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1 **Original article**

2 Understanding the legacy of widespread population translocations on the post-glacial genetic
3 structure of the European beech, *Fagus sylvatica* L.

4

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18 **Running Header**

19 Natural colonisation signals persist despite beech forest translocation

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27 **ABSTRACT**

28 **Aim** Human impacts have shaped species ranges throughout the Holocene. The putative
29 native range of beech, *Fagus sylvatica*, in Britain was obscured by its late post-glacial arrival
30 and subsequent extensive management. We sought to differentiate the interacting effects of
31 post-glacial colonization and anthropic impacts on the current genetic structure and diversity
32 of beech by contrasting phylogeographic signals from putatively natural and translocated
33 populations.

34 **Location** Samples were obtained from 42 sites throughout Great Britain.

35 **Methods** Chloroplast and nuclear microsatellite marker data were interpreted alongside
36 palynological, historical and anecdotal evidence. Genetic structure was analysed using
37 individual-based Bayesian assignment methods and colonization history was analysed using
38 an approximate Bayesian computation framework.

39 **Results** Phylogeographic patterns suggested contemporary forests originated from putative
40 native south-eastern populations. High haplotypic diversity was found near the entry point of
41 beech into Britain. Cryptic signals of isolation-by-distance persisted in the putative native
42 range, together with higher levels of gene diversity in nuclear markers. Weak regional nuclear
43 genetic structure suggested high levels of contemporary gene flow throughout the country.

44 **Main conclusions** Genetic patterns driven by natural colonization persist despite widespread
45 anthropic intervention. Forests in northerly regions were established from forests in the
46 putative native range, diminishing the credibility of any present boundary between the native
47 and non-native range of beech in Britain.

48 **Key words** Anthropogenic, Britain, colonization, *Fagus sylvatica*, gene flow, microsatellites,
49 phylogeography, post-glacial.

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51

52

53 **INTRODUCTION**

54 The migration of species during the Holocene (Taberlet *et al.*, 1998) coincided with
55 substantial human impact (Kalis & Merkt, 2003; Kalis *et al.*, 2003) shaping species
56 contemporary ranges. Historic range limits have been examined from an ecological
57 perspective and post-glacial plant migrations from a phylogeographic perspective (Comes &
58 Kadereit, 1998; Hewitt, 2000; Jump *et al.*, 2010; Magri, 2010). However, many natural
59 systems have been under profound and persistent anthropic influence, which has shaped
60 species distributions and the genetic composition of their component populations (Bradshaw,
61 2004; Alessa & Iii, 2008; Schaberg *et al.*, 2008). The influence of forest management, through
62 the selective removal of genotypes and the translocation of plant material, can impact
63 genetic diversity and its spatial distribution within populations and across regions (Savolainen
64 & Kärkkäinen, 1992; Bradshaw, 2004; Alessa & Iii, 2008; Schaberg *et al.*, 2008). However,
65 despite widespread human influences, native forests might retain genetic signals from past
66 population distribution due to natural regeneration of local stock in combination with the
67 long life of individual trees (Petit & Hampe, 2006; Bradshaw, 2004), thereby allowing natural
68 ranges to be detected.

69

70 Given its many uses, including timber, fuel and fodder, the European beech, *Fagus sylvatica*
71 L., has experienced a long history of management (Nocentini, 2009; Read *et al.*, 2010;
72 Packham *et al.*, 2012) including wide-scale historical translocations throughout Great Britain.
73 Anecdotal, historical and palynological evidence suggest that the native range of beech was
74 limited to south-east England (Rackham, 1980; Pott, 2000; Packham *et al.*, 2012). Despite this
75 commonly held view, this species has been classified as native throughout mainland Great
76 Britain, due to insufficient evidence available to accurately define the native range (Preston
77 *et al.*, 2002). At the regional scale, the species range is believed to be under broad climatic
78 control (Huntley *et al.*, 1989) and in Great Britain, beech has naturalised further north than its

79 presumed native range in the south-east.
80
81 Pollen records from Great Britain indicate that beech migrated into south-east England, with
82 its first establishment just before 3000 BP. Beech maintained a steady rate of spread of
83 100-200 m per year, whereas the majority of other tree species displayed a decrease in the
84 rate of spread. The relatively constant rate of spread suggests that beech had not reached its
85 natural climatic limit by 1000 BP (Birks, 1989). Human intervention might have manipulated
86 the species' range before it reached its climatic range limit (Watt, 1931; Packham *et al.*,
87 2012). Historical records suggests the existence of native populations that occur further north
88 than the predicted range as suggested by pollen evidence (Rackham, 1980). However,
89 confirmation of the species' presence in the pollen record does not directly indicate native
90 origin of forests that exist today. Beech therefore provides a valuable model for
91 understanding how human intervention through translocation of material might impact the
92 genetic structure and diversity of natural populations.

93
94 Studies that aim to determine the 'native status' of a species in a particular region have
95 hypothesised that introduced populations are genetically depauperate as they originate from
96 limited source propagules (Stone & Sunnucks, 1993; Fuentes-Utrilla *et al.*, 2014).
97 Consequently, we sought to determine the interacting effects of past migration and anthropic
98 impacts on the current genetic structure and diversity of beech by assessing if a
99 phylogeographic signal of natural colonization can be identified in its putative native range.
100 We used a combination of conservative, maternally-inherited chloroplast markers (Reboud &
101 Zeyl, 1994; Magri *et al.*, 2006) and highly variable nuclear markers to explore regional trends
102 in genetic variation in combination with historical and palynological data. Extensive sampling
103 was carried out in both the putative native and non-native range across Great Britain. We
104 hypothesised that; (1) patterns associated with natural colonization, such as spatial genetic

105 structure, should persist in the putative native range, and putative native sites will show high
106 genetic similarity; (2) patterns driven by natural colonization should be absent in the putative
107 non-native range due to extensive translocations of plant material by humans outside the
108 putative native range; (3) putative non-native sites should display lower levels of haplotypic
109 and genotypic diversity and greater inter-population divergence, compared to putative native
110 sites, due to the high levels of genetic drift associated with the translocation of a limited
111 number genotypes. We refer to sites as 'putative native' and 'putative non-native' as the *a*
112 *priori* assigned origins of sites used in our study are purely for analytical purposes and should
113 not be taken as precedent.

114

115 **MATERIALS AND METHODS**

116 **Study species**

117 *Fagus sylvatica* L. is a broadleaved, monoecious, primarily outcrossing tree, with pollen
118 dispersed by wind and seeds dispersed by gravity and animals (Fig 1.; Wagner *et al.*, 2010;
119 Packham *et al.*, 2012). It covers approximately 14 million ha, forming the dominant forest
120 type in much of mainland Europe. With the exception of Great Britain, the distribution of
121 beech is primarily climatically limited owing to the species' drought and late frost
122 susceptibility (Watt, 1923; Peterken & Mountford, 1996). Beech trees reach approximately
123 300 years of age, commencing flowering typically between 40 to 80 years (Firbas & Losert,
124 1949).

125

126 Palaeobotanical and genetic data indicate central and northern European populations were
127 colonized from source populations in southern France, eastern Alps-Slovenia-Istria and
128 potentially Moravia and southern Bohemia. Residual populations in the Iberian, Italian and
129 Balkan refugia are believed to have expanded relatively late and did not contribute
130 significantly to the colonization of central and northern Europe (Magri *et al.*, 2006). Recent

131 evidence found in Denmark suggests that post-glacial colonization was aided by occasional
132 long-distance dispersal events, leading to the establishment of beech and other temperate
133 tree species ahead of their main colonization fronts (Overballe-Petersen *et al.*, 2012) and
134 might have contributed significantly to the observed rates of spread of the species (Feurdean
135 *et al.*, 2013).

136

137 **Study sites**

138 Forty-two populations were sampled across Great Britain (Fig. 2). Using a combination of
139 historical records, palynological, and anecdotal evidence, study sites were designated *a priori*
140 as stands of putative native or putative non-native origin (see Table S1.1 in Appendix S1 in
141 Supporting Information). In each site, leaf samples were collected from 20 mature trees
142 within a 10 ha area, preferentially sampling the oldest trees determined by using diameter at
143 breast height (DBH) as a proxy for age. All samples were geo-referenced using a GARMIN 62s
144 handheld GPS (GARMIN, Southampton, UK).

145

146 **Molecular analysis**

147 DNA was obtained from the silica gel dried leaf samples and isolated using the QIAGEN
148 DNeasy 96 Plant Kit (QIAGEN, Venlo, Netherlands). Out of 840 samples, 802 individuals were
149 successfully genotyped using four Chloroplast DNA (CpDNA) microsatellite markers (ccmp4,
150 ccmp7 (Weising & Gardner 1999), cmcs3, and cmcs12 (Sebastiani *et al.*, 2004)). Chloroplast
151 markers were combined in one PCR multiplex, FSCplex, using 10ng of template DNA and the
152 QIAGEN Type-it Microsatellite PCR Kit with the following primer concentrations, ccmp4 at 0.5
153 μ M, ccmp7 at 0.5 μ M, cmcs3 at 3 μ M, and cmcs12 at 3 μ M. Annealing temperature was set to
154 55°C, with a total PCR reaction volume of 10 μ l. 837 individuals were successfully genotyped
155 using 13 nuclear microsatellite markers (fs1-03, fs1-15, fs3-04, fs4-46, fcm5 (Pastorelli *et al.*,
156 2003), mfc7 (Tanaka *et al.*, 1999), mfs11 (Vornam *et al.*, 2004), sfc0007-2, sfc0018, sfc0036,

157 sfc1143, sfc1061, sfc1063 (Asuka *et al.*, 2004) processed in three multiplexes as detailed in
158 Sjölund & Jump (2015). A total of three chloroplast microsatellite loci were used, excluding
159 cmcs12 as it was monomorphic. Fragment analysis was performed on an ABI 3730 (Applied
160 Biosystems, Bleiswijk, Netherlands) and allele scoring on GENEMARKER 2.4.0 (SoftGenetics,
161 State College, PA, U.S.A).

162

163 Scoring errors and null alleles in nuclear loci were checked using MICRO-CHECKER (Van
164 Oosterhout *et al.*, 2004). Analyses presented exclude fs4-46, fcm5, and fs1-15 due to null
165 allele presence and use a total of 10 nuclear microsatellite loci. Gametic disequilibrium was
166 tested between pairs of nuclear loci using FSTAT 2.9.3.2 (Goudet, 1995), identifying
167 significant associations between loci by randomly associating genotypes at pairs of loci 1100
168 times, using a 5% nominal level after Bonferroni correction. The multilocus average error
169 rates were 0.0% for the 3 chloroplast loci and 0.4% for the 10 nuclear loci included in analysis.
170 The error rate per locus was calculated as the number of erroneously assigned loci over 45
171 repeated samples.

172

173 **Measuring genetic diversity and structure using nuclear and chloroplast markers**

174 Estimators of genetic diversity were prefixed with 'c' for chloroplast and 'n' for nuclear.
175 Multilocus estimates of haplotypic and genotypic diversity were mapped in ARCMAP 10 (ESRI
176 software) against the pollen isochrones from Birks' (1989) map for the rational limit of beech
177 pollen. Chloroplast haplotypic diversity was measured as the number of haplotypes (cH_N) and
178 the number of private haplotypes (cH_p). Nuclear genetic diversity was measured as rarefied
179 allelic richness (nA_R) (Petit *et al.*, 1998), gene diversity corrected for sample size (nH_S) (Nei,
180 1978), and the inbreeding coefficient (nF_{IS}) with P -values derived from 10,000 permutations
181 of gene copies within individuals per site (Weir and Cockerham 1984), calculated in SPAGeDi
182 1.4b (Hardy & Vekemans, 2002), and rarefied private allelic richness (nA_p) calculated in ADZE

183 1.0 (Szpiech *et al.*, 2008). The minimum number of gene copies (k) used for rarefaction
184 analysis of nA_R and nA_p is 38. To map nuclear genetic differentiation, for each site we
185 calculated the percentage of total sites that it was significantly differentiated from (i.e.
186 percentage of differentiated sites, nDS (%)), based on nF_{ST} values (Weir & Cockerham, 1984).
187 nF_{ST} values were obtained from pairwise tests of genetic differentiation not assuming
188 Hardy-Weinberg, with significances determined for a 5% nominal level after Bonferonni
189 correction in FSTAT 2.9.3.2 (Goudet, 1995).

190

191 To test for differences of nuclear-based measurements, nA_R , nH_S , nF_{IS} and nF_{ST} among *a priori*
192 determined groups of putative native and putative non-native sites, we performed
193 permutation tests using FSTAT 2.9.3.2 (Goudet, 1995). The difference between putative
194 native and putative non-native groups in the remaining estimators nA_p and cN_H were tested
195 using the non-parametric Mann-Whitney U test. To quantify the distribution of variation
196 displayed by the maps of nuclear genetic diversity and chloroplast haplotypes (considering
197 haplotype distances) we tested both marker sets in a hierarchical analysis of molecular
198 variance (AMOVA) performed in ARLEQUIN 3.5.1.2 (Excoffier & Lischer, 2010) with sites
199 grouped into potential stand origin, putative native or putative non-native.

200

201 **Testing isolation-by-distance with nuclear markers**

202 Subsets of sites with putative native and putative non-native origin were tested for
203 isolation-by-distance following Rousset (1997) using nuclear markers in SPAGeDi 1.4b (Hardy
204 & Vekemans, 2002) with significance of the slope of the correlation of the natural log of the
205 linear spatial distance (Ln(Spatial Distance (km)) against $F_{ST}/(1-F_{ST})$ was computed after
206 10,000 permutations of sites among locations. IBD was analysed with and without the
207 continental datasets to check whether a geographical cline in allelic frequencies was
208 influencing clustering (Guillot *et al.*, 2009). To test for geographic gradients in genetic

209 diversity, we performed non-parametric corrected Spearman's Rank tests on all genetic
210 diversity estimators and all sites, including separate tests on subsets of putative native and
211 putative non-native sites.

212

213 **Analysing population clusters**

214 Individual-based Bayesian assignment methods were performed using data from nuclear loci
215 in STRUCTURE 2.3.4. (Pritchard *et al.*, 2000) using the correlated allele frequency model
216 (Falush *et al.*, 2007), the admixture ancestry model, and site location *a priori* (LOCPRIOR
217 option) to improve the detection of weak population structure (Hubisz *et al.*, 2009). No stand
218 origin was included *a priori* in cluster analysis. To examine relationships between British
219 samples and putative colonization sources in continental Europe, we included nuclear
220 microsatellite data from a subset of 150 samples collected from native beech forests in
221 France, Germany and Italy as detailed in Sjölund & Jump (2015). Analysis without continental
222 samples revealed a similar structure in Britain to that found with continental samples,
223 therefore we present the data including continental samples to set the results in context. K
224 was set from 1 to 20, with 10 runs each. Runs consisted of 500,000 Markov chain Monte
225 Carlo (MCMC) iterations with a burn-in period of 100,000. To observe the consensus in
226 number of clusters in the data, we plotted the log probability of the data ($\ln P(D)$), identifying
227 K where log likelihood values converged. Individual Q-matrices were computed in CLUMPP
228 1.1.2 (Jakobsson & Rosenberg, 2007), with graphics created in DISTRUCT 1.1 (Rosenberg,
229 2004). As results indicated cryptic genetic structure in the putative native range, we
230 performed a subsequent analysis on a subset of the 17 putative native sites (i.e. using the
231 putative stand origin *a priori*) under the same conditions as the main model and excluding
232 continental samples to test for further population sub-structure.

233

234 **Demographic history**

235 We used an approximate Bayesian computation (ABC) framework implemented in DIYABC
236 2.1.0 (Cornuet *et al.*, 2010) to explore scenarios of demographic history that were likely to
237 have generated current regional genetic structure of beech in Britain according to the results
238 obtained from analyses of regional genetic structure, combined with palynological and
239 historical information. Populations were grouped regionally according to Birks' (1989)
240 isochrones, with British populations grouped into southern, central and northern
241 populations, and populations in Italy, France and Germany grouped into a continental
242 population (see Fig S1.1 in Appendix S1 in Supporting Information). Three scenarios were
243 tested (Fig. 6): Scenario 1 assumes natural colonization of beech in southern and central
244 populations from continental Europe, and northern populations originating from continental
245 European stock (i.e. beech is established in northern Britain by anthropogenic translocation
246 from the continent). Scenario 2 assumes natural colonization of southern and central
247 populations from continental Europe, and northern populations originating from southern
248 and central stock (i.e. northern populations are established from material derived from the
249 putative native region). Scenario 3 assumes natural colonization of southern and central
250 populations from continental Europe, and northern populations originating from an
251 admixture of southern, central and continental populations (i.e. some northern populations
252 are established from southern and central stock, the putative native region, and some from
253 continental stock).

254

255 Prior parameters for effective population sizes and timing of events were defined based on
256 knowledge of beech colonization dynamics from palynological and historical information.
257 Prior parameter distributions were uniform and bounded between $10 \cdot 10^4$ for the effective
258 population size of southern, central and northern Britain, and $10 \cdot 10^5$ for continental Europe.
259 Population divergence time priors ranged between 10-50 for t_1 , 25-100 for t_2 and 25-200 for
260 t_3 , with the additional setting $t_3 > t_2$, and $t_2 > t_1$. Priors for admixture rates ranged between

261 0.01 and 0.99. Nuclear microsatellite loci were assumed to follow a Generalized Stepwise
262 Mutation model and a uniform prior was assumed for the mean microsatellite mutation rate
263 bounded between 10^{-3} and 10^{-4} . 36 summary statistics were used for the ABC analysis. Three
264 single sample statistics were used (mean number of alleles, mean Nei's genetic diversity
265 index (Nei, 1987) and mean allele size variance), and three between-sample statistics (mean
266 allele size variance, F_{ST} , and mean index of classification (Rannala & Mountain, 1997; Pascual
267 *et al.*, 2007). Following the methods outlined by Cornuet (2010), type I and type II errors were
268 estimated to evaluate the power of the model. The selected scenario was used to estimate
269 posterior distribution of demographic parameters.

270

271 **RESULTS**

272 **Geographic trends in genetic diversity**

273 Three variants were detected for chloroplast loci, *ccmp4*, *ccmp7* and *cmcs3*. The number of
274 haplotypes (cH_N) within sites ranged from one to four, with a total of seven haplotypes
275 recorded (Table 1, Fig. 2; see Fig. S2.1 in Appendix S2 in Supporting Information). Haplotype
276 (A) was present in all sites and was the dominant haplotype within sites. Haplotype diversity
277 was highest in the putative native sites BLE ($cH_N = 5$) and LUL ($cH_N = 3$), the two most
278 south-easterly British sites. Six out of seven haplotypes (A to F) were represented collectively
279 in these two sites. Two private haplotypes, F and G, were present in sites BLE and DEV,
280 respectively, in single individuals. Significant genetic structuring was found in chloroplast and
281 nuclear markers between sites using AMOVA (Table 2), although no significant difference was
282 found between groups of putative native and putative non-native sites. The remaining
283 variation was present within individuals.

284

285 Multilocus estimates of genetic diversity were obtained for 10 nuclear loci, with an average
286 number of 13.3 alleles per locus, and a maximum of five to 30 alleles per locus (Table 1 and

287 Fig. 3). All nuclear loci were under gametic equilibrium. Rarefied allelic richness (nA_R) varied
288 from 4.58 to 7.19 with rarefied private allelic richness (nA_p) ranging from 0 to 0.156. Gene
289 diversity estimates ranged from 0.606 to 0.750. Putative native site WYC and putative
290 non-native MAB displayed a significant homozygote excess (WYC $nF_{IS} = 0.088$, $P < 0.05$; MAB
291 $nF_{IS} = 0.089$, $P < 0.05$), whilst a heterozygote excess was found in sites BEE and CRA, both
292 putative non-native sites (BEE $nF_{IS} = -0.152$, $P < 0.001$; CRA $nF_{IS} = -0.086$, $P < 0.05$). The
293 percentage of significantly differentiated sites [nDS (%)] varied greatly, for example, site WYT
294 was significantly differentiated to 4.9% of sites, whilst site BEE was significantly differentiated
295 to 95.1% of all sites.

296

297 **Differences between groups of putative native and non-native sites**

298 Significantly higher levels of gene diversity (nH_S) were found in putative native sites,
299 compared to putative non-native sites; H_S : putative native 0.708, putative non-native 0.690 (P
300 < 0.05) (Table 1). The general trend of lower gene diversity in sites outside of the putative
301 native range can be seen in the map for gene diversity in Fig. 3. No significant differences
302 were found for other estimators; nA_R : putative native 6.225 v 6.252 putative non-native
303 ($P = 0.87$), nA_p : 0.053 v 0.037 ($U(40) = 245$, $Z = 0.84$, $P = 0.41$), nF_{IS} : 0.022 v -0.005 ($P = 0.07$),
304 nF_{ST} : 0.024 v 0.021 ($P = 0.59$), and cN_H : 1.5 v 1.4 ($U(40) = 208$, $Z = -0.155$, $P = 0.89$).

305

306 **Isolation-by-distance**

307 Significant isolation-by-distance was found in Britain amongst putative native sites (refer to
308 Fig. 4C: slope = 0.0085, $R^2 = 0.10$, $P < 0.05$). IBD was not significant amongst putative non-
309 native sites, nor was it significant in all sites combined with and without the continental
310 subset (refer to Fig. 4, A: British sites only (slope = 0.0011, $R^2 < 0.01$, $P = 0.22$), B: British and
311 continental sites (slope = 0.0032, $R^2 = 0.03$, $P = 0.06$), D: putative non-native sites (slope =
312 0.0030, $R^2 = 0.02$, $P = 0.09$). Within the putative native sites, there was a significant reduction

313 in haplotype number following an east to west gradient ($\rho = 0.70$, $P < 0.01$; Fig. 2). This
314 effect disappeared when all sites were analysed together ($\rho = 0.18$, $P = 0.241$), and was not
315 found in putative non-native sites alone ($\rho = -0.02$, $P = 0.90$). Preliminary analysis revealed
316 no significant correlations between nuclear genetic diversity estimators and geographic
317 variables (i.e. latitude and longitude) overall sites and within subsets of putative native and
318 putative non-native sites (see Table S2.1 in Appendix S2 in Supporting Information).

319

320 **Detection of further regional structuring using nuclear markers**

321 Mean log-likelihood values for each of the STRUCTURE runs on samples from Britain including
322 the continental samples, gradually ceased to converge after $K = 2$, after which they began to
323 plateau (see Fig. S3.1 in Appendix S3 in Supporting Information). Examination of Q-matrices
324 indicated that $K = 3$ provided meaningful biological clusters that followed a regional
325 distribution (Fig. 5). Although values of $\ln P(D)$ for $K = 3$ varied more than $K = 2$, assignment
326 of individuals to clusters were congruent between runs (see Fig. S3.2 in Appendix S3 in
327 Supporting Information).

328

329 Several sites throughout Britain contained highly admixed individuals, whereas individuals
330 from continental sites displayed homogenous levels of admixture among individuals within
331 site. Organising the Q-matrix according to Birks' (1989) isochrones in approximate geographic
332 order revealed some consistency in cluster assignment between neighbouring sites (Fig. 5).
333 There appeared to be a geographic gradient in the continental clusters with the predominant
334 cluster being blue in ITA, to grey in FRA and GER, with the introduction of the red cluster in
335 GER. TAN appears to have the highest assignment to the blue cluster out of any other sites in
336 Britain. Individuals from Britain were largely assigned to the grey and red clusters. The red
337 cluster appeared to be associated with sites within the putative native range and the putative
338 non-native sites in the south-west (DEV, GOL, HEM, and BRI). Similar patterns were observed

339 for $K = 2$ and $K = 3$ when analysing a subset of British samples alone and a subset of putative
340 native sites, both revealing no further population sub-structuring (see Fig. S3.3 and Fig. S3.4
341 in Appendix S3 in Supporting Information).

342

343 Model-based inference using ABC analyses indicated that scenario 2 was supported with
344 maximum probability compared to around zero support for scenarios 1 and 3 (Fig. 6). This is
345 indicative of a gradual colonization of beech from continental Europe and expanding
346 northwards into and through Britain, with northern populations being derived from the
347 putative native range of beech in Britain. Confidence in scenario choice was high, with low
348 error rates (type 1 = 0.001–0.054, type 2 = 0–0.054). For scenario 2, median values for the
349 effective population size were 6830, 4920, 6270 and 25100 for $N1$ (northern Britain), $N2$
350 (central Britain), $N3$ (southern Britain) and $N4$ (Continental Europe). The median values for
351 divergence events corresponded to 16.9, 26.5 and 170 number of generations for $t1$, $t2$ and
352 $t3$.

353

354 **DISCUSSION**

355 Analysing genetic data according to palynological and historical evidence allowed us to tease
356 apart the post-glacial history of beech in Britain despite the prolonged human impacts on this
357 species. Patterns of past colonization dynamics persisted in putative native sites, while
358 phylogeographic patterns and modelled demographic history support the hypothesis that
359 throughout Britain, beech forests are largely derived from native stock originating from trees
360 that colonized the island during the Holocene.

361

362 **Native origins of beech in Britain**

363 The distribution of chloroplast haplotypes and modelled demographic history strongly
364 support the colonization of northern beech populations from stock originating from the

365 putative native range of beech. The distribution of haplotypes in Britain matched the
366 expected phylogeographic signal of postglacial colonization, with the highest number of
367 haplotypes found in south-eastern sites, LUL and BLE (Table 1; Fig. 2) in close proximity to
368 the purported entry point of beech migration into Britain (Birks 1989). All the haplotypes,
369 except one (G), were represented in these sites, suggesting that the majority of Britain's
370 beech forests were colonized from native stock. Similar south to north patterns in haplotype
371 distribution have been found for other European tree taxa, including *Alnus*, *Quercus* and
372 *Populus* (King & Ferris *et al.*, 1998; Petit *et al.*, 2002a; Cottrell *et al.*, 2005 along with genetic
373 clines indicative of gradual recolonization of *Quercus robur* and *Quercus petraea* in Ireland
374 (Kelleher *et al.*, 2004). The loss of haplotype diversity occurring away from proposed entry
375 sites can be a result of founder effects induced by the progressive movement of the
376 migration front (Excoffier *et al.*, 2009). The clinal pattern of haplotype diversity in *F. sylvatica*
377 in Britain is in contrast with *Q. robur* and *Q. petraea*, which displayed a highly clumped
378 distribution of haplotypes indicative of long-distance dispersal events (Cottrell *et al.*, 2002a)
379 following the much earlier entry of these oaks into Britain (Birks 1989). There is little similar
380 evidence of long-distance dispersal events in *F. sylvatica* in Britain. site BLE harboured one of
381 the two private haplotypes found while haplotype (G) in site DEV may have arisen from a
382 single base mutation in haplotype A or a long-distance dispersal event (Overballe-Petersen *et*
383 *al.*, 2012).

384

385 The high number of haplotypes ($ch_N = 7$) is in contrast to that found by Magri *et al.* (2006)
386 who report one haplotype throughout Britain. This is likely a consequence of our larger
387 sample size, the significant partitioning of haplotype variation between sites, and
388 polymorphism in *cmcs3*. Restricting analysis to *ccmp4* and *ccmp7* used by Magri *et al.* (2006)
389 reduces haplotype number to four (data not shown) and matches the regional trend seen
390 with all three loci, with diversity restricted to south-eastern sites. The average levels of

391 rarefied allelic richness using nuclear markers in Britain ($nA_R = 6.25 \pm 0.08$) were lower than
392 those reported in studies using some of the same microsatellite markers, approximately
393 ranging from 8.2 to 18.2 in other studies (Jump & Peñuelas, 2006; Buiteveld *et al.*, 2007;
394 Sjölund & Jump, 2015). Sites in Britain also displayed lower levels of rarefied private allelic
395 richness ($nA_p = 0.044 \pm 0.007$) compared to sites in continental Europe sampled by Sjölund and
396 Jump (2015) where values for nA_p ranged between 1.51 and 2.36. Overall levels of gene
397 diversity were similar to those found in other studies ($nH_s = 0.696 \pm 0.004$) (Jump & Peñuelas,
398 2006; Buiteveld *et al.*, 2007; Oddou-Muratorio *et al.*, 2008; Sjölund & Jump, 2015).

399

400

401 **Genetic variation between groups of different a priori stand origins**

402 Isolation-by-distance occurs when the genetic differentiation between individuals or
403 populations increases with geographic distance (Wright, 1940). In plants, this is primarily a
404 consequence of restricted gene flow via seed or pollen (Loveless & Hamrick, 1984). Although
405 beech is assumed to show high levels of gene flow as a wind-pollinated tree, it displays
406 significant structuring at local (Chybicki *et al.*, 2009; Jump *et al.*, 2012; Piotti *et al.*, 2013;
407 Sjölund & Jump, 2015) and regional scales (Jump & Peñuelas, 2006; de Lafontaine *et al.*,
408 2013). In agreement with the significant genetic structuring found in natural populations of
409 beech, putative native sites displayed a weak but significant trend of IBD based on nuclear
410 loci, which was absent in putative non-native sites, with the addition of all sites obscuring the
411 IBD signal (Fig. 4).

412

413 Populations of beech in France with relatively recent colonization histories displayed stronger
414 IBD compared to southern refugial populations in France (de Lafontaine *et al.*, 2013). As
415 beech only arrived in Britain around 3000 BP (Birks, 1989), IBD in the putative native range is
416 likely driven by relatively recent colonization dynamics. In contrast, widespread

417 translocations are likely to have prevented the development of IBD between putative
418 non-native populations, possibly due to the anthropic movement of plant material
419 throughout the country. A similar result was found by Leonardi *et al.* (2012) in beech
420 populations of different levels of fragmentation in central Italy. IBD in less fragmented non-
421 marginal populations was obscured when populations from marginal, fragmented
422 populations were included. This finding was interpreted as a result of intense genetic drift in
423 fragmented populations.

424

425 We found a significant decrease in gene diversity (nH_s) in putative non-native sites,
426 suggesting a reduction in genetic diversity due to founder effects, although the magnitude of
427 this difference may have been limited by high gene flow associated with wind pollination.
428 However, no significant difference was found for rarefied allelic richness between sites of
429 different origins. Allelic richness is expected to be more sensitive to reductions in effective
430 population sizes, as rare alleles, which do not contribute considerably to gene diversity, are
431 more likely to be lost first (Nei *et al.*, 1975). Low levels of allelic richness throughout Britain
432 suggest a significant proportion was lost during post-glacial colonization, probably due to
433 founder effects. The lack of a pattern in allelic richness between putative native and putative
434 non-native sites may be due to a lack of sensitivity of the analysis arising from the
435 comparison of two already limited gene pools. In agreement with theoretical predictions,
436 gene diversity throughout Britain displayed similarly high levels to that found on the
437 continent (Jump & Peñuelas, 2006; Buiteveld *et al.*, 2007; Oddou-Muratorio *et al.*, 2008;
438 Sjölund & Jump, 2015). Although putative native sites displayed the highest average private
439 allelic richness (nA_p), this difference was not statistically significant.

440

441 **Regional patterns of postglacial migration of beech into Britain**

442 Within Britain, neighbouring sites displayed congruent levels of admixture with lower levels

443 of admixture in northern, putative non-native sites (Fig. 5). A gradient of admixture marked
444 the transition of continental regions to Britain, with continental sites assigned predominantly
445 to the blue and grey cluster. This is in contrast to that found in *Quercus* spp. (Petit *et al.*,
446 2002b) and *Populus nigra* (Cottrell *et al.*, 1997; Cottrell *et al.*, 2002b; Cottrell *et al.*, 2004),
447 where much longer periods of translocations have led to a decrease in genetic differentiation.
448 Individuals from the putative non-native site, TAN, may have arisen from translocation of
449 continental stock as it displayed the highest levels of assignment to the blue cluster in Britain,
450 similar to individuals in FRA. Individuals from the south of Britain displayed a relatively higher
451 probability of assignment to the red cluster, which was generally associated with the putative
452 native range, in addition to the south-west region, including putative non-native sites DEV,
453 GOL, HEM, and BRI. There appeared to be a transition between the assignment of individuals
454 to the red and grey cluster that occurred in proximity to the border for the 1000 BP
455 isochrone, with a tendency towards assigning individuals to the red cluster in regions pre-
456 1000 BP. Only one putative native site, CWw, occurred outside of the border of the 1000 BP
457 limit and was predominantly assigned to the red cluster. However, CWw is only 15km away
458 from the 1000 BP isochrones.

459

460 **Conclusion**

461 Using multiple data sources, we were able to identify signals of natural colonization in a
462 forest system that has been heavily impacted by humans. Our results build upon existing
463 palynological and historical evidence to substantially advance our understanding of the
464 contemporary genetic impact of past population translocation. Chloroplast markers suggest
465 that the majority of trees sampled were derived from putative native stock either through
466 natural regeneration or translocation, whilst nuclear markers confirm the persistence of
467 signals of the natural colonization of beech in Britain in putative native sites. Although cryptic
468 genetic signals of population expansion remain in the putative native range of beech, we

469 caution against using this evidence as a means to classify stand origins, since gene flow
470 between neighbouring regions essentially blurs the borders of the native range. Warming
471 climate is increasing the productivity of this species in more northerly parts of its range while
472 decreasing growth and reproduction are predicted in the south. Given that our data suggest
473 native origin for most of Britain's populations, it is paramount that climate-induced range
474 shifts are considered in management plans and this species managed as a whole, irrespective
475 of regional boundaries.

476

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483

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680 **Supporting Information**

681 Additional Supporting Information may be found in the online version of this article:

682 **Appendix S1** Stand origins for study sites

683 **Appendix S2** Chloroplast and nuclear genetic data

684 **Appendix S3** STRUCTURE analysis output

685

686 **Data Accessibility**

687 All microsatellite and GPS data for this study are available at DataSTORRE: Stirling Online

688 Repository for Research Data: <http://hdl.handle.net/11667/90>

689

690 **Biosketch**

691 Alistair Jump's research team has a strong focus on understanding biogeographic impacts of
692 past and present environmental changes from population genetics to demography and
693 remote sensing and how they interact with human interventions.

694 Author contributions: MJS and ASJ designed the research. MJS conducted field-based work.

695 MJS, JJM, and PGD conducted lab-based work. MJS and PGD conducted data analysis. ASJ

696 supervised the research project. MJS, ASJ, PGD, and JJM wrote the manuscript.

697 Editor: Jim Provan

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703 **Tables**

704 **Table 1 Genetic diversity estimates obtained from nuclear (*n*) and chloroplast (*c*) markers**

705 **for beech in Great Britain.** Data from chloroplast (*c*) markers include; *cN*, no. of samples; *cH_N*,

706 no. of haplotypes, † identifies private haplotypes (*cH_p*). Data from nuclear (*n*) markers

707 include; *nN*, no. of samples; *nA_R*, rarefied allelic richness; *nA_p*, rarefied private allelic

708 richness; *nH_S*, gene diversity; *nF_{IS}*, inbreeding coefficient; and, *nDS%*, percentage of

709 significantly differentiated sites. Mean±SE is given per group (Putative *Native* and *Non-native*)

710 and overall for genetic diversity estimators. Significant *P*-values are indicated as * *P* < 0.05,

711 *** *P* < 0.001.

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	Sites	<i>cN</i>	<i>cHN</i>	<i>nN</i>	<i>nA_R</i>	<i>nA_P</i>	<i>nH_S</i>	<i>nF_{IS}</i>	<i>nDS (%)</i>
724									
725	Native	321	1.5±0.2	340	6.22±0.13	0.053±0.13	0.707±0.006*	0.022±0.010	46.1±6.4
726	FEL	17	2	20	5.17	0.095	0.651	0.057	75.6
727	BED	18	1	20	5.95	0.025	0.722	0.059	17.1
728	SEC	17	1	20	6.06	0.008	0.717	-0.040	56.1
729	WYC	20	1	20	7.12	0.077	0.694	0.088*	9.8
730	LAD	20	1	20	6.72	0.110	0.736	0.033	22.0
731	BUC	20	1	20	5.33	0.000	0.682	0.010	87.8
732	CWe	20	1	20	6.64	0.146	0.750	0.055	85.4
733	CWw	20	1	20	6.00	0.000	0.710	-0.037	85.4
734	MON	19	1	20	6.71	0.156	0.716	-0.013	56.1
735	GRE	20	1	20	5.98	0.004	0.712	0.038	26.8
736	BUR	19	2	20	6.80	0.109	0.713	-0.003	22.0
737	SAV	16	1	20	6.09	0.001	0.687	0.041	43.9
738	LUL	17	3	20	6.09	0.020	0.727	-0.054	29.3
739	FRI	20	1	20	6.51	0.087	0.679	0.007	26.8
740	BLE	18	4 [†]	20	6.82	0.027	0.721	0.030	24.4
741	WEA	20	2	20	5.86	0.007	0.703	0.070	56.1
742	DEN	20	1	20	5.97	0.024	0.706	0.031	58.5
743	Non-native	481	1.4±0.1	497	6.25±0.11	0.037±0.008	0.690±0.006*	-0.006±0.010	32.9±4.5
744	APP	19	2	19	6.68	0.065	0.682	0.004	14.6
745	BAR	19	2	19	6.50	0.001	0.702	-0.012	17.1
746	BEE	19	2	20	4.58	0.000	0.606	-0.152***	95.1
747	BRI	20	1	20	6.21	0.080	0.663	-0.065	58.5
748	CAR	18	1	19	5.92	0.000	0.685	0.057	26.8
749	CLE	20	1	20	6.30	0.014	0.695	0.014	17.1
750	CRA	20	1	20	5.29	0.000	0.706	-0.086*	75.6
751	DEV	16	2 [†]	20	6.30	0.038	0.691	-0.066	22.0
752	DRU	20	1	20	6.44	0.042	0.692	-0.012	39.0
753	DUN	19	2	20	7.16	0.103	0.684	0.005	24.4
754	ECC	20	2	20	6.32	0.025	0.700	0.036	56.1
755	GEL	20	1	20	6.32	0.013	0.700	0.014	17.1
756	GOL	20	1	20	5.49	0.048	0.672	0.034	36.6
757	HEM	20	1	20	5.86	0.001	0.672	-0.042	70.7
758	KIN	20	1	20	5.87	0.013	0.675	-0.005	34.1
759	MAB	20	1	20	6.14	0.011	0.701	0.089*	9.8
760	PLO	17	2	20	6.04	0.013	0.693	0.019	24.4
761	STR	20	1	20	6.67	0.099	0.692	-0.012	12.2
762	TAL	15	2	20	6.28	0.122	0.667	0.018	17.1
763	TAN	20	1	20	7.19	0.077	0.747	0.065	51.2
764	TON	20	1	20	6.23	0.000	0.680	0.023	24.4
765	TWO	20	1	20	6.33	0.014	0.717	0.003	39.0
766	WAL	20	1	20	6.52	0.033	0.708	-0.069	7.3
767	WYT	20	2	20	6.79	0.103	0.700	-0.005	4.9
768	YEL	19	1	20	6.84	0.000	0.722	-0.012	26.8
769	Overall	802	1.4±0.1	837	6.25±0.08	0.044±0.007	0.696±0.004	0.004±0.008	38.7±3.8

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774 **Table 2 Hierarchical analysis of molecular variance (AMOVA) for chloroplast and nuclear**
 775 **markers for beech in Great Britain.** The degrees of freedom (*df*), percentage of variation
 776 explained by each level (Variation (%)), and the relevant F-statistic are presented with
 777 significant *P*-values indicated as *** $P < 0.001$.

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	Chloroplast			Nuclear		
Levels	<i>df</i>	Variation (%)	<i>F</i> -statistic	<i>df</i>	Variation (%)	<i>F</i> -statistic
Among groups	1	-0.22	<0.001	1	0.01	0.000
Among sites						
within groups	40	12.59	0.005***	40	2.25	0.022***
Within sites	763	87.63	0.032***	795	97.74	0.023***

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801 **Figures**

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803 **Fig. 1 Beech in the putative non-native range in Scotland, Great Britain (site CRA, N57.5777**

804 **W4.1435).** Photo courtesy of M. J. Sjölund.



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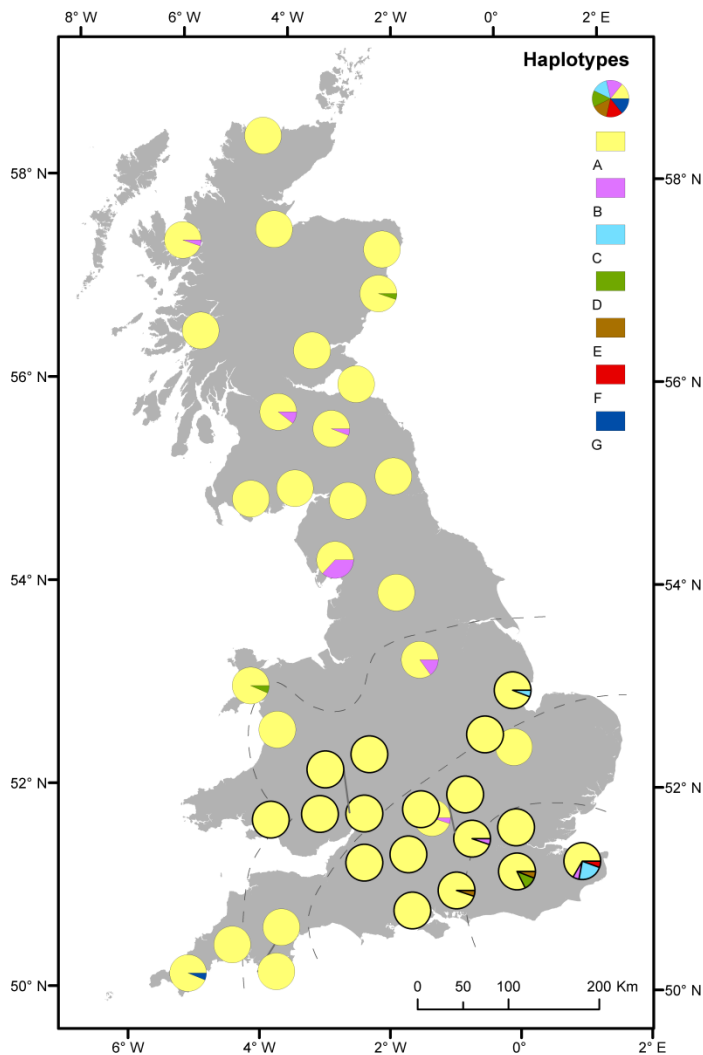
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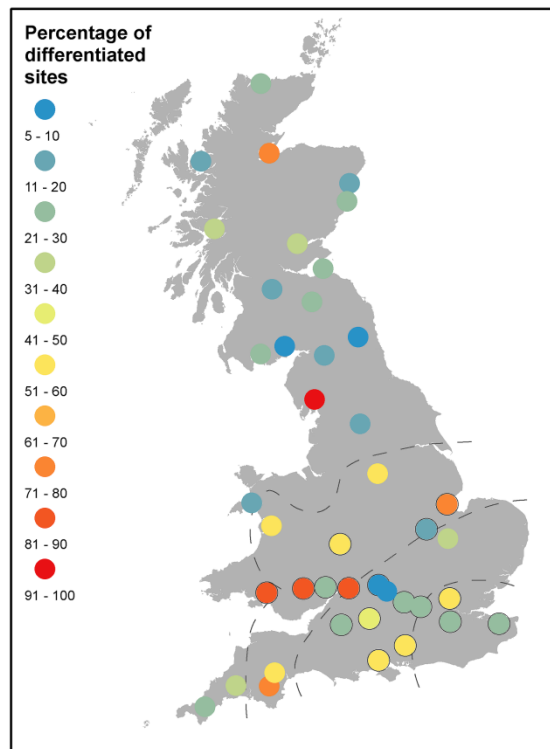
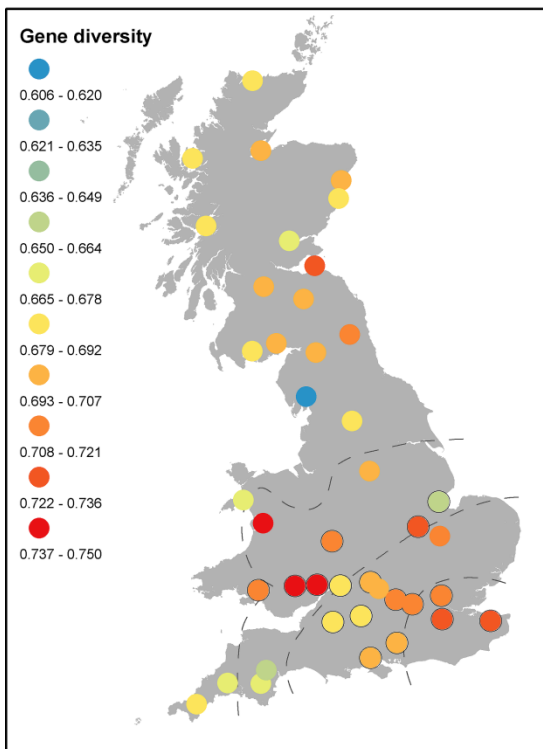
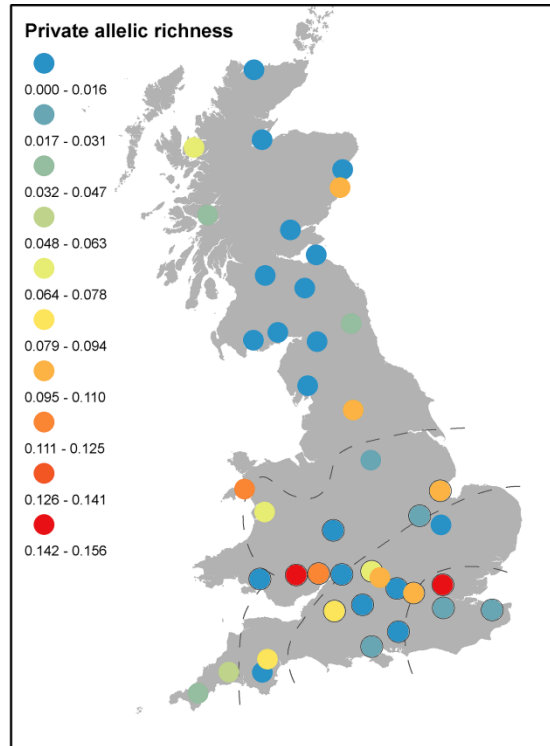
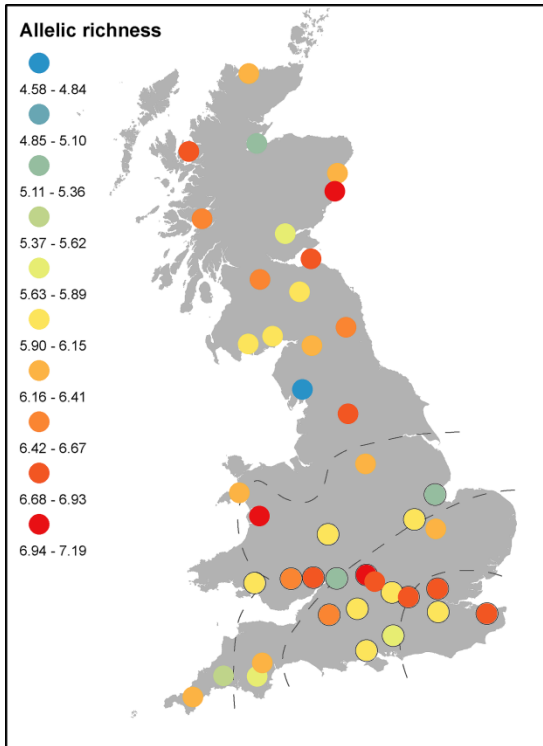
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816 **Fig. 2 Geographical variation of chloroplast haplotypic diversity of beech in Great Britain.** A
817 total of seven haplotypes are displayed against Birks' (1989) isochrones. Putative native sites
818 are outlined.
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821 **Fig. 3 Geographical variation of estimators of beech nuclear genetic diversity in Great**
822 **Britain.** Estimators include rarefied allelic richness (nA_R), rarefied private allelic richness (nA_P),
823 gene diversity (nH_S), and the percentage of significantly differentiated sites ($nDS\%$). Putative
824 native sites are outlined, with Birks' (1989) isochrones for *F. sylvatica* redrawn as broken
825 lines.

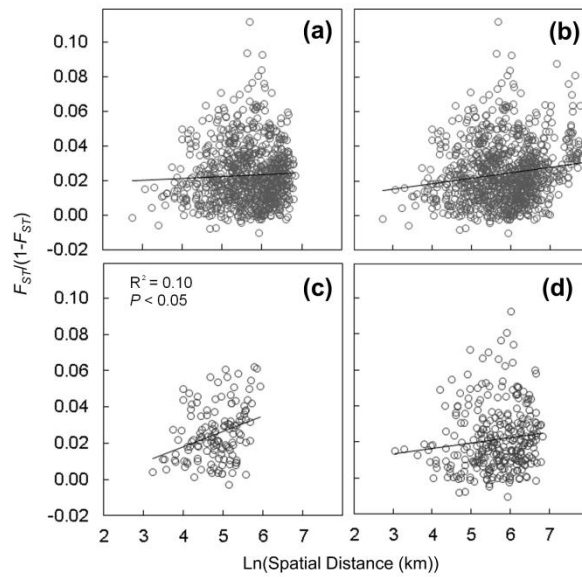


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829 **Fig. 4 Comparison of isolation-by-distance analyses amongst beech populations in putative**
830 **native and putative non-native regions in Great Britain and continental Europe. Sites**
831 **included in the analysis are as follows; A) British sites only; B) British and continental sites; C)**
832 **putative native sites; D) putative non-native sites.**



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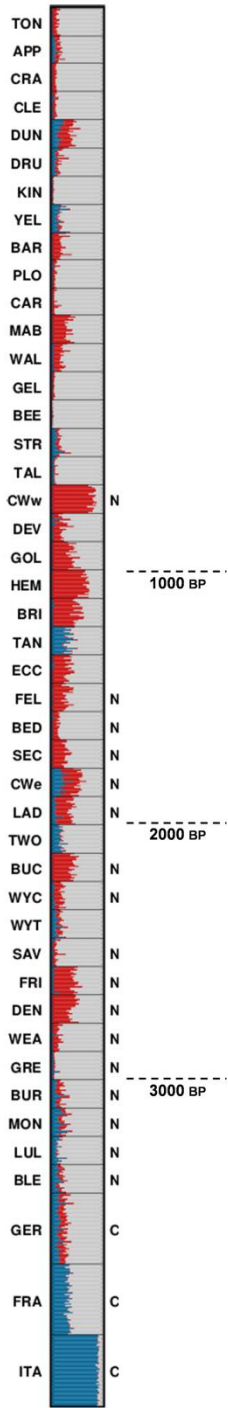
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842 **Fig. 5 Regional genetic structure of beech in Great Britain.** Three clusters are shown in blue,
843 red, and grey. Each horizontal bar represents an individual with the proportions of its genetic
844 make-up assigned probabilistically to each of the three clusters. Sites are ordered on an
845 approximate geographical gradient by ordering sites following Birks' (1989) isochrones to
846 reflect the putative migration route of Beech into Britain. Continental samples are situated at
847 the bottom of the figure, with a general northward trend to the top of the graph. Stand
848 history of the sites are indicated on the right of the Q-matrix, with continental sites labelled
849 C, putative native sites N, and putative non-native sites left blank. Approximate borders of
850 the isochrones are indicated by a dashed line with years in BP, with site codes on the left.



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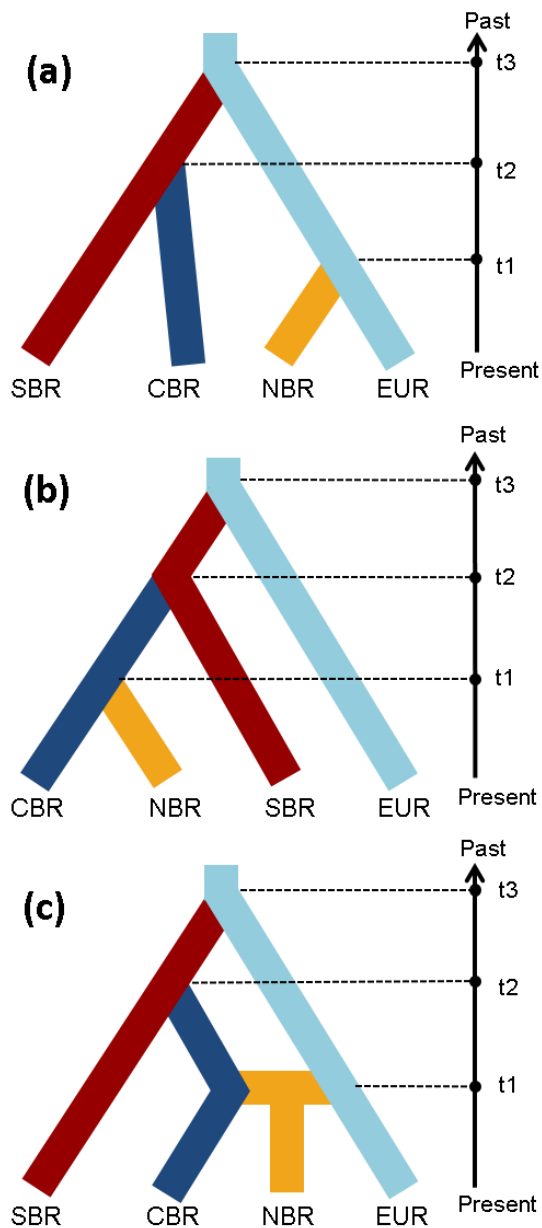
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856 **Fig. 6 Comparison of modelled colonization scenarios of beech in Great Britain.** There are
 857 four assumed populations of beech: South (SGB), Central (CGB) and North (NGB)
 858 populations of Great Britain, and continental Europe (EUR). All scenarios assume
 859 colonization from EUR towards SGB and CGB and either assumes NGB originates from EUR
 860 (scenario 1 - a); from natural colonization of SGB and CGB (scenario 2 - b); or from the
 861 admixture of both EUR and British CGB and SGB (scenario 3 - c). t_1 , t_2 , and t_3 correspond to
 862 the divergence times in generations.



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