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

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1 **Bucking the trend of pollinator decline: the population genetics of a**

2 **range expanding bumblebee**

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26   ABSTRACT

27   Recent research has shown drastic reductions in the global diversity and abundance of

28   insects. This is a major concern given the expected cascade effects on ecosystem services,

29   such as pollination. Understanding the patterns and drivers of changes in the distribution and

30   abundance of species in our rapidly changing environment is therefore urgent. Cases of

31   species showing trends that run counter to general population declines, especially when they

32   deliver key ecosystem services, are especially interesting. The tree bumblebee (*Bombus*

33   *hypnorum*), which belongs to a globally important group of pollinators, has substantially

34   expanded its range in recent years in direct contrast to many other species within this group.

35   Here we reconstructed the likely pattern of colonization of the UK based on RAD-seq

36   population genomic data combined with Bayesian population modelling. This RAD-seq

37   approach also enabled an analysis of genomic regions potentially under selection. We report

38   a complex and dynamic colonization pattern that is most likely ongoing. Current evidence

39   suggests that either a shift in its migration potential, and/or adaptive genomic changes have

40   contributed to the recent range expansion of *B. hypnorum*. Genomic areas of potential

41   adaptive significance included genes involved in regulation of transcription and gene

42   expression, circadian rhythms and innate immunity. Our results are framed within the general

43   context of understanding the factors driving successful population expansions.

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INTRODUCTION

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

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

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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

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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

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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 20th 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.

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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*

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

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

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

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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

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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).

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The mpileup file of pooled samples was converted to the sync file format and filtered for

indels using Popoolation2 (Kofler et al. 2011). Because variation in sequencing depth can

impair the accuracy of allele frequency estimates derived from pooled samples, only sites

with a sequencing coverage of ≥50 and ≤ 500 within any sample, and a maximum coverage of

3000 across all samples, were considered for pooled samples. Note that specifically for the

analysis of the distribution of genome wide polymorphism, which does not rely on allele

frequency estimates, a minimum within-sample coverage of 10 was applied. For pooled

samples, SNPs were called if they had a minimum of 3 reads per allele across all samples and

a minimum within-sample allele frequency of 0.01, as commonly used in population genetic

studies (e.g. Bruneaux et al. 2013; Fraser et al. 2014). We also applied a threshold of 0.05 for

the identification of F_{ST}-outliers to test for consistency (Roesti et al. 2012).

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Analysis of population genetic diversity and characterization of population structure

Average expected genome-wide heterozygosity was calculated following Fischer et al. (2017).

The proportion of polymorphic sites was calculated across all base pairs covered in all

samples. Pseudo-haplotypes were created based on population allele frequencies using a

custom Python script and imported into R v.3.4.2 (R Core Team, 2012). Allelic richness was

calculated in the R package Hierfstat v.0.04-22 (Goudet 2004). Pairwise population F_{ST} was

calculated using Popoolation2 (Kofler et al. 2011) and confidence intervals were created by

bootstrapping over loci for 1000 cycles using a custom Python script. A Mann-Whitney-

Wilcoxon test was used to test if there is a significant difference in the pairwise F_{ST} values

between UK-Le Havre and UK-UK populations (Mann and Whitney 1947). A Mantel test

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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

13

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

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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

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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

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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

18

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

19

437   cut-sites (57,157) of the enzyme used to digest samples (Xhol). 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 $6\pm3\%$ 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,

20

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

21

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

22

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

23

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

24

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

26

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

27

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

28

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 21st 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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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**

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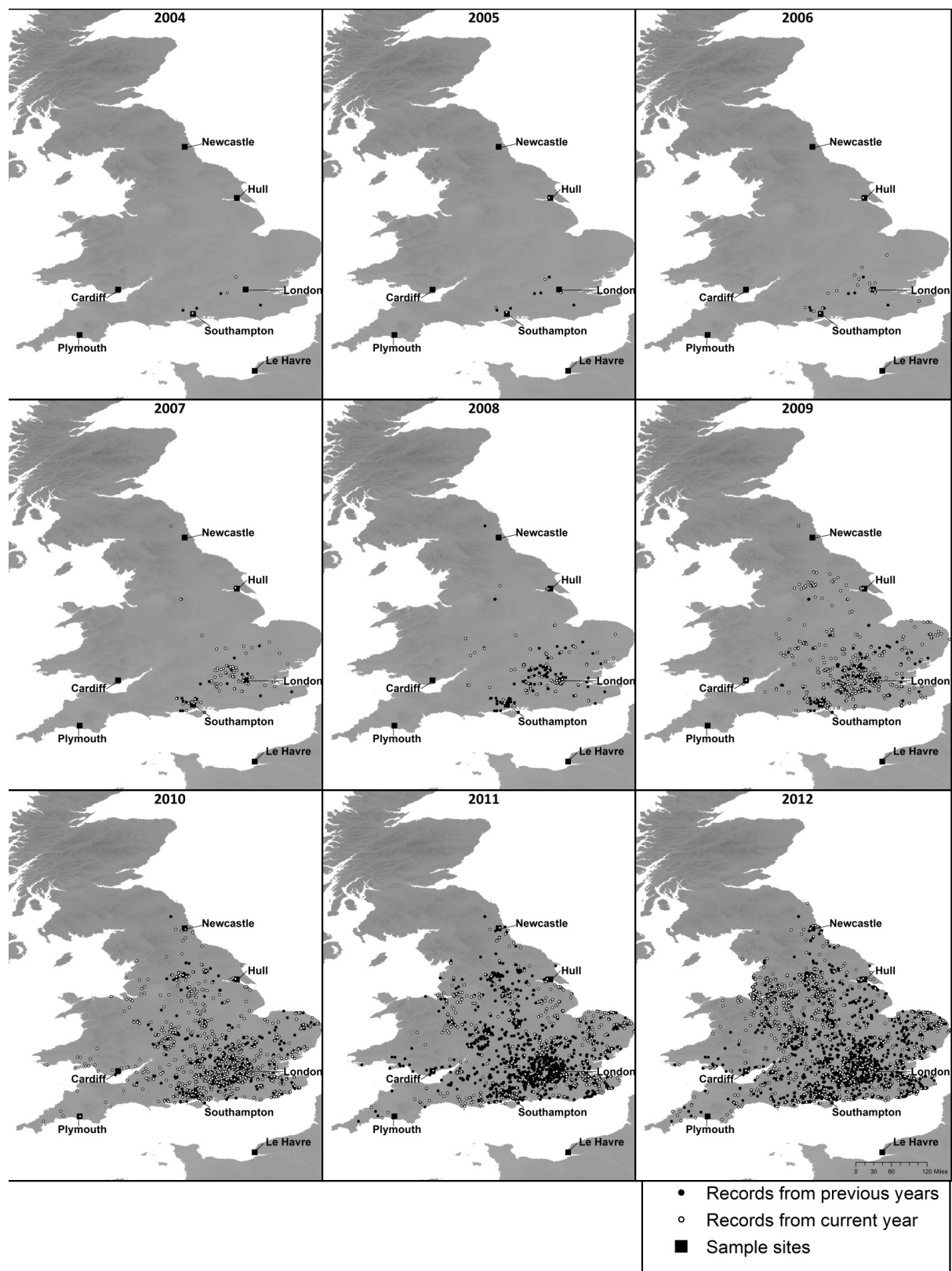
Figure 1: *Reconstruction of the UK* Bombus hypnorum *colonization from BWARS records of year-wise sightings 2004-2012.*
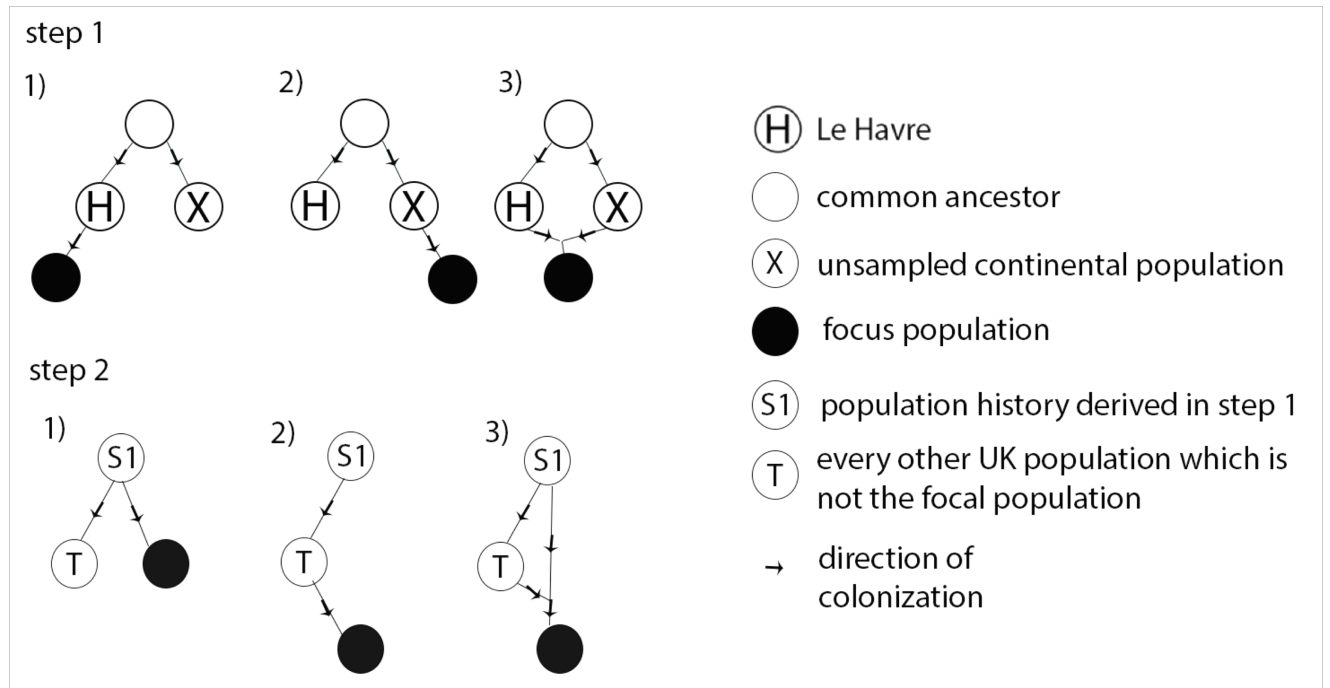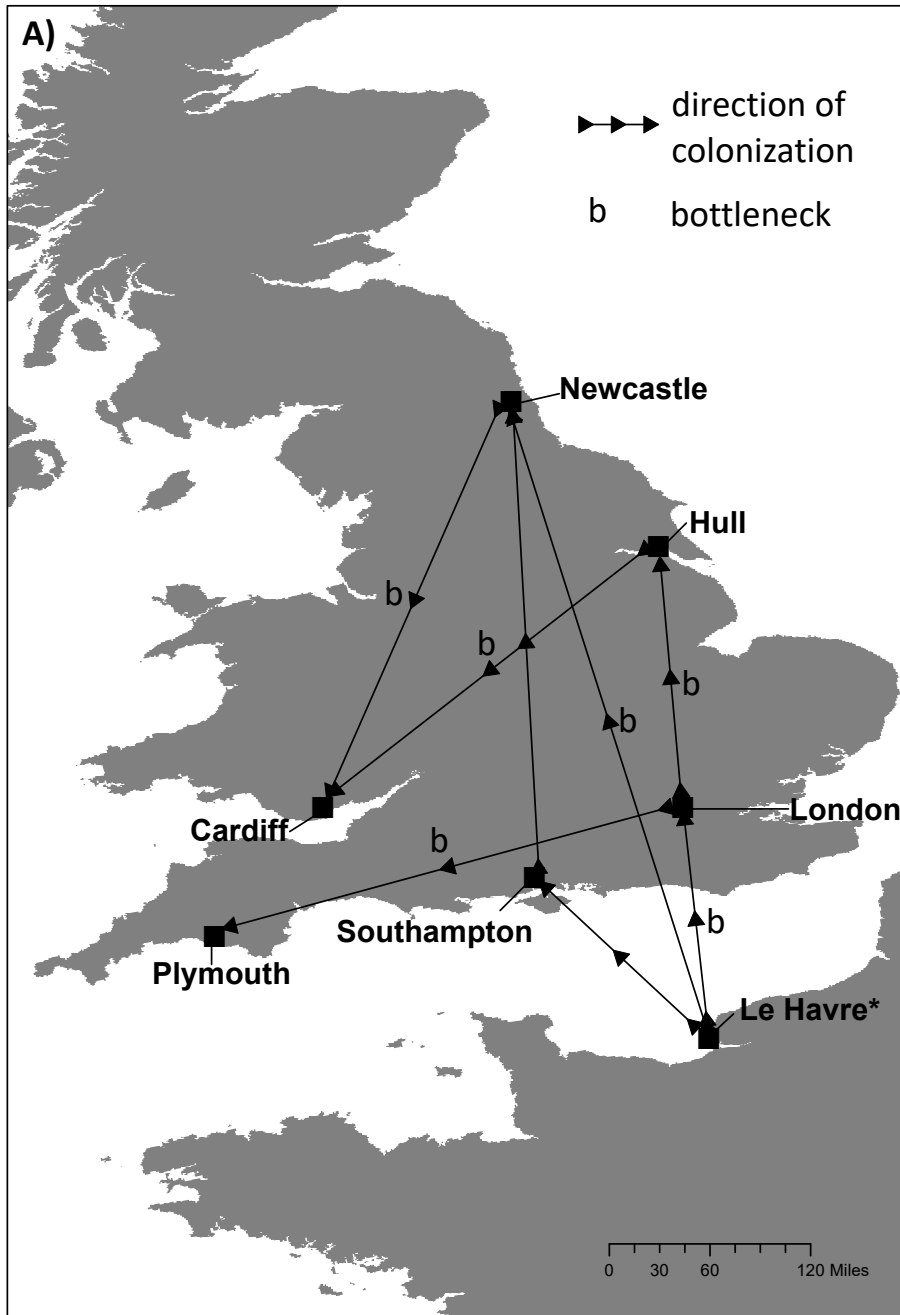
Figure 2: *schematic representation of steps 1 and 2 of the hierarchical DIYABC analysis done for each UK sampling site independently*
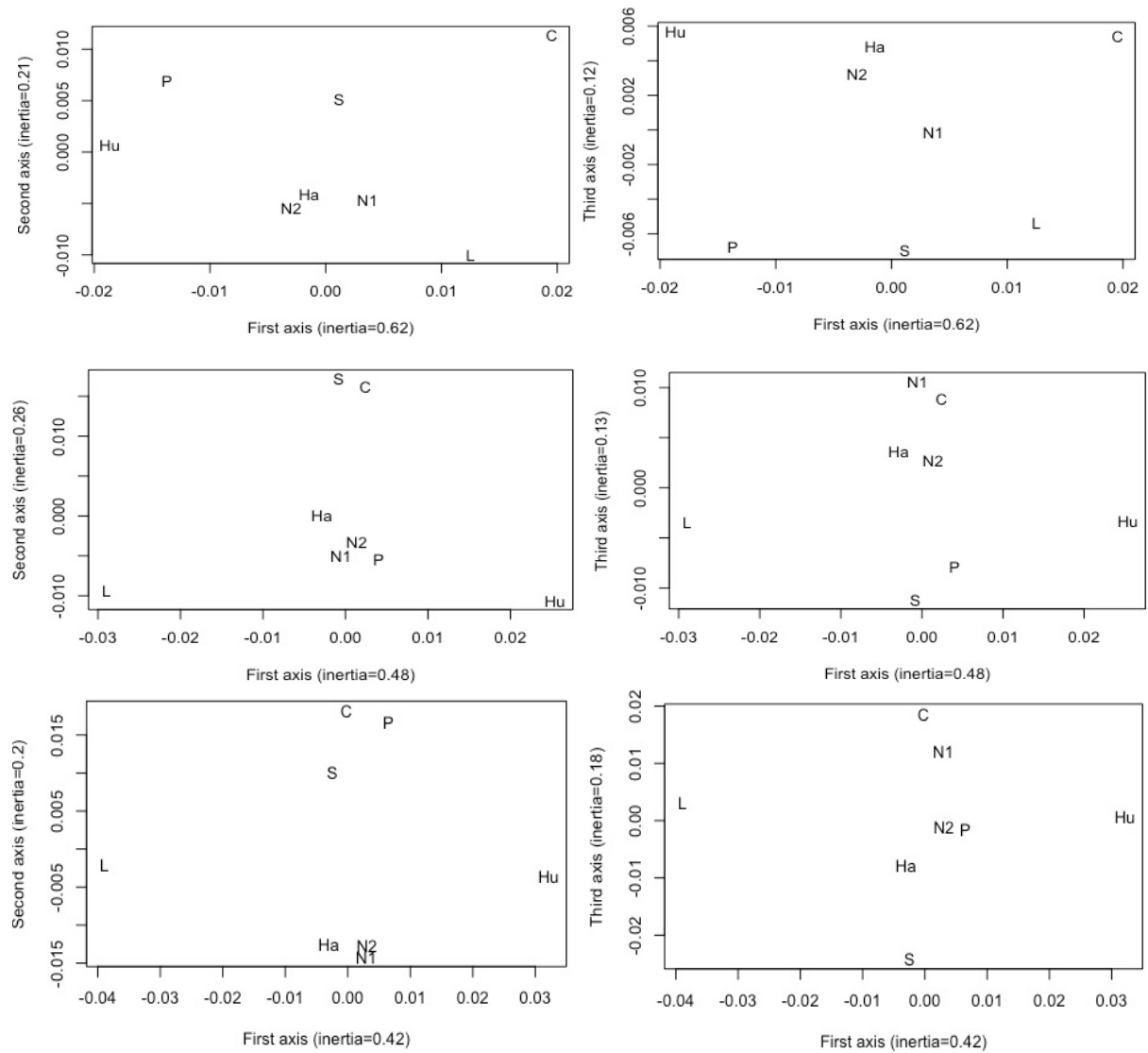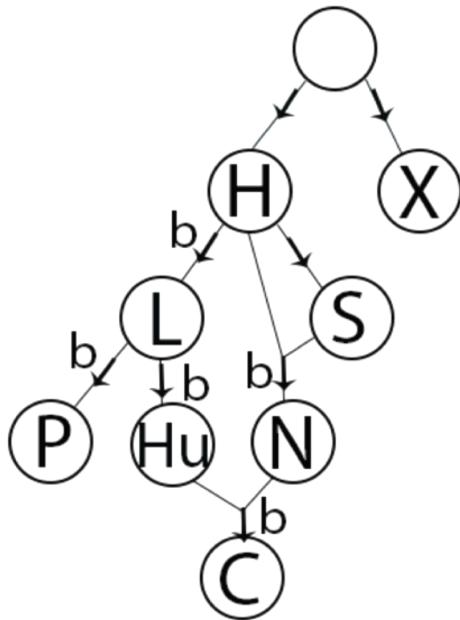
Figure 3: *Principal coordinate analysis on pairwise F_{ST} values between sampling sites for A) all SNPs covered in all sampling sites (101); B) all SNPs (2166) covered in at least six sampling sites (75%) and C) all SNPs (3475) covered in at least four sampling sites (50%); sample sites are abbreviated 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

b

b

b

b

Cardiff

London

b

Southampton

b

Plymouth

b

Le Havre*

0   30   60   120 Miles

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

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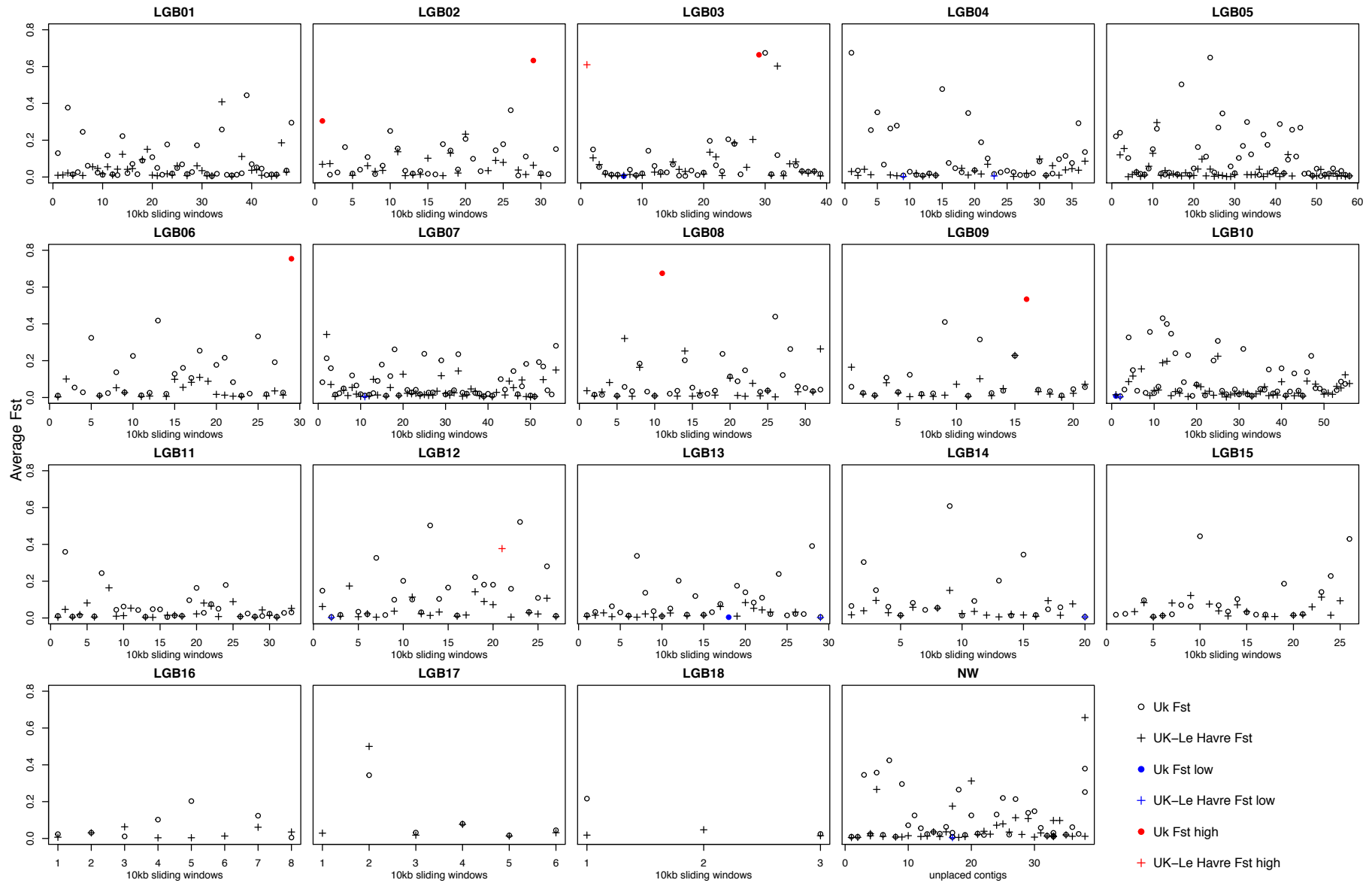
Figure 5: *Distribution of average F_{ST} -values across linkage groups across all pairwise comparisons within the UK (points) and between the UK and Le Havre (crosses) respectively. Genomic regions showing significantly high F_{ST} -values based on permutation tests are highlighted in red and regions with significantly low F_{ST} -values are highlighted in blue.*
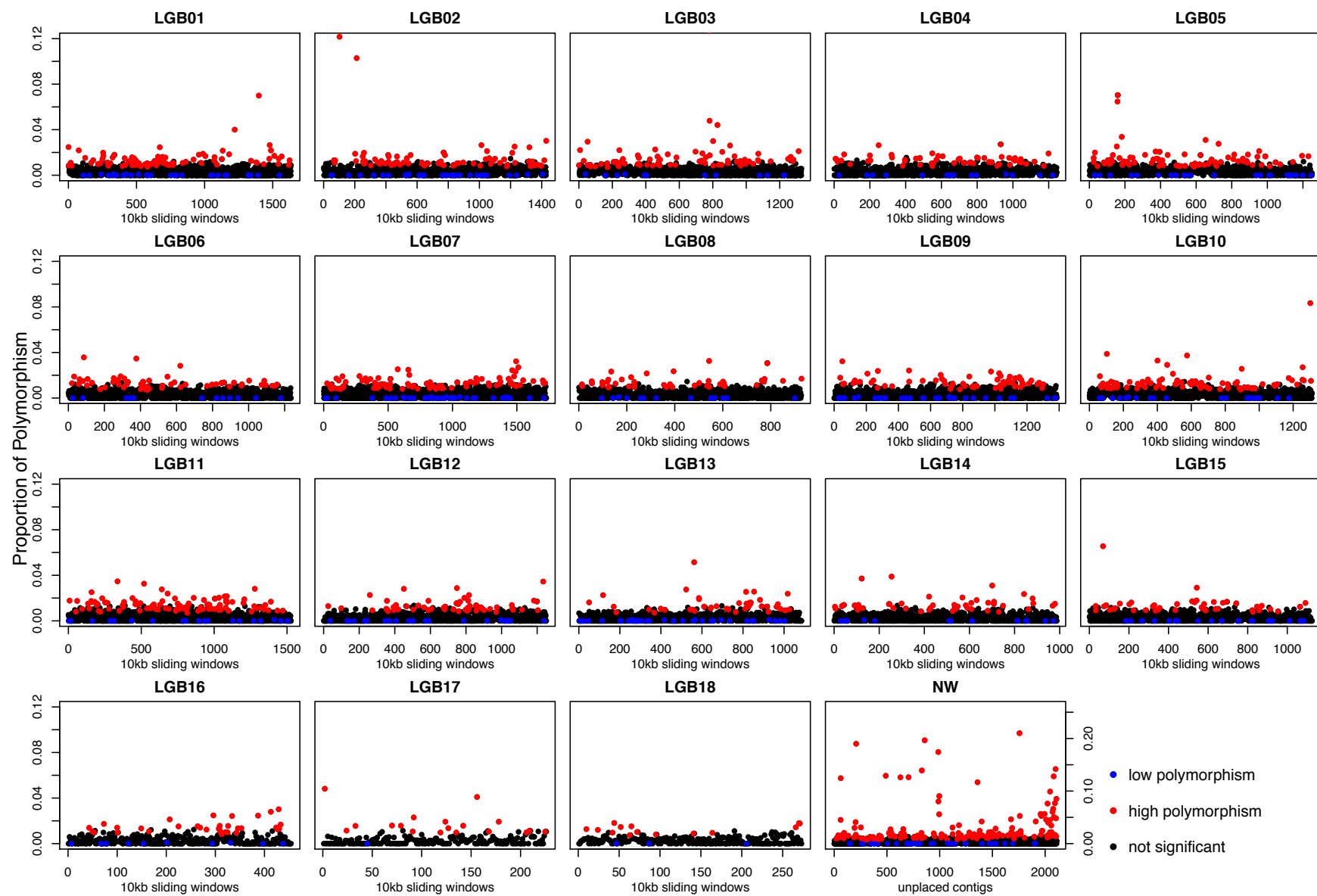
Figure 6: *Distribution of polymorphism across linkage groups. Genomic regions showing significantly elevated polymorphism based on permutation tests are highlighted in red and areas of significantly reduced polymorphism in blue. Note the different y-axis scale in the NW linkage group.*