Received: 18 February 2021 | Accepted: 18 May 2022

DOI: 10.1111/een.13174

Ecological Entomology

ORIGINAL ARTICLE

Diverging effects of geographic distance and local habitat quality on the genetic characteristics of three butterfly species

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Funding information
Fonds De La Recherche Scientifique - FNRS, Grant/Award Number: CDR grant J.0143.13F; Service Public de Wallonie

Associate Editor: Michael Bonsall

Abstract

1. The genetic characteristics of neutral loci in natural populations are shaped by the interplay between genetic drift and gene flow that themselves result from key ecological and evolutionary processes.
2. Because of their influence on population size, habitats characteristics may influence substantially populations' genetic characteristics. However, past studies focused primarily on variables related to habitat quantity while habitat quality was largely overlooked.
3. We therefore combined genetic data to detailed habitat descriptions of three sympatric butterfly species having different ecological needs to quantify the relative contribution of geographic distance and habitat quality/quantity on their genetic characteristics.
4. In all species, the genetic diversity was greater in larger and good quality patches. The genetic differentiation of *Lycaena helle* (specialist species with limited dispersal) was mostly explained by geographical distances. This effect was dampened by habitat quality as populations inhabiting low quality patches had higher genetic differentiations indicating that the constraints set by local environmental factors increased genetic drift. In *Boloria eunomia* (specialist species with greater dispersal), the genetic differentiation was only influenced by geographic distance. Finally, in *Brenthis ino* (more generalist species with greater dispersal), isolation-by-distance was weak and habitat characteristics were unrelated to genetic differentiation.
5. The different effect of habitat quality on these species' genetic characteristics suggests that fundamental ecological traits underpin their response to habitat degradation. In *L. helle*, a species with major conservation concerns, these results show that preserving habitat quality and connectivity among populations' are important to ensure the maintenance of fully functional metapopulations.

KEYWORDS
dispersal, eutrophication, genetic diversity, habitat quality, isolation-by-environment, specialist species

INTRODUCTION

The genetic diversity and differentiation in natural populations result from the interplay between gene flow and variations in effective population size that underpin the rate of genetic drift. In systems where individuals' dispersal is constrained by geography, gene flow cannot counter-balance as effectively the effect of random genetic drift in local populations (Slatkin, 1993; Wright, 1943). This results in a decline in the genetic diversity within small populations and the accumulation of genetic differences between populations with geographic distance that characterises patterns of isolation-by-distance. This process is strengthened when populations become isolated; in them, strong declines in population size may result in increasing inbreeding rates and trigger extinction vortexes (Frankham, 2010; Saccheri et al., 1998; Spielman et al., 2004). Similarly, the genetic differentiation is greater and the genetic diversity is lower in populations at the forefront of a range expansion or in recently recolonised populations of a metapopulation because of the small population size of these newly established self-sustained populations, the genetic similarity of (re)colonisers originating from a limited number of source populations, and their partial isolation (Cosentino et al., 2012; Garroway et al., 2011; Haag et al., 2005; Le Corre & Kremer, 1998; Peter & Slatkin, 2013; Whitlock & McCauley, 1990).

In addition to the geographical constraints on gene flow, the levels of genetic diversity and differentiation might also result from differences in local environmental conditions acting either through local adaptations (Hendry, 2004; Nosil et al., 2005; Wang & Bradburd, 2014) or a decline in local effective population size (Prunier et al., 2017). More specifically, adaptations to local environmental conditions can explain a substantial amount of variance in genetic diversity and differentiation through natural or sexual selection against immigrants, reduced hybrid fitness, or biased dispersal (Bradburd et al., 2013; Wang, 2013; Wang & Bradburd, 2014). The effect of declining population sizes on their genetic diversity and differentiation and the ensuing increase in extinction risks is at the heart of the field of conservation genetics (e.g. Caplins et al., 2014; Frankham, 2010; Frankham et al., 2001; Joyce & Pullin, 2003; Wellenreuther et al., 2011). However, the role of the degradation of local habitats is often not directly tested while it has been shown that habitat characteristics themselves can be associated with increased genetic differentiation in birds (Edelaar et al., 2012; Porlier et al., 2012), insects (Marcombe et al., 2013), and plants species (Giles & Goudet, 1996; Vergeer et al., 2003; De Vere et al., 2009; reviewed in Wang & Bradburd, 2014). In species with conservation concerns, for which habitat degradation and fragmentation are major issues, it is therefore important to quantify the relative contribution of geographic isolation and local habitat deterioration on their genetic diversity and differentiation to identify the most relevant conservation measures (i.e. focus on preserving habitat quality and/or favouring gene flow).

A major challenge to quantifying the relative contribution of geographic distance vs local environment on species genetic characteristics is the need to quantify accurately species habitat quality and its deterioration. Indeed, species' habitats can be defined as the areas containing all the biotic and abiotic factors enabling individuals' of a given species to complete their life cycle (Dennis et al., 2003; Hall et al., 1997). Degradations of these habitats are therefore characterised by directional changes in these biotic and abiotic factors over space and time that may result in a decline in individuals' fitness (Dennis & Eales, 1997; Thomas et al., 2001). While studies investigating functional resource-based habitats characterise individuals' multifaceted needs over their entire lifespans (e.g Bruton et al., 2015; Jugovic et al., 2017; Maes et al., 2014; Turlure et al., 2010), past studies linking local environmental conditions to spatial genetic structures often used a limited set of environmental descriptors such as habitat size (Caplins et al., 2014; Cushman et al., 2012; Sunny et al., 2014), island size (Petren et al., 2005), time elapsed since the most recent extinction (Giles & Goudet, 1996; Haag et al., 2005; Ingvarsson et al., 1997; McCauley et al., 1995; Whitlock, 1992). Studies that used a much more comprehensive set of habitat descriptors (Bradburd et al., 2013; Wang, 2013) clearly showed that quantifying accurately the relative contribution of geographic distances and local habitat characteristics on species spatial genetic differentiation requires understanding species multifaceted needs during their entire life.

These processes are often studied in single species because understanding 'habitat quality' is by definition a species-specific task, but using related species inhabiting the same area is key to understand how slightly different ecological needs can lead to different responses to the same constraints (Schwenk & Donovan, 2011). More specifically, increasing isolation of patches of favourable habitats and/or habitat deterioration might have major consequences for specialist species because of their more limited dispersal than generalist species (Koivula et al., 2002; Krauss et al., 2003) and their narrow diet/set of environmental conditions in which they can complete their life cycle (Pöyry et al., 2009; Thomas et al., 2001; Warren et al., 2001), making them particularly sensitive to environmental variation (Krauss et al., 2003). In metapopulation genetics, there has been much emphasis on founders' effects and high genetic differentiation levels and low genetic diversity have usually been found in newly (re)colonised habitat patches (Castric & Bernatchez, 2003; McCauley et al., 1995; Slatkin, 1977; Wade & McCauley, 1988). In specialist butterfly species, habitat deterioration may act like the senescence of a subpopulation, in which the decline in effective population size increases the rate of genetic drift and hence increases genetic differentiation (Blomqvist et al., 2010; De Vere et al., 2009; Malone et al., 2003; Pöyry et al., 2009). Conversely, more generalist species, thanks to the wider range of resources and habitats that they can use and their greater dispersal, can have larger population sizes and therefore may be less prone to genetic drift (Dawson, 2012; Steele et al., 2009). These processes might explain why generalist species have weaker patterns of isolation-by-distance and isolation by environment than specialist species (Krauss et al., 2003; Pöyry et al., 2009) Recent comparative analyses supported this hypothesis (Dapporto et al., 2019; Scalercio et al., 2020) but as these were based on mtDNA, there is a clear need for studies using more variable genetic markers to confirm these results. By carefully selecting species that are phylogenetically close and experience the same geographical/environmental constraints, it would be possible to understand the relative sensitivity of

each species to the environmental constraints acting on gene flow, genetic drift, and effective population size.

In this study, we quantified the relative contribution of geographic distances and local habitat characteristics on the spatial genetic differentiation of three butterfly species (the Violet copper *Lycaena helle*, the Bog fritillary *Boloria eunomia*, the Lesser Marbled fritillary *Brenthis ino*) that inhabit nutrient poor-wet meadows in the Belgian Ardenne Region. These species differ in several ways. *L. helle* is primarily found in mountains and in lowland nutrient poor wet meadows (see Habel et al., 2011). This species is considered vulnerable in Western Europe because its remnant populations have strongly declined during the last decades (Van Swaay et al., 2010). Caterpillars of *L. helle* in our study area feed on a single host plant, *Bistorta officinalis*, while adults are opportunistic in their nectar use (Turlure et al., 2009) and have limited dispersal behaviour (Bauerfeind et al., 2009). *B. eunomia* is a larger specialist species also inhabiting peat bogs and wet hay meadows (Turlure et al., 2011) whose local populations have been declining in European countries and is listed as vulnerable in Belgium (Rosès et al., 2019). *B. eunomia* has comparatively greater dispersal abilities than *L. helle* (Nève et al., 2008; Schtickzelle et al., 2013) and caterpillars and adults of this species feed on *B. officinalis* leaves and nectar, respectively. *B. ino* is a more generalist species with broader habitat requirements and good dispersal abilities (Fric et al., 2010). Its range has slightly been expanding in Western Europe because of the eutrophication of wet meadows that led to the expansion of its main larval host plant *Filipendula ulmaria* (Van Swaay & Warren, 1999; Zimmermann et al., 2005). The population dynamics of *B. ino* differ substantially from the declines observed in *L. helle* and *B. eunomia* in the studied area, making it particularly useful for comparative purposes. Using these three species, we first characterised the quality of 24 nutrient poor wet meadows using compound measures of vegetation plots and the chemical composition of *B. officinalis* leaves. We then quantified in each species: the local genetic diversity in each patch, the overall genetic differentiation, and the patterns of isolation-by-distance (hypothesising that *B. ino* has a weaker genetic differentiation and isolation-by-distance than the other two species). Finally, we tested the hypotheses that the genetic diversity in each species is higher in large area and/or good quality habitat patches, and that the genetic differentiation increases in small patches of low quality. Obviously, three species cannot enable us to directly quantify the effect of dispersal abilities and/or generalisms on their genetic characteristics, but we expected that the genetic differentiation of *L. helle* might be more strongly affected by local habitat characteristics than the other species because of its limited dispersal abilities and strong specialisation.

METHODS

Study sites and data collection

We selected 24 nutrient poor wet meadows that potentially hosted populations of these three butterfly species. These meadows were located along three different valleys of the Belgian Ardenne region (eight sites in the Bellemeuse valley, nine sites in the Bihain valley, six sites in the Lienne valley and one isolated site; Figure 1; Table S1). During the flight period of each species, we visited each site approximately every third day under suitable weather conditions (i.e. no strong wind, no or few clouds, and air temperature >16°C; flight period for *L. helle*: early May-end of June; *B. eunomia*: end of May-end of June; *B. ino*: early June-mid July). For each visit, we recorded the date, site location, and the number of adults encountered (both males and females); each encountered imago was captured with a net, marked with a unique identification number with a permanent pen, and released immediately. We collected one leg for a subset of the imagoes captured in each study population and placed them in an individual 1.5 ml microtube filled with 90% ethanol. Individual samples were stored in −20°C freezers until DNA extraction. We pooled samples collected over the three years to reach a greater statistical power (305 samples collected for *L. helle* over 2009–2012; 673 samples collected for *B. eunomia* over 2012–2014; 656 samples collected for *B. ino* over 2012–2014; Table S1).

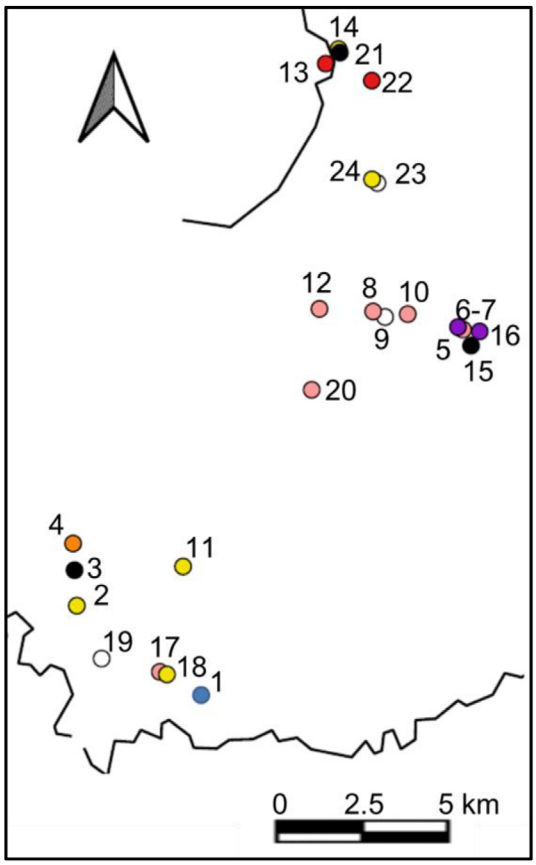

FIGURE 1 Map of the study area with the location of the wet meadows in which habitat quality was measured (see Table S1 for coordinates). The presence of each species in each patch is denoted as: Purple dots (*Lycaena helle* only); orange dots (*Boloria eunomia* only); yellow dots (*Brenthis ino* only); red dots (*B. eunomia* and *B. ino*); pink dots (*L. helle* and *B. eunomia*); blue dot (*L. helle* and *B. ino*); black dots (*L. helle*, *B. eunomia*, and *B. ino*); white dots (none of the three species present)

Habitat and host plant characteristics

In each site, several vegetation plots (1 m^2 grid) were randomly placed. Each plot was divided in 25 equal squares and plant abundance was computed based on its presence on each square (i.e. on a 0–25 scale). We summarised vegetation data of all 349 plots by using the first axis of a Detrended Correspondence Analysis (DCA1) implemented in the r-package 'vegan' (Oksanen et al., 2018); the analysis was based on the abundance of the plant species. Vegetation plots from the Pisserotte site were discarded from the analysis because this site is very large and heterogeneous, making measures of species diversity and abundance extremely variable and inaccurate using this sampling scheme; this site was therefore given the average of all DCA1 values measured across all sites.

Host plant quality can be defined as a set of chemico-physical attributes that affects the growth rate of herbivorous insects and their subsequent fitness (Mevi-Schütz & Erhardt, 2005; Teder et al., 2014). We used two chemically based measures of host plant quality: the Carbon: Nitrogen (C:N) ratio, and the concentration in two potentially harmful heavy metals (Cadmium –Cd, and lead -Pb). The C:N ratio has been linked to major changes in growth rates and fitness in multiple species (e.g. in lepidopterans: Obermaier & Zwölfer, 1999; Han et al., 2014). Full description of the methods used to quantify the C:N ratio and leaves's Cd and Pb concentrations can be found in Lebigre et al. (2018). Briefly, we randomly collected 10–15 *B. officinalis* leaves in each study site (*B. officinalis* and *F. ulmaria* being both moderately nitrophilic and the small size of each habitat patch makes it unlikely that the C:N ratio would differ substantially between species). These leaves were dried in the laboratory and passed through a mill (Retsch SK100; grid size 0.25 mm). The dried samples (10 mg) were then placed in an oven at 65°C for 24 h. We measured samples' chemical content using an elemental analyser following flash combustion (Thermo) and a spectrophotometer (Spectraa-300 Varian). The amount of Nitrogen, Carbon, Cadnium, and Lead present in the dried leaves was then measured in $mg.g^{-1}$.

The last habitat characteristic we used was the log-transformed larval host plant area of these species within each studied patch (the area occupied by *B. officinalis* for *L. helle* and *B. eunomia*, and the area covered by *F. ulmaria* for *B. ino*). This area was delineated using maps on the field and the host plant area was measured in QGIS (QGIS development team 2019) and used in the analyses following a log-transformation. As DCA1, the C:N ratio, and concentrations of Cd and Pb were correlated, we carried out a principal component analysis (PCA) using these four variables. We then extracted the first axis of this PCA, which explained 55% of the total variance, and used it as a measure of habitat quality (hereafter 'PC1'; host plant area was not related to the other parameters and was therefore used directly as explanatory variable).

Genotyping

DNA extraction was carried out using a phenol-chloroform-isoamyl alcohol method (Vandewoestijne & Baguette, 2002). All DNA extractions were treated by RNase (2 µl of RNase 100 µg/mL; incubation: 30 min at 37°C). Microsatellite loci and their amplification conditions are described for *L. helle* in Habel et al. (2008), for *B. eunomia* in Legrand et al. (2014), and for *B. ino* in Lebigre et al. (2015). Multiplex amplifications (Table S2) were carried out using QIAGEN Multiplex PCR Kit (Qiagen, Venlo, Netherlands) with the following protocol: denaturation step (95°C for 15 min), 25 DNA amplification 25 cycles (94°C for 30 s, the annealing temperatures for 90 s, 72°C for 90 s), and a final elongation step (72°C for 45 min). We then checked the amplification by loading samples on 1% agarose gels. Forward primers were labelled using Applied Biosystems' dyes NED, 6-FAM, PET, and VIC (Carlsbad, CA, USA). Individuals were genotyped using a Prism® 3100 genetic analyser and allele sizes were scored using GeneMapper 3.7® (both from Life Technologies). Allele sizes were scored using an internal standard (600-LIZ, Life Technologies).

In total, we used six, ten, and 15 loci to genotype samples of *L. helle*, *B. eunomia*, and *B. ino*, respectively (Table S2). Possible linkage disequilibrium and the Hardy–Weinberg (HW) equilibrium were assessed using FSTAT 2.9.3.2 (Goudet, 1995). We found no significant linkage disequilibrium across all loci-site combinations (adjusted alpha level for each species). In *L. helle*, there was a significant deficit of heterozygous individuals in three of the six loci (lheB06, lhe14, lheD01, Table S1). As lheB06 fitted the Hardy–Weinberg expectations in most sites (10 of the 12 sampled populations), we decided to analyse the data including this locus. For *B. eunomia* and *B. ino*, there was a significant deficit of heterozygous individuals in five and in six of the loci genotyped, respectively (Boleun01, Boleun08, Boleun11, Boleun25, Boleun33, Bi_112, Bi_115, Bi_125, Bi_128, Bi_131, Bi_135, Table S2). All subsequent analyses were carried out without these loci. Across the remaining loci, the overall mean number of alleles per locus was relatively high in each species (*L. helle*: 14.25, *B. eunomia*: 10.20, *B. ino*: 9.56; Table S2). We used the software POWSIM (Ryman & Palm, 2006) to test the power of these loci to detect small genetic differentiations (0.0050 and 0.0025) and risks of type I errors with these genetic markers. These analyses showed that our markers in each species enabled us to identify between 89%, 92%, and 99% of these levels of genetic differentiation levels depending on the method and species (Table S3) while the risks of type I error ranged between 5% and 12% (Table S3).

Allelic richness, genetic differentiation, and their links with local habitat characteristics

Estimates of the allelic richness (AR) in each population were calculated using a randomisation tests implemented in hierFSTAT (Goudet, 2005) based upon the smallest number of individuals sampled in a study site for each species. Hence, AR was calculated for each site based on six, 11, and 23 individuals for *L. helle*, *B. eunomia*, and *B. ino*, respectively (we did not estimate AR in Chapons and Grande Fange for *L. helle* and Langlire B for *B. ino* because of their particularly small sample sizes: 4, 5, and 5 individuals genotyped). To test this hypothesis that patch area and quality influenced each species'

AR, we used the AR calculated in each patch for each species' as the response variable in a linear regression with patch area (log-transformed), patch quality (PC1), and species' identities as explanatory variables (we used species identities to account for species differences in mean AR). The full model consisted in all explanatory variables and the two-way interactions between patch area and quality with species' identities. We compared the performance of this full model with a set of simplified models using AICc (the simplest model consisted in only the species identities). Model-averaged coefficient estimates with unconditional *SE* and unconditional 95%CI were calculated for models with differences in AICc lower than 2. All these steps were carried out using the r-package 'MuMIn' v1.40.4 (Barton, 2018). As spatial coordinates were not accounted for in these analyses, we tested whether there was significant spatial autocorrelations of the residuals of the best model using Moran tests (based on the two nearest sampling sites) implemented in the r-package 'spdep' (Bivand et al., 2013).

We quantified the overall population genetic differentiation within each species using Jost's D (D_{est}) estimated via the r package "diveRsity" (Keenan et al., 2013). The significance of D_{est} was estimated using permutation tests (1000 permutations). Patterns of isolation-by-distance were quantified using a Mantel test implemented in the r package 'ade4' (Dray & Dufour, 2007) with 999 randomisations using standardised matrices of genetic distance ($D_{est}/[1-D_{est}]$) and the logarithm of the Euclidean distances (Rousset, 2001). We tested whether habitat characteristics influenced the genetic differentiation of *L. helle* using a partial distance-based redundancy analysis to explain the genetic distances (*Y*) as a function of environmental variables (*X*) and Euclidian distances (*Z*) (partial db-RDA; Legendre & Andersson, 1999; McArdle & Anderson, 2001). The pairwise D_{est} values were used as dependent matrices following principal coordinate analysis (PCoA) in which the first two or three axes were retained, explaining 90% (*L. helle*, 3 axes), 98% (*B. eunomia*, 2 axes), and 91% (*B. ino*, 3 axes) of the total inertia (Legendre & Andersson, 1999). The *X* and *Z* matrices consisted in five explanatory variables associated with habitat quality and quantity for *X* (patch quality- PC1, patch area -larval host plant area, log-transformed) and the first three axes of a PCoA using matrices of Euclidian distances between all sampled populations of each species for *Z* (log-transformed Euclidian distances). These axes explained 50%, 50%, and 63% of the total variation in Euclidian distances for *L. helle*, *B. eunomia*, and *B. ino*, respectively. We followed the procedure outlined in Borcard et al. (2011) for these analyses. We therefore first carried out a stepwise backward model selection procedure on the RDAs relating $Y \sim X$ ('RDA1') and $Y \sim Z$ ('RDA2') to identify the most parsimonious models. We then estimated the variance explained by the model $Y \sim X + Z$ ('RDA3') parameterised only with the significant variables of *X* and *Z*, and calculated the adjusted R^2 for each these three first RDAs. We then partitioned the overall variance due to (i) the significant environmental variables (i.e. the difference in variance explained by RDA3 and RDA2), (ii) the Euclidian distances (i.e. the difference in variance explained by RDA3 and RDA1), (iii) the shared contributions of the environmental variables and the Euclidian distances (calculated as the amount of variance explained by RDA1-(RDA3-RDA2) or RDA2-(RDA3-RDA1)). Collinearity was limited

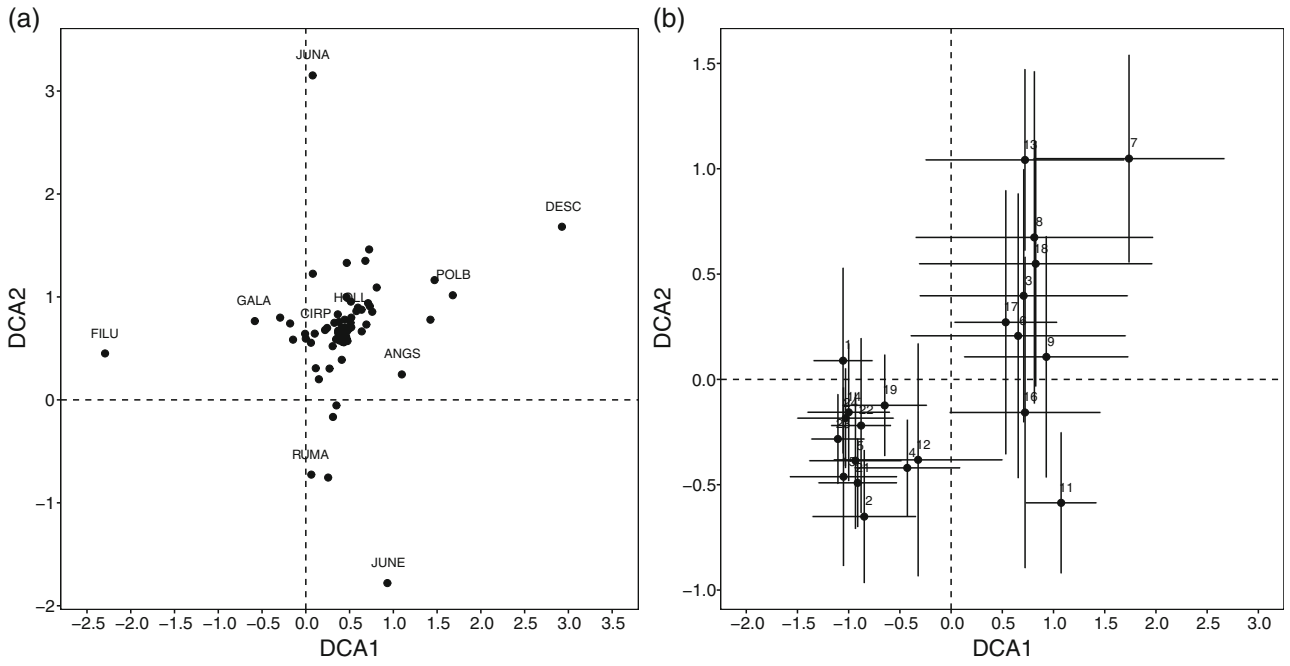

FIGURE 2 Graphical representation of the Detrended correspondence analysis for plant species (panel A) and sites (panel B, mean DCA scores across all quadrats and *SEM*). Study site identification numbers are explained in Table 1. Plant species abbreviations are: *Angelica sylvestris* (ANGS), *Cirsium palustre* (CIRP), *Deschampsia cespitosa* (DESC), *Filipendula ulmaria* (FILU), *Galium aparine* (GALA), *Holcus lanatus* (HOLL), *Juncus acutus* (JUNA), *Juncus effusus* (JUNE), *Bistorta officinalis* (POLB), *Rumex acetosa* (RUMA)

TABLE 1 Relative performance of models explaining the variance allelic richness (AR) as a function of wet meadows quality (patch quality 'PQ'; the first axis of a PCA), log-transformed area of each species' larval host plant (patch size: 'PS')

Models	*K*	AICc	ΔAICc	LogLik	w_i	Cum. w_i
PQ + Sp	6	20.8	0.00	−2.77	0.42	0.42
PS + PQ + Sp	7	20.9	0.17	−1.23	0.38	0.80
Sp	5	24.4	3.62	−6.09	0.07	0.87
PS + Sp	6	25.0	4.25	−4.90	0.05	0.92
PQ + PS*Sp	9	25.3	4.51	0.27	0.04	0.96
PQ*Sp	8	26.8	6.06	−2.42	0.02	0.98
PS + PQ*Sp	9	27.5	6.76	−0.85	0.01	0.99
PS*Sp	8	28.8	8.02	−3.40	0.01	1.00
PS*PQ*Sp	11	33.0	12.26	0.77	0.00	1.00

Notes: Species ('Sp') were included in the models as a main effect to account for systematic differences in allelic richness; sites' identities were used as a random effect to account for the non-independence of the AR measured in the same sites in different species. Table entries: Number of parameters (*K*), Akaike's information criterion for small sample sizes (AICc), difference in AICc values with the lowest AICc model (ΔAICc), log likelihood (LogLik), model weight (w_i), and cumulative model weight (cum. w_i).

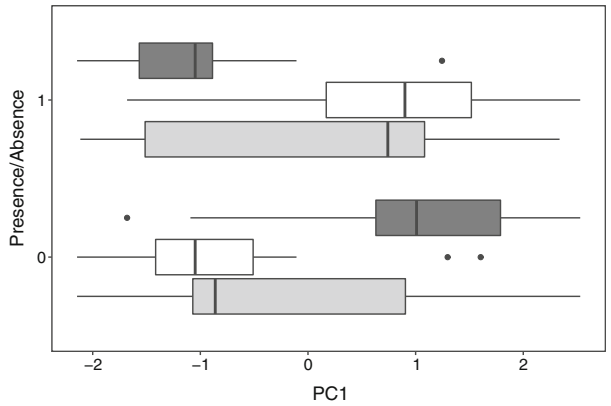

FIGURE 3 Relationship between the quality of patches of nutrient poor wet meadows (measured as the first principal component of a PCA) and the presence/absence of *Lycaena helle* (white box), *Boloria eunomia* (light grey box), and *Brenthis ino* (dark grey box)

across variables used in these analyses as PCoA axes extracted from the Euclidian distances between sites are orthogonal and the correlation coefficients between patch area and patch quality was limited (mean variance inflation factors across full models of RDA1: 1.05; range: 1.01–1.15). All these analyses were performed using the R package 'vegan' and significance tests of the variance partitioning were implemented using permutation test (999 permutations).

RESULTS

Nutrient poor wet meadows quality and species occurrence

The first axis of the DCA (DCA1) explained 62% of the total variation in plant species abundance and positive values of this axis were associated with species often found in nutrient poor wet meadows or peat bogs (e.g. *B. officinalis* and *Deschampsia cespitosa*, Figure 2a) and negative values were associated with plant species of enriched meadows (e.g. *F. ulmaria*, *Galium aparine*). Therefore, DCA1 typically reflected a gradient of enrichment of the wet meadows (Figure 2b). The first axis of the PCA (PC1) performed with DCA1, the CN ratio, and the concentrations in Cd and Pb explained 55% of the total variation across variables. This axis was positively related to DCA1 ($r = 0.51$) and the CN ratio ($r = 0.59$), and negatively related to Cd ($r = -0.43$) and Pb concentrations ($r = -0.43$, $N = 23$ in all cases). PC1 therefore captured the overall degradation of nutrient poor wet meadows. The second axis explained 23% of the overall variation and was related to the leftover variation in Cd and Pb concentrations independent of the first axis. Given the low number of study sites, we decided to solely use PC1 in the subsequent analyses. Across the 24 surveyed sites, *L. helle*, *B. eunomia*, and *B. ino* occurred in 12, 12, and 11 sites (Table 1). While there was no effect of PC1 on the presence/absence of *B. eunomia* (logistic regression: $\beta \pm SE = 0.06 \pm 0.28$, $z = 0.22$, $p = 0.83$; Figure 3), *L. helle* was present in sites with high PC1 value (logistic regression: $\beta \pm SE = 0.78 \pm 0.36$, $z = 2.16$, $p = 0.03$; Figure 3), and *B. ino* was present in sites with low PC1 values (logistic regression: $\beta \pm SE = -1.32 \pm 0.49$, $z = -2.68$, $p = 0.01$; Figure 3).

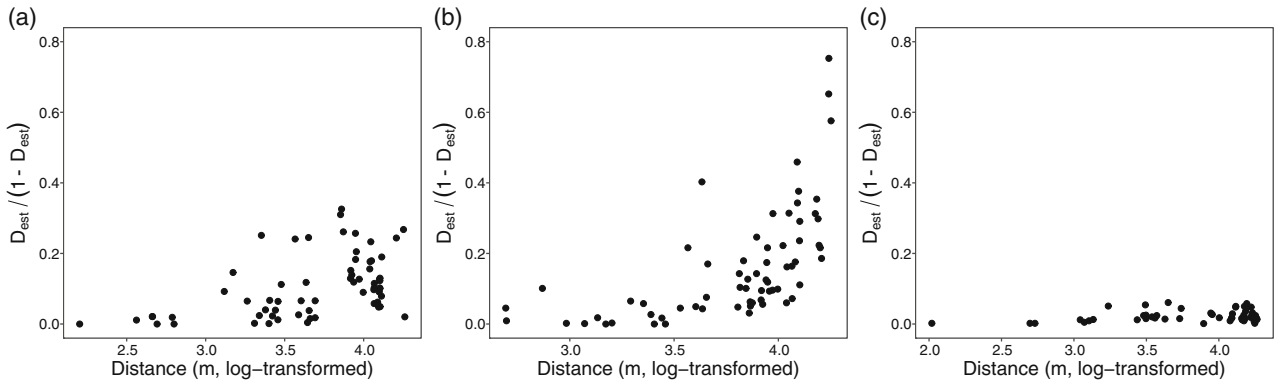

FIGURE 4 Relationship between standardised genetic distances estimated using D_{est} and the Euclidian distances between study sites for *Lycaena helle* (panel A), *Boloria eunomia* (panel B), and *Brenthis ino* (panel C)

Species' allelic richness and genetic differentiation

The levels of allelic richness (AR) differed substantially within species, ranging from 2.97 to 3.58 for *L. helle* (mean = 3.32; *N* = 11), 3.36 to 4.67 for *B. eunomia* (mean = 4.00; *N* = 12), and 4.61 to 5.54 for *B. ino* (mean = 5.12; *N* = 10). The overall genetic differentiation was significant over the entire study area in all species but such structure was substantially greater in both *L. helle* and *B. eunomia* than in *B. ino* (*L. helle*: D_{est} = 0.187; 95%CI: 0.147–0.234; *B. eunomia*: D_{est} = 0.197; 95%CI: 0.169–0.228; *B. ino*: D_{est} = 0.062; 95%CI: 0.041–0.091). Standardised pairwise D_{est} values ranged from 0.000 to 0.326 for *L. helle* (Table S4), 0.000 to 0.753 for *B. eunomia* (Table S5), and 0.001

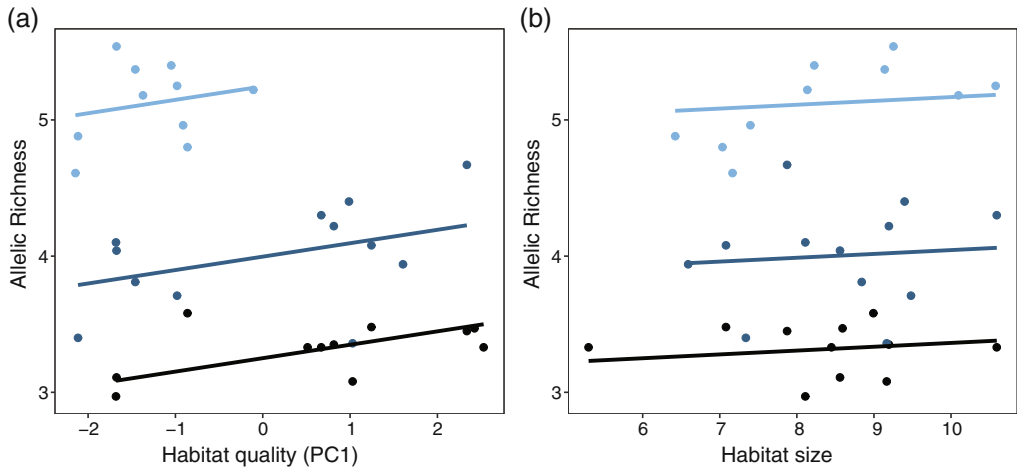

FIGURE 5 Changes in allelic richness with the degree of habitat quality of nutrient poor wet meadows (PC1) and the area of the larval host plant of *Lycaena helle* (black dots and line), *Boloria eunomia* (dark blue dots and line), and *Brenthis ino* (light blue dots and line). Lines represent the predicted values of averaged models

TABLE 2 Results of the variable selection of the distance-based redundancy analysis relating pairwise genetic differentiation of three butterfly species to their local habitats' quality

Species	Model	Removed terms	Retained terms	Df	Inertia ($\times 10^{-3}$)	F	P	Env. Var.	Dist. Var.	Shared var.
L. helle	RDA1	PS		1	4.44	0.06	0.98			
	RDA2	Eucl2		1	0.07	0.68	0.59			
	RDA2	Eucl1		1	0.09	0.96	0.44			
	RDA3		PQ	1	4.44	5.27	<0.01			
	RDA3		Eucl3	1	3.25	3.86	0.02	0.118	0.174	0.101
B. eunomia	RDA1	PS		1	0.86	0.18	0.85			
	RDA1	PQ		1	3.05	0.71	0.52			
	RDA2	Eucl3		1	0.73	0.33	0.71			
	RDA3		Eucl1	1	21.26	10.50	<0.01			
	RDA3		Eucl2	1	6.77	3.34	0.05	–	0.519	–
B. ino	RDA1	PS		1	0.04	0.83	0.49			
	RDA1	PQ		1	0.04	0.85	0.41			
	RDA2	Eucl1		1	0.04	1.09	0.38			
	RDA2	Eucl2		1	0.07	1.78	0.19			
	RDA2	Eucl3		1	0.10	2.38	0.11	–	–	–

Notes: Terms of the full models were sequentially removed according to their *p*-value. The RDA was carried out using the first axes of a PCoA loaded with matrices of genetic distance.

Abbreviations for the terms entered in the db-RDA; 'PS', Log-transformed area of each species' larval host plant; 'PQ', First axis of a PCA describing habitat quality (PCA loaded with the first axis of a DCA, the C:N ratio and the leaves' concentrations in cd and Pb); 'Eucl1-3', The first three axes of a PCoA loaded with matrices of Euclidian distance for each species; Env. Variance: Adjusted R^2 for environmental effects alone; Dist. Var, Adjusted R^2 for Euclidian distance effects alone; shared variance: Adjusted R^2 for the shared effects of environmental and geographic variables. Models consisted in only environmental variables (RDA1), only PCoA axes of Euclidian distances (RDA2), and a final model retaining only the significant effects of RDA1 and RDA2 to partition the variance explained by each component.

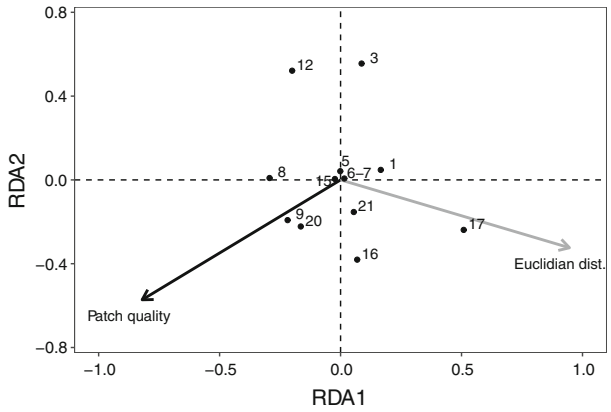

FIGURE 6 Biplot of the distance-based redundancy analyses quantifying the relative contributions of Euclidian distance (grey arrow) and the quality of patches of nutrient poor wet meadows (measured as the first axis of a PCA; black arrows) for *Lycaena helle*

to 0.061 for *B. ino* (Table S6). Geographic distance between pairs of sites explained a substantial amount of the overall genetic variation in *B. eunomia* (Mantel test, $r = 0.589$, $p = 0.001$), and *L. helle*, ($r = 0.466$, $p = 0.003$) but was weaker in *B. ino* (Mantel test: $r = 0.255$, $p = 0.011$; Figure 4). In *L. helle*, there are several cases of nearby sites with strong genetic differentiation while some very distant sites have low genetic differentiation (Figure 4) while in *B. ino*, there is a weak but consistent increase in genetic differentiation (Figure 4).

Effect of the local environment on species' allelic richness and genetic differentiation

The model that best explained the variance in AR was the one with patch quality and species identity as fixed effects (Table 1). Another model with habitat size, habitat quality, and species identities had an AICc difference to the best model lower than 2 (Table 1). We therefore averaged their parameter estimates that showed that both patch area and quality have positive effects on AR of these butterfly species (Table S7; Figure 5), patch quality having a slightly greater effect on AR than patch area. The magnitude of these effects was similar in each species as none of the best models included the interaction terms and there was no clear sign of spatial autocorrelation of the residuals across species ($I = 0.04$, $p = 0.27$).

The stepwise backward selection procedure showed that both the third PCoA axis of the Euclidian distance and habitat quality, but not the host plant area, were significantly related to the genetic differentiation in *L. helle* (Table 2). In this species, habitat quality explained a substantial amount of the overall variation in genetic differentiation among sites as 12% of the overall variance in pairwise D_{est} was explained by differences in patch quality alone and 10% was explained by the shared effects of habitat quality and Euclidian distance (Table 2). Overall, patches with greater habitat quality had lower genetic differentiations and degraded patches had higher genetic differentiation (Figure 6). Only the Euclidian distance (first and second PCoA axes) between sites was significant for *B. eunomia*, explaining 52% of the overall variation of the genetic differentiation and none of the explanatory variables was related to the genetic differentiation of *B. ino* (Table 2).

DISCUSSION

Local populations of many butterflies inhabiting wet meadows have strongly declined due to the effects of climate change and the degradation of their habitat (Fischer et al., 1999). Here, we showed that populations of *L. helle* and *B. eunomia* had a very strong local spatial genetic differentiation at a relatively small spatial scale (20 × 20 km) while the genetic differentiation of *B. ino* (preferring enriched/degraded meadows) was limited. While the allelic richness increased with both patch quality and area in all three species, the effect of local habitat characteristics and geographic distance on species' genetic differentiation differed probably because of their different ecological needs and mobility.

Generalist species often have greater genetic diversity and weaker genetic differentiation than specialist species because of the greater range of environmental conditions in which they can complete their life cycle (Brouat et al., 2004; Habel et al., 2013). Our results completely support the idea that the genetic differentiation is greater in specialist species as there was a clear gradient of isolation-by-distance in our three species (weak in *B. ino*, and strong in *B. eunomia* and *L. helle*). Increases in genetic differentiation with distance between sites are typically caused by individuals' limited dispersal and/or the presence of barriers to gene flow that result in the formation of isolated groups (Guillot et al., 2009). Hence, our results indicate that gene flow is limited in *L. helle* and *B. eunomia*, even at a very small spatial scale, and that dispersal is not sufficient to dampen the effects of genetic drift. This is supported in *L. helle* by field studies that showed that this species is highly sedentary, partly due to males' territorial behaviour (Fischer et al., 1999). In *B. eunomia*, dispersal distances are clearly greater than those of *L. helle* (Schtickzelle et al., 2007) but the small perceptual range of this species makes it particularly sensitive to the presence of landscape structures (this species is able to locate favourable habitats at <30 m, Schtickzelle et al., 2006). Therefore, dispersal in this species strongly declined in fragmented habitats (Schtickzelle et al., 2007) and this pattern of isolation-by-distance clearly shows that there are major costs to dispersal in this area as it has recently been shown (Legrand et al., 2021). Finally, the effect of geographic distance on the genetic differentiation of *B. ino* (more generalist species compared with *L. helle* and *B. eunomia*) was very weak, suggesting substantial gene flow. This result is consistent with past observations that showed the high emigration propensity and rather large dispersal distances of this species (Fric et al., 2010).

The loss of rare alleles in small and/or isolated populations is well established theoretically and the relationship between population size and genetic diversity is empirically well-supported (Leimu et al., 2006). Therefore, many studies have investigated the role of environmental

factors on the distribution of species' genetic diversity because of its key role in populations' evolutionary potential and extinction processes (Lenormand, 2002; Spielman et al., 2004). Hence, the positive relationship between patch area and quality on the genetic diversity of our three butterfly species is not surprising as these two characteristics likely underpin local population sizes in these species (Turlure et al., 2009, 2010). It has been shown that the genetic diversity in generalist species is less influenced by local habitat size or quality because the broader range of environmental conditions in which they can complete their life cycle enables them to have greater abundances (Habel et al., 2009; Koivula et al., 2002; Krauss et al., 2003). The patterns of increase in AR with patch area and quality were not statistically different between species even though they have clearly different ecological requirements. This lack of difference might stem from a lack of statistical power to detect small differences in these relationships between species, the overall low abundance of all studied populations, and maybe a too narrow range of ecological needs across species (*B. ino* in this area is a larval specialist species and more generalist species might have been better suited to test these differences). Note that we are only interested here in the slopes of the relationships as the differences in AR between species—i.e. intercepts—cannot be directly compared as AR was measured using different number of individuals, and different loci with different number of alleles. Finally, we found that the influence of patch quality was greater than that of patch area, indicating that patch quality is really a key determinant of local population sizes and is in itself a critical factor influencing their dynamics.

These effects of local habitat characteristics on species' within population genetic diversity were partly translated into among populations genetic diversity as the genetic differentiation decreased with habitat quality in *L. helle*. This increase of genetic differentiation with habitat degradation is consistent with the hypothesis that local habitat quality underpins butterfly's variation in population size (Dennis & Eales, 1997; Dennis & Hardy, 2007) which in turns influences the magnitude of genetic drift and the genetic differentiation among populations. Hence, the gene flow from large populations with good habitat quality is probably not sufficient to dampen the effect of genetic drift in the small populations of this species inhabiting degraded meadows. Habitat quality in *L. helle* is likely strongly associated with individuals' fitness as we have previously shown that strong enrichment of *B. officinalis* leaves with nitrogen resulted in a decline in individuals' growth in *L. helle* (Lebigre et al., 2018) Finally, the measures of habitat quality we used had no effect on the genetic differentiation among populations of *B. eunomia* and *B. ino*. Overall habitat quality explains the variation in *B. eunomia* population size but our measurement of habitat quality is chemically based and we did not record specifically the amount of dense cover of *B. officinalis* with tussocks of *Deschampsia cespitosa* (Turlure et al., 2009, 2010). Hence, it is likely that the good dispersal abilities of *B. eunomia* and *B. ino* led to lower variances in pairwise genetic distances that effectively dampens any potential effect of local habitat quality. Indeed, the main factor driving the genetic differentiation in *B. eunomia* was the Euclidian distance between sites while in *L. helle* some particularly distant patches had low genetic differentiation in spite of the overall pattern of isolation-by-distance. For instance for distances >12 km, the smallest D_{est} value was 0.019 for *L. helle* and such particularly low D_{est} values enable the detection of the effect of habitat quality on this species' genetic differentiation while the strong overall increase in genetic differentiation with geographic distance in *B. eunomia*, leaves little room for habitat quality to influence its genetic differentiation. Larval host plant area was not related to genetic differentiation in any of the three species. This might seem counterintuitive as we found a positive relationship between AR and patch area and population size in butterflies is often related to the quantity of their host plant(s) (e.g. Turlure et al., 2010). However, many studies have shown that patch quality influences local abundances at least as much as habitat area (Mcintire et al., 2007; Thomas et al., 2001) and the effect of patch quality on AR in these species was greater than that of patch area. Altogether, these results show that species' ecological requirements determine their genetic characteristics and that these effects can be particularly strong in a specialist species with limited dispersal behaviours.

Defining and quantifying habitat quality precisely are challenging, and many other environmental factors influencing local population sizes (and hence genetic diversity and differentiation) could be considered. For instance, individuals' fitness can be affected by other host plant chemicals compounds, the mean and variance in microclimatic conditions, the altitude of habitat patches, the presence/absence of a key limiting resource, host plant density, and the abundance of predators/parasites. Yet, adding extra habitat descriptors should be associated with an even more important increase in the number of investigated sites/populations to be able to extract meaningful compound variables from multivariate analyses. In *L. helle*, the shared effect of geographic distance and habitat quality shows that it can be difficult to disentangle these effects. Hence, it would be necessary to sample more sites of varying quality at very short distances to reduce the strength of the autocorrelation in habitat quality. When comparing species with different number of loci, there is a risk that having more or fewer loci in one species affects the ability to detect IBD and/or the effect of habitat quality on their genetic differentiations. This is however unlikely in our case as the species with the greater statistical power (*B. ino*) is the one with the lower pattern of IBD and lower effect of habitat quality. Subsampling this dataset to obtain quantitatively comparable measures of genetic differentiation across species is therefore unlikely to change our conclusions. When species habitat is or is becoming fragmented, the fitness costs of crossing unfavourable environments lead to a decrease in gene flow (Lawler et al., 2006; Vitousek et al., 1997). Landscape features, such as patches of unsuitable vegetation types (e.g. Piertney et al., 1998) or man-made structures (e.g. de Telles et al., 2007) can substantially increase the costs of dispersal (Storfer et al., 2007, 2010), making such measurements of functional connectivity more accurate than a simple geographical distance. In a comparative context, this would require estimating the costs of dispersal in each species (e.g. Engler et al., 2014) to estimate the relative contribution of functional connectivity and local environmental factors as this has been done for single species (e.g. Cosentino et al., 2012). As we lacked accurate estimates of the costs of crossing specific landscape features and there are clear risks of using expert opinion in a comparative purpose, we could not determine

the degree to which functional connectivity influenced the genetic differentiation of these three species. Finally, using only three species is not enough to quantify differences in species sensitivity to geographical and environmental factors and extending the number of species sampled in a given area and using nuclear markers would clearly enable us to rigorously undertake a comparative study (e.g. using mitochondrial DNA: Dapporto et al., 2019; Scalercio et al., 2020).

Overall, our study clearly showed that local habitat quality can influence the genetic characteristics of butterfly species even at a small spatial scale. This effect of habitat quality on species' genetic characteristics is particularly strong in *L. helle*, a species with major conservation concerns. Current conservation attempts of this species focus primarily on stopping habitat deterioration to limit the declines in population sizes, and our results show that such measures must be sustained to preserve local populations of this emblematic glacial relict species. Moreover, other studies have shown that habitat fragmentation can lead to the extinction of small and isolated populations (Mattila et al., 2012), and that patch connectivity can mediate genetic response to a demographic collapse (Caplins et al., 2014). As the geographic distance explained most of the variance in genetic differentiation in *L. helle*, it is clear that improving the level gene flow in addition to the preservation of local habitat quality is critical if we are to maintain a fully functional metapopulation in this species.

AUTHOR CONTRIBUTION

Christophe Lebigre, Camille Turlure, Jan Christian Habel, and Nicolas Schtickzelle conceived the study; Christophe Lebigre, Camille Turlure, Hélène Vandewalle, and Fabian Binard sampled the butterflies; Camille Turlure and Fabian Binard collected the vegetation plots; Christophe Lebigre Hélène Vandewalle and Jan Christian Habel collected the genetic and chemical data; Christophe Lebigre and Hélène Vandewalle analysed the data; Christophe Lebigre wrote the manuscript and all co-authors provided feedback.

ACKNOWLEDGEMENTS

We thank the Belgian's Fonds de la Recherche Scientifique (F.R.S.-FNRS) for funding of post-doctoral fellowships to Camille Turlure and Christophe Lebigre and the funding of the field and laboratory works through CDR grant J.0143.13F. This work on legally protected species and sites was undertaken with the permission of Service Public de Wallonie SPW and Département de l'Etude du Milieu Naturel et Agricole (DEMNA). We thank Viktoriia Radchuk, Quentin Dubois, and Justine Vandendorpe for their help during the fieldwork, and we thank Caroline Vanderbeken and Karine Hénin for their assistance in measuring the chemical properties of *Bistorta officinalis* leaves. This is publication BRC392 of the Biodiversity Research Centre. Authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

All the data necessary to replicate these analyses are available in the supporting information of the article.

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SUPPORTING INFORMATION

Additional supporting information may be found in the online version of the article at the publisher's website.

Table S1 Location of the habitat patches and number of adults genotyped of *Lycaena helle*, *Boloria eunomia*, and *Brenthis ino*

Table S2 Characteristics of the loci genotyped for the three species used in this study

Table S3 Summary of simulations testing the power and risks of type I errors based on the genetic markers used in the analyses

Table S4 Standardised pairwise genetic distances (D_{est}) between study sites for *Lycaena helle*

Table S5 Standardised pairwise genetic distances (D_{est}) between study sites for *Boloria eunomia*

Table S6 Standardised pairwise genetic distances (D_{est}) between study sites for *Brenthis ino*

Table S7 Averaged parameter estimates with unconditional standard errors and 95% unconditional confidential intervals (CI) obtained for the three best models (ΔAICc <2) linking environmental parameters with species' allelic richness

How to cite this article: Lebigre, C., Turlure, C., Vandewalle, H., Binard, F., Habel, J.C. & Schtickzelle, N. (2022) Diverging effects of geographic distance and local habitat quality on the genetic characteristics of three butterfly species. *Ecological Entomology*, 47(5), 842–854. Available from: https://doi.org/10.1111/een.13174