most extreme f_d peaks that are consistent across all three domestic chicken groups for further
214 investigation (Fig. 4): a 26 Mb region on chromosome 1 at chromosomal position 141287737
215 - 167334186 bp, a 9 Mb region on chromosome 2 at position 11022874 - 19972089 bp, and a
216 2.8 Mb region on chromosome 4 at position 76429662 - 79206200 bp (Supplementary Table
217 S2*A*; Supplementary Fig. S2*A* – S4*A*). Both the haplotype tree and network show nesting of
218 some Grey junglefowl haplotypes within the domestic chicken lineage, consistent with

219 introgression from domestic chicken/Red junglefowl into Grey junglefowl (Supplementary
220 Fig. S2 – S4 (*B* – *C*)). A result further supported by *Twisst* which indicates localised reductions
221 in the weighting of the species topology and increases in the weightings for both the topologies
222 (((Grey junglefowl, domestic), Red junglefowl), common Pheasant) and (((Grey junglefowl,
223 Red junglefowl), domestic), common Pheasant) (Supplementary Fig. S2*D* – S4*D*).
224 Furthermore, at the candidate introgressed region, *dxy* and *Fst* are reduced between domestic
225 chicken and Grey junglefowl, but not between domestic chicken and Red junglefowl
226 (Supplementary Fig. S2 – S4 (*E* – *F*)). These large tracts therefore show all the signals
227 expected of recent introgression from domestic chicken/Red junglefowl into the Grey
228 junglefowl.

229 Next, we investigated candidate introgression signals that are not consistent across the three
230 domestic chicken group comparisons, i.e. peaks present in one or two comparisons but absent
231 in the other(s). Eight candidate regions were randomly analysed and reported here
232 (Supplementary Table S2*B*). These regions are characterised by fragment sizes ranging from
233 100 kb to 500 kb. Haplotype trees and networks reveal that, unlike the large tracts introgressed
234 into Grey junglefowl, these shorter tracts show some domestic chicken haplotypes (referred to
235 here as targetDom) nested within or close to the Grey junglefowl, indicating introgression from
236 Grey junglefowl into domestic chicken (Fig. 5*A*; Supplementary Fig. S5 – S11). *Twisst* results
237 indicate localised increases in the weighting for the topology (((Grey junglefowl, targetDom),
238 Red Junglefowl), common Pheasant) with proportions ranging from 61% to 80%, much higher
239 than the species topology (((Red junglefowl, targetDom), Grey junglefowl), common
240 Pheasant) ranging from 14% to 28%, and the other alternative topology (((Grey junglefowl,
241 Red junglefowl), targetDom), common Pheasant) ranging from 6% to 11%. These loci are also
242 characterised by reduced *dxy* and *Fst* values between Grey junglefowl and domestic chicken
243 and by increased *dxy* and *Fst* between Red junglefowl and domestic chicken (Fig. 5;
244 Supplementary Fig. S5 – S11 (*E* – *F*)). These Grey junglefowl introgressed haplotypes are most
245 commonly found in Ethiopian chickens, where all eight candidate genomic regions show signal
246 of introgression, followed by Sri Lankan chicken (4 regions), Saudi Arabian chicken (3
247 regions), Sumatran chicken (2 regions) and chicken from one region in Kedu Hitam and in Red
248 junglefowl (Supplementary Table S2*B*). The introgression found on chromosome 5 was also
249 present in two European fancy chicken breeds (Poulet-de-Bresse and Mechelse-koekoe,
250 Supplementary Fig. S8). No Grey junglefowl introgression was observed in the Langshan
251 chicken. Across these eight regions, a single candidate for bidirectional introgression was
252 observed in the 100 kb region of chromosome 12, where a single Grey junglefowl haplotype
253 is nested within the domestic/Red junglefowl lineage (Supplementary Fig. S11).

254 A smaller number of candidate regions are detectable in f_d between domestic chicken and
255 Ceylon junglefowl (Supplementary Fig. S12). In most of the candidate regions investigated,
256 haplotype trees and networks indicate unresolved relationships, whereas, some show
257 introgression from Grey rather than Ceylon junglefowl into domestic chicken. By further
258 analysing every peak in the plot, we identified four candidate introgressed regions from Ceylon
259 junglefowl into domestic chicken: three on chromosome 1, spanning 6.52 Mb, 3.95 Mb and
260 1.38 Mb; and one on chromosome 3, spanning 600 kb (Supplementary Table S2*B*). Both the
261 haplotype trees and networks show introgression of one haplotype into the two different
262 domestic chicken from Sri Lanka for the three candidate regions on chromosome 1
263 (Supplementary Fig. S13), and two haplotypes into two Sri Lankan domestic chicken for the
264 chromosome 3 region (Fig. 6*B*; Supplementary Fig. S14). The 1.38 Mb region on chromosome
265 1 also shows introgression from domestic/Red junglefowl into Grey junglefowl

266 (Supplementary Fig. S13*C*). For the four introgressed regions, *Twisst* shows the highest
267 weighting for a topology grouping the target domestic samples with Ceylon junglefowl
268 (Supplementary Fig. S13). Only one candidate region of 100 kb on chromosome 5 shows
269 evidence of introgression from domestic/Red junglefowl into Ceylon junglefowl, supported by
270 both the haplotype network and topology weightings (Fig. 6*C*).

271 There were several peaks of elevated f_d between Green junglefowl and one or more groups of
272 domestic chicken (Supplementary Fig. S15). However, both the haplotype tree and network
273 clearly support introgression only in a single case, from the Green junglefowl into domestic
274 chicken in a 100 kb region on chromosome 5 at position 9538700 - 9638700 bp (Fig. 6*D*).
275 Here, the Green junglefowl is introgressed into 10 of 16 Langshan domestic chicken haplotypes
276 (Supplementary Table S2*B*). As for the candidate regions described above, this case is
277 supported by strong weighting for the topology grouping the target domestic samples with the
278 Green junglefowl, as well as reduced d_{XY} and F_{ST} between domestic chicken and Green
279 junglefowl (Supplementary Fig. S16).

280 **Functional annotations for the enriched genes within the introgressed regions**

281 We identified gene classes that are overrepresented in the regions affected by introgression
282 (Fisher's exact test, $P < 0.05$). These genes, their gene ontology (GO) terms, functions and
283 their *P*-values are catalogued in Supplementary Table S3. The overrepresented GO terms
284 include some which are linked to the immune responses (e.g. 'Positive regulation of B cell
285 proliferation' for the domestic chicken introgression into Grey junglefowl) but also to the
286 regulation of gene expression (e.g. 'Double-stranded RNA binding' for the introgression of
287 Grey junglefowl to domestic chicken; 'Post-embryonic development and Alternative mRNA
288 splicing, *via* spliceosome' for the introgression of Ceylon junglefowl into domestic chicken).

289 **Discussion**

290 The Red junglefowl has long been known to be the ancestor of domestic chicken [2-4].
291 However, one molecular study has shown the presence of an autosomal DNA fragment from
292 the Grey junglefowl in the genome of some domestic chicken [10], and Red junglefowl –
293 domestic chicken mitochondrial DNA have been found in Grey junglefowl [8, 9]. Also, F1
294 crossbreeding of domestic birds with Green junglefowl is common [5] and captive breeding
295 experiments have reported, although at a very low rate, hatching of eggs and survival of chicks
296 from F1 female Grey x Red junglefowl birds backcrossed to male parental birds from each
297 species [6, 7]. These studies suggest that other species within the genus *Gallus* may have
298 contributed to the diversity of the domestic chicken gene pool. Here, we report for the first
299 time an analysis of the full genomes of the four wild junglefowl species to assess their level of
300 contribution to the domestic chicken.

301 We first established the species phylogeny of the genus *Gallus.* Both autosomes and Z
302 chromosomes places the Red/Javanese red junglefowl equally close to the Grey and Ceylon
303 junglefowls, which show a sister species relationship. Both also indicate that the Green
304 junglefowl lineage was the first to separate from the common ancestry of the genus.
305 Interestingly, the separation of the Javanese red junglefowl occurs at the root of other Red
306 junglefowl samples studied here, noting that the latter did not include any representative of *G.*
307 *gallus murghi*, the Red junglefowl subspecies with the largest geographic distribution on the
308 Indian subcontinent. The *Gallus* phylogeny (autosomal) supports a South-East Asian origin of

309 the genus, with a first speciation event separating the Green junglefowl lineage on the present-
310 day Indonesian Islands, occurring ~ 4 MYA, at the time boundary between the Pliocene and
311 early Pleistocene. Then, a North and North-West dispersion of the continental Red junglefowl
312 ancestral population led to the separation, possibly on the Indian subcontinent, of the lineages
313 leading to the Grey and Ceylon junglefowls ~ 2.6 to 2.8 MYA. This was followed by the
314 speciation of Grey and Ceylon junglefowls ~ 1.7 MYA. The South-East Indonesian Islands
315 saw a second arrival of ancestral Red junglefowl ~ 1.1 MYA, which led to the separation of
316 the Javanese red junglefowl lineage. Using the same approach, we estimated that the
317 domestication of chicken from Red junglefowl likely occurred ~ 8,000 years ago (95% CI:
318 7,014 - 8,768 years), earlier than the archaeological evidence (at least 4,000 BP) on the North
319 of the Indian subcontinent [26] but within the Neolithic time and in agreement with the dating
320 of the early chicken remains found from 16 Neolithic sites in China (6,000 BC) [27].

321 The phylogeny of the genus *Gallus* reported here differ from some other studies [28-30], which
322 are based on short fragments of the genome. In particular, we show here a sister relationship
323 between Grey and Ceylon junglefowls, rather than between Grey and Red junglefowls [28, 30]
324 or between Green and Red junglefowls [29]. A sister relationship between the Grey and Ceylon
325 junglefowls agrees with the today geographic distribution of these two species in South India
326 and Ceylon, respectively. Other studies also indicate more ancient divergence times between
327 the different *Gallus* lineages than the ones reported here (see TimeTree, www.timetree.org).
328 For example, the separation between Grey and Ceylon junglefowl ~ 1.7 MYA (CI: 1.52 – 1.91
329 MYA) in this study is more recent than the 8.05 MYA (CI: 3.94 - 12.15 MYA) reported in
330 TimeTree. Several reasons for such discrepancy may be advocated here, e.g. the use of full
331 genome information rather than fragmentary ones as well as different mean Galliformes neutral
332 mutation rates between studies.
333
334 Topology weighting analysis, while supporting the same species tree as the primary also show
335 considerable discordance in relationships across the genome, with weightings for topologies
336 that group Red junglefowl/domestic chicken alleles closely with other *Gallus* species. Also,
337 we observe high weighting in *Twisst* analysis for the topology showing relationship between
338 the sister species Grey - Ceylon junglefowls and Green junglefowl, although lower compared
339 to the relation for these two-former species with the Red junglefowl All these results are
340 indicative of presence of incomplete lineage sorting and/or introgression during the history of
341 the genus. Interestingly, while the three non-red junglefowls (i.e. Grey, Ceylon and Green) are
342 allopatric, the fluctuating climatic changes of the Pliocene and early Pleistocene geological era
343 may have not only triggered speciation events within the genus but could have also led to
344 subsequent geographic contact between incipient species, providing opportunities for
345 hybridization at the time. Also, these topologies are supporting introgression resulting from
346 intentional crossbreeding among the genus including between domestic chicken and the Grey,
347 Ceylon and Green junglefowl in modern times.

348 Several lines of evidence support recent introgression into domestic chicken from other *Gallus*
349 species. Comparison of the *D*-statistic for the autosomes and the Z chromosome show higher
350 levels of admixture on the former than the latter. This trend is not unusual for introgression
351 between species, as species barriers to introgression are often stronger on the sex-chromosomes
352 compared to the autosomes [31].We also report larger genomic tracts showing evidence of
353 introgression than expected under incomplete lineage sorting considering the times for
354 common ancestry reported in this study. This is consistent with recent introgression events
355 where the introgressed haplotypes have not yet been fully broken down by recombination [32].

356 Typically, haplotypes subject to ILS will be expected to be of smaller size given their antiquity
357 [33]. Obviously, the timeframe of introgression between domestic chicken and the non-red
358 junglefowl species cannot be more ancient than the domestication time and the subsequent
359 dispersion time of domestic birds. At candidate introgressed fragments, we also show excess
360 sequences shared variation between the donors and recipient species, low absolute divergence
361 index with the donor species and genealogical nesting of the candidate introgressed haplotypes
362 within or close to the donor species in both the phylogenies and networks analyses. Together,
363 all the evidence strongly support that these haplotypes represent true introgressed regions from
364 the three non-red junglefowl species into domestic chicken.

365 Our results also show extensive introgression from domestic chicken/Red junglefowl into Grey
366 junglefowl with introgressed tracts at least as long as 26 Mb in size. It supports recent
367 introgression events into the Grey junglefowl examined here, which originated form captive
368 breed population. The close relationship between domestic chicken and Red junglefowl makes
369 it difficult to pinpoint the source (domestic or red junglefowl) of these introgressed alleles in
370 Grey junglefowl. Specifically, the introgression of Grey junglefowl might have originated in
371 the wild from the Red junglefowl or following the domestication and the dispersion of domestic
372 chicken, considering the long history of sympatry between domestic chicken and the Grey
373 junglefowl across India. Detailed genome analysis of candidate introgressed regions in the wild
374 Grey junglefowl as well as the inclusion in further studies of the Red junglefowl subspecies
375 from the Indian subcontinent *G. g. murghi* may further clarify these issues. Interestingly,
376 amongst the introgressed haplotype regions in the Grey junglefowl, we found several
377 previously proposed chicken domestication genes (e.g. *DACH1, RAB28*) [34, 35] favouring
378 domestic chicken introgression events. Our results highlight the need for further studies of wild
379 Grey junglefowl populations to assess whether their genetic integrity is being threatened by
380 domestic chicken introgression.
381
382 We identified introgression from the Grey junglefowl into all domestic chicken populations
383 except in the Langshan, a breed originating from China. It supports the Indian subcontinent as
384 a major centre of origin and dispersion of domestic chicken towards Africa (Ethiopia), the
385 Arabian Peninsula (Saudi Arabia), Sri Lanka, Indonesia and Europe. Interestingly, Ethiopia is
386 the region with the largest proportion of introgressed Grey junglefowl haplotypes in domestic
387 chicken (Supplementary Table S2*B*), possibly a consequence of direct trading routes between
388 the Southern part of the Indian subcontinent and East Africa. It requires further investigation.
389 Surprisingly, we also find evidence of Grey junglefowl introgression into one of the wild Red
390 junglefowl. This Red junglefowl sample originated from the Yunnan Province in China [36],
391 well outside the geographic distribution of the Grey junglefowl confined to India. Here this
392 signature of introgression is likely the result of crossbreeding between domestic chicken and
393 local wild Red junglefowl. Introgression between domestic chicken and wild Red junglefowl
394 has been shown in the past using microsatellite loci in Vietnam [37]. By extension, this result
395 supports a movement of domestic chicken from the centre of origin on the Indian subcontinent
396 towards East and South-East Asia. This hypothesis is also supported by mtDNA analysis which
397 indicates the presence at low frequency of a mtDNA haplogroup in East Asia likely originated
398 from the Indian subcontinent [4].

399 Our results also highlight the limitations of the current approaches for introgression analysis
400 when dealing with closely related species, the need to include all candidate donor species for
401 the correct interpretation of the introgression patterns, and the importance to complement
402 genome-wide analysis of introgression with locus specific analyses including phylogenetic

403 analysis of haplotypes. The *Gallus* species phylogeny indicates that the Grey and the Ceylon
404 junglefowls are sister species, which speciated after the separation of the Red
405 junglefowl/domestic chicken lineages. Signatures of shared variation suggest that both species
406 have introgressed domestic chicken. However, detailed analysis of candidate introgressed
407 regions reveal that a majority of the Ceylon junglefowl candidate f_d correspond to introgression
408 events involving the Grey junglefowl. This highlights a limitation of both genome wide *D*-
409 statistics and local admixture proportions estimates when there are multiple closely-related
410 donor species. Only a detailed assessment of all the significant f_d candidates using phylogenetic
411 analyses allowed us to identify regions showing introgression from Ceylon junglefowl into
412 domestic chicken. It should also be noted that the genome-wide estimated admixture
413 proportions observed here between the domestic chicken and the Grey, Ceylon and Green
414 junglefowls are likely underestimation following reference genome bias, with all samples
415 aligned against Red junglefowl and in the case of the Grey junglefowl, the use of introgressed
416 reference samples.

417 At the scale of individual candidate regions, we also observe a different pattern of introgression
418 for Grey and Ceylon junglefowls. While we identify several strong cases of introgression from
419 Grey junglefowl into domestic chicken, evidence for Ceylon junglefowl introgression are
420 limited to one or two Sri Lankan domestic haplotypes at each introgressed region. Similarly,
421 we only reveal one case of introgression from domestic into wild Ceylon junglefowl, a
422 somewhat surprising result considering the potential for introgression in Sri Lanka and the
423 sister relationship between the Ceylon and Grey junglefowls. While we cannot exclude a
424 sampling artefact, the findings suggest that the effect of introgression from Ceylon junglefowl
425 into domestic chicken is likely limited to Sri Lankan domestic birds only. Ceylon junglefowl
426 produce fertile hybrids in captivity with both the Red and Grey junglefowls [5], and there is
427 also anecdotal evidence of human-mediated crosses between male Ceylon junglefowl and
428 female domestic chicken in Sri Lanka (Pradeepa Silva personal communication) to increase
429 the cockfighting vigour of roosters [9].

430 Crosses between the Green junglefowl and domestic chicken are common in Indonesia [5]
431 suggesting that introgression may have occurred in either direction between these species. The
432 autosomal estimated admixture proportion (*f*) between the domestic chicken and the Green
433 junglefowl is ~9%. It is ~7% for the Z chromosome (Table 2). However, our results support
434 only a single compelling example of introgression from the Green junglefowl into domestic
435 chicken. This signal is limited to the Langshan, a Chinese chicken breed. It may represent a
436 legacy of movement of domestic birds from the Indonesian Islands to the East Asian continent.
437 No candidate introgressed regions were detected in the Indonesian domestic chickens (Kedu
438 Hitam and Sumatra). Introgression between the Green junglefowl and domestic chicken may
439 be impeded by genetic barriers, given the greater time since divergence compared to that
440 between Red junglefowl/domestic chicken and the Grey and Ceylon junglefowls.

441 There is increasing evidence for "adaptive" cross-species introgression amongst mammalian
442 domesticates [38] as well as in humans [33]. A previous study suggests that the chicken yellow
443 skin phenotype is the consequence of introgression event(s) from the Grey junglefowl into
444 domestic chicken [10], a phenotype favoured by some chicken breeders and now fixed in
445 several fancy and commercial breeds [10, 35]. Here, besides some traditional breeds
446 (Langshan, Kedu Hitam Sumatra) with fixed morphological phenotypes, we analysed village
447 chicken populations that are typically characterized by a high level of phenotypic diversity
448 (e.g. plumage colour and pattern, morphology). Introgressed regions were not found fixed or

449 approaching fixation in any of the indigenous village chicken populations examined. Gene
450 ontology (GO) analysis reveals several biological functions related to the control of gene
451 expression (see Supplementary Table S3). Undoubtedly, these candidate introgressed regions
452 are contributing to the genome diversity of the domestic chicken and while we have no
453 evidence of positive selection at these introgressed regions [34], other selection pressures (e.g.
454 heterozygote advantage - balancing selection) may possibly be acting. Whether or these
455 introgression events influence the phenotypic diversity in village chickens is unclear, but it is
456 likely that variation in expression during post embryonic development as revealed by GO
457 analysis contributes to the wide variety of morphological phenotypes observed within and
458 across domestic chickens. For example, among several genes within haplotypes introgressed
459 from Grey junglefowl are *NOX3* and *GSC* involved in ear development and biogenesis of
460 otoconia supporting balance and gravity detection [39, 40]. Moreover, *CPEB3,* which is
461 associated with thermoception and enhancing memory [41, 42] could play central roles in
462 adaptation to new environmental challenges. *MME,* which plays a role in stimulating cytokine
463 production [43] and *RAP2B,* which is mainly expressed in the neutrophils for platelet activation
464 and aggregation [44] might also affect the immune system of introgressed chickens. Other
465 genes of interests include *CDC5L* and *FOXP2* introgressed from Ceylon junglefowl. The
466 former is a key mitotic progression regulator involved in DNA damage response [45] and the
467 latter is a gene involved in song learning in birds [46]. *IPO7* which is introgressed from Green
468 junglefowl is involved in the innate immune system [47].

469 In conclusion, our study reveals a polyphyletic origin of domestic chicken diversity with major
470 contributions from the Red junglefowl, but also introgression from the Grey, Ceylon and Green
471 junglefowls. These findings provide new insights into the domestication and evolutionary
472 history of the species. Considering the present geographic distributions of the non-red
473 junglefowl species and the dispersal history of domestic chickens, it is unsurprising that the
474 level of introgression amongst domestic populations varies from one geographic region to the
475 other as it will likely reflect their genetic histories. Similarly, analysis of more domestic
476 populations on a wider geographic scale may provide us with a detailed geographic map of the
477 presence and frequency of introgressed regions across the domestic chicken distribution. Our
478 results shed new light on the origin of the diversity of our most important agricultural livestock
479 species and illustrates the uniqueness of each local domestic chicken population across the
480 world.

481 **Materials and Methods**

482 **Sampling and DNA extraction**

483 Details of the 87 samples studied here and their geographic location of sampling distributions
484 are provided in Supplementary Table S1. Blood samples were collected from the wing vein of
485 27 indigenous village domestic chickens from three countries (i.e. Ethiopia (n = 11), Saudi
486 Arabia (n = 5) and Sri Lanka (n = 11)) [9, 34, 48], eight Chinese Langshan chicken sampled in
487 the United Kingdom, and 11 non-red junglefowl *Gallus* species (i.e. Grey (n = 2), Ceylon (n =
488 7) and Green (n = 2) junglefowls). Blood samples from five of the Ceylon junglefowls were
489 obtained from the wild in Uva province of Sri Lanka while the remaining two Ceylon
490 junglefowls blood were sampled from Koen Vanmechelen collection. The two common
491 Pheasants, *Phasianus colchicus* were sampled from the wild in the United Kingdom. Genomic
492 DNA was extracted following the standard phenol-chloroform extraction procedure method

493 [49]. For all, genome sequencing was performed on the Illumina HiSeq 2000/2500/X platforms
494 with an average depth of 30X coverage.
495
496 This dataset was complemented with genome sequences from two domestic fancy chicken
497 breeds (Poule de Bresse and Mechelse Koekoek), one Mechelse Styrian, a 16th generation
498 crossbred bird from the Cosmopolitan Chicken Research Project (CCRP:
499 https://www.koenvanmechelen.be/) and as well as one Red, Grey, Ceylon and Green
500 junglefowls sequences also from Koen Vanmechelen collection (www.koenvanmechelen.be/).
501 The publicly retrieved genome sequences of 15 Indonesian indigenous chickens (Sumatra, n =
502 5 and Kedu Hitam, n = 10) [50], three Javanese red junglefowl *G. g. bankiva* and nine Green
503 junglefowls [50], and five Red junglefowls, sampled in Yunnan or Hainan Provinces (People's
504 Republic of China)[36] were also included in our dataset. Genome sequence depth for these
505 birds ranges from 8X to 14X.

506 In total, these 87 genomes include 53 domestic chicken, 6 Red junglefowl, 3 Javanese red
507 junglefowl, 3 Grey junglefowl, 8 Ceylon junglefowl, 12 Green junglefowl and 2 common
508 Pheasants. The newly sequence reads of these birds are accessible at
509 https://www.ncbi.nlm.nih.gov/sra/PRJNA432200 or in the NCBI with the accession number
510 PRJNA432200.
511
512 **Sequence mapping and variants calling**

513 Raw reads were trimmed of adapter contamination at the sequencing centre (i.e BGI/Edinburgh
514 Genomics) and reads that contained more than 50% low quality bases (quality value ≤ 5) were
515 removed. Reads from all genomes were mapped independently to the *Galgal* 5.0 reference
516 genome [51] using the Burrows-Wheeler Aligner bwa mem option version 0.7.15 [52] and
517 duplicates were marked using Picard tools version 2.9.0
518 (http://broadinstitute.github.io/picard/). Following the genome analysis toolkit (GATK)
519 version 3.8.0 best practises [53], we performed local realignment around INDELs to minimize
520 the number of mismatching bases across all reads. To apply a base quality score recalibration
521 step to reduce the significance of any sequencing errors, we used a bootstrapping approach
522 across both the wild non-red junglefowls species and common Pheasants that has no known
523 sets of high-quality database SNPs. We applied same approach to the red junglefowl for
524 consistency. To do this, we ran an initial variant calling on individual unrecalibrated BAM files
525 and then extracted the variants with highest confidence based on the following criteria: --
526 filterexpression "QD < 2.0 || FS > 60.0 || MQ < 40.0". We then fed this initial high-quality
527 SNPs as input for known set of database SNPs. Finally, we did a real round of SNPs calling on
528 the recalibrated data. We ran these steps in a loop for multiple times until we reach convergence
529 for each sample.

530 To improve the genotype likelihoods for all samples using standard hard filtering parameters,
531 we followed the multi-sample aggregation approach, which jointly genotypes variants by
532 merging records of all samples using the '-ERC GVCF' mode in 'HaplotypeCaller'. We first
533 called variants per sample to generate an intermediate genomic (gVCF) file. Joint genotype
534 was performed for each species separately using 'GenotypeGVCFs' and then subsequently
535 merged with BCFtools version 1.4 [54]. Variants were called using Hard filtering "--
536 filterExpression "QD < 2.0 || FS > 60.0 || MQ < 40.0 || MQRankSum < -12.5 ||
537 ReadPosRankSum < -8.0". All downstream analyses were restricted to the autosomes, the Z
538 chromosome and the mitochondrial DNA.

539 The percentage of the mapped reads and read pairs properly mapped to the same chromosome
540 were calculated using SAMtools "flagstat" version 1.4 [54] while the number of SNPs per
541 sample were identified using VCFtools "vcf-stats" version 0.1.14 [55]. Summary statistics for
542 read mapping and genotyping are provided in Supplementary Table S1.

543 **Population genetic structure**

544 Principal component analysis was performed on the SNPs identified across the autosomes,
545 filtered with "--indep-pairwise 50 10 0.3", to visualise the genetic structure of the junglefowl
546 species using PLINK version 1.9 (http://pngu.mgh.harvard.edu/purcell/plink/) and was
547 complemented with analysis using ADMIXTURE version 1.3.0 [56], performed unsupervised
548 with default (folds = 5) for cross-validation in 5 runs with different number of clusters (*K*).

549 **Species tree**

550 To unravel the species tree of the genus, we constructed an autosomal Neigbour-Joining
551 phylogenetic tree using Phyml version 3.0 [57] and network using NeigborNet option of
552 SplitsTree version 4.14.6 (*splitstree.org)*. First, the dataset was filtered to sites separated by at
553 least 1 kb and then converted to a phylip sequence file using scripts from
554 https://github.com/simonhmartin. We also constructed maximum likelihood tree on the exon
555 variants. This was done by first annotating the entire whole-genome vcf file with SnpEff and
556 then extracted different variants effect within the exons using SnpSift [58]. As with the above,
557 all trees including the Z chromosome were based on polymorphic sites but not for the mtDNA
558 (i.e. all consensus sequences were used). All trees were plotted using the General Time
559 Reversible (GTR) model of nucleotides substitution following its prediction by jModeltest
560 2.1.7 [59] and then viewed in MEGA 7.0 [60].

561 After phasing all the autosomal SNPs using SHAPEIT[61], we next performed "Topology
562 Weighting by Iterative Sampling of Sub-Trees" (*Twisst*) [22] which summarized the
563 relationships among multiple samples in a tree by providing a weighting for each possible sub-
564 tree topology. Neighbour-joining trees were generated for fixed 50-SNP windows using Phyml
565 3.0 [57]. Topologies were plotted in R using the package "APE" version 5.1 [62]. We ran the
566 TreeMix [63] in a block size of 1000 SNPs per window after having filtered the vcf file with
567 "maf 0.01" using PLINK version 1.9 (http://pngu.mgh.harvard.edu/purcell/plink/).

568 **Species divergence time**

569 To estimate the divergence time between the junglefowl species as well as with the common
570 Pheasant, we first estimated the approximate coalescence time, which include the divergence
571 that has accumulated from the present to the period of split and the divergence among the
572 average pair of individuals that was present in the ancestral population at the time of split,
573 using the equation:

574 $T = K/2r$ [64]

575 where K is the average sequence divergence for pairwise species. We included both the variant
576 and non-variant sites in the analysis of K which was run in every 100 kb region of the genome
577 with 20 kb step size. r is the Galliformes nucleotide substitution rate per site per year 1.3 (1.2
578 – 1.5) x 10^{-9} [65], T is the time in year.

579 Assuming the average diversity (π) across the descendant of junglefowl species is similar to
580 the diversity present in their ancestral population before each split, we estimated the divergence
581 time as below:

582 $T = (K - \pi)/2r$

583 Using the commonest species topology, the average $\pi = (\pi_{Pheasant} + (\pi_{Green} + ((\pi_{Grey} + \pi_{Ceylon})/2$
584 $+ (\pi_{Javanese\ Red} + \pi_{Red})/2)/2)/2$

585 **Estimating tract lengths for shared haplotypes under incomplete lineage sorting**

586 Using the approach of Huerta-Sánchez et al [66], we estimated the likely length of shared
587 haplotypes across the genome following incomplete ancestral lineage sorting. This was done
588 with the equation

589 $L = 1/(r \times t)$

590 where L is expected length of a shared ancestral sequence, r is recombination rate per
591 generation per bp (3×10^{-8} for chicken on the autosomes) [67] and t is the expected divergence
592 time across the junglefowls (~ 4 MYA), assuming one year generation time.

593 **Detecting introgression**

594 First, we computed *D*-statistics [23, 24] to test for a genome-wide excess of shared derived
595 allele(s) between two in-groups using the outgroup as representative of the ancestral state.
596 Considering the three in-groups, P_1 (Red junglefowl), P_2 (domestic chicken) and P_3 (Grey or
597 Ceylon or Green junglefowls), and an out-group *O* (common Pheasant), the expected
598 phylogeny is $(((P_1, P_2), P_3), O)$. ABBA denotes sites where the derived allele 'B' is shared
599 between the domestic chicken 'P_2' and the Grey or Ceylon or Green junglefowls 'P_3', while
600 the Red junglefowl 'P_1' shares the ancestral allele 'A' with the common Pheasant 'O'. BABA
601 denotes sites where the Red junglefowl 'P_1' shares the derived allele 'B' with 'P_3' while the
602 domestic chicken 'P_2' shares the same ancestral state with the outgroup 'O'. The majority of
603 ABBA and BABA patterns are due to incomplete lineage sorting but an excess of one over the
604 other can be indicative of introgression [23-25]. *D* is the relative excess computed as the
605 difference in the number of ABBA and BABA sites divided by the total number of ABBA and
606 BABA sites. Under the assumption of no gene flow and a neutral coalescent model, count of
607 both ABBA and BABA should be similar and *D* should tend to zero. We used the approach of
608 Durand et .al [24] to compute ABBA and BABA counts from allele frequencies, in which each
609 SNP contributes to the counts even if it is not fixed. We used the jackknife approach, with a
610 block size of 1 Mb to test for a significant deviation of *D* from zero (i.e consistent with
611 introgression), using a minimum Z score of 4 as significant. We then estimated the proportion
612 of admixture, f [23, 24]

613 **Identifying introgression at particular loci and inferring the direction of introgression**

614 To identify specific regions showing introgression between the domestic chicken and the non-
615 red junglefowl species, we used a combination of analyses. First, we estimated f_d [25], which
616 is based on the four-taxon ABBA-BABA statistics and was designed to detect and quantify
617 bidirectional introgression at particular loci [25], f_d was computed in 100 kb windows with a

618 20 kb step size. Each window was required to contains a minimum of 100 SNPs. The strongest
619 candidate regions of introgression (highest elevated *fd* values) were visually assessed to
620 determine the introgression size. We avoided the use of extreme tail end or cut-off approach to
621 prevent losing out peaks harbouring introgression into domestic chicken as the majority of
622 candidate introgression signals supports introgression from domestic chicken into Grey
623 junglefowl. These f_d regions were then extracted and further investigated using *Twisst* [22] to
624 test for a deviation in topology weightings in the candidate regions. Here, we used only four
625 taxa: domestic chicken, Red junglefowl, common Pheasant, and either the Grey, Ceylon or
626 Green junglefowl.

627 Next, we constructed haplotype-based gene trees and networks to make inferences about the
628 direction of gene flow. The expectation is that introgressed regions in domestic chicken from
629 any of the non-red junglefowls will be indicated by finding chicken haplotypes nested within
630 the donor species, or with the donor species haplotypes at the root of the introgressed ones. For
631 regions in non-red junglefowls that are introgressed from domestic chicken, the expectation is
632 that the introgressed haplotypes will be nested within the domestic chicken clade. To do this,
633 sequences from the candidate introgressed regions were phased using SHAPEIT [61]. The
634 phased haplotypes were converted into a variant call format file and subsequently formatted in
635 Plink 1.9 [68] with the 'beagle recode' option, the output from which was provided as an input
636 to a custom bash script to generate a FASTA file. The optimal molecular evolutionary model
637 was inferred using jModeltest 2.1.7 [59] based on the Akaike information criterion (AIC).
638 Phyml 3.0 [57] was used to compute the approximate likelihood ratio score for each branch
639 using the best predicted model. For the network, we used the NeigborNet option of SplitsTree
640 version 4.14.6 (*splitstree.org)*. The input file for the network was a distance matrix created
641 using 'distMat.py' accessible at https://github.com/simonhmartin/genomics_general.

642 Finally, we examined levels of divergence between species to further validate our candidate
643 regions. Introgression between domestic chicken and either the Grey, Ceylon or Green
644 junglefowls is expected to reduce genetic divergence between the two species, regardless of
645 the direction of introgression. Introgression into domestic chicken is expected to also increase
646 divergence between domestic chicken and Red junglefowl, whereas introgression from
647 domestic chicken into the Grey, Ceylon or Green junglefowl should not affect divergence
648 between domestic chicken and Red junglefowl. We therefore computed relative (F_{ST}) and
649 absolute (d_{XY}) measures of divergence between pairs using the script popgenWindows.py
650 available at https://github.com/simonhmartin/genomics_general.

651 **Remapping of candidate introgressed regions to GRCg6a**

652 Following the recent release of new reference genome (GRCg6a), all candidate introgressed
653 regions obtained from *Galgal* 5.0 were remapped using the NCBI remapper tool. All
654 remapping options were set to the default threshold. The GRCg6a coordinates for the candidate
655 introgressed regions and genes are reported here throughout the manuscript.

656 **Gene ontology analysis**

657 All candidate genes within the introgressed regions for different pairwise comparison and in
658 different introgressed directions were used to determine their biological cluster functions using
659 DAVID version 6.8 (https://david.ncifcrf.gov/summary.jsp). Only gene ontology with Fisher
660 exact $P < 0.05$ default threshold were retained in this study.

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Author contributions

O.H. and R.A.L. designed and supervised the project with major contributions from S.H.M for the data analysis. P.S. contributed the DNA of five Ceylon junglefowls and domestic birds from Sri Lanka. R.M.A., R.S.A., and J.M.M. collected the samples and provided the DNA of the Saudi Arabian birds. All the captive junglefowl blood samples were collected from K.V. farm while their DNA preparation was performed by R.A.L., D.W. and O.H. The DNA preparation of the Ethiopian chickens were performed by J.M.M. The genome sequences of the fancy birds, one Red, Grey, Ceylon and Green junglefowls were contributed by K.V. and A.V. Langshan samples were collected by P.M.H with genome sequences information provided by D.D.W. and Y-P.Z. The genome sequence of Pheasant was provided by J.S. All data analyses were performed by R.A.L. The manuscript was prepared by R.A.L and substantially revised by O.H and S.H.M. All other authors reviewed and accepted the final draft of the manuscript.

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Figures and Tables

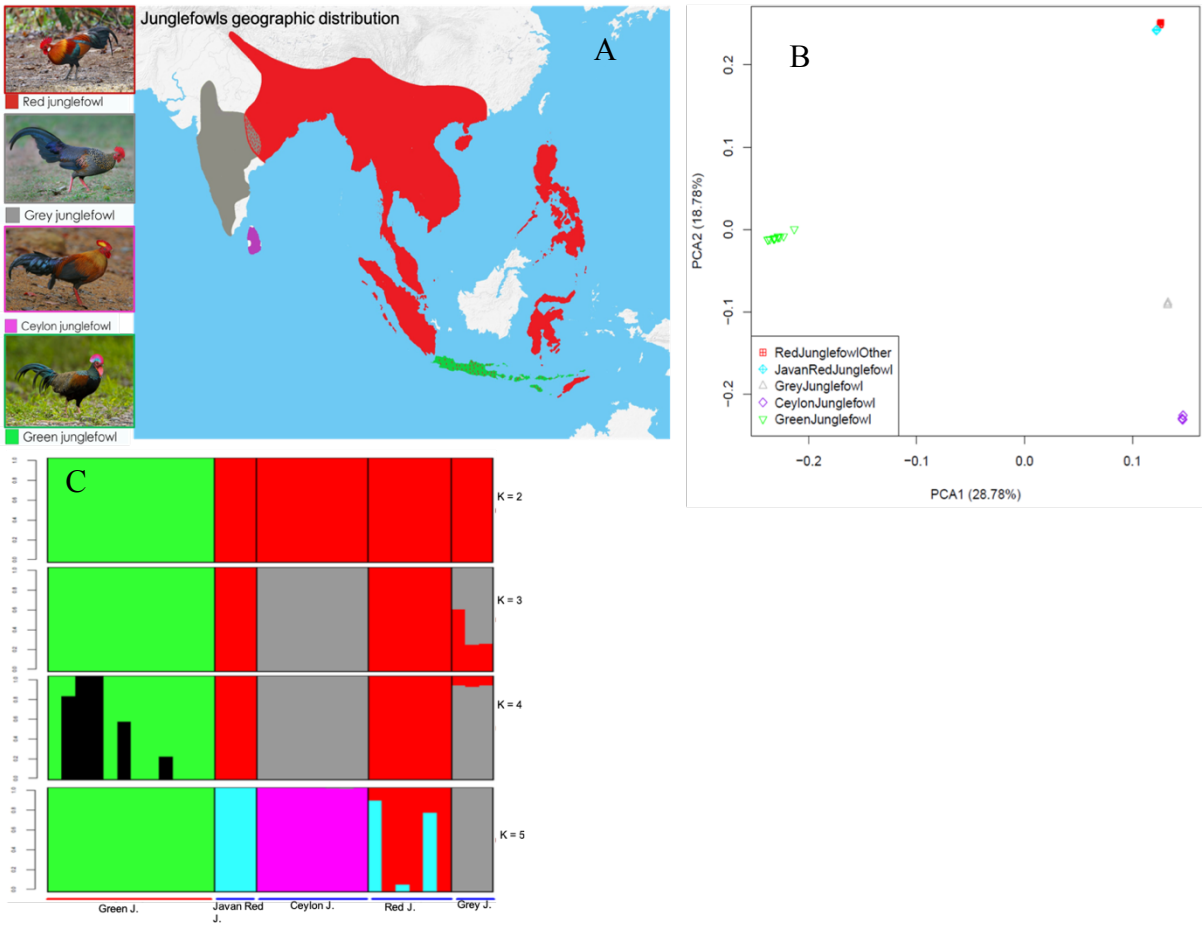

Fig. 1: (***A***) The geographic distribution of the four junglefowl species. The sympatric zones where Indian red junglefowl (*Gallus gallus murghi*) overlap with the Grey junglefowl on the Indian subcontinent and Javanese red junglefowl (*Gallus gallus* bankiva) overlap with the Green junglefowl on the Indonesian Islands are annotated with red dots on the map. The map was drawn by overlaying the distribution map of each species obtained from the Handbook of the Birds of the World (consulted in December 2018). Junglefowl species photo credits: Peter Ericsson (Red junglefowl), Clement Francis (Grey junglefowl), Markus Lilje (Ceylon junglefowl), Eric Tan (Green junglefowl). (***B***) Principal component and (***C***) Admixture analysis to establish the species relatedness and genetic structures from the autosomal sequences. ◆: Red junglefowl, ◆: Javanese red junglefowl, ◆: Grey junglefowl, ◆: Ceylon junglefowl, ◆: Green junglefowl

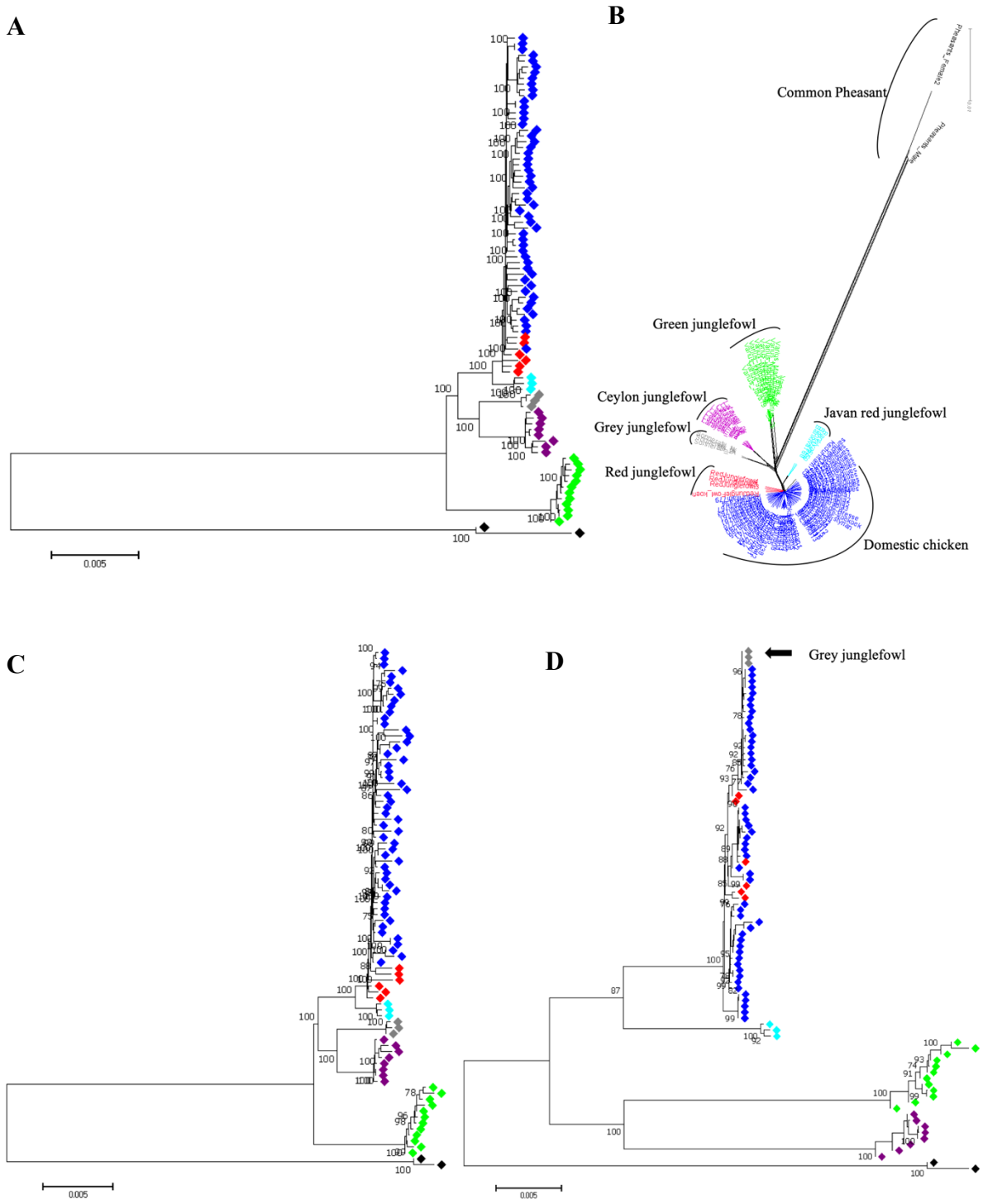

Fig. 2. The genome-wide phylogeny of the genus *Gallus.* The figures (***A***), (***C***) and (***D***) are based on Neighbour-Joining phylogenetic trees on the autosomes, Z chromosome and mitochondrial DNA, respectively. The figure (***B***) is the distance matrix of the autosomes constructed from the NeighbourNet network of SplitsTree4. The three Grey junglefowl mtDNA haplotypes in (***D***) are embedded within the domestic/Red junglefowl lineage, indicated with the black arrow. All the trees were rooted with the common Pheasant *Phasianus colchicus*. The colours defining each species are; ◆: Domestic chicken, ◆: Red junglefowl, ◆: Javanese red junglefowl, ◆: Grey junglefowl, ◆: Ceylon junglefowl, ◆: Green junglefowl, ◆: common Pheasant.

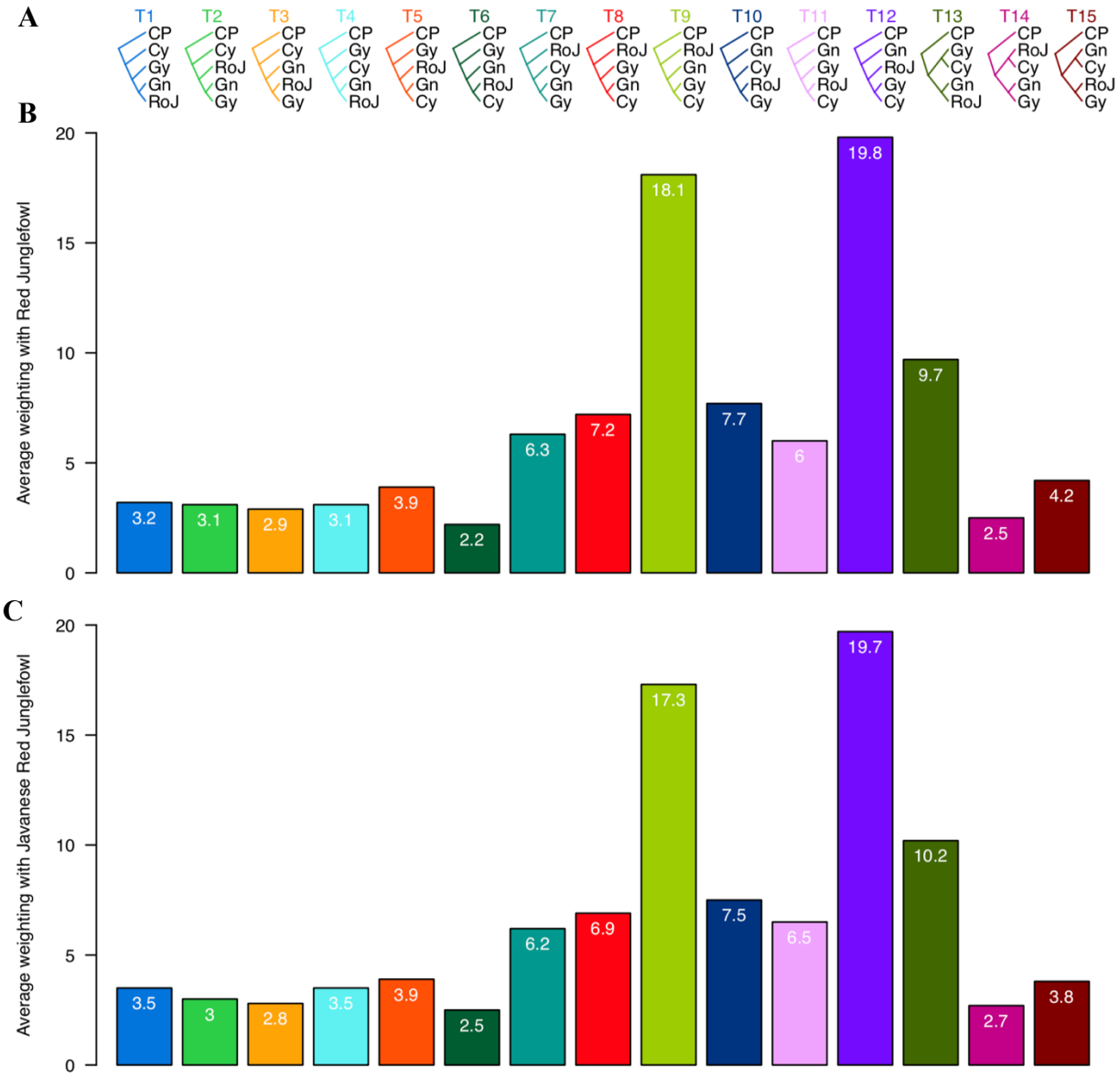

Fig. 3. Topology weighting by iterative sampling of sub-trees (*Twisst*) for all the (***A***) 15 possible topologies (T1 – T15) from five taxa: Red junglefowl 'or' Javanese red junglefowl (RoJ), Grey junglefowl (Gy), Ceylon junglefowl (Cy), Green junglefowl (Gn) and Common Pheasant (CP). As the number of possible topologies work best for maximum of five taxa (Martin & Van Belleghem, 2017) and with the presence of six taxa in this study, we ran the analysis twice; one with (***B***) Red junglefowl (R) 'or' with (**C**) Javanese Red junglefowl (J). (see materials and methods for detail on how this was done). The average weighting (%) for each of the 15 topologies is included in each bar and as well indicated on the *Y* axis.

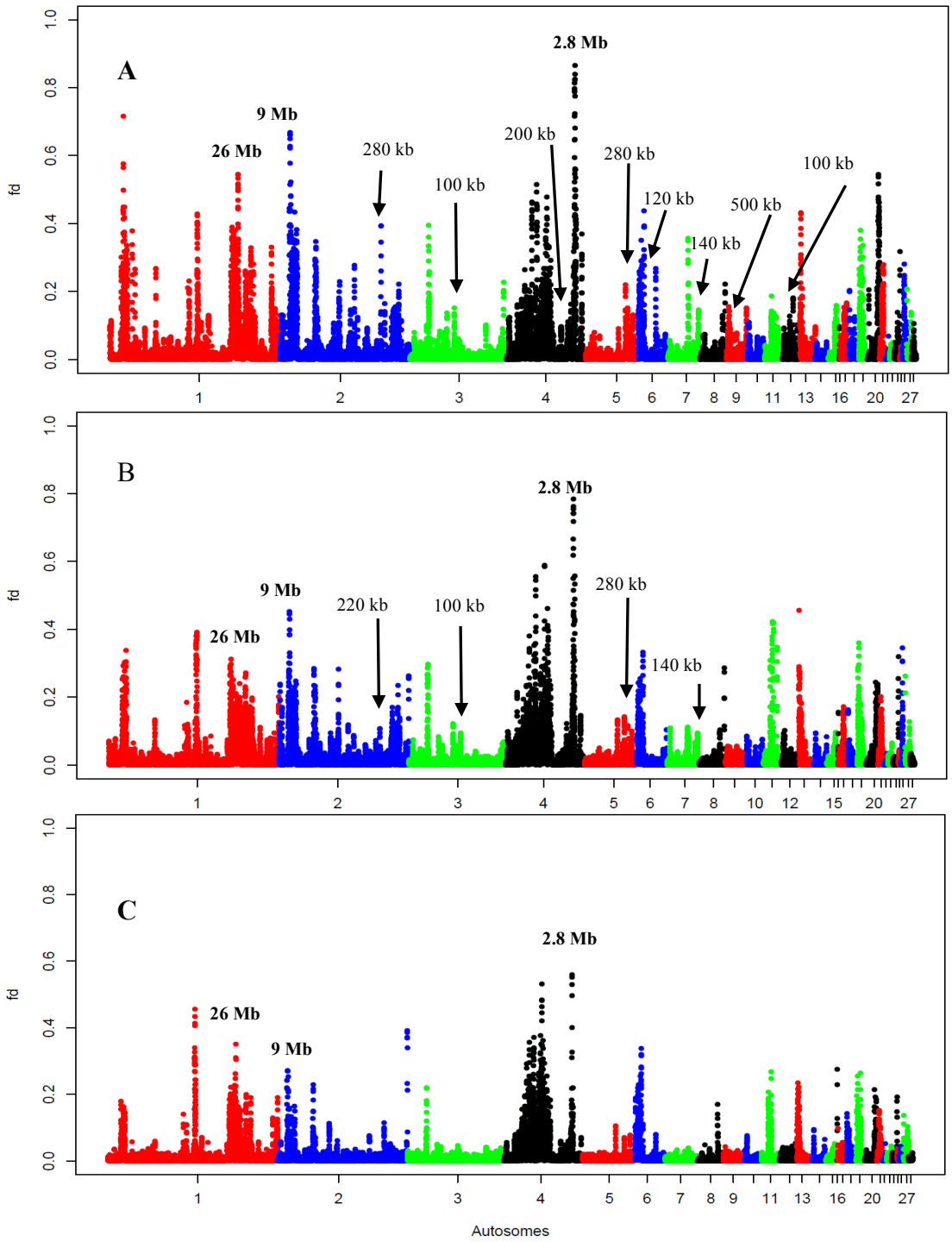

Fig. 4. The f_d plots test for the comparison between Grey junglefowl and domestic chicken populations from (***A***) Ethiopia and Saudi Arabia, (***B***) Sri Lanka (***C***) South-East and East Asia. The candidate introgressed regions reported here and their sizes are indicated above each peak (see also Supplementary Table S2). Bold values are introgressed regions from domestic chicken/Red junglefowl into Grey junglefowl, plain values are introgressed regions from Grey junglefowl into domestic chicken. *Y* axis: f_d value spanning 0 to 1, *X* axis: autosomal chromosomes numbers from 1 - 28. See Supplementary figures S12 and S15 for the domestic – Ceylon comparison and domestic – Green junglefowl comparison, respectively.

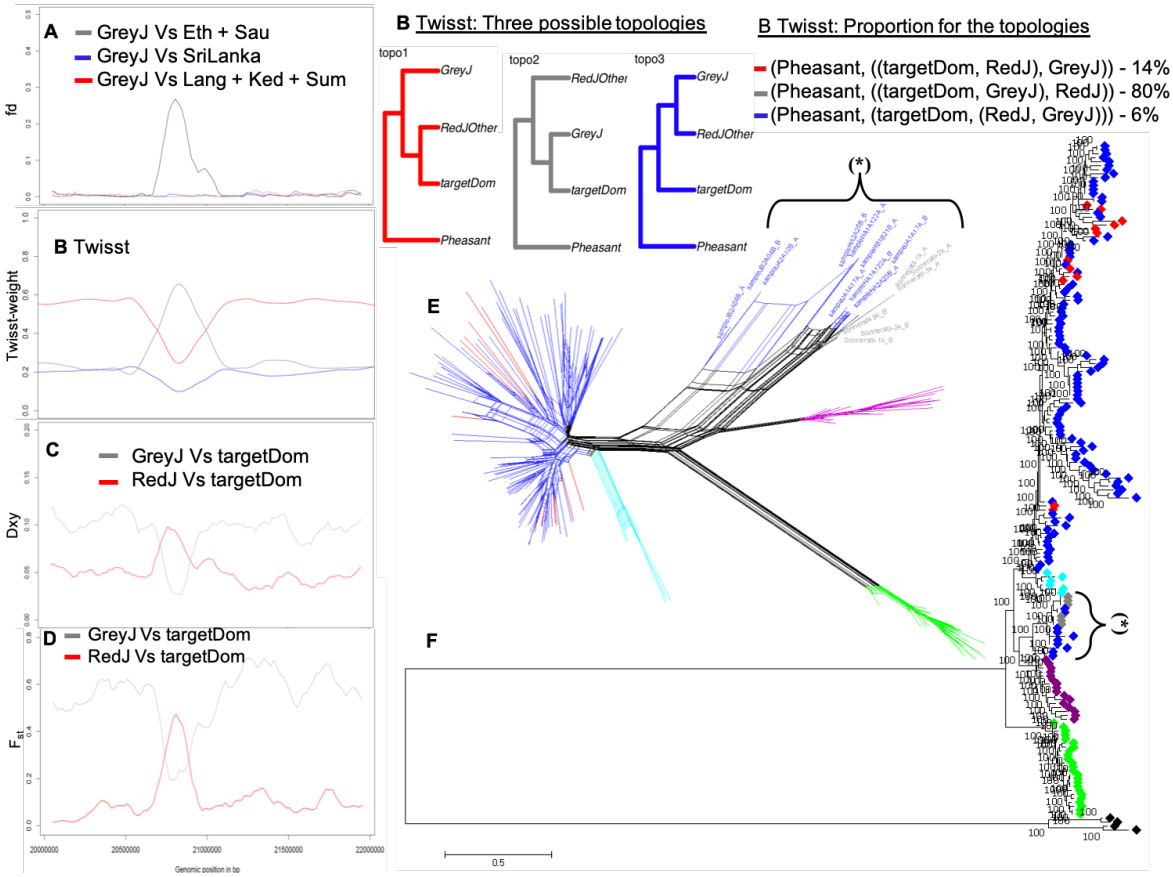

Fig. 5. A 120 kb (Chr 6: 21729370 – 21849500 bp) introgressed region from Grey junglefowl into domestic chicken. (***A***) f_d plot for the zoom region, (***B***) *Twisst* plot, its topologies and their proportions. The most consistent topology (80%) has monophyletic relationship between targetDom (introgressed domestic haplotypes) and Grey junglefowl. (***C***) *dxy* and (***D***) *Fst* for the zoom region. Eth, Sau, SriLanka, Lang, Ked and Sum are domestic chickens from Ethiopia, Saudi, Sri Lanka and Langshan, KeduHitam and Sumatra breeds, respectively. targetDom are the introgressed domestic chicken haplotypes from Grey junglefowl (GreyJ) denoted as (*) in (***E***) haplotype-based network and (***F***) maximum likelihood tree for the region. The colours defining each population in (***E***) and (***F***) are: ◆: domestic chicken; ◆: Red junglefowl, ◆: Javanese red junglefowl, ◆: Grey junglefowl, ◆: Ceylon junglefowl, ◆: Green junglefowl, ◆: common Pheasant.

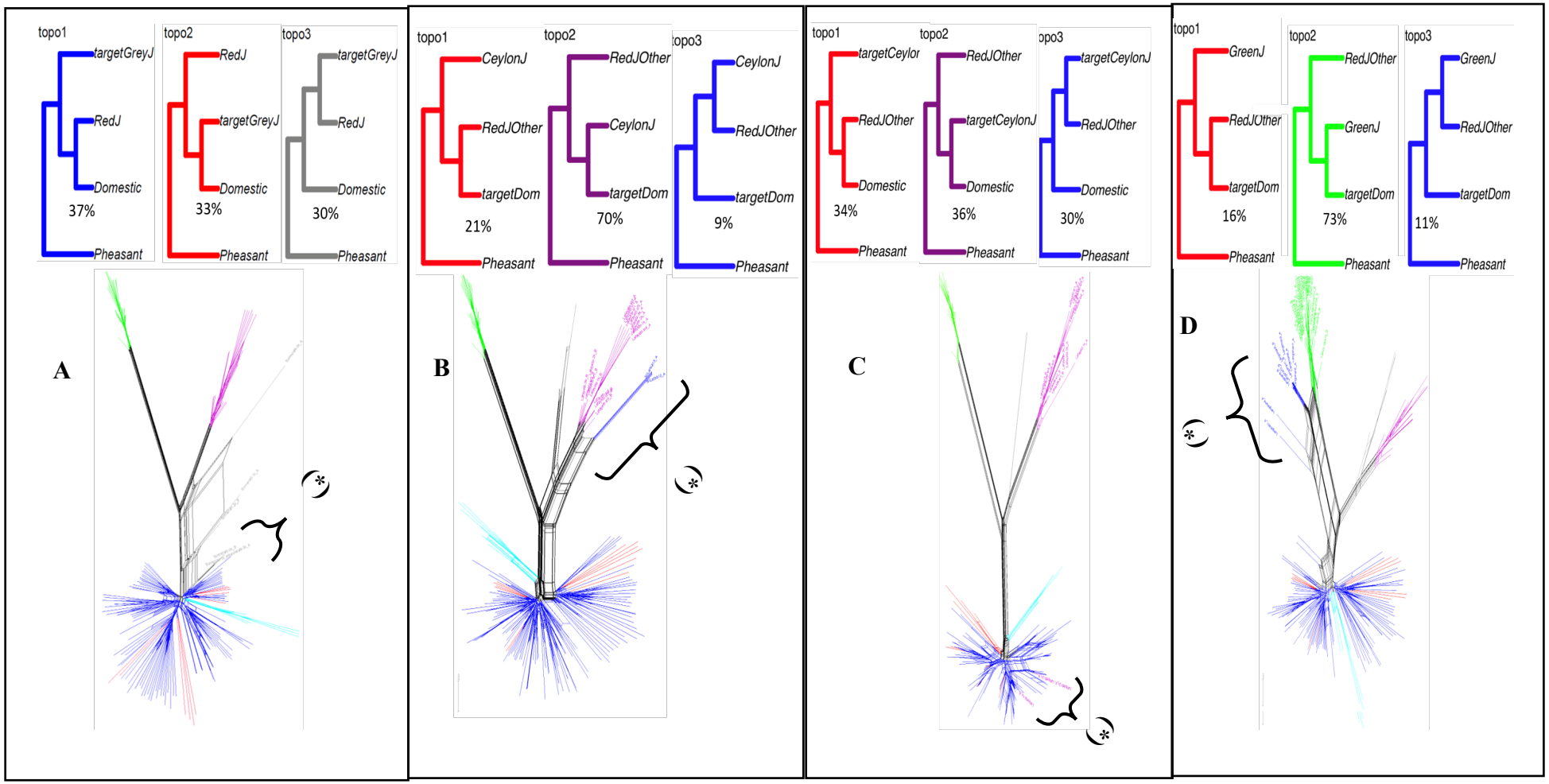

Fig. 6. Topologies (*Twisst*), their estimated proportions and network analyses for the introgression from (***A***) domestic chicken to Grey junglefowl (2.8 Mb, Chr 4: 76429662 - 79206200 bp), (***B***) Ceylon junglefowl to domestic chicken (600 kb, Chr 3: 108325801 - 108925700 bp), (***C***)) domestic chicken/Red junglefowl to Ceylon junglefowl (100 kb, Chr5: 49333700 - 49433700 bp), and (***D***) Green junglefowl to domestic chicken (100 kb, Chr 5: 9538700 - 9638700 bp). (*) introgressed haplotypes. The targetGreyJ, targetDom and targetCeylon in the *Twisst* are the introgressed Grey junglefowl, domestic chicken and Ceylon junglefowl haplotypes, respectively as revealed by the network.

Table 1. Divergence time estimates between junglefowl species and with the Common Pheasant. Time in "years". CT is the coalescence time (i.e. sum of time before and after split), DT is the divergence time (i.e. time from the split to the present).

Pairwise species comparison	**CT (years)**	**95% confidence interval (years)**	**DT (years)**	**95% confidence interval (years)**
Domestic chicken - Red junglefowl	2,060,419	1,785,697 ≥ CT ≤ 2,232,121	8,093	7,014 ≥ DT ≤ 8,768
Red junglefowl - Javanese red junglefowl	2,679,697	2,322,404 ≥ CT ≤ 2,903,005	1,164,612	1,009,331 ≥ DT ≤ 1,261,663
Red junglefowl - Grey junglefowl	4,072,106	3,529,159 ≥ CT ≤ 4,411,448	2,557,021	2,216,085 ≥ DT ≤ 2,770,106
Javanese red junglefowl - Grey junglefowl	4,161,441	3,606,582 ≥ CT ≤ 4,508,228	2,646,356	2,293,509 ≥ DT ≤ 2,866,886
Grey junglefowl - Ceylon junglefowl	3,282,030	2,844,426 ≥ CT ≤ 3,555,533	1,766,945	1,531,352 ≥ DT ≤ 1,914,191
Red junglefowl - Ceylon junglefowl	4,357,225	3,776,262 ≥ CT ≤ 4,720,327	2,842,140	2,463,188 ≥ DT ≤ 3,078,985
Javanese red junglefowl - Ceylon junglefowl	4,379,681	3,795,723 ≥ CT ≤ 4,744,654	2,864,596	2,482,650 ≥ DT ≤ 3,103,312
Red junglefowl - Green junglefowl	5,211,015	4,516,213 ≥ CT ≤ 5,645,266	4,057,810	3,516,769 ≥ DT ≤ 4,395,961
Javanese red junglefowl - Green junglefowl	5,212,814	4,517,772 ≥ CT ≤ 5,647,215	4,059,609	3,518,328 ≥ DT ≤ 4,397,910
Grey junglefowl - Green junglefowl	5,145,901	4,459,781 ≥ CT ≤ 5,574,726	3,992,696	3,460,337 ≥ DT ≤ 4,325,421
Ceylon junglefowl - Green junglefowl	5,150,532	4,463,794 ≥ CT ≤ 5,579,743	3,997,328	3,464,351 ≥ DT ≤ 4,330,438
Red junglefowl - Common Pheasant	21,885,883	18,967,766 ≥ CT ≤ 23,709,707	20,736,660	17,971,772 ≥ DT ≤ 22,464,715
Javanese red junglefowl - Common Pheasant	22,083,637	19,139,152 ≥ CT ≤ 23,923,940	20,934,414	18,143,159 ≥ DT ≤ 22,678,949
Grey junglefowl - Common Pheasant	22,136,134	19,184,649 ≥ CT ≤ 23,980,812	20,986,911	18,188,656 ≥ DT ≤ 22,735,820
Ceylon junglefowl - Common Pheasant	22,174,484	19,217,886 ≥ CT ≤ 24,022,357	21,025,261	18,221,892 ≥ DT ≤ 22,777,366
Green junglefowl - Common Pheasant	22,510,922	19,509,466 ≥ CT ≤ 24,386,832	21,361,699	18,513,472 ≥ DT ≤ 23,141,840

Table 2. Patterson's D statistics and quantification of admixture proportion

Domestic	**Junglefowls**	Patterson's *D*-statistics			Admixture proportion (*f*)	
		D	**Jackknife SD**	**Z score**	***f* estimates**	**95% confidence interval**
Autosomes (chromosomes 1 – 28)						
Domestic	Grey junglefowl	0.069	0.056	37.854	0.124	$0.107 \geq f \leq 0.141$
Domestic	Ceylon junglefowl	0.055	0.046	36.776	0.142	$0.134 \geq f \leq 0.150$
Domestic	Green junglefowl	0.052	0.047	34.236	0.086	$0.081 \geq f \leq 0.091$
Z chromosome						
Domestic	Grey junglefowl	0.041	0.089	4.177	0.059	$0.031 \geq f \leq 0.086$
Domestic	Ceylon junglefowl	0.042	0.086	4.514	0.099	$0.056 \geq f \leq 0.141$
Domestic	Green junglefowl	0.041	0.088	4.246	0.071	$0.038 \geq f \leq 0.103$