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Received: 21 November 2022 | Accepted: 28 September 2024

DOI: 10.1111/icad.12786

ORIGINAL ARTICLE

Monitoring spatiotemporal patterns in the genetic diversity of a European butterfly species

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Funding information
Horizon 2020: eLTER PLUS, Grant/Award Number: 871128; Estonian Research Council, Grant/Award Number: PRG741; Internal Grant Agency of the Faculty of Environmental Sciences, Czech University of Life Sciences Prague, Grant/Award Number: 42900/1312/3141; SCENARIO NERC Doctoral Training Partnership, Grant/Award Number: NE/L002566/1; UKCEH National Capability Fund WP2: Mechanisms of Resilience across ecological scales, Grant/Award Number: NEC05106; Horizon 2020: EUROPABON, Grant/Award Number: 101003553; 15. Juni Fonden, Grant/Award

Abstract

1. The importance of genetic diversity has been recognised by the Convention on Biological Diversity but attempts at monitoring or improving the genetic diversity of populations have been minimal.
2. Here, we investigate changes over time in the genetic diversity of a wild insect species, *Maniola jurtina* (Lepidoptera: Nymphalidae) and present a large-scale investigation into contemporary spatial genetic diversity.
3. Using microsatellite markers, we calculate multiple measures of genetic diversity and divergence for *M. jurtina* populations over 8 years in the UK and compare these findings with long-term abundance trends.
4. We also conduct a large-scale spatial analysis into the genetic diversity and population structuring of *M. jurtina* across Europe.

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Number: 2023-0140; Aage V Jensens Naturfond, Grant/Award Number: N2023-0017; Butterfly Conservation (BC); UK Centre for Ecology and Hydrology (UKCEH); British Trust for Ornithology (BTO); Joint Nature Conservation Committee (JNCC)

Editor/Associate Editor: Nusha Keyghobadi

5. All UK populations sampled have high levels of gene flow and genetic diversity, with genetic diversity stable over time.
6. Across Europe, we find some population structuring between populations in the UK and the European mainland, suggesting restricted geneflow between the two regions.
7. The monitoring of a wild species' genetic diversity is an achievable aim, and one that could be carried out for many species, particularly Lepidoptera. Future approaches may aim to develop higher resolution genetic markers and cover a wider range of species.
8. The use of abundance data offers additional insight, and we find that concurrent, dedicated genetic monitoring can provide effective tracking of biodiversity trends.

KEYWORDS
genetic diversity, *Maniola jurtina*, meadow brown, microsatellites, monitoring

INTRODUCTION

Genetic diversity is important for the long-term persistence of species (Hoban et al., 2014). Despite the need for genetic diversity monitoring being increasingly recognised (Boettcher et al., 2010), and genetic diversity being regarded as a key measure of biodiversity (Pereira et al., 2013), studies quantifying temporal changes in the genetic metrics of a population remain relatively limited outside of endangered or socio-economically important species. However, technological advances have allowed an increase in the frequency of such studies over recent years (Hoban et al., 2014; Leroy et al., 2018), for examples, see Jangjoo et al. (2020) for Rocky Mountain Apollo butterfly, *Parnassius smintheus* (Lepidoptera: Papilionidae); Nakahama and Isagi (2018) for *Melitaea protomedia* (Lepidoptera: Nymphalidae).

In 2011, the Convention on Biological Diversity (CBD) stated the aim that by 2020 there would be development and implementation of methods for maintaining the genetic diversity and minimising the genetic erosion of cultivated plants, farmed and domesticated animals and of wild relatives, including other socio-economically and culturally valuable species (CBD, 2011). In 2020, this aim to enhance knowledge of the genetic diversity of the above species groups was pushed back to 2030 but was also expanded to include 90% of species by 2050 (CBD, 2020a, 2020b). These aims have come under criticism, as most species globally do not fall into these narrow categories and such knowledge is currently lacking for the vast majority of wild species (Laikre, 2010; Laikre et al., 2020).

The ability to maintain genetic diversity is dependent upon a baseline level of knowledge of how genetically diverse populations are. This knowledge is severely lacking for the vast majority of wild species. Here, we explore the potential for genetic monitoring of a non-endangered, non-socio-economically important wild butterfly species; the meadow brown butterfly (*Maniola jurtina*; L. 1758). *Maniola jurtina* is an ideal species for a pilot genetic monitoring scheme for multiple reasons. First, long-term population monitoring data are available across Europe. Our sampling sites are co-located with long-term abundance monitoring data ('Pollard' transect walks) from national partners within the framework of the European Butterfly Monitoring Scheme (www.butterfly-monitoring.net), providing an opportunity to link genetic and abundance biodiversity metrics. Although long-term population monitoring data are available for other taxa, for example, birds, the ability to sample genetic material is far more practical using a common butterfly species, in addition to the fact that insects tend to be under-represented in syntheses of biodiversity change (Titley et al., 2017). Second, much is already known about *M. jurtina* biology including evolutionary genetics (e.g., Brakefield & Shreeve, 1992 and references within), ecology and life history (e.g., Brakefield, 1982a, 1982b; Greenwell et al., 2021; Lebeau et al., 2018), dispersal and movement (e.g. Conradt et al., 2000; Delattre et al., 2013; Evans et al., 2019, 2020; Merckx & Van Dyck, 2002; Schneider et al., 2003), historical distributions (Dapporto et al., 2011) and spatial genetic diversity and landscape genetics (Baxter et al., 2017; Richard et al., 2015; Villemey et al., 2016). Third, a number of microsatellite loci are already available for population genetics studies (Richard et al., 2015). In combination, these factors mean we are able, not only to determine levels of genetic diversity within this species, but also to demonstrate how population abundances could be affected by genetic diversity, through the direct comparison of trends in genetic variability and population dynamics over time.

Previous studies into *M. jurtina* spatial genetic diversity and historical distribution provide valuable information (Baxter et al., 2017; Dapporto et al., 2011; Goulson, 1993; Habel et al., 2009; Kreuzinger et al., 2015; Richard et al., 2015; Schmitt et al., 2005; Tauber, 1970; Thomson, 1973; Thomson, 1987; Villemey et al., 2016). However, it is not possible to infer changes in genetic diversity from multiple independent studies over time due to differences in locations and differences in molecular technologies.

Here, we introduce the Butterfly Genetics Monitoring Scheme (BGEMS), a pilot study investigating the feasibility of monitoring annual changes in the genetic diversity of a target species (*M. jurtina*). We report on the first 8 years of this study in the United Kingdom, as well as results from a large-scale spatial investigation spanning over 2500 km across continental Europe. Specific long-term studies, using

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a consistent, comparable methodology, are required to monitor genetic diversity and contribute towards addressing CBD commitments.

MATERIALS AND METHODS

The BGEMS

The BGEMS (https://butterfly-monitoring.net/project/bgems) began in 2012 in a partnership between the University of Reading and the UK Centre for Ecology & Hydrology. The project aimed to develop a spatially replicated, long-term genetic monitoring scheme linked with population abundance data. Between 2012 and 2019 the scheme expanded to include 12 institutions from 10 countries (Table S1).

Study species

Maniola jurtina is a Satyrine butterfly species with a western Palearctic distribution (Tolman and Lewington, 1997; Figure S1), classified as 'Least Concern' under the Red Lists of British and European butterfly species (Van Swaay et al., 2010; Fox et al., 2011). Individuals typically move around an area with a radius of less than 500 m, although longer distance movements of up to 2.1 km have been recorded (Schneider et al., 2003). Microsatellite studies in France determined that distance has little effect on pairwise differentiation between *M. jurtina* populations (Richard et al., 2015) and that linear grassland elements enhance *M. jurtina* gene flow between populations (Villemey et al., 2016).

Sample collection

Over the 8-year study period (2012–2019), a total of 1024 individual *M. jurtina* samples were collected from 15 sites in the South of England, UK (Figure 1, Table S1). Between 2017 and 2019, additional samples were collected from 24 sites across 10 mainland European countries (only the 2017 samples ($n = 523$) are used in this analysis), with sampling carried out by trained volunteers and/or scheme coordinators from 12 collaborating organisations (Figure 1, Table S1). Collaborators were engaged through the European Butterfly Monitoring Scheme, a partnership of national butterfly abundance monitoring schemes (BMSs). Lethal sampling was carried out to provide redundancy in the analysis, in the event of DNA extraction failure. The samples remain stored at −80°C at the University of Reading for future, additional analyses. This integrated approach provides a link between genetic monitoring and long-term abundance time series, enabling novel assessments of population dynamics.

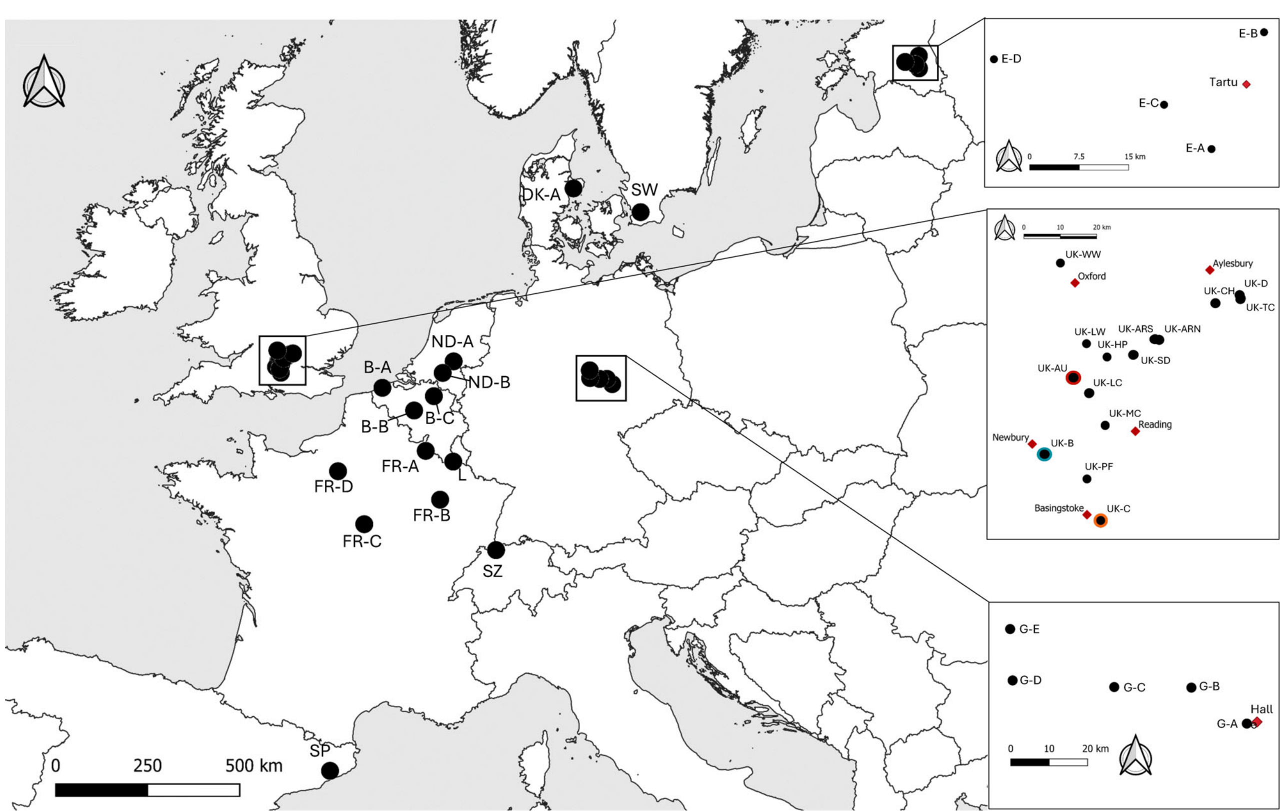

FIGURE 1 Site locations of 39 sites across Europe from which samples were collected for genetic analysis over the study period. Inset maps show Estonian, German and UK sites in closer detail, with local towns and cities (red diamonds) for location reference. UK sites with coloured rings are the three sites used for the time series analysis. Location data are available in Table S1.

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Population monitoring data

Abundance data from 1412 long-term monitoring sites (2012 onwards) of the United Kingdom Butterfly Monitoring Scheme (UKBMS; Botham et al., 2020) were used to compare abundance with genetic diversity at the UK sample sites over time. UKBMS data are collected by volunteers using the 'Pollard walk' method (Pollard and Yates, 1993). The UKBMS uses a two-step method, fitting Generalised Additive Models to the data to produce fitted weekly counts and an overall collated annual index as a relative measure of abundance at each site (Dennis et al., 2013).

Molecular analysis

DNA extractions were carried out using only leg tissue. DNA from samples collected in 2012 and 2013 were extracted using DNeasy Blood and Tissue Kits (Qiagen), following the manufacturer's guidelines. DNA from samples from sites named 'Aston Upthorpe' (UK-AU), 'Bowdown' (UK-B) and 'Crabtree Plantation' (UK-C) from 2014 onwards and samples from all sites in 2017 were extracted using prepGEM Universal (MicroGEM), following the manufacturer's guidelines, optimised by halving the reaction volumes resulting in 20 μL eluted DNA. Samples were genotyped over 2 years using the following six microsatellites: Mj7232, Mj5522, Mj0247, Mj4870, Mj7132 and Mj5331 (Richard et al., 2015). PCRs were carried out in an Eppendorf Mastercycler nexus eco (for PCR conditions and reaction concentrations see Data S2). All PCR products were diluted by 100× and run on a single Applied Biosystems 3730 DNA Analyser. Allele peaks were then scored using GeneMarker® version 1.5 (SoftGenetics) using the standard default settings for animal fragments. Any individuals for which there were more than two loci with missing data were removed from the analysis (5.96% of samples). Further samples were then removed to ensure that all populations had a maximum of 5% missing data per locus, ensuring that no loci were dropped in the analysis due to insufficient data.

Statistical analysis

To conduct a temporal analysis of genetic diversity across UK populations, all analyses were undertaken separately for samples in 2012 (252 individuals, 14 sites), 2017 (287 individuals, 15 sites) and a subset of samples from three sites (UK-AU, UK-B, UK-C) every year from 2012 to 2019 (432 individuals). These datasets are herein referred to as '2012', '2017' and 'All-Years', respectively. The 2012 and 2017 datasets had the greatest number of sites and longest gap between years available at the time of the analysis. Subsequent samples were collected in 2018 and 2019, a subsample of which were included in the All-Years dataset. All 39 sampling sites across Europe in 2017 were used to conduct a spatial analysis of contemporary genetic diversity. This dataset is herein referred to as 'Europe'. Unless otherwise specified analyses were conducted in R (R Core Team, 2024).

Microsatellite analysis

Genotyping errors, including stuttering and large allele dropout, were computed using MICROCHECKER V2.2.3 (Van Oosterhout et al., 2004) Linkage disequilibrium and null allele frequencies were tested for in Genepop v4.7 (Rousset, 2008). Observed and expected heterozygosities (H_o and H_e) across each dataset, for individual loci and averaged across all loci, were calculated using the R package PopGenReport (Adamack & Gruber, 2014; Gruber & Adamack, 2015). Deviations from the Hardy–Weinberg equilibrium (HWE) were also calculated using PopGenReport, via a Chi-square test and Bartlett test of homogeneity of variance used to compare observed versus expected heterozygosity. Whereby a significant result indicates that the population departs from HWE. For each locus, Wright's F statistics (Wright 1965; Excoffier, 2007) were calculated across all sampling locations using Genepop.

Population genetic diversity

The number of private alleles (A_p) per site were calculated using PopGenReport, as were the expected (H_e) and observed (H_o) heterozygosity for each locus and the percentage differences between H_e and H_o. H_e for each site and across all sites were calculated using Arlequin v 3.5.2.2 (Excoffier and Lischer, 2010). Differences in genetic diversity between the UK and mainland Europe were investigated by comparing mean H_e from the 2017 and Europe datasets using two-sample t-tests. Additionally, to determine whether abundance had any effect on genetic diversity over the study period a linear mixed effects model was fitted using the All-Years data with H_e as a function of annual abundance. Site was included as a random intercept. To explore possible lag effects, whereby multiple generations can occur between an environmental perturbation occuring and a genetic response being detected (Epps & Keyghobadi, 2015), two additional models were fitted using the previous year's and 2 year's previous abundances respectively instead of the current year's abundance.

Genetic structure, differentiation and geneflow

Population structure was investigated using STRUCTURE v.2.3.4 (Pritchard et al., 2000, Falush et al., 2003, 2007; Hubisz et al., 2009). For all models, the parameter set used an admixture model and correlated allele frequencies with a 100,000 burn-in and 1,000,000 Markov Chain Monte Carlo (MCMC) replications per chain. To determine the most probable number of clusters (K) in the different datasets, 20 chains were run. The most likely value of K within the sample sets was estimated using STRUCTURE Harvester (Earl and VonHoldt, 2012) which estimates an ad hoc statistic, Delta K, to determine the most likely value of K (Evanno et al., 2005).

For measures of genetic distance, only the data sets 2012, 2017 and Europe were analysed. Individuals were pooled on a per-site basis and populations with fewer than 15 individuals were removed from

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the analysis. Weir and Cockerham's pairwise F_{ST} values were calculated using FSTAT, followed by a correction for false discovery rates (FDR), accounting for multiple testing, using the Benjamini and Hochberg (1995) method. F_{ST} was chosen over Gst to allow greater comparison with non-microsatellite analyses (Meirmans & Hedrick, 2011). Euclidean distances between all sites were calculated from the GPS coordinates of each site using the function *distm* in the R package Geosphere (Hijmans, 2017). Estimates of isolation by distance (IBD) via spatial autocorrelation were then calculated by plotting pairwise F_{ST} values against (log) Euclidean distances between sites, using Mantel correlograms using the *mantel.correlog* function from the R package Ecodist (Goslee and Urban, 2007). Mantel correlograms were performed using Pearson's correlations, 10,000 permutations with an $\alpha = 0.05$ and the Holm correction for testing multiple *p*-values. Five distance classes were assigned per test with unequal distance intervals to keep the pairs of populations per class similar (Diniz-Filho et al., 2013). Owing to the unevenness of the distribution of distances between sites in the Europe dataset (distance between sites: $\mu = 728$ km, min = 0.8 km, max = 2525 km, var. = 346,031), estimates were also calculated with the UK sites removed, the Estonian sites removed and again with the Spanish site removed (distances between sites with Estonian sites removed: $\mu = 501$ km, min = 0.8 km, max = 1742 km, var. = 121,266, distances between sites with Spanish site removed: $\mu = 698$ km, min = 0.8 km, max = 1979 km, var. = 335,279). A final test using only mainland European sites was also run ($\mu = 846$ km, min = 14 km, max = 2525 km, var. = 359,002). Breakpoints for distance classes were set to 2012 and 2017: 0, 10, 20, 30, 40, 100; Europe (All), Mainland Europe and Europe with Spain removed: 0, 250, 500, 1000, 1500, 3000; Europe with Estonia removed: 0, 250, 500, 750, 1000, 2000.

An additional investigation into the effects of landscape on *M. jurtina* geneflow across the UK populations in 2017 was carried out via a resistance analysis using the R package ResistanceGA (Peterman, 2018) (Data S3; Figure S2).

RESULTS

Microsatellite analysis

MICROCHECKER found no evidence for large allelic dropout, scoring errors due to stuttering or null alleles. Alleles were randomly associated among loci, indicated by no evidence of linkage disequilibrium between pairs of loci (Table S4). Most site locus combinations (523 out of 552) displayed a low frequency (<0.2) of null alleles (Table S5); however, Mj4870 displayed high levels of null alleles within multiple populations, particularly in the Europe dataset (Table S5d). This led to all analyses being re-done with the locus Mj4870 removed. With the exception of the STRUCTURE analysis (see below), this had no significant effect on the overall results therefore the locus was included. The microsatellites displayed high levels of variability (Table 1), with the highest values of H_o occurring at Mj5331 (0.877, 0.902, 0.905 and 0.862 for 2012, 2017, All-Years and Europe respectively) and the lowest occurring at Mj4870 (0.262, 0.279, 0.277 and 0.237). Across each dataset, all loci showed a non-significant level of heterozygote deficit. The greatest occurred at Mj4870 with deficits of 31.4% (2012), 24.6% (2017), 24.3% (All-Years) and 62.5% (Europe; Table 1).

Minimal genetic differentiation (F_{ST}) occurred within the 2012, 2017 and All-Years datasets, suggesting high levels of gene flow between populations (Table 1). However, F_{ST} values were significantly greater than zero at Mj7232 and Mj4870 in the Europe dataset suggesting lower levels of gene flow and some differentiation. F_{IS} values were significantly greater than zero at multiple loci (Table 1), indicating some (low-level) inbreeding within populations.

Population genetic diversity

Full genetic diversity (H_e) metrics for each dataset can be found in Table S6. In summary, genetic diversity was high across all datasets, with H_e 0.772 (2012), 0.764 (2017), 0.764 (All-Years) and 0. 821 (Europe) (Figure 2). Expected heterozygosity (H_e) ranged from 0.704 (UK-B-2014) to 0.831 (B-B). Genetic diversity was significantly higher across mainland European populations than UK populations (Europe $H_e = 0.804$, UK $H_e = 0.764$; $p > 0.001$ [Figure 2a]). Across all four datasets (39 sites in total), five private alleles occurred at one site (UK-PF-2017), two occurred at eight sites and one private allele occurred at 23 sites (Table S6). According to the LMM, site abundance had no effect on genetic diversity (H_e: coef = 0.000006, SE = 00002, $p = 0.509$), including when lagged by one (coef = 000009, SE = 0.00001, $p = 0.486$) and 2 years (coef = 0.000005, SE = 0.00002, $p = 0.608$).

Genetic structure, differentiation and gene flow

No evidence of spatial genetic structuring was found between populations for the UK datasets (2012, 2017) using the six microsatellites described. No population was found to be genetically distinct from any other population (Figure S3). Across Europe, initial STRUCTURE analysis suggested four clusters as optimal (Figure 3). However, subsequent analysis using the Evanno method (Evanno et al., 2005) suggested just two clusters (Figure 4; Figure S4). Re-running the STRUCTURE analyses with the removal of locus Mj4870 resulted in optimal *K*s of $K = 1$ and $K = 5$ using the above methods respectively (Figure S5). However, retaining Mj4870 and removing Mj5522 (low frequency (<0.2) of null alleles within all populations) had the same result. As such the differing results with the removal of Mj4870 are likely due to a lack of power in the analysis with only five loci, rather than locus Mj4870 driving the differentiation between populations when included.

Within each of the UK datasets (2012, 2017) there was little genetic differentiation among populations, indicated by F_{ST} scores being close to zero (2012: mean $F_{ST} = 0.004$, variance = 0.00005, 2017: mean $F_{ST} = 0.002$, variance = 0.00004; Tables S7 and S8).

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TABLE 1 Samples sizes, population-wide expected and observed heterozygosity and percentage differences ((*E*−*O*)/*E* × 100), F_{IT}, F_{IS} and F_{ST} at each locus.

	Locus	Number of samples	Number of alleles	H_e	H_o	H_e versus H_o % difference	F_{IT} (*p*-Value)	F_{ST} (*p*-Value)	F_{IS} (*p*-Value)
2012	Mj7232	251	13	0.804	0.733	−8.8	0.091 (0.001)	0.006 (0.967)	0.085 (0.002)
	Mj5522	251	11	0.860	0.813	−5.6	0.058 (0.007)	0.012 (0.700)	0.046 (0.057)
	Mj0247	250	29	0.941	0.808	−14.2	0.144 (0.000)	0.003 (1.000)	0.141 (0.000)
	Mj4870	248	5	0.382	0.262	−31.4	0.316 (0.000)	−0.015 (1.000)	0.326 (0.000)
	Mj7132	248	10	0.768	0.758	−1.3	0.015 (0.342)	0.002 (0.997)	0.014 (0.380)
	Mj5331	252	26	0.894	0.877	−1.9	0.021 (0.186)	0.002 (1.000)	0.019 (0.211)
	Mean	250	15.7	0.775	0.709	−10.5	0.107 (–)	0.002 (–)	0.105 (–)
2017	Mj7232	285	12	0.798	0.762	−4.5	0.048 (0.035)	0.002 (0.999)	0.046 (0.049)
	Mj5522	281	12	0.862	0.809	−6.2	0.064 (0.008)	0.000 (1.000)	0.064 (0.006)
	Mj0247	283	31	0.941	0.842	−10.6	0.105 (0.000)	0.000 (1.000)	0.105 (0.000)
	Mj4870	282	6	0.370	0.279	−24.6	0.252 (0.000)	0.000 (0.982)	0.252 (0.000)
	Mj7132	282	10	0.741	0.752	1.5	−0.013 (0.692)	0.007 (0.911)	−0.020 (0.770)
	Mj5331	286	22	0.894	0.902	0.9	−0.007 (0.667)	0.002 (1.000)	−0.009 (0.692)
	Mean	283	15.5	0.768	0.724	−7.3	0.075 (–)	0.002 (–)	0.073 (–)
All years	Mj7232	426	14	0.819	0.719	−12.2	0.125 (0.000)	0.016 (0.429)	0.111 (0.000)
	Mj5522	426	12	0.865	0.806	−6.9	0.071 (0.000)	0.012 (0.739)	0.059 (0.001)
	Mj0247	424	31	0.936	0.758	−19.1	0.190 (0.000)	0.002 (1.000)	0.188 (0.000)
	Mj4870	428	6	0.367	0.277	−24.3	0.247 (0.000)	0.006 (0.949)	0.243 (0.000)
	Mj7132	427	10	0.737	0.707	−4.1	0.042 (0.048)	0.003 (0.999)	0.039 (0.064)
	Mj5331	430	24	0.898	0.905	0.8	−0.006 (0.680)	0.008 (0.988)	−0.014 (0.808)
	Mean	427	16.2	0.770	0.695	−11.0	0.112 (–)	0.008 (–)	0.104 (–)
Europe	Mj7232	720	16	0.837	0.738	11.9	0.120 (0.000)	0.054 (0.000)	0.070 (0.000)
	Mj5522	709	13	0.845	0.794	6.1	0.062 (0.000)	0.012 (0.818)	0.051 (0.000)
	Mj0247	715	33	0.940	0.779	17.1	0.171 (0.000)	0.017 (0.232)	0.156 (0.000)
	Mj4870	712	8	0.632	0.237	62.5	0.630 (0.000)	0.102 (0.000)	0.588 (0.000)
	Mj7132	710	11	0.771	0.737	4.4	0.046 (0.008)	0.017 (0.241)	0.030 (0.070)
	Mj5331	717	23	0.895	0.862	3.6	0.038 (0.002)	0.017 (0.124)	0.021 (0.060)
	Mean	714	17.3	0.820	0.691	17.6	0.178 (–)	0.036 (–)	0.153 (–)

Note: Bartlett's *K*-squared: 2012 = 0.049, df = 1, *p*-value = 0.82; 2017 = 0.03, df = 1, *p*-value = 0.86; All years = 0.006, df = 1, *p*-value = 0.94; Europe = 2.29, df = 1, *p*-value = 0.13.

Pairwise F_{ST} scores were significantly greater than zero for seven site-pair combinations in the 2012 dataset. No pairwise F_{ST} scores were significantly greater than zero for 2012 and 2017. More differentiation among populations was observed within the Europe dataset (mean F_{ST} = 0.033, variance = 0.0007), with pairwise F_{ST} being significantly greater than zero for 473 out of 630 pairwise combinations across Europe (Table S9).

Testing for spatial autocorrelation in the data using mantel correlograms it was possible to observe both negative and positive autocorrelation in different distance classes (Figure 5). In 2012 and 2017 (UK) no spatial autocorrelation was found over any distance class. Across Europe, when including UK sites, positive spatial autocorrelation was found below 200 km, but then negative spatial autocorrelation between 300 km and 400 km and 700 and 800 km. The exception to this was when Estonian sites were removed and only positive spatial autocorrelation was found below 100 km. Removal of the UK sites resulted in positive spatial correlation at all points.

The results of the resistance analysis (Data S3; Tables S2 and S3) found no evidence of either IBD or isolation by resistance (IBR) in *M. jurtina* populations across the study area and suggest that landscape features do not affect *M. jurtina* gene flow at the study scale.

DISCUSSION

This work represents a contribution towards the aims of the CBD by showcasing an example of genetic monitoring for a terrestrial insect species in combination with population abundance data. Only through monitoring genetic diversity can we determine if mitigation measures

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are required to maintain or minimise the erosion of genetic diversity, as targeted by the CBD (CBD, 2011, 2020a, 2020b). Furthermore, despite *M. jurtina* being extensively studied over the past century, previous investigations into the population genetics of *M. jurtina* (Habel et al., 2009; Richard et al., 2015; Schmitt et al., 2005; Thomson, 1987; Villemey et al., 2016), have not looked at temporal changes, as has been done for other, well-studied Lepidopteran species (Han & Caprio, 2002; Keyghobadi et al., 2013; Kim et al., 2009; Saarinen et al., 2010, 2016; Vandewoestijne et al., 1999). By sampling *M. jurtina* individuals yearly from the same sites, this study monitors genetic diversity across a wide spatial extent. The addition of population monitoring data offers a novel insight, assisting in understanding the associations between genetic diversity and population abundance in real-world natural systems. Such evidence is required to determine if conservation actions are necessary or not.

As butterflies are a well-studied taxonomic group, with standardised monitoring schemes in place across Europe (Brereton et al., 2011, 2017; Van Swaay et al., 2008), combining traditional population monitoring with genetic monitoring data opens the door for revealing spatiotemporal patterns of biodiversity across multiple levels. For example, average *M. jurtina* abundances show relatively stable trends (Figure 2), with the UKBMS reporting a 36.5% increase in abundance over the past 20 years (up to 2020; https://ukbms.org/species/meadow-brown). Therefore, it is unsurprising that we found no significant difference between genetic diversity (H_e) in 2012 and 2017. However, obvious fluctuations are apparent at the single-site level. Whilst sites UK-B and UK-C show little change in abundance, UK-AU has two large peaks during the study period. The first of these peaks (2013) matches with a peak in expected heterozygosity, whereas the second matches with a trough. It is important, however, not to infer causality with these observations, as many factors both contemporary and historic affect butterfly abundance and genetic diversity (Keyghobadi et al., 2005; Roy et al., 2001), and these comparisons are limited by the number of years and sites in the study. Instead, a more robust interpretation is the comparisons between 2012 and 2017 described above. It would be interesting to compare trends in the genetic diversity of species with unstable population trends, for example, species that are susceptible to population crashes due to extreme events, such as the ringlet butterfly, *Aphantopus hyperantus* (Oliver et al., 2013).

This work also highlights specific details about our pilot species, *M. jurtina*. Our results show negligible differences in levels of genetic diversity both spatially and temporally for UK populations. We found no evidence to suggest a changing trend in the genetic diversity of *M. jurtina* in UK populations over the eight-year study period. The results from the 2012 and 2017 datasets are highly consistent, as are the results for the three sites from 2012 to 2019. We also find minimal effects of landscape composition on gene flow and no evidence of IBD within the UK study landscape. The results from the UK landscape analysis are consistent with the other findings in this study. As a result, we can conclude that populations within the study area can be considered as one large population with high levels of gene flow between them.

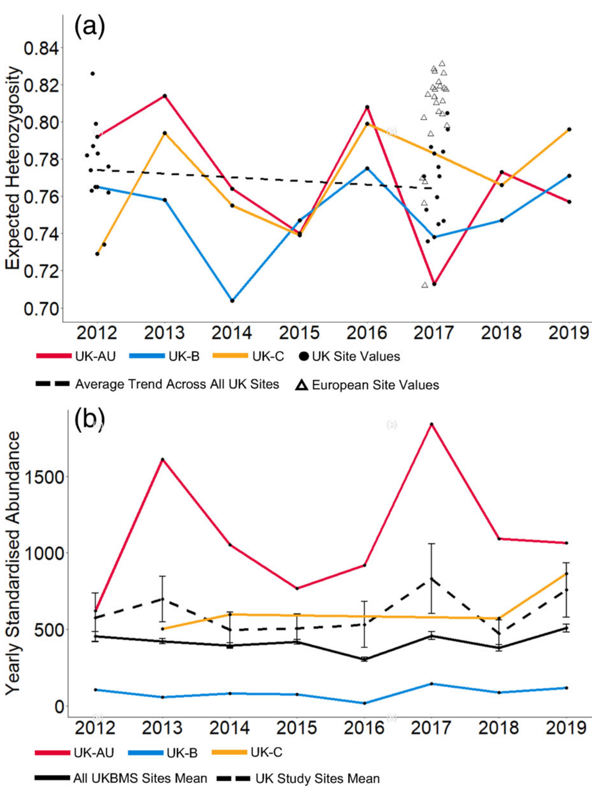

FIGURE 2 (a) Changes in expected heterozygosites for *Maniola jurtina* sample populations between 2012 and 2019 at three sites in the UK: UK-AU, UK-B and UK-C (Table S1). Expected heterozygosites for populations at 14 UK sites in 2012 and all sites (15 UK and 24 mainland Europe) in 2017 are also shown. (b) Changes in *M. jurtina* yearly standardised abundance at three UK sites: UK-AU, UK-B and UK-C. Mean *M. jurtina* yearly standardised abundance across all 15 sample sites are shown, as well as mean yearly standardised abundance across all United Kingdom Butterfly Monitoring Scheme (UKBMS) sites. Error bars show standard error.

Our results contribute to the body of evidence that shows *M. jurtina* to have high levels of genetic diversity across much of its European range (Goulson 1993; Habel et al., 2009; Richard et al., 2015; Schmitt et al., 2005; Villemey et al., 2016). Genetic differentiation was low at the continental scale, however, a large number of population pairs displayed significant, moderate levels of differentiation, many of these pairs including one UK and one mainland European population. Examples of significantly greater than zero pairwise F_{ST} scores between pairs of mainland European sites are distributed throughout the data. However, in most cases, pairwise F_{ST} scores were below 0.2, that is, differentiation can be deemed as negligible (Freeland et al., 2011). The majority of cases occurred when one population was from Estonia or Spain - the two most geographically separated sampling areas. The Mantel correlograms for Europe suggest that sites that are closer together exhibit higher levels of gene flow, than those further apart. However, inferring IBD should be done with caution, owing to the uneven distribution of sampling sites (Wright, 1943), with our data being made up of clusters of nearby sites separated from other clusters by large distances. Interestingly,

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FIGURE 3 Bayesian cluster analysis in STRUCTURE (a) Individual membership coefficients for *Maniola jurtina* across Europe where $K = 4$ (four clusters shown as red, blue, grey and orange) and (b) subsequent geographic distribution of these clusters. Initial estimation of K for *M. jurtina* using median values of LnPr(X|K) (Pritchard et al., 2000) and estimation of Delta K (Evanno et al., 2005) can be seen in Figure S4.

the UK 2012 and 2017 data sets do not show any spatial autocorrelation despite a more even sampling structure.

For the UK, we found no evidence to suggest any spatial patterning or population structuring, however, at the continental scale distinct population clustering was observed. The initial analysis using the methods described in Pritchard et al. (2000) determined an optimum of four distinct genetic clusters across Europe. However, the resultant barplots (Figure 3) show high levels of admixture making it difficult to distinguish clusters. Instead, the Evanno method (Evanno et al., 2005) suggests that two clusters are optimal (Figure 5.), a result much easier to interpret, with one cluster originating from the UK populations and another from mainland Europe. Whilst this makes some biological sense, with populations being separated by a sea barrier, it should be noted that the Evanno method can be biased towards $K = 2$ and should therefore be interpreted with caution (Meirmans, 2015). In this instance, $K = 2$ supports the other findings of the study indicating some level of isolation between the UK and mainland Europe. Whilst UK populations displayed significantly lower genetic diversity to the mainland, as is often seen with island populations (Frankham, 1997), levels in UK populations are still very high, possibly due to the relatively minimal isolation and large size of the UK, reducing the effects of inbreeding and genetic drift (Furlan et al., 2012).

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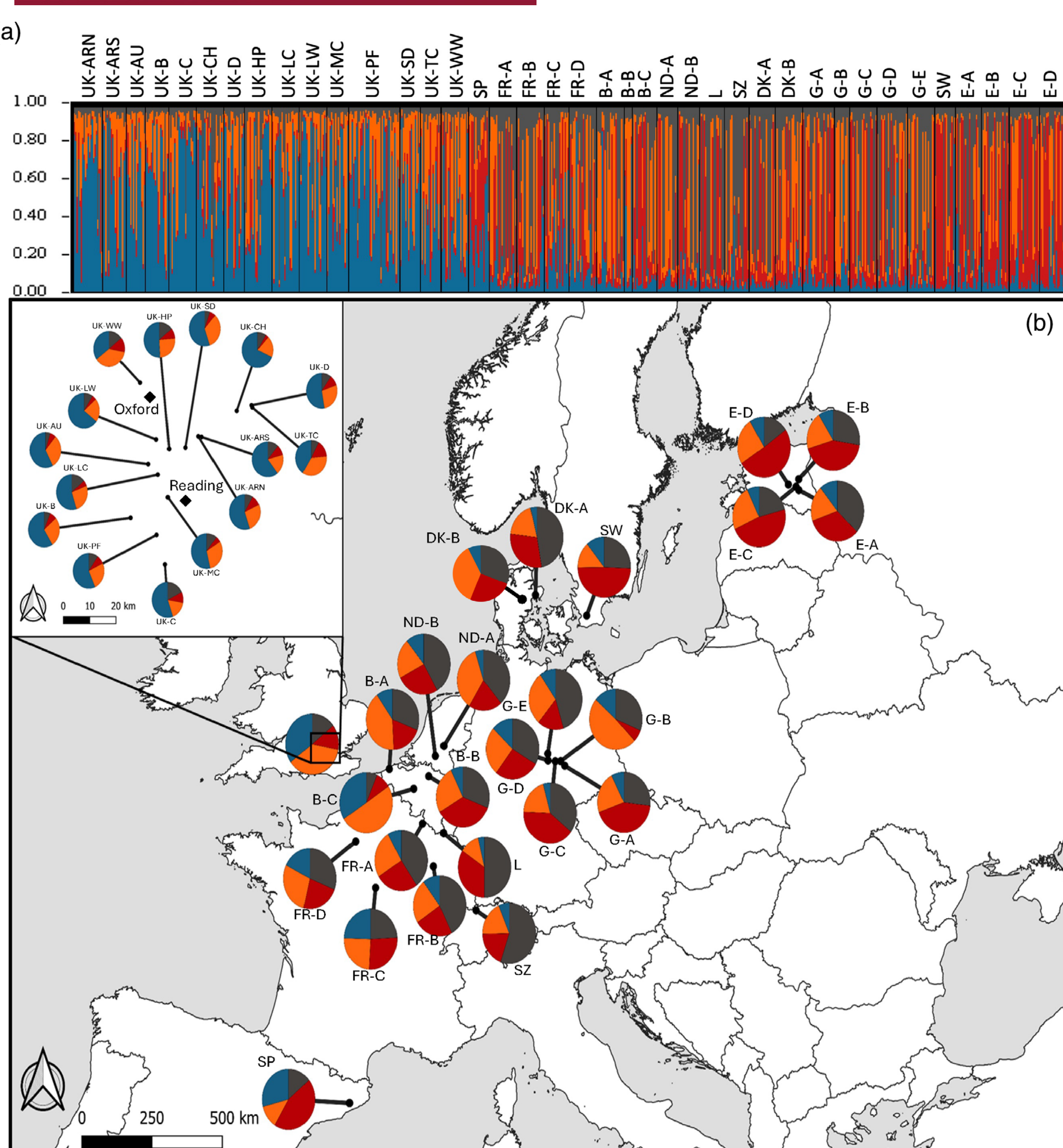

FIGURE 4 Bayesian cluster analysis in STRUCTURE (a) Individual membership coefficients for *Maniola jurtina* across Europe where $K = 2$ (two clusters shown as red and blue), and (b) subsequent geographic distribution of these clusters. Initial estimation of *K* for *M. jurtina* using median values of LnPr(X|K) (Pritchard et al., 2000) and estimation of Delta *K* (Evanno et al., 2005) can be seen in Figure S4.

Limitations, considerations and recommendations

Despite the consistency of our findings, some limitations should be noted. All analyses are restricted by the low number of microsatellites. This is most apparent in the results of the STRUCTURE analysis of European populations, where the removal of one locus resulted in the removal of any meaningful population clustering. Additionally, low sample numbers at some sites should be noted. Future studies would benefit from either more microsatellites or the application of next-generation sequencing techniques such as single nucleotide polymorphisms (Helyar et al., 2011; Pertoldi et al., 2021).

Collecting and archiving whole samples provides redundancy in the case of failed DNA extractions, as well as further research opportunities, for example, wing pattern analysis, and future-proofing, but whole sample collection is not always possible or practical (Hamm et al., 2010). Instead, lower-impact methods could be employed including the sampling of larvae as in Pertoldi et al. (2021) or the use of non-lethal sampling techniques (De Ro et al., 2021; Hamm et al.,

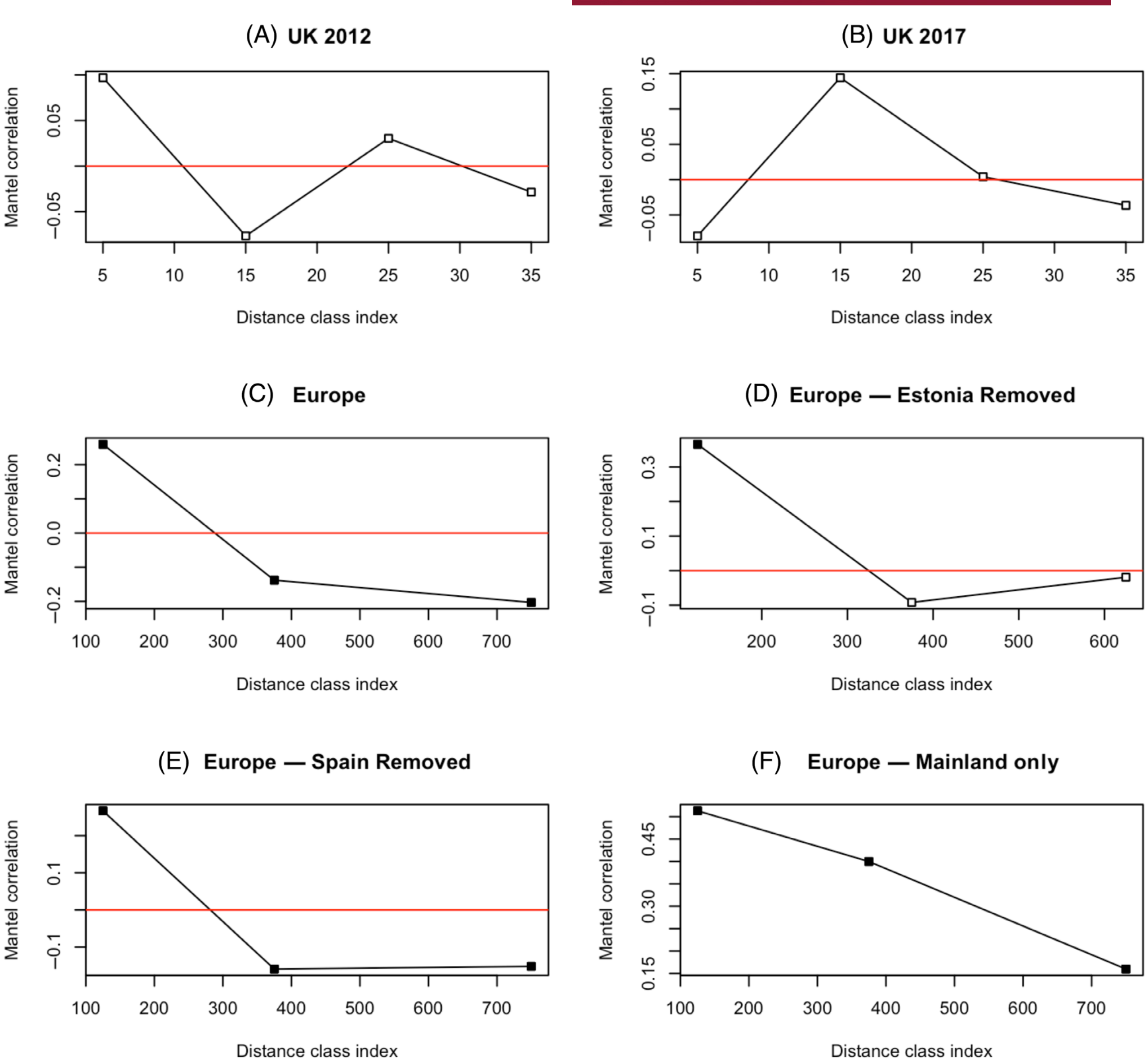

FIGURE 5 Mantel correlogram showing isolation by distance correlations across distance classes (distance classes in KM). (a) = 2012 data set. (b) = 2017 data set. (c) = Full Europe data set. (d) = Europe data set with Estonian sites removed. (e) = Europe data set with Spanish site removed. (f) = Europe data set with UK sites removed. Black squares indicate statistically significant spatial autocorrelation in that distance class. Mantel correlation values >0 indicate positive spatial autocorrelation and mantel correlation values <0 show negative spatial autocorrelation.

2010; Keyghobadi et al., 2009; Koscinski et al., 2011; Broeck et al., 2017; Vila et al., 2009). In this study, DNA extractions were carried out using leg tissue, with only a few samples requiring repeated extractions. This suggests that the level of redundancy provided by whole organism sampling may be unnecessary and a more conservative collection approach, for example, leg sampling could be employed. This would allow the monitoring of genetic diversity in rare Lepidoptera species and could also increase public support and involvement for similar monitoring schemes, as volunteer collectors are more likely to engage with projects using non-lethal sampling. Biodiversity monitoring schemes depend on citizen scientists and therefore if genetic diversity monitoring is to become a part of the standard monitoring toolbox, the potential trade-off in increased training requirements required for non-lethal sampling would likely be outweighed by an increased number of volunteer samplers.

In addition to revealing spatiotemporal patterns in the genetic diversity of *M. jurtina*, the BGEMS pilot has been successful in providing information that could inform future genetic monitoring schemes. First, if genetic diversity monitoring schemes are to be established, they should, where possible, utilise already existing monitoring networks. For example, the UKBMS has over 3000 monitoring sites and genetic material, collected from just a fraction of these sites by the same volunteers recording species, could have a huge impact on our understanding of species' genetic diversity. Additionally, current monitoring schemes have well-established communication and logistical networks that if shared could reduce the effort needed to set up an

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additional scheme from scratch. A major, additional benefit to working with pre-existing monitoring schemes is the availability of long-term abundance data, allowing for direct comparisons between genetic diversity and population abundance. Whilst combining with pre-established schemes would be hugely beneficial, it would also bring significant issues. Aside from the potential need to train volunteers, expanding monitoring schemes will require dedicated lab protocols and facilities, specifically funded for the purpose. Such facilities exist for DNA barcoding (e.g., Bold Systems v4, 2024) and genome sequencing (e.g., Darwin Tree of Life, 2024), suggesting a willingness to fund genetic initiatives.

Assuming funding for a full genetic diversity monitoring scheme is secured, the efficient use of these funds will be crucial. One element to consider is the regularity at which samples should be collected. Our analysis showed minimal changes in genetic diversity over time, therefore annual monitoring may not be required for a common species with relatively stable population dynamics such as *M. jurtina*. Using pre-existing monitoring data (e.g., UKBMS) to determine levels of population fluctuations would be a valuable starting point, as species with unstable population dynamics are more likely to show changes in genetic diversity over shorter periods and therefore require more frequent monitoring. Additionally, the choice of molecular marker should be considered, not just in terms of cost, but required recorder effort. The reducing costs of next-generation sequencing (Bruford et al., 2017) means a wider range of molecular techniques could be implemented, either through the characterisation of microsatellite markers via methods including Illumina sequencing (e.g., Kroeze et al., 2022) or the use of alternative markers such as Single Nucleotide Polymorphisms (SNPs) (Saarinen, 2015). SNPs offer a further advantage in that fewer samples are required to attain reliable estimates of population genetics (Nazareno et al., 2017).

A valuable addition to this work would be the inclusion of historical specimens, to allow the comparison of historical and contemporary genetic diversity, as has been done for other Lepidoptera (Saarinen and Daniels, 2012; Ugelvig et al., 2011). This would provide evidence as to whether the perceived high levels of genetic diversity reported here are an example of shifting baseline syndrome (Papworth et al., 2009).

Conclusion

The approach used here has merit in revealing spatiotemporal patterns of biodiversity change at multiple levels, however, many studies of a similar nature are required if we are to develop the capacity to track the progress of biodiversity targets over the next decade. The refinement and upscaling of such initiatives are urgently needed if the commitments of the Convention on Biodiversity are to be fulfilled.

AUTHOR CONTRIBUTIONS

Matthew P. Greenwell: Investigation; writing – original draft; formal analysis; project administration; data curation; methodology. **Marc S. Botham:** Conceptualization; data curation; writing – review and editing; methodology. **Michael W. Bruford:** Conceptualization; funding acquisition; methodology; writing – review and editing; supervision. **John C. Day:** Conceptualization; funding acquisition; writing – review and editing; methodology; supervision; investigation. **Melanie Gibbs:** Investigation; writing – review and editing. **Toke T. Høye:** Writing – review and editing; investigation. **Dirk Maes:** Writing – review and editing; investigation. **Ian Middlebrook:** Writing – review and editing; investigation; data curation. **Martin Musche:** Investigation; writing – review and editing. **Lars B. Pettersson:** Investigation; writing – review and editing. **David B. Roy:** Conceptualization; methodology; writing – review and editing; data curation. **Josef Settele:** Investigation; writing – review and editing. **Constantí Stefanescu:** Investigation; writing – review and editing. **Tiit Teder:** Investigation; writing – review and editing. **Nia E. Thomas:** Writing – review and editing; formal analysis. **Kevin Watts:** Conceptualization; investigation; methodology; writing – review and editing; supervision; funding acquisition. **Tom H. Oliver:** Conceptualization; investigation; funding acquisition; methodology; writing – review and editing; formal analysis; supervision.

ACKNOWLEDGEMENTS

Thanks to Johan Broidioi, Anne Eskildsen, Luke Evans, Lisbeth Hordley, Elisabeth Kühn, Xavier Mestdagh, Matthias Plattner, Chris van Swaay, Pieterjan Vervecken and Irma Wynhoff for sample collection. Thanks to David Greenwell for additional computational support. Matthew P. Greenwell acknowledges PhD studentship funding from the SCENARIO NERC Doctoral Training Partnership grant NE/L002566/1. Melanie Gibbs was funded through the UKCEH National Capability Fund NEC05106 WP2: Mechanisms of Resilience across ecological scales. Martin Musche was supported by the projects eLTER PLUS (Grand Agreement No. 871128) and EUROPABON (Grand Agreement No 101003553) funded under the EU's research and innovation funding programme HORIZON 2020. Toke T. Høye Acknowledges funding from 15. Juni Fonden (grant no. 2023-0140) and Aage V Jensens Naturfond (grant no. N2023-0017). Tiit Teder was funded by the Estonian Research Council (grant no. PRG741) and the Internal Grant Agency of the Faculty of Environmental Sciences, Czech University of Life Sciences Prague (grant no. 42900/1312/3141). The UK Butterfly Monitoring Scheme is organised and funded by Butterfly Conservation (BC), the UK Centre for Ecology and Hydrology (UKCEH), the British Trust for Ornithology (BTO) and the Joint Nature Conservation Committee (JNCC). The UKBMS is indebted to all volunteers who contribute data to the scheme.

CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are openly available in Mendeley Data at DOI: 10.17632/kfz2fbrkdx.1 and in the Environmental Information Data Centre available at DOI: https://doi.org/10.

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5285/6c6c9203-7333-4d96-88ab-78925e7a4e73 and DOI: https://doi.org/10.5285/180a1c76-bceb-4264-872b-deddfe67b3de.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

Data S1: Site location data and collaborating organisations.

Table S1. *M. jurtina* sampling locations across Europe.

Figure S1. *M. jurtina* European distribution (reproduced from Tolman, 1997) and sampling locations.

S2. PCR conditions and concentrations.

S3. Landscape Genetics Analysis.

Figure S2. Landscape surfaces used in the ResistanceGA analysis of Southern England.

Table S2. Model selection results the from ResistanceGA analysis.

Table S3. Summary results of the bootstrap analysis conducted using the *resist.boot* function from *ResistanceGA*.

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S4. Microsatellite analysis.

Table S4. Composite linkage disequilibrium test outputs.

Table S5. Locus by populations estimated null allele frequencies.

S5. Population genetic diversity.

Table S6. Sample sizes and genetic diversity for *M. jurtina* UK populations.

S6. Genetic structure, differentiation and gene flow.

Figure S3. STRUCTURE individual assignment bar plots.

Figure S4. (a) Initial estimation of *K* for *M. jurtina* using median values of LnPr(X|K). (b) Estimation of Delta *K*.

Figure S5. Comparison of STRUCTURE assignments and estimations of *K* when using all loci available and removing the locus Mj4870.

Table S7. Pairwise F_{ST} values between twelve pairs of sites in 2012.

Table S8. Pairwise F_{ST} values between fifteen pairs of sites in 2017.

Table S9. Pairwise F_{ST} values between 36 pairs of sites across Europe.

How to cite this article: Greenwell, M.P., Botham, M.S., Bruford, M.W., Day, J.C., Gibbs, M., Høye, T.T. et al. (2025) Monitoring spatiotemporal patterns in the genetic diversity of a European butterfly species. *Insect Conservation and Diversity*, 18(1), 80–94. Available from: https://doi.org/10.1111/icad.12786

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