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# Bucking the trend of pollinator decline: the population genetics of a range expanding bumblebee

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ABSTRACT

Recent research has shown drastic reductions in the global diversity and abundance of insects. This is a major concern given the expected cascade effects on ecosystem services, such as pollination. Understanding the patterns and drivers of changes in the distribution and abundance of species in our rapidly changing environment is therefore urgent. Cases of species showing trends that run counter to general population declines, especially when they deliver key ecosystem services, are especially interesting. The tree bumblebee (*Bombus hypnorum*), which belongs to a globally important group of pollinators, has substantially expanded its range in recent years in direct contrast to many other species within this group. Here we reconstructed the likely pattern of colonization of the UK based on RAD-seq population genomic data combined with Bayesian population modelling. This RAD-seq approach also enabled an analysis of genomic regions potentially under selection. We report a complex and dynamic colonization pattern that is most likely ongoing. Current evidence suggests that either a shift in its migration potential, and/or adaptive genomic changes have contributed to the recent range expansion of *B. hypnorum*. Genomic areas of potential adaptive significance included genes involved in regulation of transcription and gene expression, circadian rhythms and innate immunity. Our results are framed within the general context of understanding the factors driving successful population expansions.

49 INTRODUCTION

50 Current rates of species extinction are substantially elevated relative to the historical record
51 (Ceballos et al. 2015) and many species are undergoing declines in range and abundance
52 (Hallmann et al. 2017). However, some species remain widespread or are even undergoing
53 range expansion. Such contrasting patterns of population decline and success are especially
54 interesting when they occur among species within close phylogenetic groups (McKinney and
55 Lockwood 1999; Angert et al. 2011; Moran and Alexander 2014). This allows identification of
56 traits that potentially contribute to species' resilience or extinction risk (Purvis 2008;
57 Chichorro et al. 2019) and therefore provides ideal opportunity to gain traction on the factors
58 driving diversity changes (Sax et al. 2007; Moran and Alexander 2014).

59

60 A key aspect of understanding these rapid changes in global biodiversity patterns is
61 unravelling the dynamics of colonization. This has historically been achievable only through
62 reconstructing events from observational records. While useful, these are not always
63 available and are typically limited in their capacity to trace historical events with accuracy.
64 However, the progressive accessibility of genome-scale data, and development of population
65 modelling tools (Cornuet et al. 2014; Cabrera and Palsbøll 2017), have offered a powerful
66 approach that is yielding significant insights into the routes and biological signatures of
67 colonizations (e.g. Guzinski et al. 2018). These tools have been successfully used in a range of
68 contexts, such as inference of the evolutionary history of speciation (Momigliano et al. 2017),
69 measuring the success of translocation programmes (Puckett et al. 2014), and investigating
70 meta-population dynamics (Stillfried et al. 2017).

71

72 Advances in sequencing technology also facilitate the investigation of the wider genetic
73 effects associated with range expansions. For example, new colonizations are often
74 characterized by a limited number of founders, resulting in a population bottleneck and small
75 initial population sizes (Dlugosch and Parker 2008). However, colonizing species often
76 successfully establish within their new environment despite the predicted loss of genetic
77 diversity and harmful inbreeding effects, posing a genetic paradox (Allendorf and Lundquist
78 2003; Sax & Brown 2000; Estoup et al. 2016). One potential explanation is that bottlenecks
79 may not lead to a substantial loss of genetic variance in quantitative traits (Lewontin 1965); it
80 may even increase when dominance interactions are considered (Robertson 1952). The loss
81 of genetic diversity during colonization may also be overcome if there are multiple
82 colonization events or high migration rates from the source population, which can eliminate
83 founder effects (Lockwood et al. 2005; Roman and Darling 2007; Dlugosch and Parker 2008).

84

85 In parallel with developments in sequencing technologies, there have been theoretical
86 advances in the understanding of range expansions. Facon et al. (2006) proposed a useful
87 framework to consider colonization scenarios, identifying three scenarios that are not
88 mutually exclusive. In the first, 'migration change', a species is limited only by its capacity to
89 migrate to an existing habitat that meets its requirements. Here, colonization may occur if
90 barriers to migration are removed through, for example, human activities. The second
91 scenario, 'environmental change', describes the situation where suitable habitat becomes
92 newly available. The third scenario, 'evolutionary change', assumes genetic changes in the
93 colonizing species that either occur prior to, or during, the colonization, conferring a fitness
94 advantage in the new environment. Successful range expansions following evolutionary

95 change have been linked to adaptations to anthropogenically modified habitats, coined as
96 'Anthropogenically Induced Adaptations to Invade' (Hufbauer et al. 2012).
97
98 Bumblebees (*Bombus* spp.) are a highly pertinent group to investigate factors driving changes
99 in diversity as the group contains taxa with strikingly different population trends. In addition,
100 globally, patterns of decline are predominant, which is an issue of particular concern because
101 of their role as ecologically and economically important pollinators (Goulson et al. 2005;
102 Williams 2005; Williams and Osborne 2009).
103 Patterns of declines with the *Bombus* group are, at least to some degree, phylogenetically
104 structured. For example, members of the subgenus *Thoracobombus* appear to show
105 increased vulnerability to population decline (Cameron et al. 2011; Arbetman et al. 2017), as
106 do several others (Goulson et al. 2008), but those of the subgenus *Pyrobombus*, in contrast,
107 seem to exhibit increased resilience (Arbetman et al. 2017; Richardson et al. 2019). Several
108 *Pyrobombus* species are reported to be increasing in abundance and/or expanding their
109 range, for example *Bombus haematurus* in central Europe (Biella 2020), *Bombus pratorum*
110 and *Bombus monticola* which have colonized Ireland during the last century (Speight 1974;
111 Fitzpatrick et al. 2007), *Bombus bimaculatus, Bombus impatiens, Bombus ternarius,* and
112 *Bombus vagans* in Vermont (Richardson et al. 2019) and *Bombus hypnorum* across the
113 western part of its distribution (Rasmont et al. 2015). These contrasting patterns suggest that
114 this group exhibits traits that make it less vulnerable to threats that are causing declines in
115 the majority of other bumblebees. Investigating the underlying mechanisms for this increased
116 resilience in this group are therefore important to improve understanding of the drivers of

117 diversity change, and in turn inform conservation efforts, in this important group of
118 pollinators.

119

120 A notably successful species among the *Pyrobombus* group is the tree bumblebee, *Bombus*
121 *hypnorum*, which has recently substantially expanded its range (Goulson and Williams 2001;
122 Prŷs-Jones et al. 2016). *Bombus hypnorum* is one of the most widespread bumblebee species
123 across Europe and Asia (Williams 1991; Goulson and Williams 2001), with a broad palaearctic
124 distribution from Iceland to Japan (Williams 1991; Rasmont et al. 2015). It has been present
125 along the north-western coast of Belgium and France from at least the early 20$^{th}$ century
126 (Rasmont 1988). A recent study showed a lack of genetic structuring of *B. hypnorum*
127 populations in Belgium, indicating a large panmictic population across western parts of
128 Europe (Maebe et al. 2019). Significant differentiation was observed between western
129 European and Baltic populations, suggesting population structure at much larger geographical
130 scales (Maebe et al. 2019). In the last decades, *B. hypnorum* has expanded its range
131 significantly in the western part of its distribution (Rasmont et al. 2015), reaching Iceland in
132 2010 (Prŷs-Jones et al. 2016) and Ireland in 2017. It was first recorded in the south of England
133 in 2001 (Goulson and Williams 2001) and has since spread rapidly northwards, arriving in
134 Scotland in 2012. It is now one of the most common bumblebee species in the UK, both
135 abundant and widely distributed (BWARS 2019). Given no published data to-date on any
136 negative impacts of this population expansion, we refer to this throughout as a colonization
137 rather than an invasion.

138

139 There are a number of characteristics of *B. hypnorum* that may indicate its potential as a
140 successful colonizer. It has a wide distribution across Europe and Asia, where it is found across
141 diverse types of habitats (Goulson and Williams 2001). This reflects its broad niche, including
142 dietary and climatic requirements (BWARS 2019), attributes generally associated with
143 successfully colonizing species (Baker 1965; Willamson and Fitter 1996; Vazquez 2006). It
144 exhibits a facultative bivoltine colony cycle (Edwards and Jenner 2005), which may contribute
145 to an increased rate of population growth facilitating an accelerated spread (Sakai et al. 2001).
146 It also shows facultative polyandrous mating patterns, at least in some parts of it range, which
147 may increase the genetic diversity within colonies (Paxton et al. 2001). Finally, *B. hypnorum*
148 is unique amongst UK *Bombus* species in nesting high above ground (Benton 2006), often in
149 buildings, bird nest boxes and roof structures, which may give it a competitive advantage in a
150 highly urbanized environment (Crowther et al. 2014). However, what has enabled its rapid
151 spread since 2001 remains unclear: these are long-standing characteristics and *B. hypnorum*
152 has nonetheless only very recently colonized the UK. Information on continental populations
153 is incomplete, but available information points to *B. hypnorum* being well established and
154 abundant across western continental Europe for some time (>100 years) prior to its
155 colonization of the UK (Rasmont et al. 1988; Maebe et al. 2019). Although there are intrinsic
156 lag times in the introduction, growth, expansion and detection of colonizing species (Crooks
157 et al 2005), this lag would be particularly prolonged (up to 200 generation since *B. hypnorum*
158 is bivoltine) in this case if the colonization of the UK represents the continuation of a range
159 expansion dating back to over a century ago. Thus, evidence to-date points to a significant
160 change in either the environment, which includes an increase in opportunities for migration,
161 e.g. through human aided transport, or the intrinsic biology of this species that have
162 contributed to its sudden and highly successful range expansion.

163

164 Here, we explore the genetic signatures associated with this rapid expansion of *B. hypnorum*
165 into the UK using a RAD-seq population genomic dataset. Our specific goals were to: i) identify
166 whether the UK population was founded from a single event or from multiple and potentially
167 ongoing events; (ii) assess population structure and whether there has been any significant
168 loss of genetic diversity as a consequence of the population expansion; (iii) identify
169 preliminary indicators of any signatures of selection before or during the colonization that
170 may have promoted its success.

171

172 MATERIALS AND METHODS

173 *Establishing the spread of* Bombus hypnorum *across the UK*

174 The likely geographical spread of *Bombus hypnorum* across the UK from its first record in 2001
175 (Goulson and Williams 2001) was reconstructed using the BWARS database (Bees, Wasps &
176 Ants Recording Society 2019), which collates recorded sightings as part of an ongoing
177 dedicated mapping program (Figure 1). This database is reliant on records being sent in by
178 the public, amateur groups and specialists. Although it will inevitably contain some gaps and
179 biases (e.g. towards human population density), all records are verified by specialists within
180 BWARS for quality assurance (van der Wal et al. 2015). While not systematic, it is nevertheless
181 likely to estimate broad patterns reasonably, and has been used previously for such purposes
182 (e.g. Potts et al. 2010; Ollerton et al. 2014).

183

184 *Tissue samples and RAD library preparation*

185 *Bombus hypnorum* was sampled from seven localities representing different 'fronts' of the
186 range expansion of this species (as established from the BWARS database, see also Results).
187 Six localities were sampled in the UK (Cardiff, Hull, London, Newcastle, Plymouth and
188 Southampton), representing different stages of the expansion, and one in France (Le Havre)
189 representing its already well-established distribution in continental Europe (Maebe et al.
190 2019).

191

192 Forty individual samples were collected across a large area (approximately 10 x 10km) at each
193 site using standard population sampling methods for colony-living Hymenopteran species
194 (see e.g. Goulson et al. 2011) where individual samples were collected a minimum of 200m
195 apart to avoid any significant sampling of sisters (belonging to the same nest). Samples were
196 collected between May and July of 2013 and 2014 and stored in 100% ethanol.

197

198 DNA was extracted from the thoracic muscle tissue of all sampled individuals using an
199 ammonium acetate protocol (Nicholls et al. 2000) and quantified on a Qubit 3.0 Fluorometer
200 using a broad range assay (Thermo Fisher Scientific). DNA (500ng) was digested in 20µl
201 volumes after RNase treatment with 40 units of the restriction enzyme Xhol (New England
202 Biolabs) at 37°C for 3 hours with a 20 minute heat deactivation stage at 80°C. Digested DNA
203 was purified using AmpureXP (Beckmann and Coulter, 1.4X ratio of beads to DNA) and
204 quantified on a Qubit 3.0 Fluorometer (Thermo Fisher Scientific). RAD libraries were prepared
205 using the IonXpress Plus gDNA Fragment Library Kit (Life Technologies). Library preparation
206 was carried out using a pooled approach with population-specific barcodes using equimolar
207 concentrations from each individually digested sample for each sampling site. A pooled

208 sequencing design represents a well established and cost-effective alternative to individual
209 sequencing to reliably obtain genome wide allele frequency data (Futschik and Schlötterer
210 2010; Gautier et al. 2013; Schlötterer et al. 2014) and has been used in a wide range of
211 systems (e.g. Guo et al. 2015; Kahnt et al. 2018). (We note that a limitation of this approach
212 is the lack of individual genotypes and heterozygosity necessary for analysis such as
213 assignment tests or estimation of inbreeding coefficients (Andrews et al. 2016)). For quality
214 control, library preparation of the Newcastle sample was performed twice independently
215 using the pooled approach. Additionally, both the Newcastle and Le Havre samples were
216 prepared and sequenced using an individual barcoding approach with twenty individual
217 samples for each sampling site (Gautier et al. 2013). A barcoded Ion Torrent adapter A was
218 ligated using 0.1 µM of barcode adaptor, 200 U of T4 DNA ligase (New England Biolabs), 100
219 mM of ATP and 2µl of NE 4 Buffer in 40µl volumes for 2 hours at 22°C, followed by heat
220 deactivation at 65°C for 20 minutes, either on an individual or pooled basis for each sampling
221 site. Purification was repeated twice after this step (1.2X ratio of beads to DNA) and prior to
222 shearing using Ion Shear Plus Enzyme Mix II (Life Technologies) following the manufacturer's
223 protocol. After further AmpureXP purification (1.4X ratio of beads to DNA) the Ion Torrent
224 adapter P1 (Thermo Fisher Scientific) was ligated in 49µl volumes for 20 minutes at 25°C
225 followed by heat deactivation at 72°C for 5 minutes following the manufacturer's guidelines.
226 After another step of AmpureXP purification (1.2X ratio of beads to DNA), library amplification
227 was achieved through: 5 minutes at 95°C, followed by 18 cycles of a heat denaturation at 95°C
228 for 15 seconds, annealing at 58°C for 15 seconds and extension at 70°C for 1 minute. Pippin
229 Prep (Sage Science) was employed to select for a fragment size range of 159 and 164bp prior
230 to AmpureXP purification (1.5X ratio of beads to DNA) and quantification was then estimated

231 by qPCR. Libraries were run on an Ion Torrent PGM using one 318 chip for each individually

232 barcoded sample or for two pooled samples respectively.

233

234 *Data processing and SNP calling*

235 Raw reads were trimmed from both ends to excise low quality base-calls (average Q-score <

236 15 across 4bp sliding windows) and filtered for a minimum length of 10bp using Trimmomatic-

237 0.36 (Bolger et al. 2014). Filtered reads were aligned to the *Bombus terrestris* genome

238 (Bter_1.0 assembly, Ensembl) using the Burrow-Wheeler Aligner (BWA) and the MEM

239 algorithm (Li 2013), which has been shown to perform best in the presence of indel errors

240 typical of the Ion Torrent (Ziemann 2016). We chose the *Bombus terrestris* genome, and not

241 that of the more closely related *Bombus impatiens*, because linkage group annotations are

242 available for this species. This allowed us to investigate patterns of diversity across genomic

243 regions. We tested if there was evidence for bias caused by this choice through comparison

244 of mapping statistics against the *Bombus impatiens* genome. Aligned reads were only retained

245 if they mapped uniquely to the reference genome and had a minimum mapping quality score

246 of 20 (Cibulskis et al. 2013). Aligned BAM files were sorted and converted into the mpileup

247 format allowing a maximum of 1,000 reads at a given position using Samtools 0.1.5 (Li et al.

248 2009). SNP calling (excluding indels) for individually barcoded samples was performed using

249 VarScan v.2.3.9 (Koboldt et al. 2009) using all sites that had a minimum coverage of 10 in at

250 least 10 individuals. For these individual samples, requirements for SNP acceptance were a

251 minimum of two reads (default) for the minor allele and a p-value of ≤0.05 derived from

252 Fisher's exact test on read counts (coverage) supporting the reference and variant allele

253 respectively (Koboldt et al. 2009; Koboldt et al. 2013).

254

255 The mpileup file of pooled samples was converted to the sync file format and filtered for
256 indels using Popoolation2 (Kofler et al. 2011). Because variation in sequencing depth can
257 impair the accuracy of allele frequency estimates derived from pooled samples, only sites
258 with a sequencing coverage of ≥50 and ≤ 500 within any sample, and a maximum coverage of
259 3000 across all samples, were considered for pooled samples. Note that specifically for the
260 analysis of the distribution of genome wide polymorphism, which does not rely on allele
261 frequency estimates, a minimum within-sample coverage of 10 was applied. For pooled
262 samples, SNPs were called if they had a minimum of 3 reads per allele across all samples and
263 a minimum within-sample allele frequency of 0.01, as commonly used in population genetic
264 studies (e.g. Bruneaux et al. 2013; Fraser et al. 2014). We also applied a threshold of 0.05 for
265 the identification of $F_{ST}$-outliers to test for consistency (Roesti et al. 2012).

266

267 *Analysis of population genetic diversity and characterization of population structure*

268 Average expected genome-wide heterozygosity was calculated following Fischer et al. (2017).
269 The proportion of polymorphic sites was calculated across all base pairs covered in all
270 samples. Pseudo-haplotypes were created based on population allele frequencies using a
271 custom Python script and imported into R v.3.4.2 (R Core Team, 2012). Allelic richness was
272 calculated in the R package Hierfstat v.0.04-22 (Goudet 2004). Pairwise population $F_{ST}$ was
273 calculated using Popoolation2 (Kofler et al. 2011) and confidence intervals were created by
274 bootstrapping over loci for 1000 cycles using a custom Python script. A Mann-Whitney-
275 Wilcoxon test was used to test if there is a significant difference in the pairwise $F_{ST}$ values
276 between UK-Le Havre and UK-UK populations (Mann and Whitney 1947). A Mantel test

277 (Mantel 1967) was implemented in Genepop 4.2 (Raymond and Rousset 1995) to test for
278 correlation between geographic and genetic distance. A principal coordinate analysis was
279 performed in the R package Hierfstat v.0.04-22 (Goudet 2004). This was carried out for SNPs
280 covered in all sample sites, a minimum of six sample sites (75%) and a minimum of four
281 sample sites (50%) to assess the effect of missing data on the resolution of population
282 structure.

283

284 *Testing hypotheses of colonization history*

285 The approximate Bayesian computation software DIYABC v2.0 (Cornuet et al. 2014) was used
286 to assess the relative probability of different colonization scenarios. These were derived using
287 a 'bottom up' hierarchical nested approach to identify the most likely colonization for each
288 UK sample site independently (described in detail below). The highest probability scenario for
289 each individual sampling site was combined into a final consensus model. The advantages of
290 this approach are that it allows the most robust model to be constructed from the data
291 available. No prior assumptions based on the observed BWARS records were required and
292 the history of each sampling site is built independently. It also allowed model construction
293 using the maximum number of available SNPs at each stage. This approach involved the
294 following steps:

295 Step 1. For each UK sampling site separately, the likelihood of the panmictic Western
296 European population (sampled at Le Havre, France) as a colonization source was tested in
297 DIYABC against a simulated population, where the Western European population and the
298 simulated population share the same common ancestral population (Figure 2). This was
299 carried out to test for the contribution of a potential other source that is more divergent to

300 the Western European population, e.g. from Scandinavia. The possibility of simulating
301 unsampled populations (with shared ancestry to test populations) is an effective feature of
302 the DIYABC software implementation to account for the possible contribution of other genetic
303 lineages that have not been sampled (Estoup and Guillemaud 2010). Simulated populations
304 are constructed using coalescence theory by generating a genealogy based on the defined
305 order of events in a given scenario (Cornuet et al. 2014).

306 Step 2. This next step tested whether each UK sampling site was (i) founded independently
307 from the most likely source identified in step 1; (ii) was founded sequentially via the most
308 likely source in step one and through another UK sampling site (e.g. Le Havre colonized
309 Southampton which colonized London); (iii) was founded by ongoing colonization from the
310 source identified in step one and concurrent colonization by another UK sampling site (Figure
311 2).

312 Step 3: In the case that more than one UK sampling site was more likely to be the source of
313 another UK sample than the continental sample in step 2, these were tested against each
314 other as well as against a dual (concurrent) colonization from each of them. As an example:
315 for the Cardiff sampling site, the most likely scenario derived from step 1 was a colonization
316 from Le Havre. Testing this scenario against the probability of colonization from every other
317 UK sampling site directly or in combination with Le Havre resulted in both Hull and Newcastle
318 being more likely as a source of colonization than Le Havre. In step 3 a colonization from Hull
319 was tested against a colonization from Newcastle and a dual colonization from both sites with
320 the latter showing highest probability.

321

322 Step 4. The most likely scenarios from steps 1- 3 were then combined into a final consensus
323 model that included all sample sites.

324 All scenarios implemented were tested with or without a bottleneck.

325

326 Each individual scenario was tested using a total of 100,000 iterations, following
327 recommended guidelines (Cornuet et al. 2014). For Newcastle (which included a technical
328 replicate), only the "Newcastle 2" replicate was considered (see Results). Model evaluation
329 was based on summary statistics for all biallelic SNP markers covered in the sampling sites
330 considered. Summary statistics consisted of the mean of non-zero values, variance of non-
331 zero values and mean of the complete distribution for genic diversity and pairwise $F_{ST}$ and
332 Nei's distance. Uniform simulation priors were applied to all demographic parameters. Prior
333 constraints on simulation parameters consisted of the definition of the order of events
334 (t3>t2>t1) and an initial effective population size between 10 and 100, where bottlenecks
335 were simulated. Model scenarios were compared using a subset of 1% of all simulations,
336 which matched the observed data best (Cornuet et al. 2014). Using a logistic regression
337 approach, deviations from the summary statistics among the selected subset of simulations
338 were used to predict the probability of a given scenario (Estoup et al. 2012). In order to reduce
339 correlation among explanatory variables, summary statistics were transformed by linear
340 discriminant analysis prior to logistic regression (Estoup et al., 2012).

341

342 *Identification of genomic regions under selection*

343 A commonly adopted approach to investigate genomic regions that may be under strong
344 selective regimes is to look for $F_{ST}$ outliers in population genomic datasets where several

345 populations or locations have been sampled (e.g. Vandepitte et al. 2014; Lin et al. 2017;
346 Leydet et al. 2018; Theodorou et al. 2018). This is based on the expectation that areas of
347 reduced or elevated differentiation are subject to balancing and directional selection
348 respectively (e.g. Lotterhos and Whitlock 2014). Other commonly used approaches to
349 detecting selection in population genomic studies follow the expectation that directional
350 selection decreases genetic variation in the genomic region of the selected site and balancing
351 selection increases it (Oleksyk et al. 2010). Levels of genetic diversity can then be compared
352 to the genomic background to infer candidate regions under selection (Hohenlohe et al. 2010,
353 Bruneaux et al. 2013).
354

355 *Approach 1: $F_{ST}$-outlier approaches*

356 To identify signatures of selection, we tested for elevated differentiation by first considering
357 all pairwise comparisons between UK sampling sites and secondly for all pairwise UK-Le Havre
358 comparisons. This allows distinction between patterns of differentiation across the UK and/or
359 between UK and a continental population. Two approaches were used: a permutation
360 approach (Bruneaux et al. 2013) and BayeScan (Foll & Gaggiotti 2008).
361

362 For the permutation approach, observed $F_{ST}$ -values averaged across all pairwise comparisons
363 of sampling sites were shuffled 1000 times and compared to the observed $F_{ST}$ values averaged
364 across SNP sites within 10kb sliding windows. A 10kb window size was chosen because linkage
365 has been shown to decrease rapidly over these distances in *Bombus* (Sadd et al. 2015). To
366 avoid single SNPs or RAD-tags driving the average across windows, a minimum SNP density of
367 3 SNPs was required across at least two independent RAD-tags for a window to be included

368 in the analysis (Purfield et al. 2017; Jacobs et al. 2018). P-values were generated as the
369 proportion of permutations being lower/higher than the observed estimates and corrected
370 for multiple testing using a FDR approach implemented in the qvalue package in R (Storey et
371 al. 2015). For the BayeScan approach (Foll and Gaggiotti 2008), the program was run using
372 default parameters. BayseScan implements a basic regression model to differentiate between
373 locus and population specific effects on the distribution of $F_{ST}$ -values (Foll and Gaggiotti
374 2008). A likelihood ratio test is then used to assess if the population specific component is
375 sufficient to explain the observed variation (no selection) or if a SNP specific component
376 (selection) is supported (Foll and Gaggiotti 2008). This allows assessment of population
377 specific demographic effects in contrast to the permutation approach, which identifies larger
378 genomic areas with elevated $F_{ST}$ values averaged across all sampling sites considered.

379 To reduce the rate of false positives (De Mita et al. 2013), genomic regions were only
380 considered as outliers if they were identified using both the permutation and the BayeScan
381 approach.

382

383 *Approach 2: Distribution of polymorphic sites*

384 Using the approach of Bruneaux et al. (2013), which evaluates the distribution of
385 polymorphism across the genome against a random null-distribution and does not rely on
386 allele frequency estimates, the proportion of polymorphic sites was calculated. This was
387 performed across non-overlapping 10kb sliding windows or across the length of the contig
388 sequence in the case of genomic regions not placed within the assembled reference genome
389 of *B. terrestris*. To generate a null-distribution 1,000 permutations were applied to the data
390 and p-values were generated as the proportion of permutations being lower/higher than the

391 observed estimates (Bruneaux et al. 2013). Only windows that had a minimum coverage of
392 100bp were considered in the analysis (Cooper et al. 2004). For the analysis of low
393 polymorphism, the hypergeometric test was used to derive the minimum coverage in base
394 pairs needed across a window to obtain a probability below 5% of not sampling a SNP within
395 a sliding window given our observed SNP density (Lentner, 1972; Fontanillas et al., 2010). In
396 line with other studies, a false discovery rate (FDR) of 10% (q-value < 0.1) was applied (e.g.
397 Krehenwinkel et al. 2015; Rane et al. 2015) as an appropriate balance between the false
398 discovery rate and statistical power where large numbers of tests are involved (van den Oord
399 2008). Genes that were found within windows of significantly high/low polymorphism were
400 considered for gene ontology analysis.
401
402 *Gene ontology analysis*
403 Gene ontology (GO) terms for the *B. terrestris* genome were obtained from the Ensembl
404 database. Enrichment tests based on gene count (the 'classic' algorithm, Alexa et al. 2006)
405 were conducted using the R package topGO (Alexa and Rahnenfuhrer 2016). These were
406 assessed using Fisher's exact test and a minimum node size of 10 in order to prune our
407 hierarchy from nodes with the support of less than 10 annotated genes, a frequently applied
408 threshold (e.g. Ahrens et al. 2013; Rademacher et al. 2017). Correction for multiple testing
409 (FDR < 5%, q-value < 0.05) was carried out using the qvalue package in R (Storey et al. 2015).
410 Additionally, the 'weight' and 'elim' algorithms were used, which account for dependencies
411 within the gene ontology hierarchies (Alexa et al. 2006). Here, multiple testing theory does
412 not directly apply as tests are not independent and raw p-values ≤0.05 were considered as
413 significant (Alexa and Rahnenfuhrer 2016). Genes within sliding windows that were identified
414 as $F_{ST}$ outliers or that showed significantly high or low polymorphism were subject to gene

415 ontology analysis to investigate if outliers show significant enrichment for specific biological
416 or molecular functions.
417
418 In order to classify SNPs as synonymous or non-synonymous, the annotation for the *B.*
419 *terrestris* genome was obtained from the Ensembl database, which is assembled across 18
420 linkage groups.
421
422 RESULTS
423 *The range expansion of* Bombus hypnorum *into and across the UK*
424 After the first record of *B. hypnorum* near Southampton in 2001 (Goulson and Williams 2001)
425 more sightings followed across the south of the UK, with records increasing rapidly thereafter
426 (Figure 1). The sharpest increase in records was observed in 2009, where sightings increased
427 more than four-fold compared to the previous year (likely to have been at least partly
428 influenced by an increasing public awareness of the existence of *B. hypnorum* in the UK). The
429 expansion was first in a north-eastward direction with some isolated records as far north as
430 Hull in 2005 and Newcastle in 2007. The expansion then extended to the west with the first
431 sightings of *B. hypnorum* in Cardiff in 2009, and in Plymouth in 2010.
432
433 *RAD-seq data summary*
434 Ion Torrent sequencing generated 15,513,192 raw reads for all sample pools. After mapping,
435 quality filtering and the application of coverage thresholds 2,469,636bp were covered in total
436 among pools, representing ~1% of the expected genome size. This covers 40% of all expected

437 cut-sites (57,157) of the enzyme used to digest samples (XhoI). Within each sampling site, the
438 range of coverage was 152,225 to 953,719bp (Table 1). After stringent filtering, 12,823 high
439 confidence SNPs were identified in total, ranging from 464 to 3,695 within each sampling site.
440 Mapping success was on average 6±3% higher against the *B. impatiens* genome but the
441 proportion of raw reads that were uniquely mapped per sampling site was highly correlated
442 (rho 0.94, p<0.001) between the *B. terrestris* and *B. impatiens* genome, indicating minimal
443 bias. We therefore used the *B. terrestris* genome to take advantage of annotated linkage
444 groups, which has been shown to generally increase the power to detect selection across
445 genomic regions compared with single site comparisons (Shafer et al. 2017). Across all
446 genomic sites that were covered in all sampling sites(1,886, including non-polymorphic and
447 polymorphic sites), the proportion of polymorphic markers varied from 0.006 (London) to
448 0.014 (Le Havre, Table 1), which fall within the range reported from other studies (e.g.
449 Catchen et al. 2013). Deviation in the proportion of polymorphic markers between replicate
450 pools was 0.001.

451

452 *Allele frequency validation*

453 In line with other studies we tested the consistency of our genotyping approach by comparing
454 replicate allele frequency estimates (Anand et al. 2016, Guo et al. 2016; Dorant et al. 2019).
455 A total of 3,065 SNP sites were covered in both Newcastle replicates and allele frequencies
456 were highly correlated between them (rho 0.95, p $<2.2$ e-$^{16}$) with an average difference in
457 allele frequencies of 0.06. SNP allele frequencies within the individually barcoded Newcastle
458 and Le Havre replicates compared to the pooled samples were high (rho 0.87, p $<2.2$ e-$^{16}$
459 across 361 SNP sites for Newcastle 1, rho 0.97, p $<2.2$ e-$^{16}$ across 507 sites for Newcastle 2,

460 and rho 0.97, p <2.2 e-[16] across 439 sites for Le Havre). The average allele frequency difference
461 was 0.14 for the Newcastle 1 replicate, 0.07 for the Newcastle 2 replicate and 0.06 for the Le
462 Havre pool in comparison to the respective individual data set.

463

464 *Characterization of population structure*

465 Average heterozygosity ranged from 0.12 in London and Hull to 0.2 in Le Havre and Newcastle
466 (Table 1). Allelic richness ranged from 1.11 in London and 1.26 in Le Havre (Table 1). The
467 deviation in average heterozygosity between the replicate pools was 0.02. Principal
468 coordinate analysis on pairwise $F_{ST}$ values was done for SNPs covered across all sample sites
469 (101), a minimum of six sample sites (2166 SNPs) and a minimum of four sample sites (3475
470 SNPs) The pattern obtained was similar, with the Newcastle replicates clustered closely
471 together with the Le Havre sampling site irrespective of the threshold of missing data that
472 was applied (Figure 3). $F_{ST}$ -values were generally low (Table 2), and no significant isolation by
473 distance was detected using a Mantel test ($R^2$ = $-2.3^{-5}$, p=0.87). There was no significant
474 difference in average $F_{ST}$ between within-UK comparisons and UK–Le Havre comparisons
475 (Mann-Whitney-Wilcoxon test, p-value >0.05). The average $F_{ST}$ for within-UK comparisons
476 was 0.023 ± 0.008 and 0.016 ± 0.007 for the UK–Le Havre comparison.

477

478 *Evaluation of colonization history*

479 The continental reference sample (Le Havre), which we have assumed to be representative
480 of the panmictic Western European population, was more likely to be the source of
481 colonization than the simulated unsampled population in all cases (Figure 4). Modelling
482 results gave high support for multiple colonization events, indicating a direct colonization

483 from continental Europe into Southampton, London and Newcastle and complex patterns of
484 colonization within the UK (Figure 4, Table 3). A bottleneck was supported for all populations
485 with the exception of Southampton (Figure 4, Table 3). The highest relative probability was
486 observed for the colonization history of the London, Newcastle and Plymouth sites (Table 3).
487 For the Cardiff, Hull and Southampton sites relative probabilities of the second most likely
488 scenario were more similar and confidence intervals were overlapping (Table 3). In the cases
489 of Cardiff and Southampton, the two top scenarios differed in their support for a dual
490 colonization history, whilst for the Hull site the probability of a colonization from London was
491 closely followed by the probability for a direct colonization from the Western continental
492 population (Table 3).

493

494 *Detection of selection*

495 Of all sites covered (2,469,636bp), 136,626bp fell within coding sequences, of which 557 were
496 polymorphic. Of these polymorphisms, 346 were non-synonymous and 211 synonymous. A
497 total of 75 $F_{ST}$-outliers (66 under directional selection and 9 under balancing selection) were
498 identified by BayeScan. The permutation approach identified 8 windows with significantly
499 elevated $F_{ST}$-values (6 for within UK comparisons and 2 for UK-Le Havre comparisons) and 11
500 windows with significantly low $F_{ST}$-values (3 for within UK comparisons and 8 for UK-Le Havre
501 comparisons) (Figure 5). The analysis of patterns of polymorphism across the genome
502 revealed 1,219 windows with significantly elevated polymorphism (Figure 6). Given an
503 observed SNP density of 4.5 per kB, the minimum coverage in base pairs required to obtain a
504 probability below 5% of not detecting any SNP within a sliding window was 645bp, as shown

505 by the hypergeometric test. This resulted in a total of 383 windows exhibiting significantly
506 reduced polymorphism (Figure 6).

507

508 Genes that showed evidence of directional selection from both approaches and all three
509 methods (i.e. $F_{ST}$-outlier permutation, BayeScan *and* the polymorphism approach) were the
510 protein vestigial (Le Havre-UK comparison), the circadian locomotor output cycles protein
511 (kaput), and one gene important in signal transduction (serine/threonine-protein kinase NLK),
512 both from within-UK comparisons (Table 4). None of the outliers identified by BayeScan to be
513 under balancing selection fell within windows of low $F_{ST}$ in any of the data sets.

514

515 GO analysis

516 GO analysis revealed areas of high polymorphism that were significantly associated with
517 biological processes, including regulation of transcription and gene expression, signaling and
518 developmental processes (Table 5A). Further, areas of high polymorphism were significantly
519 associated with the molecular functions of protein and sequence specific DNA binding, DNA
520 binding transcription factor activity and zinc ion binding (Table 5A). Areas of significantly low
521 polymorphism were associated with the molecular functions of transmembrane signaling
522 receptor activity and extracellular ligand-gated ion channel activity (Table 5B). GO analysis did
523 not reveal any significant associations for molecular functions or biological processes for
524 windows identified as outliers within any of the $F_{ST}$ data sets.

525

526 DISCUSSION

527 The colonization of the UK by *Bombus hypnorum* is an important and interesting counter-
528 example to the reported widespread decline in bumblebees across the northern hemisphere
529 (Goulson et al. 2008). Here we report (i) evidence for multiple entries into the UK (ii) similar
530 levels of genetic diversity in the sampled UK and continental population, despite indications
531 of initial bottlenecks in some sites, and (iii) preliminary evidence of selection in some genomic
532 regions.

533

534 *The population expansion of* B. hypnorum *into and across the UK*

535 For the first time, we report population genomic evidence that supports multiple colonization
536 of the UK by *B. hypnorum*. Collated records from the BWARS database indicate an initial
537 colonization of the UK in the south, with a subsequent rapid spread north and east and a later
538 spread westwards. Our modelled scenarios generally support the colonization pattern
539 suggested by the BWARS database although some sample sites were founded from multiple
540 sites. There is a high likelihood of migration into multiple sites from continental Europe across
541 the south and north-east, combined with ongoing and rapid migration from neighboring sites
542 already colonized. In bumblebees, queens are the founders of new nests and therefore drive
543 the effective dispersal of the species (Lepais et al. 2010). Our results suggest that both jump-
544 dispersal over a longer distance outside the native range, as well as diffusion dispersal, the
545 gradual dispersion over shorter distances (Pielou 1979), are playing a role in explaining the
546 colonization pattern of *B. hypnorum* queens into the UK. A pattern of multiple founders from
547 both local and continental sources is supported further by patterns of genetic differentiation.
548 For example, the Newcastle sample clustered most closely with Le Havre in a PCoA on

549 pairwise $F_{ST}$ (Figure 3), which again suggests an independent introduction from the Western
550 continental population rather than colonization from within the UK only.

551

552 *Evidence for loss of genetic diversity in colonizing populations*

553 Whether or not colonization is accompanied by a loss of genetic diversity is important in terms
554 of understanding both the colonization event itself, and the potential evolutionary constraints
555 and vulnerability of the newly established populations (Dlugosch and Parker 2008), especially
556 in haplodiploid species where effective population size is reduced (Lester and Selander 1979).
557 Here, we observed generally comparable proportions of polymorphic markers and
558 measurements of heterozygosity within the continental Le Havre and UK locations (Table 1).
559 This is consistent with a previous report that found no evidence for the loss of genetic
560 diversity in a single UK population (Crowther 2017). The levels of genetic diversity observed
561 here in *B. hypnorum* are similar to other long-established bumblebee populations in North
562 America based on SNP data (Lozier 2014). This is despite the fact that DIYABC analysis suggests
563 that bottleneck events have occurred during the establishment of *B.hypnorum* in the UK
564 (Figure 4). However, the likely occurrence of multiple introductions through the presumed
565 successive influx of incoming dispersing queens, the dynamic patterns of migration within
566 colonized UK sites and the rapid expansion will mitigate a loss of genetic diversity (Nei et al.
567 1975; Pannell and Charlesworth 2000; Zenger et al. 2003; Dlugosch and Parker 2008).

568

569 As we sampled only one continental location, the level of variation in genetic diversity across
570 potential source populations is unknown and so could be under-represented in our study.
571 Studies on other bumblebee species that are widespread and abundant across continental

572 Europe (*B. terrestris* and *B. pascuarum*) did not reveal population structuring on a continental
573 scale (Estoup et al 1996, Widmer and Schmid-Hempel 1999). This suggest that across
574 continental Europe there are no significant barriers to gene flow for bumblebee species with
575 ample dispersal abilities, consistent with other studies suggesting that connectivity is
576 generally high for mainland populations of bumblebees (Lozier 2014). It is therefore likely that
577 population structuring is minimal across the coast of north-western continental Europe in *B.*
578 *hypnorum*. Indeed, a very recent study did not find population structure across populations
579 of *B. hypnorum* in Belgium (Maebe et al. 2019).

580

581 Jones and Brown (2014) used an indirect approach of diploid male production as a preliminary
582 assessment of genetic diversity of *B. hypnorum* in the UK. They reported lower than expected
583 genetic diversity within the UK compared to continental European populations, in contrast to
584 our results here. However, that study focused exclusively on samples from the London area,
585 and here we also report this area as having the lowest diversity of all of the locations sampled
586 (both in terms of heterozygosity and in polymorphic sites), raising the possibility that the
587 London site may be less representative of the wider UK population.

588

589 Our finding of no major loss of genetic diversity in a successfully colonizing species is
590 consistent with the majority of cases documented (Roman and Darling 2007; Dlugosch and
591 Parker 2008). However, there are other contrasting examples, such as the colonization of
592 North America by the solitary bee *Lasioglossum leucozonium,* which was likely initialized by
593 the introduction of a singly-mated female (Zayed et al. 2007). Different patterns of loss of
594 genetic diversity through founding events are likely explicable by the severity and length of

595 periods of reduced population size, consistent with established population genetic theory.
596 This suggests that despite initial small population sizes in the UK as indicated by the detection
597 of bottlenecks in the DIYABC analysis, founding populations expanded quickly, which in
598 combination with the likely ongoing independent colonization events, minimized the loss of
599 genetic diversity. This is further supported by a very recent study using microsatellites that
600 reports no significant increases in diploid males in the UK population (Brock et al. in review).

601

602 *Evidence for selection*

603 The limitation in the identification of signatures of selection across the sampled sites using a
604 RAD-seq approach is that only a portion of the genome is screened (here 1%), so many loci
605 under selection are likely to be missed (Tiffin and Ross-Ibarra 2014; Lowry et al. 2017).
606 Further, we used the genome of a different species to infer functionality of genomic regions.
607 Although this is common practice when working with non-model species (Shafer et al. 2017),
608 the annotation is likely to be incomplete and areas of high divergence to the reference
609 genome may fail to be annotated. The complex demographic history and rapid expansion may
610 also leave genomic signatures similar to those expected under selection (Excoffier and Ray
611 2008; Li et al. 2012). By taking a stringent approach and only highlighting those genes
612 identified from all our approaches (two $F_{ST}$–outlier approaches *and* genomic regions showing
613 significantly elevated or low polymorphism) three genes were identified as putatively showing
614 signatures of selection. Only one gene, encoding for the protein 'vestigial' and involved in
615 wing formation, showed significantly elevated differentiation between Le Havre and UK sites.
616 Geographic variation in wing shape and size is common in insects (Hoffmann and Shirriffs
617 2002; Kandemir et al. 2009) and the establishment of geographic clines has been documented

618 within short time scales (Gilchrist et al. 2001), which may also be the case here. Differences
619 in wing morphology may also relate to flight and dispersal abilities. Spatial sorting theory
620 predicts that dispersal ability is a trait under strong selection during range expansions (Shine
621 et al. 2011; Berthouly-Salazar 2012). This is explained by the expectation that those
622 individuals with the highest dispersal abilities will be spatially assorted at the expanding range
623 front. This creates a positive feedback loop as expansion continues and accelerates the speed
624 at which new areas are colonized (Berthouly-Salazar 2012).

625

626 Among UK-UK site comparisons, two further genes were highlighted as being potentially
627 under strong directional selection: the CLOCK gene, which has an important role in the
628 regulation of circadian rhythms (Darlington et al. 1998) and has been linked with adaptive
629 responses to environmental conditions across a range of taxa, including invertebrates (Tauber
630 and Kyriacou 2005; O'Malley and Banks 2008; Liedvogel et al. 2009) and serine/threonine
631 protein kinase NLK, which is associated with innate immune function and apoptosis (cell
632 death) (Mirkovic et al. 2002; Li et al. 2014). A serine/threonine protein kinase region was also
633 identified as an $F_{ST}$ – outlier by Theodorou et al. (2018) in a recent study using a RAD-seq
634 approach in *Bombus lapidarius,* where it was suggested as a signature of an adaptive response
635 to increasing urbanization. Colonizing *B. hypnorum* suffer higher prevalence of highly virulent
636 parasites than native species (Jones and Brown 2014; Lloyd et al. unpublished data), which
637 might also be explanatory.

638

639 Further, GO analysis identified areas of significantly high polymorphism were associated with
640 biological processes or molecular functions, largely regulating transcription and gene

641 expression (Table 5). This highlights research into the role of genetic variation in regulatory
642 genes in the adaptability of *B. hypnorum* to new locations as a potentially important area for
643 further studies. The GO analysis of genes within genomic regions of low polymorphism
644 revealed a significant association with ion channel and signalling receptor activity (Table 5)
645 and the investigation of the adaptive role of these genomic regions similarly warrants further
646 research across a wider group of *Bombus* species.

647

648 *Why now?*

649 Our study has revealed a probable pattern of multiple colonization events from western
650 continental Europe of *B. hypnorum*, in addition to ongoing gene flow and spread from within
651 established UK sites. We also report evidence of some genomic areas that may show signals
652 of directional selection. Given the likely pattern of colonization, a key question is why this
653 species has been such a successful and successive colonizer now, when it has been present
654 on continental Europe for many decades prior to the turn of the 21$^{st}$ century. Answers to this
655 can only remain speculative but our study gives some potential insight by suggesting a pattern
656 of multiple, possibly ongoing, colonization routes with no evidence of a reduction in genetic
657 diversity. Following the colonization success framework of Facon et al. (2006) our results
658 highlight a possible role of 'migration change' where barriers to dispersal have recently and
659 relatively suddenly opened. Possible changes to anthropogenic transport routes are one
660 potential explanation. Anthropogenic rates of trade and transport are subject to continuous
661 growth (Hulme 2009) and although there have been no obvious significant changes over the
662 period in question, increased opportunities for assisted introduction may have facilitated the
663 colonization of the UK. Shifts in climatic conditions are another potential explanation, which

664 may facilitate dispersing queens reaching the UK. While temperature changes alone are
665 unlikely to be responsible, given that the UK sits well within the current climatic range of *B.*
666 *hypnorum*, shifts in wind patterns (e.g. Hu et al. 2016; Weber et al 2018) are among these
667 plausible scenarios. There is less compelling current evidence for a role of 'environmental
668 change'. While there are noted ecological differences between *B. hypnorum* and other UK
669 species, both in nesting site preferences and also both habitat and foraging associations
670 (Crowther et al. 2014), there is little evidence to date of any marked change in availability of
671 either nesting sites or habitat over the period in question. Neither is there any evidence for
672 'enemy release' occurring, from recent comparative study of *Bombus* parasite communities
673 (Jones and Brown 2014). Our preliminary results highlighting genes already suggested to be
674 involved in adaptive responses to urbanized environments in similar or other insect groups
675 lend some traction to the third 'evolutionary change' scenario as a contributing factor
676 underpinning the remarkable expansion of this species. Such adaptations to
677 anthropogenically modified habitats within the native range, have been put forward as an
678 important feature to promote range expansions (Hufbauer et al. 2012). Finally, biogeographic
679 factors could play a role: with declines of native species on an island (some UK bees have been
680 completely extirpated and many others are declining), it might be expected that novel species
681 might colonize. Further investigation into the potential drivers of this population expansion,
682 particularly focusing on migration and/or evolutionary changes, are likely to yield key insights
683 to our understanding of these recent population changes. Notably, establishing whether
684 these are ubiquitous signals across a much wider taxonomic range, or whether this particular
685 system has been successful because it is an exception to the norm will give important insights
686 into the rapid changes in distribution and abundance of species currently being witnessed.

687

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Declarations

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Consent to participate
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Consent for publication
Not applicable

Ethics approval
Not applicable

Declaration of conflicts of interest
The authors declare no conflicts of interest.

Data Accessibility

Raw reads generated in this study are available on GenBank (SUB9082181)

Code availability

A custom python script used for variant calling is available on GitHub: https://github.com/janavanhu/genotyping_pools_from_sync

Author Contributions

MEK, JSE and MJFB conceived the project and secured funding; KL carried out the sampling; KL, CMB and MK generated the raw data; JVH conducted the analysis in discussion with MEK and JSE; JVH, MEK and JSE drafted the manuscript, with input from the other authors; MEK led the project.

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Tables

**Table 1:** *Summary of RAD-seq data, including basic measures of genetic diversity.*

| Sampling site | raw reads | uniquely mapped reads | bp covered[a] | number of SNPs (polymorph within populations) | proportion of polymorphic sites[b] | Allelic richness[b] | Average heterozygosity[c] |
|---|---|---|---|---|---|---|---|
| Cardiff | 1,832,315 | 1,306,780 | 655,120 | 2,139 | 0.012 | 1.22 | 0.15 |
| Le Havre | 2,350,459 | 1,726,971 | 323,767 | 2,251 | 0.014 | 1.26 | 0.20 |
| Hull | 2,394,796 | 1,508,923 | 593,141 | 2,384 | 0.008 | 1.16 | 0.12 |
| London | 1,159,972 | 619,972 | 245,067 | 851 | 0.006 | 1.11 | 0.12 |
| Newcastle 1 | 2,788,910 | 1,781,942 | 953,719 | 3,695 | 0.012 | 1.23 | 0.18 |
| Newcastle 2 | 2,466,615 | 2,012,501 | 322,409 | 1,929 | 0.011 | 1.21 | 0.20 |
| Plymouth | 714,223 | 397,903 | 152,225 | 464 | 0.01 | 1.18 | 0.13 |
| Southampton | 1,805,902 | 1,225,225 | 587,552 | 1,995 | 0.009 | 1.17 | 0.16 |
| All UK populations | 13,162,733 | 8,853,246 | 2,443,350 | 11,845 | 0.048 | 1.46 | 0.16 |
| All populations | 15,513,192 | 10,580,217 | 2,469,636 | 12,823 | 0.054 | 2 | 0.17 |

[a] all bps with a coverage 50-500 within pop and <= 3,000 across sampling sites
[b] across sites covered in all sampling sites (1,886 polymorphic and non-polymorphic sites; 101 polymorphic sites)
[c] across all SNPs found at the sampling site

**Table 2:** *Pairwise $F_{ST}$ –values between sampling sites (bottom) and confidence intervals generated across 1000 bootstrap cycles (top) across 101 SNPs covered in all sampling sites.*

| | **Cardiff** | **Hull** | **Le Havre** | **London** | **Newcastle 1** | **Newcastle 2** | **Plymouth** | **Southampton** |
|---|---|---|---|---|---|---|---|---|
| **Cardiff** | 0.000 | 0.020,0.066 | 0.014, 0.039 | 0.012, 0.040 | 0.011, 0.040 | 0.014, 0.044 | 0.019, 0.056 | 0.010, 0.038 |
| **Hull** | 0.040 | 0.000 | 0.010, 0.029 | 0.009,0.066 | 0.012, 0.040 | 0.010, 0.030 | 0.010, 0.027 | 0.009, 0.043 |
| **Le Havre** | 0.026 | 0.019 | 0.000 | 0.006, 0.035 | 0.006, 0.015 | 0.003, 0.007 | 0.010, 0.034 | 0.007, 0.031 |
| **London** | 0.025 | 0.035 | 0.018 | 0.000 | 0.007, 0.025 | 0.006, 0.035 | 0.009, 0.057 | 0.006, 0.040 |
| **Newcastle 1** | 0.024 | 0.025 | 0.010 | 0.014 | 0.000 | 0.006, 0.015 | 0.012, 0.036 | 0.006, 0.025 |
| **Newcastle 2** | 0.028 | 0.018 | 0.005 | 0.018 | 0.010 | 0.000 | 0.009, 0.034 | 0.007, 0.030 |
| **Plymouth** | 0.036 | 0.018 | 0.020 | 0.032 | 0.023 | 0.019 | 0.000 | 0.009, 0.027 |
| **Southampton** | 0.023 | 0.023 | 0.017 | 0.018 | 0.014 | 0.017 | 0.017 | 0.000 |

**Table 3:** *relative probabilities and confidence intervals for the top two scenarios in the DIYABC analysis for each test site*

| test site | most likely source of colonization | relative probability [CI] | second most likely scenario | relative probability [CI] |
|---|---|---|---|---|
| **London** | Le Havre bottlenecked | 0.92 [0.75,1.00] | Hull bottlenecked | 0.0660 [0.00,0.24] |
| **Cardiff** | Combined colonization Hull-Newcastle bottlenecked | 0.5811 [0.14,1.00] | Newcastle bottlenecked | 0.4189 [0.00,1.00] |
| **Hull** | London bottlenecked | 0.5463 [0.35,0.74] | Le Havre | 0.4537 [0.26,0.65] |
| **Southampton** | Le Havre | 0.6065 [0.13,1.00] | Combined colonization Le Havre-Hull | 0.3917 [0.00,0.86] |
| **Newcastle** | Combined colonization Le Havre-Southampton bottlenecked | 1.0000 [1.00,1.00] | NA | 0.0000 [0.00,0.00] |
| **Plymouth** | London bottlenecked | 0.9661 [0.92,1.00] | Le Havre | 0.0339 [0.00,0.08] |

**Table 4:** *Genes within genomic regions of significantly elevated differentiation for UK-UK and UK-Le Havre comparisons. Genes that were also identified in areas of significantly high polymorphism are highlighted in bold.*

| GeneID | description | chromosome | Exon count | comparison | function |
|---|---|---|---|---|---|
| **100651612** | **protein vestigial** | **LG B12** | **6** | **Le Havre – UK** | **wing formation/ wing specific gene expression** |
| **100650446** | **circadian locomotor output cycles protein kaput** | **LG B09** | **12** | **UK populations** | **circadian clock** |
| **100651912** | **serine/threonine-protein kinase NLK (nemo-like kinase)** | **LG B03** | **11** | **UK populations** | **involved in signal transduction pathways that regulate growth factor response, proliferation, apoptosis and innate immune response** |
| 110119491 | uncharacterized LOC110119491 | LG B08 | 2 | UK populations | Unknown |

**Table 5:** *Gene ontology enrichment analysis of Molecular Function (MF) and Biological Processes (BP) for all regions showing significantly elevated/reduced polymorphism across all sampling sites. Results are shown where at least two of the three algorithms used ('classic', 'elim' and 'weight') supported significance for regions with significantly elevated (A) and reduced (B) polymorphism. Significant p-values are highlighted in bold.*

| | GO.ID | Term | Annotated | Significant | Expected | classic | elim | weight |
|---|---|---|---|---|---|---|---|---|
| **A)** | | | | | | | | |
| **BP** | GO:0050794 | regulation of cellular process | 373 | 118 | 86 | **0.000002** | **0.025080** | **0.022640** |
| | GO:0050789 | regulation of biological process | 381 | 119 | 87.85 | **0.000003** | **0.034850** | 0.376710 |
| | GO:0065007 | biological regulation | 394 | 121 | 90.84 | **0.000008** | **0.048680** | 0.809450 |
| | GO:0007166 | cell surface receptor signaling pathway | 37 | 19 | 8.53 | **0.000130** | **0.000130** | **0.000500** |
| | GO:0010468 | regulation of gene expression | 151 | 53 | 34.82 | **0.000200** | 0.458540 | **0.000200** |
| | GO:0006355 | regulation of transcription, DNA-templated | 144 | 51 | 33.2 | **0.000210** | **0.000210** | 0.162160 |
| | GO:0032502 | developmental process | 27 | 15 | 6.23 | **0.000220** | 0.130260 | **0.000220** |
| | GO:0007154 | cell communication | 230 | 73 | 53.03 | **0.000440** | **0.021020** | **0.036350** |
| | GO:0023052 | signaling | 229 | 72 | 52.8 | **0.000680** | **0.029120** | **0.049620** |
| | GO:0007275 | multicellular organism development | 21 | 12 | 4.84 | **0.000700** | **0.000700** | 1.000000 |
| | GO:0007165 | signal transduction | 221 | 69 | 50.96 | **0.001150** | **0.045140** | 0.540420 |
| **MF** | GO:0005515 | protein binding | 500 | 137 | 112.33 | 0.001060 | **0.001100** | **0.002110** |
| | GO:0003700 | DNA-binding transcription factor activity | 94 | 34 | 21.12 | 0.001300 | **0.001300** | **0.001300** |
| | GO:0043565 | sequence-specific DNA binding | 110 | 37 | 24.71 | 0.003610 | **0.003600** | **0.003610** |
| | GO:0008270 | zinc ion binding | 96 | 29 | 21.57 | 0.043440 | **0.043400** | **0.043440** |
| **B)** | | | | | | | | |
| **MF** | | | | | | | | |
| | GO:0004888 | transmembrane signaling receptor activity | 37 | 17 | 8.28 | 0.000940 | **0.000940** | **0.000940** |
| | GO:0005230 | extracellular ligand-gated ion channel activity | 16 | 8 | 3.58 | 0.013090 | **0.013090** | **0.013090** |

**Figure 1:** *Reconstruction of the UK* Bombus hypnorum *colonization from BWARS records of year-wise sightings 2004-2012.*

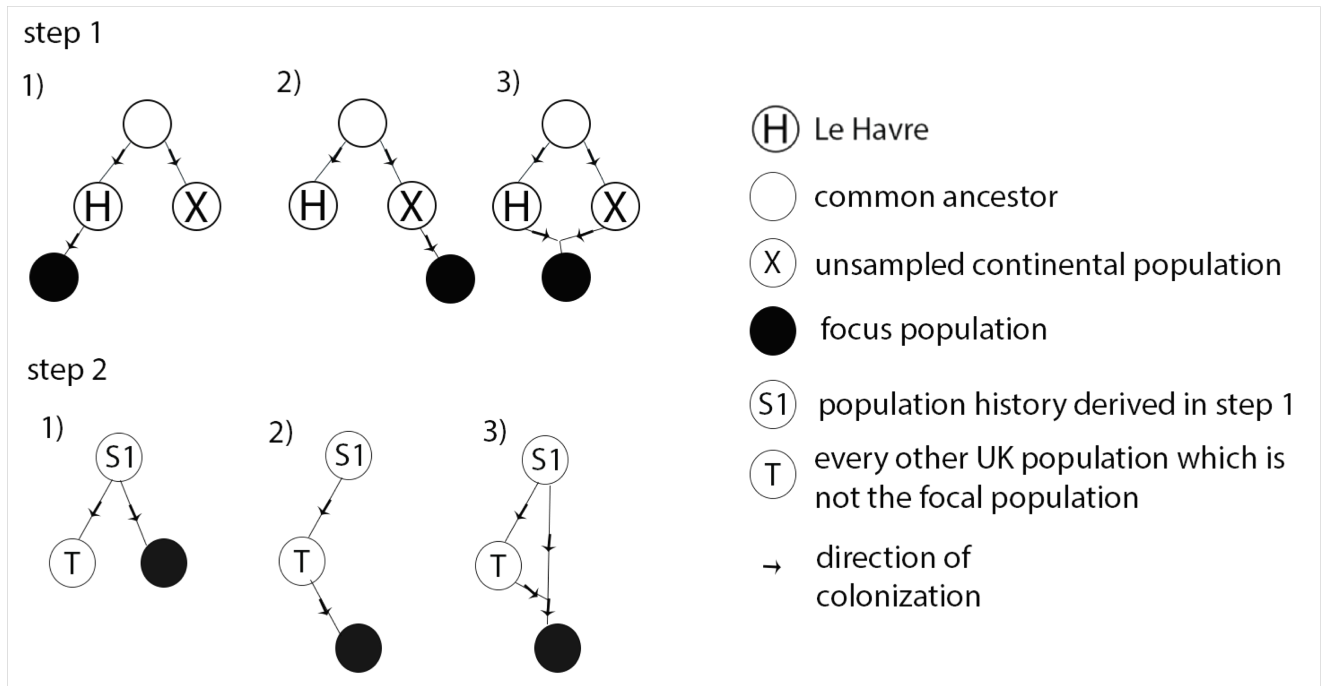



**Figure 2:** *schematic representation of steps 1 and 2 of the hierarchical DIYABC analysis done for each UK sampling site independently*

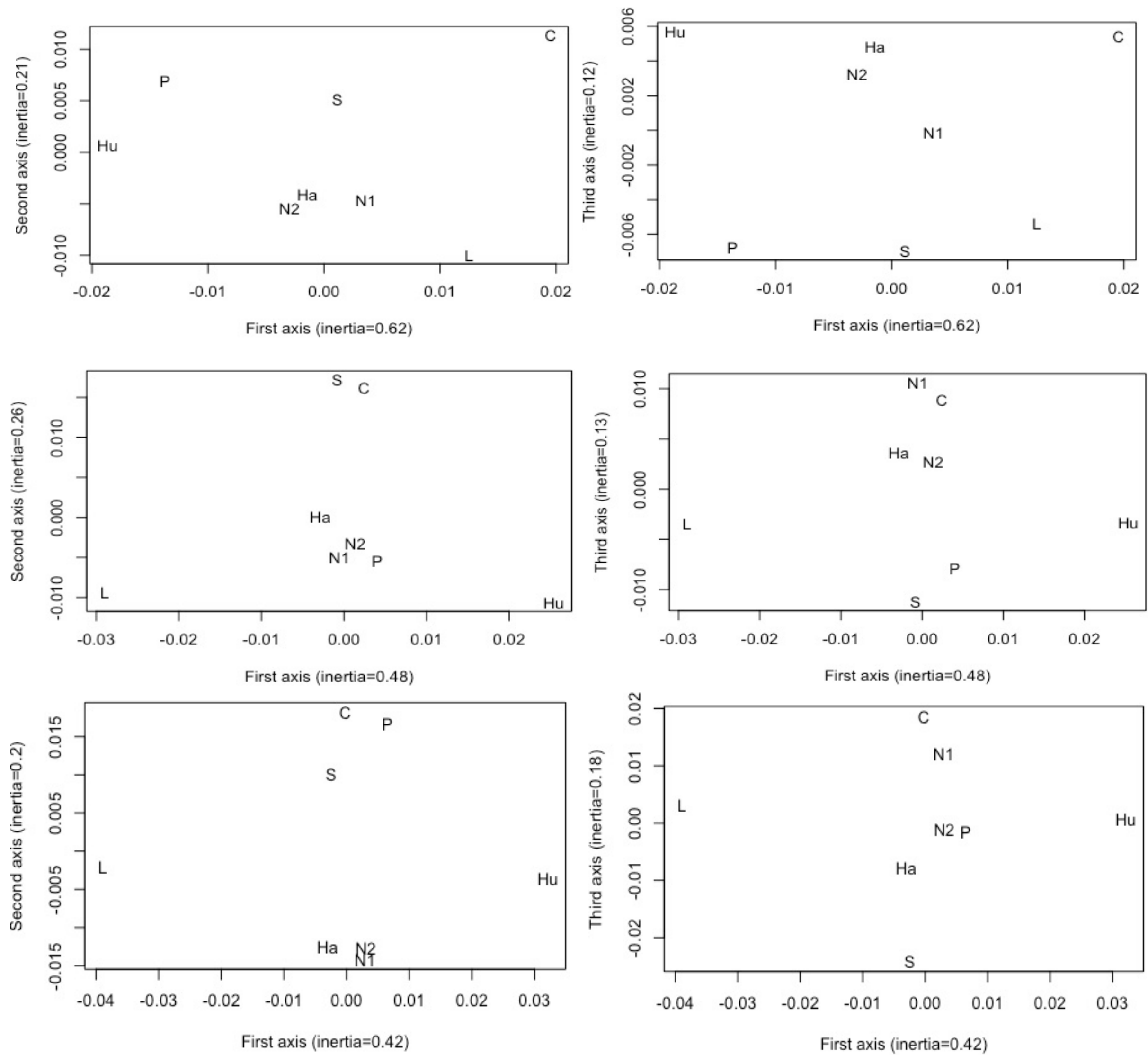
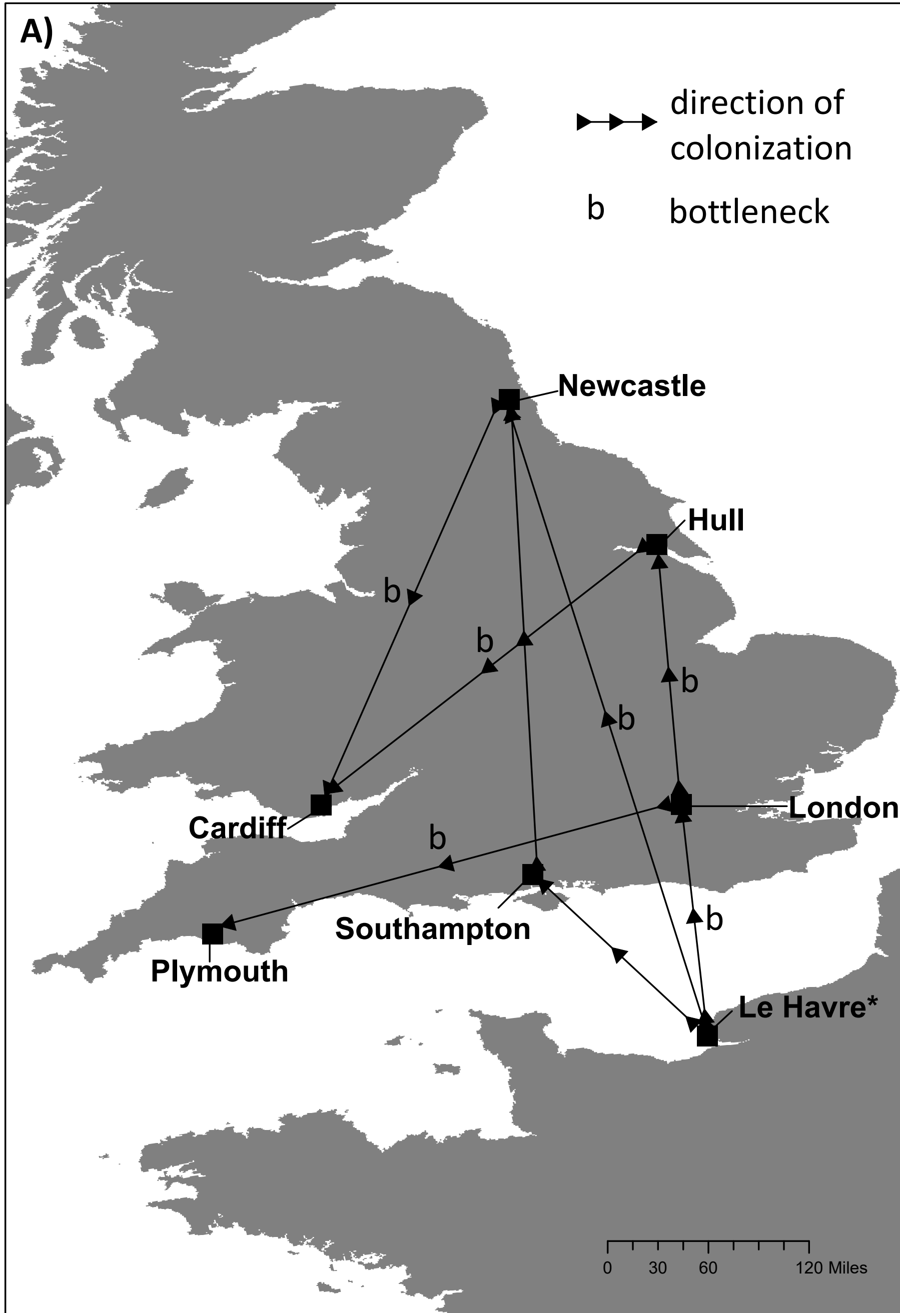
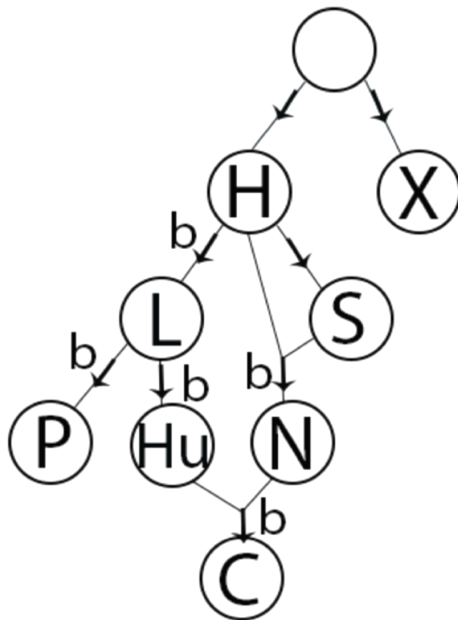



**Figure 3:** *Principal coordinate analysis on pairwise $F_{ST}$ values between sampling sites for A) all SNPs covered in all sampling sites (101); B) all SNPs (2166) covered in at least six sampling sites (75%) and C) all SNPs (3475) covered in at least four sampling sites (50%); sample sites are abbreviated as follows: Le Havre (H), Southampton (S), London (L), Hull (Hu), Newcastle (N), Cardiff (C) and Plymouth (P)*


A)
direction of colonization
b
bottleneck
Newcastle
Hull
Cardiff
London
Southampton
Plymouth
Le Havre*
b
b
b
b
b
b
0
30
60
120 Miles

**B)**



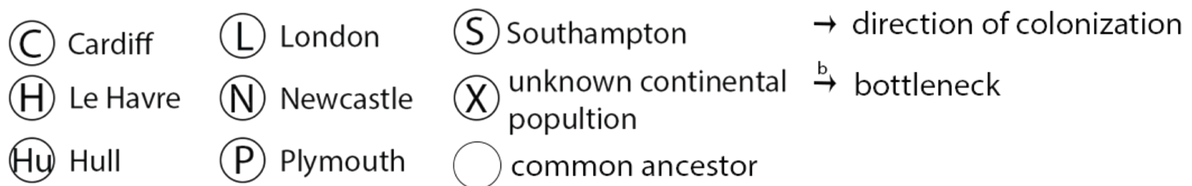
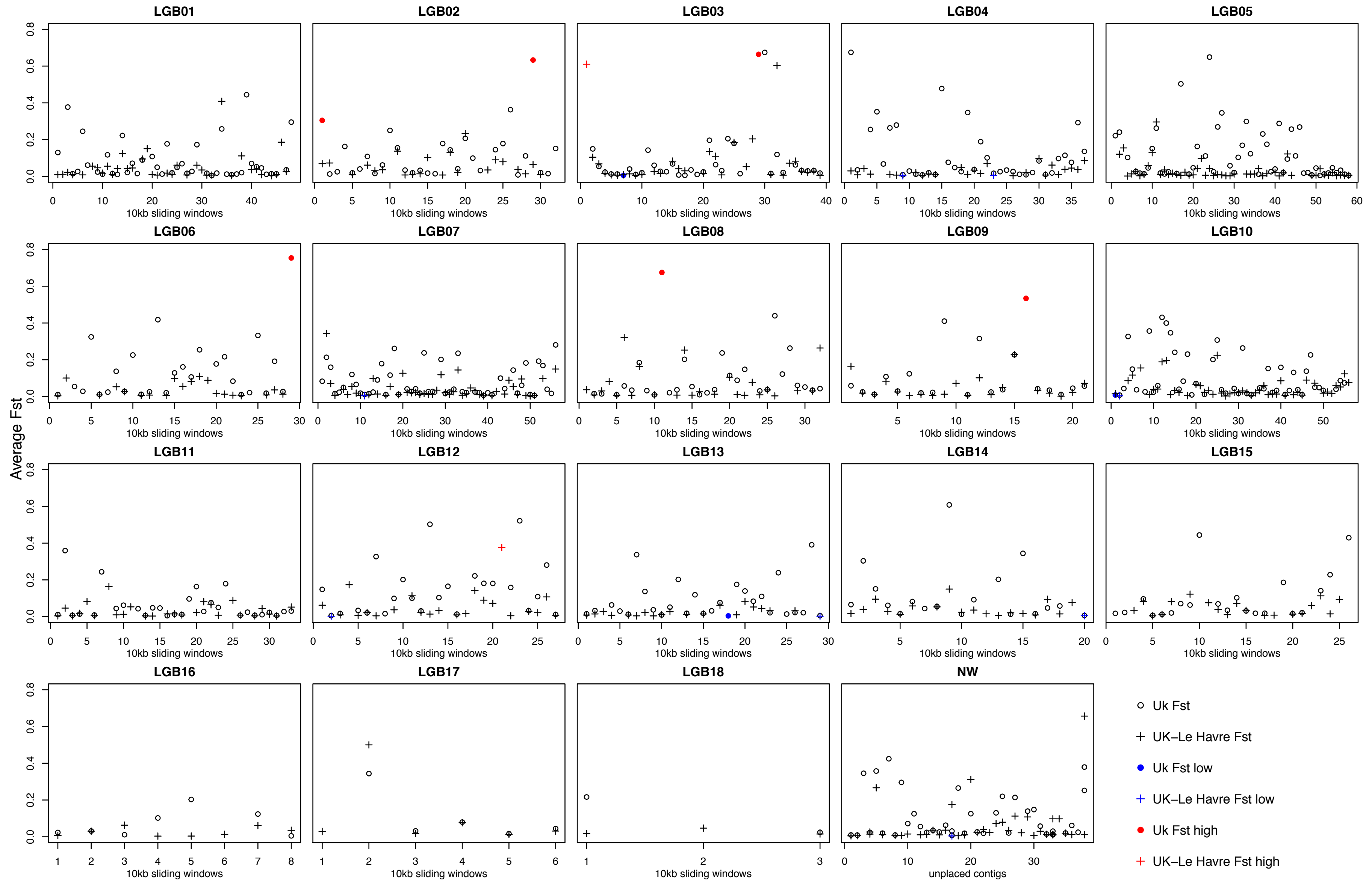




**Figure 4:** *A) Geographic representation of the DIYABC consensus model B) Schematic consensus model, derived by combining the scenarios with highest likelihood for each UK sampling site. Three independent colonization events from the continental reference site, Le Havre (H), one to Southampton (S) one to London and one to Newcastle (N) are apparent. Further, founders from close UK sampling sites (London (L), Hull (Hu), Southampton (S) and Newcastle (N)) are also involved in the establishment of populations. Note that, whilst the succession of events implies a certain timeline, time is not explicitly evaluated here and not represented by the length of connecting lines.*

**representing the continental European reference population*



LGB01
LGB02
LGB03
LGB04
LGB05
LGB06
LGB07
LGB08
LGB09
LGB10
LGB11
LGB12
LGB13
LGB14
LGB15
LGB16
LGB17
LGB18
NW
Average Fst
10kb sliding windows
unplaced contigs
Uk Fst
UK–Le Havre Fst
Uk Fst low
UK–Le Havre Fst low
Uk Fst high
UK–Le Havre Fst high

**Figure 5:** *Distribution of average $F_{ST}$ -values across linkage groups across all pairwise comparisons within the UK (points) and between the UK and Le Havre (crosses) respectively. Genomic regions showing significantly high $F_{ST}$ -values based on permutation tests are highlighted in red and regions with significantly low $F_{ST}$ -values are highlighted in blue.*

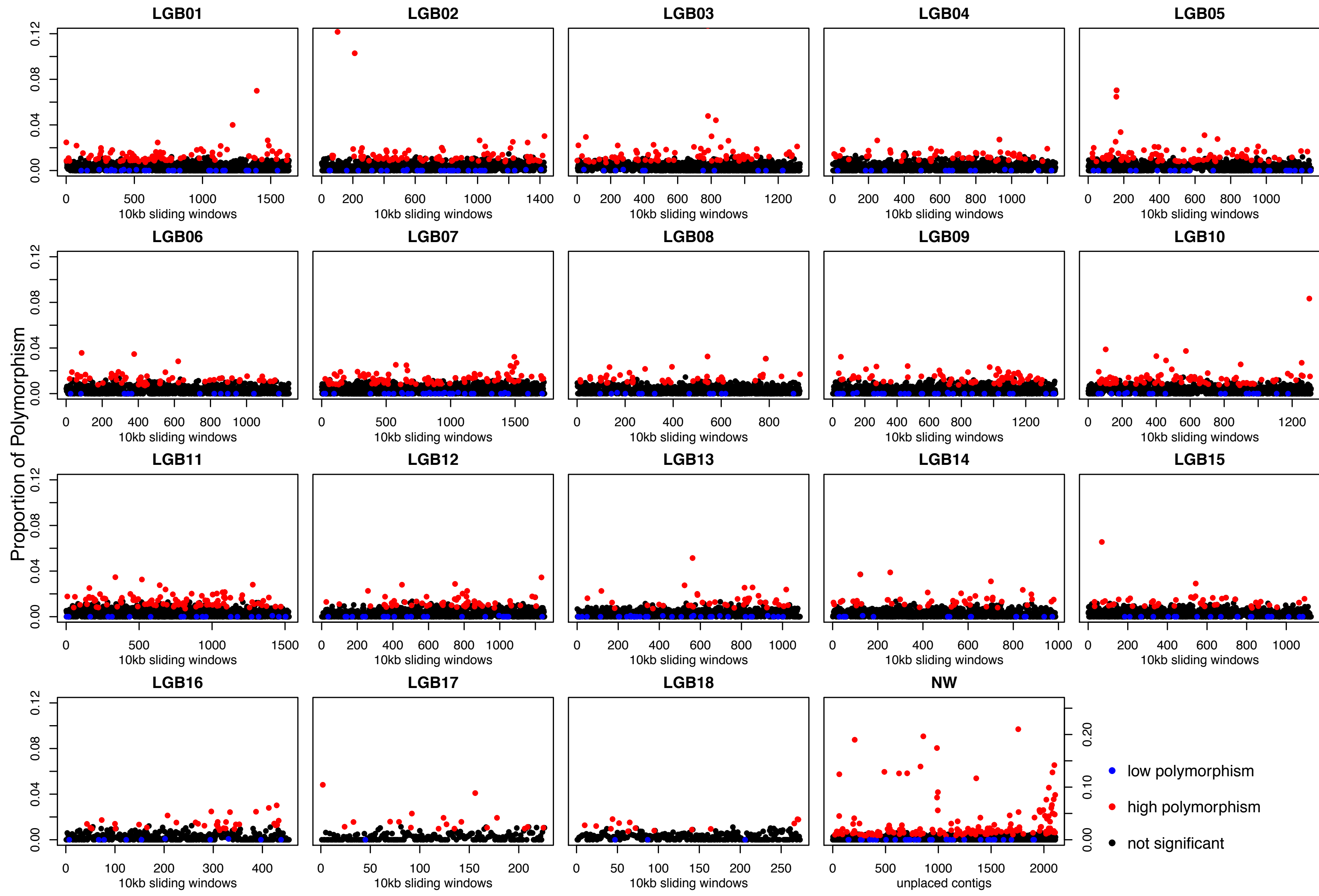

LGB01
LGB02
LGB03
LGB04
LGB05
LGB06
LGB07
LGB08
LGB09
LGB10
LGB11
LGB12
LGB13
LGB14
LGB15
LGB16
LGB17
LGB18
NW
Proportion of Polymorphism
10kb sliding windows
unplaced contigs
low polymorphism
high polymorphism
not significant

**Figure 6:** *Distribution of polymorphism across linkage groups. Genomic regions showing significantly elevated polymorphism based on permutation tests are highlighted in red and areas of significantly reduced polymorphism in blue. Note the different y-axis scale in the NW linkage group.*