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Low but contrasting neutral genetic differentiation shaped by winter temperature in European great tits

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Abstract

Gene flow is usually thought to reduce genetic divergence and impede local adaptation by homogenising gene pools between populations. However, evidence for local adaptation and phenotypic differentiation in highly mobile species, experiencing high levels of gene flow, is emerging. Assessing population genetic structure at different spatial scales is thus a crucial step towards understanding mechanisms underlying intraspecific differentiation and diversification. Here, we studied the population genetic structure of a highly mobile species – the great tit *Parus major* – at different spatial scales. We analysed 884 individuals from 30 sites across Europe including 10 close-by sites (< 50 km), using 22 microsatellite markers. Overall we found a low but significant genetic differentiation among sites (F_{ST} = 0.008). Genetic differentiation was higher, and genetic diversity lower, in south-western Europe. These regional differences were statistically best explained by winter temperature. Overall, our results suggest that great tits form a single patchy metapopulation across Europe, in which genetic differentiation is independent of geographical distance and gene flow may be regulated by environmental factors via movements related to winter severity. This might have important implications for the evolutionary trajectories of sub-populations, especially in the context of climate change, and calls for future investigations of local differences in costs and benefits of philopatry at large scales.

Introduction

Gene flow is generally thought to impede local adaptation by introducing locally maladapted genotypes into populations exchanging individuals. Consequently, micro-evolutionary processes at small scales are predicted to be rare in highly mobile organisms with high gene flow over large spatial scales, due to spatial genetic homogenisation. However, evidence for genetic differentiation and local adaptation at small scales despite high levels of gene flow at large scales has recently started to accumulate in different taxa (e.g. mammals: Musiani et al., 2007; marine invertebrates: Sanford & Kelly, 2011; birds: Charmantier et al., 2015; fish: Junge et al., 2011; trees: Savolainen, Pyhäjärvi & Knürr, 2007). This evidence suggests that dispersal is not a diffusion-like movement process and that ultimately gene flow may vary in space.

Spatial variation in gene flow is probably common, especially in relation to environmental factors in highly mobile species. High mobility and long distance dispersal facilitate spatial spread and the colonization of new habitats (Nathan et al., 2003). As a consequence, highly mobile species are likely to experience a large set of environmental conditions that may shape locally adaptive processes. In addition, high mobility combined with the ability to cross physical barriers such as seas or mountains may minimize the influence of geographical factors. Increased mobility may also reduce the impact of historical factors on gene flow by homogenising gene pools, increasing local population size and counteracting genetic drift (Slatkin, 1987). In this case, environmental factors may become the main force shaping gene flow (e.g. Pilot et al., 2006). Assessing gene flow between populations at small and large spatial scales in highly mobile species and the links between gene flow and environmental factors is crucial to understand the ecological mechanisms leading to intraspecific differentiation and diversification. When dispersal movements and immigration rate do not provide reliable estimates of gene flow, such as in highly mobile

species, a population genetic approach may help investigating patterns of gene flow at different spatial scales (Nathan et al., 2003).

The great tit *Parus major*, a widespread passerine bird across Eurasia (Snow & Perrins, 1998), is a particularly interesting biological model to address such questions. This species is considered to be an "evolutionary winner", given its ability to colonize and rapidly adapt to new habitats. Its rapid spread across Europe since the last glaciation period (Kvist et al., 2003; Pavlova et al., 2006) suggests high dispersal ability and gene flow among sub-populations (Caswell, Lensink & Neubert, 2003; Pilot et al., 2006 but see Peterson & Denno, 1998). Conversely, long-term monitoring studies provide evidence for small-scale local adaptation (Garant et al., 2005; Postma & van Noordwijk, 2005) with a considerable fraction of individuals dispersing over short distances (e.g. Verhulst, Perrins & Riddington, 1997). Thus, although great tits are considered highly mobile and forming a homogeneous population across Europe, microevolutionary processes linked with limited gene flow occur at small scales and with it, the detection of subtle fine-scale genetic structures (Björklund, Ruiz & Senar, 2010; Garroway et al., 2013; Van Bers et al., 2012). These conflicting observations call for investigating genetic differentiation using microsatellite markers at different spatial scales in this species. Indeed microsatellite markers generating multi-locus diploid genotypes provide an ideal resolution to study recent or ongoing micro-evolutionary processes occurring both at small and large scales (e.g.Wang, 2010).

Moreover, the environmental heterogeneity over the species' range combined with its colonisation history provides excellent conditions to study the influence of environmental factors on population genetic structure in this species. Indeed, phylogeographic studies based on mitochondrial DNA (mtDNA) suggest that other tit species colonized Europe from different glacial refugia, each harbouring distinct mitochondrial lineages and forming secondary contact zones within Europe (Kvist et al., 2004; Päckert, Martens & Sun, 2010;

Pentzold et al., 2013). In contrast, all western-European great tits share a common haplotype, suggesting that they originate from a single glacial refugium located in southern Europe (Kvist et al., 2007; Kvist et al., 1999; Pavlova et al., 2006, Fig. S1, Table S1). Therefore genetic differentiation in great tits estimated with microsatellites that evolve faster than mtDNA and are more powerful to detect recent and local micro-evolutionary processes among populations, are less likely to result from past genetic discontinuities across different glacial refugia as is the case for many other species (e.g. Hewitt, 2000; Kvist et al., 1999).

Using 22 microsatellite markers, we investigated population genetic diversity and structure, as well as the scale of genetic differentiation, in great tits by sampling 30 sites across Europe including 10 close-by (i.e. up to 50 km) sites. We expected the genetic differentiation to be correlated with the geographical distance either at small or large scales: the studied geographical scale should allow us to determine at which scale isolation-by-distance would occur in great tits. In addition, a signal of historical range expansion from the South to the North should result in decreased genetic diversity with increasing latitude. In a second step, we explored the influence of environmental factors on the observed genetic diversity and differentiation patterns, focusing on factors that can be expected to affect individual movement. In particular, temperature may strongly shape genetic differentiation among populations by acting on both dispersal movements (e.g. Parn et al., 2012) and establishment success (i.e. survival and reproductive success after settlement) of long-distance immigrants (e.g. Van Doorslaer et al., 2009). Three different patterns may thus be predicted in relation to temperature. First, because temperature can be positively correlated with survival and population density (Ahola et al., 2009; Garant et al., 2004; Parn et al., 2012) that increase dispersal propensities (Forsman & Monkkonen, 2003; Matthysen, 2005), genetic diversity could increase and genetic differentiation decrease with increasing temperature. Second, a negative relationship between temperature and dispersal propensities may be expected in the case of partial migration (e.g. Nilsson et al., 2006). In this case, temperature should relate to

environmental conditions during winter, triggering partial migration and favouring dispersal in general or the establishment of migrants in non-natal breeding areas. Genetic diversity should consequently decrease while genetic differentiation should increase with temperature (e.g. Miller et al., 2012). Third, if the establishment success of immigrants is linked to adaptation to temperature, we predicted that genetic differentiation should increase with the difference of temperature between sites.

Material and Methods

Species description, sampling and genotyping

The great tit is a hole-nesting passerine bird that readily breeds in nest boxes, providing easy access to breeding pairs. In this study, all individuals from all but one site (FI.TU, see Table S2) were breeding adults caught in nest boxes during the nestling period. Thirty woodland sites across Europe were sampled between 2005-2010 (Fig.1, Table S2), 10 of which were within a range of 50 km on the island of Gotland (57°10'N, 18°20'E). Overall, our studied populations fell along a south-west – northeast gradient (Fig.1). Either blood or feather samples were obtained. Most sites were sampled once, except when the sample size was too low for statistical analysis (in 10 sites). In this case, samples of two consecutive years were pooled. The number of sampled individuals per site ranged from 18 to 47 with an average of 29.

DNA was extracted with magnetic beads (MagneSil Blue, Promega AG, Dübendorf, Switzerland) and genotyped at 22 microsatellite loci (Table S3, Saladin & Richner, 2012). These 22 microsatellite markers were developed using individuals from CH.BE, a site in the geographical centre of our sampling scheme. For details on the PCR protocols and allele

scoring procedure, see Saladin & Richner, 2012). Twelve individuals with missing alleles or atypical profiles at different loci were excluded from all analyses. None of the individuals shared the same multilocus genotype indicating that none of the individuals was sampled twice. Overall, 884 individuals were analysed. Allelic dropout, scoring errors and null alleles were checked for each locus per site with MICRO-CHECKER (Van Oosterhout et al., 2004). Among all loci, no evidence for allelic dropout was detected and only one locus in one sampling site showed scoring errors. Moreover, null alleles were randomly distributed, and present at only 19 (i.e. 2.9%) locus × site combinations. Genotypic linkage disequilibrium and departure from Hardy-Weinberg equilibrium (HWE) were tested with probability tests per locus per site. In addition, departure from HWE for the overall population, i.e. across loci and sites, was tested using a multisample score test. All tests were performed using GENEPOP on the web (Rousset, 2008). *P*-values for multiple tests were corrected with a sequential Bonferroni procedure (Rice, 1989).

Genetic diversity and differentiation among sites

To assess genetic diversity at each sampling site, both the observed and unbiased expected heterozygosity (H_O and H_E) were calculated using GENALEX v6 (Peakall & Smouse, 2006). In addition, the mean allelic richness per site (A_R) based on 18 individuals, corresponding to the smallest number of individuals sampled in a given site, was estimated with FSTAT v2.9.3 (Goudet, 1995). Genetic differentiation among sites was quantified using pairwise and global F_{ST} calculated in FSTAT with 10,000 permutations to assess significance. Because F_{ST} estimates may be strongly affected by the polymorphism of the markers used (Meirmans & Hedrick, 2011), standardized estimators G''_{ST} and *D* were calculated with GENODIVE 2.0B27 (Meirmans & Van Tienderen, 2004).

To test for a spatial pattern of genetic differentiation among sites, two methods were used: (i) a principal coordinate analysis (PCoA), based on codominant genotypic distance among sites with a standardized covariance matrix, using GENALEX 6.5 and (ii) a neighbour-joining (NJ) phenogram based on Nei's genetic distance between sites, using PHYLIP v3.68 (Felsenstein, 2008). The presence of genetic clusters was also tested using two methods. First, an individual-based Bayesian cluster analysis was implemented in STRUCTURE v2.2 (Pritchard, Stephens & Donnelly, 2000). Ten runs of an admixture model with correlated allele frequencies among sites and LOCPRIOR were performed for each value of putative population number (K) between 1 and 40 with a burn-in of 50,000 iterations followed by 100,000 iterations in the Markov chain. The most likely number of genetically different populations was determined from the posterior probability of the data for a given K and the ΔK (Evanno, Regnaut & Goudet, 2005). To test for a potential bias due to the inclusion of 10 close-by sites from Gotland, the PCoA and STRUCTURE analyses were run once using individuals from all 30 sites and once using individuals from 21 sites including only a single site from Gotland (SE.OG). Since the results did not qualitatively differ (Fig.S2-S6, Table S4), we presented only the results based on 30 sites. In addition, assignment probabilities of individuals to their original site (P_A) were calculated using a discriminant analysis of principal components (DAPC - Jombart, Devillard & Balloux, 2010) in R 3.0.1 (R CORE TEAM, 2013). Second, the clustering of sites into groups was investigated by a K-Means clustering using an analysis of molecular variance (AMOVA) with 40 independent Markov chains with 50,000 iterations each assuming 2 to 15 clusters with GENODIVE. The most likely number of clusters was determined from the smallest bayesian information criterium (BIC). Furthermore, genetic differentiation was quantified between groups and among sampling sites within groups using an AMOVA with 10,000 permutations to assess significance using GENODIVE. Additionally, within-group global F_{ST} values were calculated and compared with 10,000 permutations using FSTAT.

To test for the presence of isolation-by-distance patterns, a decomposed pairwise regression analysis (DPR) was conducted in R to account for potential between-site differences in the gene flow-drift equilibrium (Koizumi, Yamamoto & Maekawa, 2006). Briefly, DPR first detects outlier sites based on the distribution of residuals from the overall regression between genetic and geographical distances. In a second step, genetic distances are regressed against geographical distances for each site against all other non-outlier sites to obtain a regression intercept and slope per site. The intercept and slope of the decomposed regressions measures genetic differentiation to other populations and isolation-by-distance (IBD) respectively for each site (see Koizumi et al., 2006 for details).

Testing for the influence of environmental factors on differences among sites

To investigate potential mechanisms underlying differences in genetic diversity, the relationships between indices of genetic diversity per site and the following environmental factors, which may be expected to influence individuals' movements, were tested: (i) geographical location (latitude and longitude); (ii) vegetation type (deciduous or coniferous trees; excluding SP.MU and ES.KI, where birds were sampled in orange tree plantations or mixed areas); (iii) temperature and (iv) minimal distance to the sea. Latitude, longitude and minimal distance to the sea were obtained using GOOGLE EARTH v5.2.1. Using the position along a southwest – northeast axis as a geographical location did not affect the results, and thus only results including latitude and longitude are reported. Temperatures were obtained from the European photovoltaic geographical information system (Huld et al., 2006). The measures based on temperature were (i) average daily temperature per month, (ii) temperature variance per year, (iii) difference between the most extreme annual temperatures and (iv) average temperature during autumn-winter (September - February) and spring-summer (March- August). In addition to the indices of genetic diversity per site, we calculated an

estimate of effective population size (N_e) with the linkage disequilibrium method using a threshold of 0.05 for the exclusion of rare alleles in NeESTIMATOR v2 (Do et al., 2014) and the kinship coefficient of Loiselle et al. (1995) averaged per site with GENODIVE. The relationships between genetic diversities (A_R and H_E), assignment probabilities, kinship coefficients, effective population sizes and environmental factors were tested using linear models since all indices were normally distributed (residuals were checked for normality and homoscedasticity). Because the environmental factors were correlated with each other (correlation coefficients ranging from 0.31 to 0.86, all $P < 0.001$, results not shown), Akaike's information criterion (AIC) values of models including each factor separately were compared in order to identify the environmental factor(s) that best explained the data using the package AICmodavg (Mazerolle, 2015) in R. The best models included the model with the smallest AIC and all models with a difference in AIC (ΔAIC) to this model of less than 2 (Burnham, Anderson & Huyvaert, 2011). Once the best models were identified, the significance of the effects retained was assessed with an *F* test.

In a second step, the influence of the following environmental factors on genetic differentiation among sampling sites was tested: (i) geographical distance between sites, (ii) mean geographical location of sites, (iii) absolute difference in average daily temperature between sites and (iv) mean of the average daily temperature of sites. Because previous analyses showed that genetic diversity was best explained by temperatures in autumn-winter (see Results section), only the difference in average autumn-winter temperatures between sites (hereafter called autumn-winter temperature difference) and the mean of the average daily temperature in autumn-winter of sites (hereafter called mean autumn-winter temperature) were tested in the analyses of genetic differentiation. Similarly, only the latitude was retained here to characterize geographical location for analyses on genetic differentiation since site latitude and longitude were correlated in our study (i.e. sites were distributed along a south-west / north-east axis). The difference between values for the two sites in pairwise

comparisons provides a measure of the environmental contrast between sites, whereas the mean value gives a measure of the position of the pair of sites in each pairwise comparison along the environmental gradient considered (geographical position or winter severity). The genetic differentiation between sites was calculated for each pair of sites and summarized in a pairwise matrix; the same approach was used for the differences and mean values of the environmental factors between sites. Correlations between levels of pairwise genetic differentiation based on either F_{ST}, G''_{ST} or D and pairwise differences in environmental factors were investigated with Mantel tests (or partial Mantel tests when more than two matrices were compared) with 10,000 permutations using the package vegan (Oksanen et al., 2011) in R . By homogenising the genetic composition of connected populations, gene flow should reduce both the mean level and the variability of genetic differentiation between populations (Hutchison & Templeton, 1999). Consequently, a factor affecting gene flow should be correlated with both the level of genetic differentiation and the absolute values of residuals of the linear regression between the factor and the level of genetic differentiation (hereafter called residual pairwise F_{ST} , G''_{ST} or D respectively) Hutchison & Templeton, 1999). Therefore, the correlation between matrices of environmental factors and their residual pairwise genetic differentiation was also tested.

Results

Genetic diversity and equilibrium

No evidence for linkage disequilibrium at any locus in any site or departure from HWE was found after correction for multi-comparisons. Pooling all sites, a significant deviation from HWE was observed (score-test: $P < 0.001$), suggesting the existence of sub-populations. The number of alleles per locus ranged from 4 to 41 with an average of 16 alleles across loci.

316 Mean allelic richness per site ranged from 6.32 to 7.66 (Table S2). Expected heterozygosity
317 varied between 0.60 and 0.68 and the number of effective alleles between 3.94 and 4.92
318 (Table S2). F_{IS} per site ranged from -0.049 to 0.047 (Table S2), but no F_{IS} value differed
319 significantly from zero after correcting for multiple tests, as expected under within-site HWE.

320

321 *Genetic differentiation among sampling sites*

322 Genetic differentiation among sampling sites across Europe was low, but significant (global
323 F_{ST} = 0.008, G''_{ST} = 0.024, D = 0.016, all P < 0.001). Pairwise F_{ST} ranged from -0.004 to
324 0.040 (Table S3). Out of 435 pairwise F_{ST} comparisons, 147 (i.e. 33.8%) were significantly
325 different from zero after sequential Bonferoni correction. Interestingly, the majority of
326 significant comparisons (134 out of 147, i.e. 91.1%) involved six (out of seven) sampling sites
327 located in the south-western part of Europe, i.e. below 47°N (CH.BE, FR.MO, SP.MU,
328 SP.FR, SP.MA and PO.CO), indicating different levels of genetic differentiation between
329 northern and southern sites (Fig. 2). FR.RO was the only site located in the southern region
330 for which pairwise F_{ST} values were non-significant. Results of both the PCoA analysis and NJ
331 phenogram based on Nei's genetic distance were congruent with the observed pairwise F_{ST}
332 pattern for six out of the seven southern sites (Fig. 3). The PCoA accounted for 62% of the
333 total genetic variation on the first 3 axes (26.5%, 18% and 17.5% respectively). Independently
334 of the axes considered, PO.CO, SP.MU, SP.MA, SP.FR, FR.MO were identified as being
335 rather distinct from all other sites (i.e. outside the 50% and close to the 95% limit of the
336 confidence interval; Fig. 3a-b). These south-western sites were also differentiated from each
337 other, except SP.MA and SP.FR, which also showed lower pairwise F_{ST} values. Only CH.BE,
338 which had relatively low F_{ST} values, was not identified as a differentiated site by the PCoA
339 and the NJ phenogram analyses. Furthermore the central cluster was randomly distributed on
340 each PCoA axis, in particular with no clumping of the 10 close-by sampling sites located on

Gotland (Fig. 3a-b), which was confirmed on the NJ phenogram. In fact, populations on Gotland showed similar levels of differentiation among themselves as among the other sites from northern Europe (Fig. 3c). Depending on the method used, some of the northern sites appeared differentiated from the central cluster (e.g. SE.LO, Fig. 2b and 3a; SE.SA, Fig. 3a-c; or NE.LA, Fig. 3a) suggesting that they could be distinct from the central cluster yet less differentiated than the south-western sites. Overall, the results indicate that (i) genetic differentiation among sampling sites was low (Fig. 2 and 3); (ii) many sites (including close-by ones) presented similar and low levels of genetic differentiation without spatial structure (e.g. a centred star-like pattern; Fig. 3c); and (iii) at least five southern sites were differentiated from the central cluster and differentiated from each other, except SP.MA and SP.FR (Fig. 2 and 3).

STRUCTURE identified three genetic clusters (K=3) following the Evanno correction (Fig. S4-S5). Two of these clusters were mainly associated with the four Iberian sites, where the Portuguese site (PO.CO) was further distinct from all Spanish sites (SP.MU, SP.MA and SP.FR), however no individual was fully assigned to either cluster (Fig. S6). All other sites were predominantly assigned to a third cluster except for CH.BE, which showed evidence for introgression from south-western Europe. Concordantly, the AMOVA based K-means clustering identified two groups: one comprising the four Iberian sites and CH.BE and a second including all other sites (all northern sites and the two sites in France). The AMOVA using south-western (i.e. below 47° latitude: PO.CO, SP.MU, SP.MA, SP.FR, FR.MO, FR.RO, CH.BE) and northern (above 47° latitude) sites as grouping variable suggested low but significant genetic differentiation between these groups ($F_{\text{group-total}} = 0.002$, $P < 0.001$) and among sites within groups ($F_{\text{sites-group}} = 0.008$, $P < 0.001$). In addition, the differentiation was higher within southern sites than other sites (global $F_{ST} = 0.016$ and 0.005, $G''_{ST} = 0.052$ and 0.014, $D = 0.034$ and 0.009, for southern sites and other sites, respectively; $P < 0.001$).

Excluding CH.BE, FR.MO and FR.RO, did not change qualitatively the results of the hierarchical AMOVA and the level of differentiation, suggesting that the observed clustering was mainly driven by the four Iberian sites, which are more differentiated than the other south-western sites. Interestingly, the weak overall differentiation among the northern sites did not result from differentiation between specific sampling sites since 19 sites had to be excluded one after the other (starting from the sites with the highest mean pairwise F_{ST} value and going downwards) for the overall differentiation to become non-significant (results not detailed). Moreover, differentiation among the close-by sites on Gotland (with distance ranging from 3 to 50 km) was not lower than among other northern sites (global F_{ST} = 0.006 and 0.004 respectively, P = 0.646; Fig. 5a).

Finally, the DPR analysis identified FR.MO (the only urban site) as an outlier, since the model excluding this site had a lower AIC (-94.78) and higher R^2 (0.17) values, although other models (either comprising all sites or with additional outliers) could not be excluded (ΔAIC < 1.28). Overall, the DPR divided sampling sites into five groups (see Fig. 1 for location, Table S2): (1) two southern sites (FR.MO and FR.RO) showed a significant atypical negative IBD pattern and significant differentiation from other sites; (2) the four Iberian sites (SP.MU, SP.MA, SP.FR and PO.CO) and CH.BE showed no significant IBD but significant differentiation from other sites; (3) ten northern sites in Fennoscandia showed both significant differentiation from other sites and an IBD pattern; (4) nine northern sites from different locations showed no differentiation from other sites but significant IBD; and (5) four central sites (UK.WY, UK.CA, BE.CE, BE.BO) showed no differentiation and no IBD. Interestingly, all but two close-by sites on Gotland showed both significant differentiation from other sites and an IBD pattern.

Exploring the influence of environmental factors on genetic differences among sites

Models including latitude, longitude, and the variance and difference in daily temperature were retained for none of the five indices (allelic richness A_R, expected heterozygosity H_E, assignment probability P_A, kinship coefficient and effective population size N_e, $\Delta AIC > 2$ in all cases; Table S5). Conversely, models with average daily temperature for months September to January, and consequently average autumn-winter temperature, were among the models best explaining the data for P_A, A_R, H_E ($\Delta AIC < 2$ in all cases; Table S5). For kinship coefficient, models with average daily temperature for months August, September and December were among the models best explaining the data ($\Delta AIC < 2$) but not the model with average autumn-winter temperature despite a relative low AIC ($\Delta AIC < 2.5$). The model including vegetation type was the only best model in explaining the data for the effective population size. Allelic richness decreased ($F_{1,28}$ = 6.90, P = 0.014, R^2 = 0.20) while assignment probabilities and kinship coefficients increased ($F_{1,28}$ = 10.57, P = 0.003, R^2 = 0.27; $F_{1,28}$ = 17.04, $P < 0.001$, R^2 = 0.36 respectively) with increasing average autumn-winter temperature (Fig. 4). Expected heterozygosity and effective population size were not correlated with average autumn-winter temperature ($F_{1,28}$=0.81, P = 0.38; $F_{1,24}$ = 0.56, P = 0.46 respectively, Fig. 4). Effective population size was similar for coniferous and deciduous forests ($F_{1,22}$ = 0.03, P = 0.87). Models with other factors were retained for part of the indices only: temperatures in summer months (July to August) for A_R and H_E, temperature in February for H_E, average spring-summer temperature for H_E, vegetation type for A_R and distance to the sea for H_E (Table S5). However, allelic richness was similar in coniferous and deciduous forests ($F_{1,26}$ = 0.08, P = 0.77), and expected heterozygosity was not correlated with spring-summer temperature or distance to the sea ($F_{1,28}$<2.5, $P > 0.12$). Based on these results, only the average autumn-winter temperature was retained among temperature measures for the analyses of genetic differentiation.

All pairwise genetic differentiation indices increased with geographical distance between sites, autumn-winter temperature difference between sites and mean autumn-winter

417 temperature of the two sites in pairwise comparisons, and decreased with mean latitude of the
418 two sites (Table 2; Fig. 5). Each environmental factor explained 36 to 57% of the variation in
419 pairwise genetic differentiation. Furthermore, both mean autumn-winter temperature and
420 latitude, but not geographical distance or autumn-winter temperature difference, were
421 correlated with their respective residual pairwise genetic differentiation (Table 2). This
422 suggests that genetic differentiation is mainly driven by site characteristics (latitude, mean
423 autumn-winter temperature) rather than environmental contrast between sites. Mean autumn-
424 winter temperature remained significantly correlated with genetic differentiation after
425 correcting for latitude (partial Mantel test: $r_M = 0.31$, $P = 0.019$), whereas mean latitude was
426 not correlated with genetic differentiation anymore after correcting for mean autumn-winter
427 temperature (partial Mantel test: $r_M = -0.03$, $P = 0.534$). This suggests that mean autumn-
428 winter temperature was the best predictor of genetic differentiation among the tested
429 environmental effects.

430

431 **Discussion**

432 *Biological relevance of the observed genetic differentiation*

433 The low but significant global genetic differentiation based on microsatellite markers suggests
434 extensive gene flow among great tit populations across Europe. Nevertheless, the overall
435 deviation from Hardy-Weinberg equilibrium, the absence of inbreeding within sites (as
436 revealed by heterozygosity) and the overall population differentiation support a Wahlund
437 effect, i.e. a substructure among sites. Individual-based clustering methods failed to
438 characterise discrete genetic groups, yet found some indication for substructure among south-
439 western sites. This is consistent with the high proportion of the genetic variance (> 98%)
440 observed within populations (e.g. Chen et al., 2007; Latch et al., 2006). We are nevertheless

confident about the validity of the significant global genetic differentiation given the relatively large sample sizes and because none of the analyses suggested a bias in both global and pairwise genetic differentiation due to variation in sample size among sites or being associated by specific loci and sites.

In general, a significant IBD supports the biological relevance of low genetic differentiation among populations (e.g. F_{ST} values around 0.003), especially in species characterised by large population sizes and high gene flow such as birds (e.g. Prochazka et al., 2011) or marine fish (e.g. Purcell et al., 2006). But low genetic differentiation even in absence of IBD may also reflect heterogeneity in gene flow affecting ongoing microevolutionary processes in highly mobile organisms. This is illustrated by the case of a physically isolated island population of great tits, where immigrants from the mainland can be easily identified (Postma & van Noordwijk, 2005). In this population, direct (i.e. observed movements of individuals) and indirect (i.e. genetic, based on microsatellite markers) measures of gene flow were compared. The genetic differentiation between resident and immigrant individuals was low but significant (F_{ST}=0.007; Postma et al., 2009). Consistent with a higher immigration rate in the western part (43%) compared to the eastern part (13%) of the study island, a low but significant genetic differentiation was found between the two parts (F_{ST}=0.011; Postma et al., 2009). Because mainland individuals lay larger clutches, immigration was shown to impede local adaptation in the western but not the eastern part of the island (Postma & van Noordwijk, 2005). Using similar microsatellite markers in the present study, we also found comparable levels of genetic differentiation between populations, supporting the biological implications of our findings. Lastly, using a restricted set of microsatellite markers, we retrieved a comparable level of genetic differentiation between two sites (NE.HO and UK.WY; F_{ST} = 0.005) as has been observed with several thousand SNP markers for the same sites (van Bers *et al.* 2012; F_{ST} = 0.010). The slightly higher level of genetic differentiation in their study could be due to the inclusion of some highly divergent outlier loci. Another study

467 also using the same SNP set further identified cryptic genetic differentiation within the
468 UK.WY site, which was similarly driven by few (<1%) markers (Garroway et al., 2013). Thus
469 our microsatellite data set seems to be suitable to accurately calculate population genetic
470 estimates that resemble average genome wide patterns (i.e.Van Bers et al., 2012), whereas
471 few genomic regions may exist that underlie patterns of local adaptation (Garroway et al.,
472 2013; Van Bers et al., 2012).

473 Our analyses revealed higher genetic differentiation in south-western compared to
474 northern European sites. This finding suggests decreased gene flow between south-western
475 and northern Europe as well as within south-western Europe. Subsequent generalisations
476 towards other southern European populations need to be done with caution since our sampling
477 design focused only on south-western populations. A similar pattern was reported for
478 different passerine species as well as for plants and mammals (Hewitt, 2000; Kvist et al.,
479 2004; Pentzold et al., 2013; Prochazka et al., 2011) and is generally interpreted as the result of
480 postglacial recolonisation. In the present case, the higher divergence of southern populations
481 compared to northern ones could be due to the fact that both groups may have derived from
482 different glacial refugia (Hewitt, 2000). Such scenario has been suggested for other tit species,
483 for which distinct glacial refugia may have existed in the Mediterranean region (Kvist et al.,
484 2004) and across Europe (Pentzold et al., 2013). However, for several reasons, the genetic
485 differentiation observed in great tits using microsatellite markers seems unlikely to result
486 from the occurrence of one or several genetic lineages that have recolonized northern Europe
487 from distinct refugia. First, the presence of several glacial refugia would have led to the
488 existence, at least in southwestern populations, of genetic variations specific to the multiple
489 refugia causing a higher genetic diversity within the Iberian Peninsula (Pentzold et al., 2013;
490 Prochazka et al., 2011). In contrast, the Iberian Peninsula harboured a level of allelic richness
491 at microsatellite markers that was comparable to all other sites (7.26 and 7.32 alleles
492 respectively). Similarly, phylogenetic studies showed in great tits a homogeneous

493 mitochondrial diversity from northern to southern Europe (Fig S1), which is consistent with a
494 colonisation from a single refugium and the absence of strong geographical barriers to
495 dispersal (Kvist et al., 2003; Kvist et al., 1999; Pavlova et al., 2006). Second, a rapid post-
496 glacial range expansion from a single refugium is likely to result in lower genetic diversity
497 within the colonized range as opposed to the ancestral refugium (Antoniazza et al., 2014;
498 Pavlova et al., 2006). In contrast, Iberian populations had a slightly lower allelic richness per
499 site compared to all other sites (6.71 ± 0.29 and 7.11 ± 0.29 alleles respectively, Table S2).
500 Interestingly haplotype diversity was lower in all south-western populations than in the north-
501 eastern populations in coal tits (Pentzold et al., 2013) suggesting that a lower genetic diversity
502 in southern regions could have arisen long time ago. However such pattern was not detected
503 with mitochondrial DNA in great tits (Pavlova et al., 2006). Therefore, the observed patterns
504 of genetic differentiation at microsatellite loci among great tit populations are unlikely to
505 result from post-glacial recolonization processes from one or several refugia but rather
506 represent other historical and/or recent processes. Further studies using genetic modelling
507 approaches combined with increased genomic coverage are however necessary to elucidate
508 the factors underlying the pattern observed here.

509

510 *How could gene flow be shaped by temperature?*

511 Latitude and (autumn-winter) temperature were significantly correlated with both the level of
512 genetic differentiation among populations and its level of variation in contrast to the
513 geographical distance and the difference of temperature that explained only the level of
514 genetic differentiation among populations. Moreover, only temperature was significantly
515 associated with the level of genetic differentiation after taking into account latitude. Finally,
516 temperature but not latitude explained the decrease of genetic diversity from the South to the
517 North. The effect of temperature on different components of the genetic variation suggests a

strong relationship between temperature and neutral genetic structure among great tit populations. However we cannot exclude that temperature is correlated with additional environmental factors such as photoperiod or irradiance cues (De Frenne et al., 2013) and then the correlation between temperature and genetic differentiation is a by-product of the effect of environmental factors on genetic variation that we did not measure here. Nonetheless the relationship between temperature and neutral genetic structure suggests that genetic differentiation, and hence gene flow, may be related to winter local movements and partial migration (Nilsson, Alerstam & Nilsson, 2008; Nowakowski & Vähätalo, 2003). This could also be associated with winter severity: food availability may be especially restricted in northern Europe (Newton, 2011 but see Nilsson et al., 2008; Nowakowski & Vähätalo, 2003) when insect abundances are lowest and great tits become mainly granivorous (Vel'ky, Kanuch & Kristin, 2011). Great tits are considered to be resident in southern and western Europe, but partial migrants in northern Europe, as shown in particular by captures at migratory passage sites in autumn and spring (Nowakowski & Vähätalo, 2003; Poluda, 2011; Gosler, 2002). Part of the birds (especially juveniles) may move during winter over short to long distances (up to > 1000 km; Nilsson et al., 2008; Nowakowski & Vähätalo, 2003). In spring, these migrants may either stay on the wintering grounds or return to their natal region to breed more or less close to their natal site (Gosler, 2002; Nilsson et al., 2008; Nowakowski & Vähätalo, 2003). Partial migration could therefore generate on average longer dispersal distances, associated with higher variance, in the northern compared to southern European populations (see Orell et al., 1999). Although part of the immigrant individuals (often around 50% of local breeders in monitored populations) may originate from the surroundings of study areas (e.g. Verhulst et al., 1997), differences in immunological, behavioural and/or life-history traits between potential immigrants (i.e. not previously captured in the population) and locally born individuals (e.g. Postma & van Noordwijk, 2005; Snoeijs et al., 2004) may support the existence of long-distance immigration in great tits. Because obtaining additional information

544 on the origin of immigrant individuals in the field is highly challenging, this hypothesis
545 however remains difficult to test.

546 Interestingly, similar genetic structures across Europe have been found in other small
547 passerine species, i.e. for the bluethroat (*Luscinia svecica*; Johnsen et al., 2006) and the pied
548 flycatcher (*Ficedula hypoleuca*; Lehtonen et al., 2009). In the latter case,, no large-scale
549 differentiation was observed in north-eastern Europe but small-scale differentiation was found
550 in southern Europe. Because the pied flycatcher is an obligatory migratory species, wintering
551 in Sub-Saharan Africa, the lower genetic differentiation of northern sites cannot be explained
552 by differences in winter movements linked to winter severity. Nevertheless, lower philopatry
553 and local recruitment rates, and thus higher dispersal rates, have been suggested in northern
554 compared to southern sites for several migratory species, including the pied flycatcher
555 (Lehtonen et al., 2009) and the barn swallow (Balbontin et al., 2009). In these species
556 dispersal may be linked to other environmental factors such as e.g. habitat stability,
557 fragmentation or elevation. Both here and in the study by Lehtonen et al. (2009), southern
558 populations were sampled in specific habitats, including high elevation sites (great tits:
559 SP.MA, SP.FR and CH.BE > 500 m.a.s.l.; pied flycatchers: Lehtonen et al., 2009), urban
560 environment (FR.MO) or plantations (SP.MU), in contrast to northern sites located mainly in
561 temperate lowland forests. In southern Europe, stable habitat heterogeneity, niche
562 specialisation or high temperature may promote local adaptation (e.g.Husby, Visser & Kruuk,
563 2011). This could increase local genetic differentiation and select against dispersal to a higher
564 degree than in the northern regions (Van Doorslaer et al., 2009), where the availability of
565 large and/or homogeneous habitat patches may reduce dispersal costs (Travis & Dytham,
566 1999) in both migratory and sedentary species. Individuals of the southern populations may
567 therefore be less prone to accept breeding in new sites, leading to lower gene flow.
568 Consequently, intraspecific differentiation might be more likely than neutral differentiation in
569 southern sites (e.g. Johnsen et al., 2006; Lehtonen et al., 2011).

Conclusion

Non-random dispersal and genetic structure in great tits have previously been investigated at small scales, providing evidence for local adaptation (i.e. within a few km; Garant et al., 2005; Garroway et al., 2013; Postma et al., 2009; Postma & van Noordwijk, 2005). Here, we compared populations across Europe and found low but significant genetic differentiation among populations. This differentiation was unrelated to geographical distance between sites but was influenced by geographic location and environmental factors, in particular autumn-winter temperature. This might have important implications for the evolutionary trajectories of great tit populations and other species showing similar patterns. The northern populations may represent a single large population in which gene flow drives demographic and evolutionary processes. In this case, habitat choice and assortative mating may play a central role in local adaptation processes (e.g. Postma & van Noordwijk, 2005). In contrast, the southern populations may be more isolated and experience stronger genetic drift and/or higher selective pressures (e.g. Lehtonen et al., 2011). Studying potentially ongoing intraspecific diversification may be particularly relevant in these populations.

The association between genetic differentiation and winter severity may have further implications in the context of climate change. If the increase of winter temperatures favours increased philopatry in northern populations (e.g. Van Vliet, Musters & Ter Keurs, 2009), the latter may reach a gene flow-drift equilibrium. As a consequence, increased genetic differentiation and IBD could arise, favouring neutral genetic differentiation and/or local adaptation. Conversely, southern populations may become extinct if genetic adaptation or phenotypic plasticity fail to allow to adapt sufficiently fast (Visser, 2008; Boeye et al., 2013). Alternatively, an increase of philopatry among northern populations, induced by warmer winters could intensify competition especially during the breeding season, leading to a

population decline (Kokko, 2011 but see Stenseth et al., 2015). And southern populations may persist if climate change combined with habitat fragmentation select for less emigration but larger dispersal distances (Boeye et al., 2013; Fronhofer et al., 2014). If global warming results in population extinction, proportionally more genetic diversity would be lost in the South than in the North of Europe. Because most studies on great tits have been conducted in north-central Europe, further work is needed to assess both the large-scale variation of philopatry, its relation to local and regional winter partial migration movements and its consequence in terms of gene flow between populations.

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Table 1. Decomposed pairwise regression (DPR) of the genetic differentiation with geographic distance for each sampling site. Intercepts indicate the level of differentiation of sites, and slopes indicate isolation-by-distance (IBD). FR.MO was identified as an outlier site and was therefore excluded to calculate the pairwise regressions of other sites. Significant values are indicated in bold**.**

Site	Intercept ± SE (10^{-2})		*P*	Slope ± SE (10^{-6})		*P*	R^2	Genetic differentiation pattern
FR.MO	2.57	0.26	**0.000**	-3.87	1.71	**0.032**	**0.159**	Negative IBD, differentiated sites
FR.RO	0.95	0.27	**0.002**	-3.75	1.77	**0.044**	**0.147**	
SP.MU	1.79	0.21	**0.000**	0.07	1.06	0.950	0.000	No IBD, differentiated sites
PO.CO	1.70	0.22	**0.000**	0.34	0.97	0.729	0.005	
SP.MA	1.28	0.23	**0.000**	-0.24	1.09	0.828	0.002	
SP.FR	1.21	0.21	**0.000**	-0.58	0.99	0.561	0.013	
CH.BE	0.61	0.22	**0.010**	1.73	1.80	0.344	0.035	
SE.SA	0.92	0.14	**0.000**	2.65	1.13	**0.027**	**0.175**	IBD, differentiated sites
SE.LO	0.87	0.12	**0.000**	4.35	1.01	**0.000**	**0.418**	
SE.BO	0.52	0.12	**0.000**	2.86	1.02	**0.010**	**0.232**	
FI.TU	0.50	0.17	**0.006**	2.36	1.11	**0.043**	**0.148**	
NO.DA	0.44	0.13	**0.002**	2.59	1.02	**0.017**	**0.199**	
SE.OG	0.42	0.11	**0.001**	3.50	0.92	**0.001**	**0.355**	
SE.JA	0.35	0.11	**0.005**	5.31	0.93	**0.000**	**0.558**	
SE.GE	0.33	0.12	**0.009**	2.51	0.97	**0.016**	**0.203**	
SE.SI	0.30	0.13	**0.035**	3.34	1.08	**0.005**	**0.269**	
SE.BI	0.26	0.11	**0.021**	2.61	0.86	**0.005**	**0.264**	
NE.LA	0.43	0.24	0.081	6.43	2.27	**0.009**	**0.235**	IBD, undifferentiated sites
FI.KO	0.20	0.17	0.251	2.38	0.94	**0.018**	**0.197**	
SE.ET	0.10	0.12	0.407	3.23	0.99	**0.003**	**0.292**	
NE.HO	0.07	0.21	0.754	4.33	2.02	**0.042**	**0.150**	
SE.DT	0.01	0.13	0.944	4.13	1.06	**0.001**	**0.369**	
NE.WE	0.00	0.17	0.994	3.94	1.55	**0.018**	**0.199**	
ES.KI	0.00	0.14	0.997	2.14	0.97	**0.036**	**0.159**	
PL.PU	-0.16	0.25	0.520	4.30	1.98	**0.039**	**0.154**	
HU.PI	-0.21	0.29	0.490	5.21	2.24	**0.028**	**0.172**	
UK.WY	0.45	0.25	0.089	0.80	1.96	0.687	0.006	No IBD, undifferentiated sites
BE.BO	0.38	0.22	0.098	2.83	2.03	0.175	0.070	
BE.CE	0.27	0.17	0.127	2.76	1.57	0.090	0.106	
UK.CA	0.22	0.23	0.331	2.84	1.83	0.132	0.085	
All	0.45	0.06	**0.000**	3.21	0.40	**0.000**	**0.130**	IBD, differentiated sites

Table 2: Effects of environmental factors on the genetic differentiation between sampling sites across Europe and its variation based on Mantel tests (r_M). Genetic distance was measured as pairwise F_{ST}, G''_{ST} and D and their variation was investigated using the residuals of a linear regression between each environmental factor and the respective pairwise genetic distances. See text for details. Significant correlations are indicated in bold.

Response variable:	F_{ST}		Residuals on F_{ST}		G''_{ST}		Residuals on G''_{ST}		D		Residuals on D	
Explanatory variable:	r_M	P	r_M	P	r_M	P	r_M	P	r_M	P	r_M	P
Mean autumn- winter temperature	**0.57**	**<0.001**	**0.17**	**0.022**	**0.57**	**<0.001**	**0.16**	**0.025**	**0.57**	**<0.001**	**0.15**	**0.023**
Latitude	**-0.50**	**0.002**	**-0.23**	**0.010**	**-0.50**	**0.002**	**-0.22**	**0.010**	**-0.51**	**0.001**	**-0.22**	**0.007**
Geographic distance	**0.36**	**<0.001**	0.06	0.205	**0.37**	**0.001**	0.07	0.188	**0.37**	**<0.001**	0.07	0.166
Difference in autumn-winter temperature	**0.39**	**0.002**	0.10	0.125	**0.39**	**0.002**	0.10	0.120	**0.39**	**0.002**	0.10	0.114

Figure legends:

Figure 1. Location of the 30 sampling sites across Europe. The inset shows the 10 sampling sites on the island of Gotland, Sweden. The dashed line shows the 47° latitude. IBD analysis treats all populations into a single quantity assuming that all local populations have similar characteristics. In contrast, the DPR analysis extracts the elements of individual local population from the information on an entire metapopulation and identifies five groups differing in relative strengths of gene flow and genetic drift patterns (i.e. different patterns of genetic differentiation and IBD summarised by different colours).

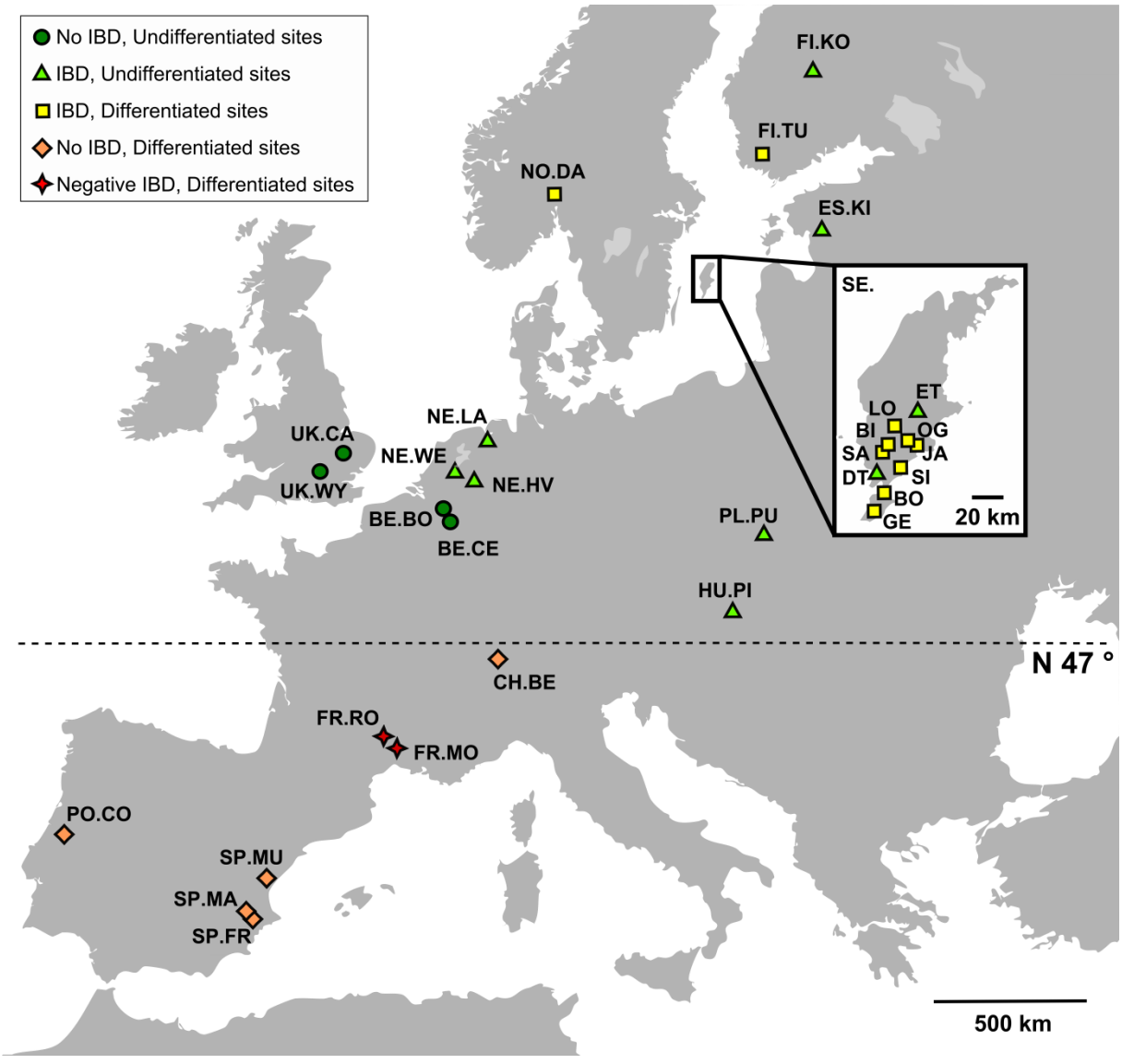


Figure 2. Heatmap of the pairwise F_{ST} values between all sites. Sites are ordinated by pairwise F_{ST} values. Black bars highlight the sites located below the 47° latitude (i.e. south-western sites).

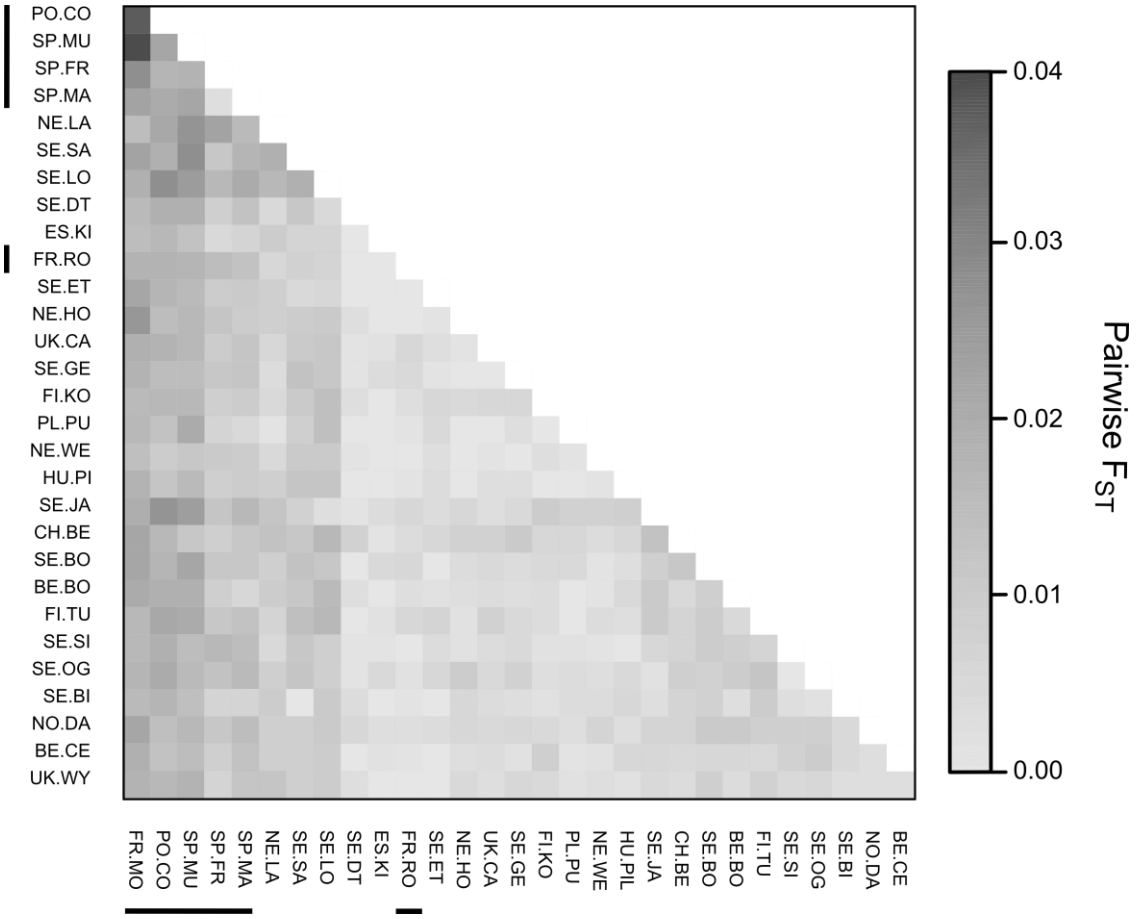


Figure 3. (a, b) Principal coordinate analysis (PCoA) contrasting axes 1 vs. 2 (a) and 1 vs. 3 (b), and (c) NJ phenogram based on Nei's genetic distance, with bootstrap values of specific clusters. (a,b) On the PCoA plots, the smallest and largest ellipses represent the 50% and 95% confidence intervals respectively; black dots represent the five sites identified as satellites, grey dots potential other satellites and white dots non-differentiated sites. (c) On the NJ phenogram, the three grey circles indicate identified clusters and the five sites identified as satellites are indicated in bold.

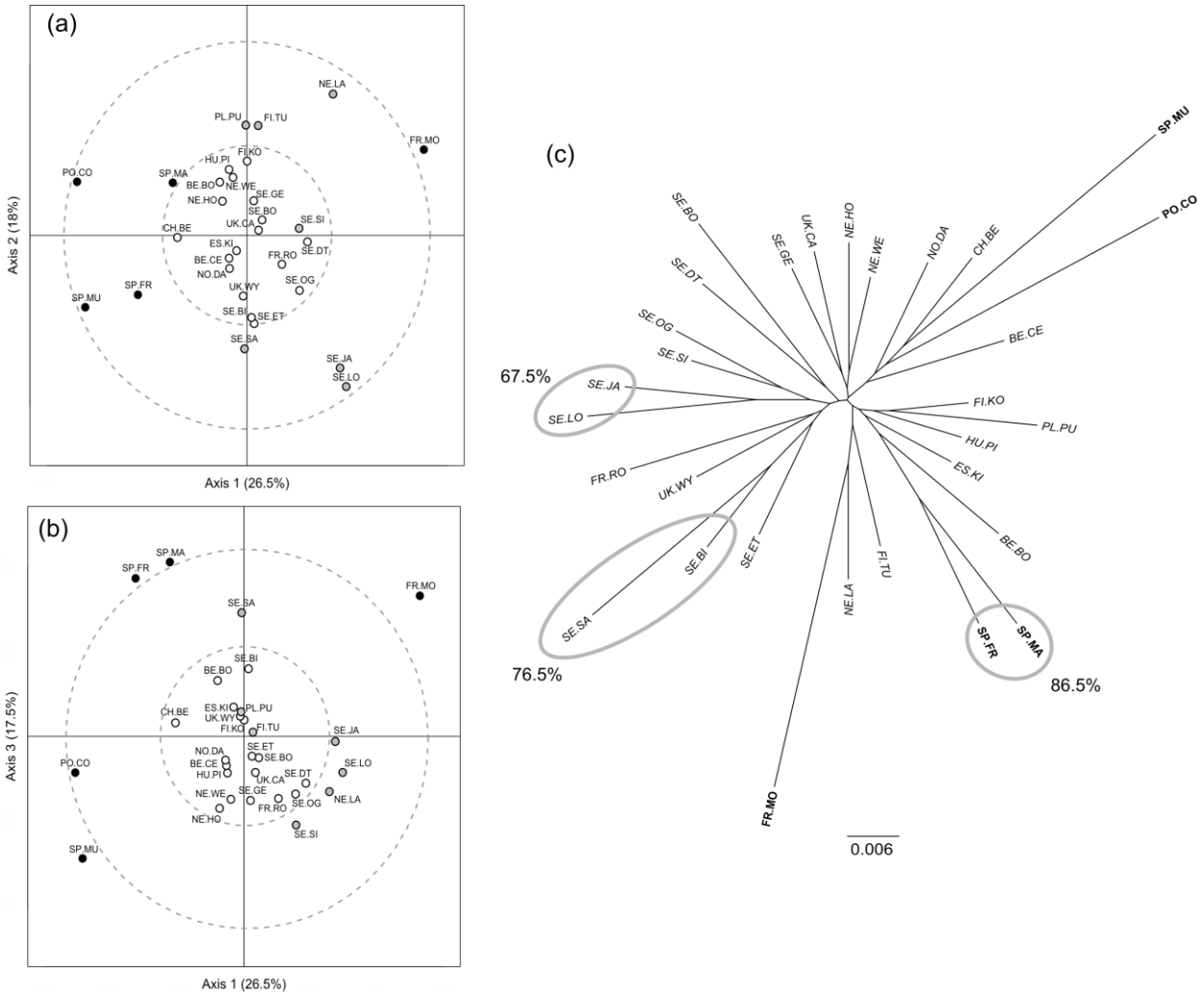


Figure 4. Relationships between average autumn-winter temperature and (a) latitude and (b-f) different population indices: assignment probability (b), allelic richness (c), mean pairwise kinship (d), unbiased expected heterozygosity (e), effective population size (f).

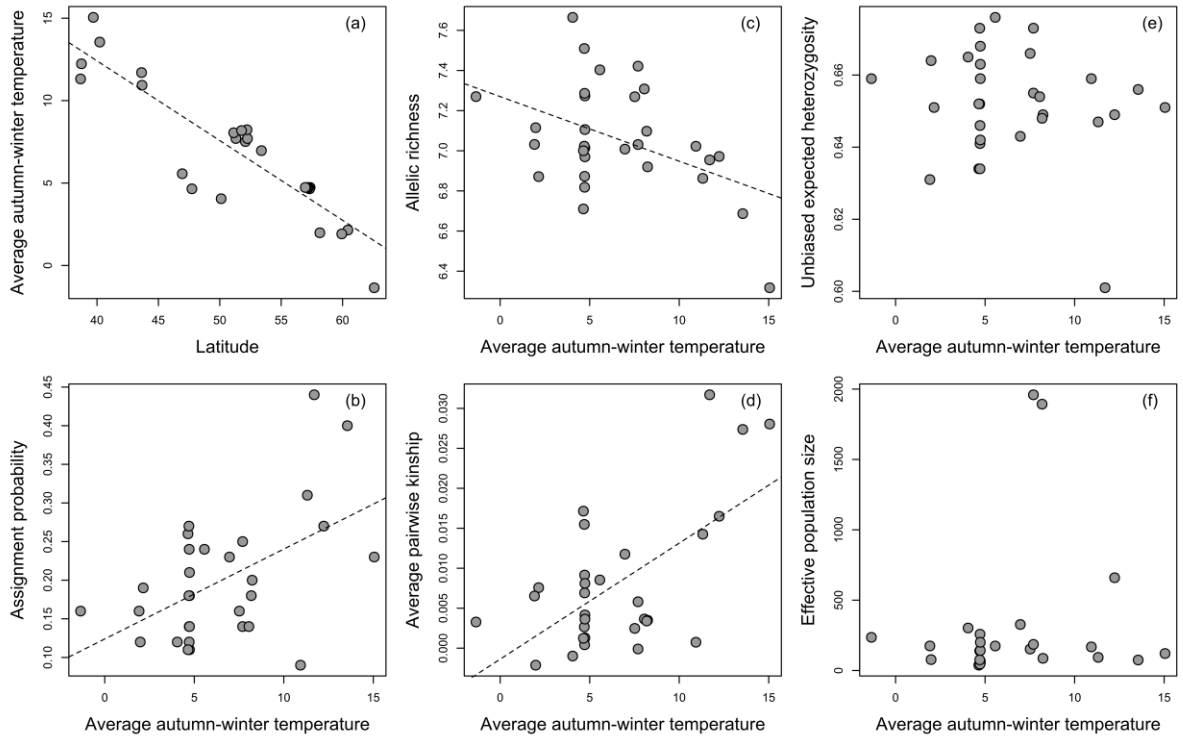
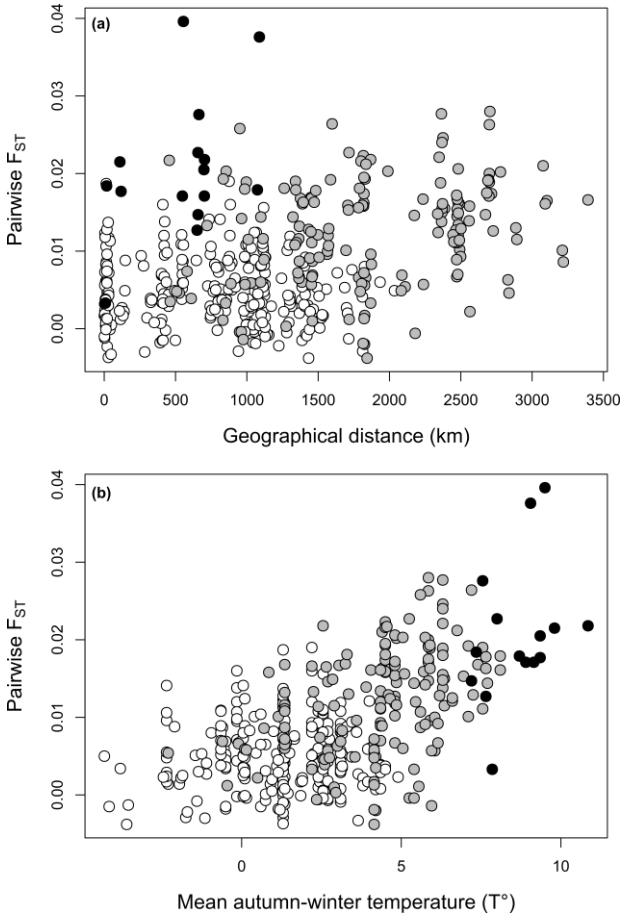


Figure 5. Relationship between pairwise F_{ST} values and (a) geographic distance between sites and (b) mean autumn-winter temperature of the two sites in pairwise comparisons. White dots: pairwise F_{ST} values between northern sites; grey dots: pairwise F_{ST} values between one northern and one south-western site; black dots: pairwise F_{ST} values between south-western sites.



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