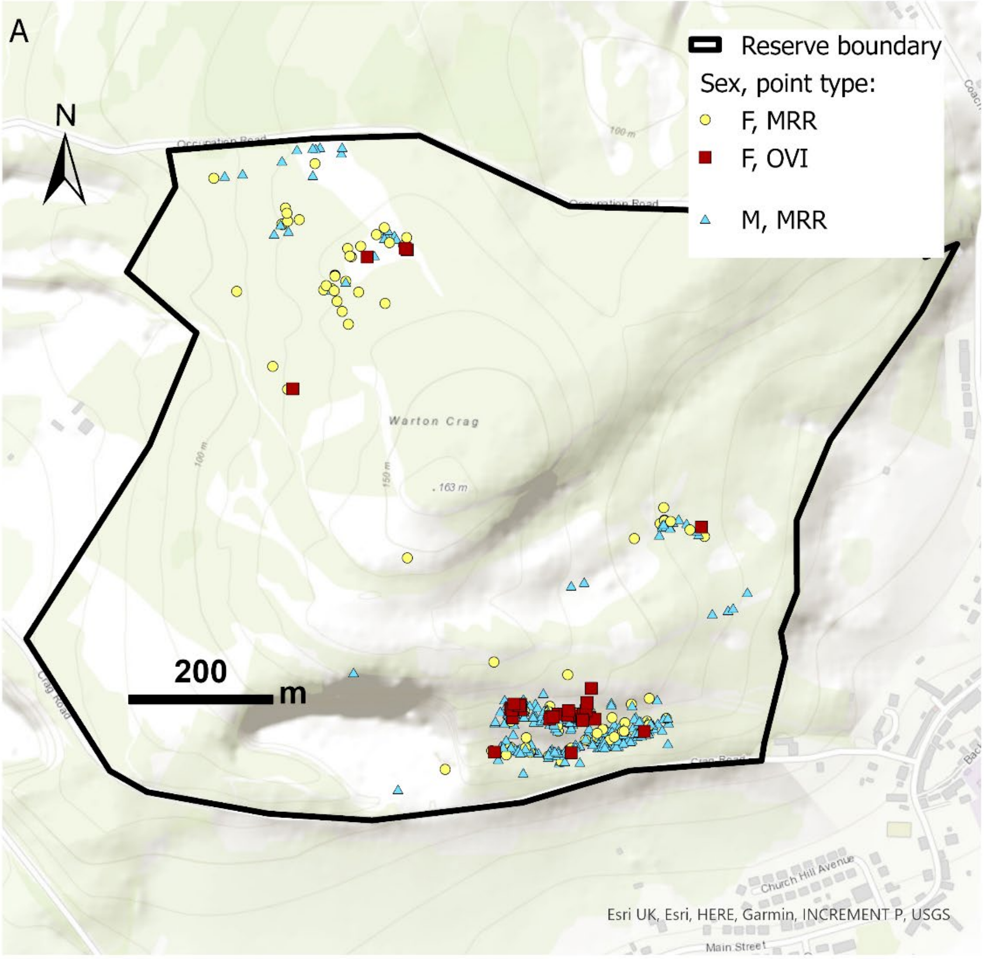

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Contrasting responses to microhabitat and temperature determine

breeding habitat differentiation between two *Viola*-feeding butterflies

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Running header: Fritillary breeding habitat differentiation

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Abstract

1. Since widespread monitoring began in 1976 in the U.K., habitat-specialist butterfly populations have declined dramatically. The main driver is habitat degradation, caused primarily by land-use change, perhaps interacting with changes in vegetation phenology.
2. Here, we focus on two declining species: *Boloria selene* [Dennis & Schiffermüller] and *B. euphrosyne* L., Lepidoptera: Nymphalidae. We hypothesise that these species differ in their preferred breeding habitat, and this is driven by differences in their temperature preferences, mediated by vegetation cover.
3. We use mark-release-recapture techniques and oviposition observations to characterize and compare adult distribution, habitat use and oviposition site preferences of the two species.
4. Egg-laying females of both species are shown to occur in areas with relatively high abundance of the larval food plants, *Viola* spp. (violets), principally *V. riviniana,* and they oviposit where *Viola* spp. abundance is locally high. However, in contrast to *B. selene*, ovipositing *B. euphrosyne* tend to occur in areas with relatively short and sparse cover of vegetation.
5. *B. euphrosyne* oviposit in sites with a relatively high plant surface temperature irrespective of ambient temperatures, in contrast with *B. selene* in which the temperature of oviposition sites increases as ambient temperature increases. These differential temperature strategies likely underlie differences in breeding habitat preference.
6. Microclimatic cooling caused by increased vegetation growth in spring may be one reason *B. euphrosyne* is declining in the U.K., while both *B. euphrosyne* and *B. selene* may be affected by declining *Viola* spp. availability. Our data provide further evidence that drivers of butterfly declines can be multi-factorial, and paradoxically, that thermophilic species do not necessarily benefit from climate warming if responses of other species result in cooling of their habitats.

Introduction

In recent decades, much of the globe has seen a marked decline in insect biomass (van Klink et al., 2020, Wagner et al., 2021, Wagner, 2020), leading to predictions that 40% of insect species may be at risk of extinction, with habitat loss identified as the principal driver (Sánchez-Bayo and Wyckhuys, 2019). Globally, Lepidoptera are reported to be one of the taxa most at risk (Sánchez-Bayo and Wyckhuys, 2019), and in north-west Europe butterfly diversity declines since the 1930s have been documented, but with some evidence that the rate of decline has slowed in recent decades (Carvalheiro et al., 2013). In the U.K. over the past three decades, 80% of butterfly species have declined in either abundance or distribution. On average, across all species, abundance has declined 6%, while distributions have constricted by 42% (Warren et al., 2021; Fox et al., 2022). Similarly, a study of c. 900 larger moth species in Britain concluded that 41% had declined, while total abundance decreased by 33% since 1968 (Fox et al., 2021). Although some butterfly species exhibit expanding range and increasing abundance, and those species without tight habitat requirements are only moderately affected, habitat specialists have experienced a serious abundance and distribution declines of 27% and 68%, respectively since 1976 (Warren et al., 2021; Fox et al., 2022).

In common with insects in general, habitat degradation is thought to be the principal cause of lepidopteran decline, and the main drivers are intensification of land use alongside abandonment of traditional land-management practices. However, it is likely that these direct anthropogenic effects on habitats occur in parallel with indirect effects mediated by climate change and pollution. Recent decades have seen advancement of spring in Britain with significant shifts in the flowering time (Fitter and Fitter, 2002) and photosynthetic activity, while the start of growing season has advanced 1.4 days per decade across the northern hemisphere resulting in higher spring productivity (Gonsamo et al., 2018). Advancement and increased productivity of vegetation can result in microclimatic cooling, whereby the cooling effect of tall green vegetation affects the development of species, particularly those overwintering as eggs or larvae whose development in the spring is highly temperature dependent (WallisDeVries and van Swaay, 2006). Vegetation structure is particularly important for early-successional species and for thermophilous species at the edge of their range that may require warm microenvironments within the landscape for larval development. The availability of suitable habitats for egg and larval development is usually more limiting than adult habitat (Bourn and Thomas, 2002; WallisDeVries and van Swaay, 2006). Evidence from the Netherlands shows that the negative impact of spring microclimatic cooling is aggravated by nitrogen deposition, and such effects have been implicated in the decline of species such as *Lasiommata megera* (Klop et al., 2015). Climate-driven changes in vegetation likely interact with climate-driven changes in the phenology of butterflies as pupal duration and the rate of larval development have also been shown to respond to climate change (Stalhandske et al., 2015). In such a period of changing annual dynamics of vegetation growth in response to environmental change, it is crucial that we revisit the requirements of declining species, re-define breeding habitat, and develop management strategies to ensure a persistent supply of such habitat.

Land-use change resulting from abandonment of traditional management practices is of particular importance for woodland species, many of which are associated with woodland edges or open spaces within woodland, such as rides or clearings. Such open habitats are features of most managed woodlands where access rides are present or in coppicing, for example, which entails rotational harvesting of woodland plots (or coupes), resulting in a mosaic of regrowth ages providing spatial variation in canopy cover, understorey structure, floral composition and abiotic conditions. Widespread cessation of coppice management and dense re-planting of coniferous trees has, therefore, reduced the availability of suitable habitat for many woodland species (Fartmann et al., 2013). Similarly, in more marginal grasslands, abandonment of managed grazing has allowed scrub encroachment causing changes to butterfly communities (Eriksson, 2021; Mora et al., 2022). The *Viola*-feeding fritillaries is one group of species affected by land-use change, with several species, particularly those associated with early successional or woodland edges and clearings, in severe decline in the U.K.. Two such species, *Boloria euphrosyne* (L.) and *B. selene* [Dennis & Schiffermüller] typically occur in seral stages produced by rotational coppice management, though both species also persist in more open moist habitats (*B. selene*) or in scrub and bracken-dominated habitats (Eeles, 2019). While *B. selene* and *B. euphrosyne* larvae both feed exclusively on *Viola* spp., their larval niches are distinct with *B. selene* reported to prefer larger plants on moist soils, whereas *B. euphrosyne* is reported to prefer smaller plants in warm microsites (Thomas et al., 2011; Randle, 2009). Both species are relatively widespread in Europe occurring as far north as northern Scandinavia, though *B. euphrosyne* extends further south than *B. selene* into Italy and Greece (Kudrna et al., 2011). As in the U.K., in continental Europe *B. euphrosyne* tends to occur in more xerothermic habitats and inhabits earlier successional stages in forest clearings than *B. selene,* although in Scandinavia *B. euphrosyne* is reported to inhabit bogs utilising alternative foodplants (*Fragaria, Rubus, Vaccinium uliginosum*) (Eliasson et al., 2005). *B. selene* also occurs in humid grasslands, fens, and bog edges, as well as forest clearings (Eeles, 2019).

Attempts to conserve habitat specialists in the U.K. have focused on the definition of high-quality habitat followed by targeted management to increase the quality of habitat and its availability at the landscape scale (Ellis et al., 2011; Ellis et al., 2019). Site-scale habitat quality is of importance in butterfly metapopulations because productive sites have more stable local populations and act as source populations for colonisation on other sites in the network (Thomas et al., 2001, Thomas et al., 2011). To halt the decline of *Viola*-feeding fritillaries, conservation management has focused on habitat management to improve site quality as well as improving connectivity in the landscape (Ellis et al., 2011; Ellis et al., 2019). For most temperate-zone butterflies, habitat quality is defined by the availability of the larval host plant in the optimum growth form or micro-habitat, often governed by the amount of light and shade (Thomas et al., 2011). Defining optimal habitat for *Viola*-feeding fritillaries is therefore a key prerequisite of effective conservation action.

Despite good understanding of the larval requirements of *B. selene* and *B. euphrosyne* and the implementation of management intended to provide suitable habitat, species declines are still evident in many regions. In England, national population indices reveal a long-term trend (1978 - 2019) of -55% for *B. selene* and -72% for *B. euphrosyne*, alongside a decline in distribution of -76% and -95% respectively (Fox et al., 2022). These population trends have resulted in *B. euphrosyne* designated as a priority A species (requiring urgent action across all occupied sites) and *B. selene* as a priority B species (action necessary on some sites) by Butterfly Conservation U.K. The Morecambe Bay limestone habitats, in the northwest of England, have seen extensive management for *Viola*-feeding fritillaries covering 185 ha over 70 different sites (Ellis et al., 2019). The region is regarded as a national stronghold for *B. euphrosyne* (Ellis et al., 2012) and the species is the subject of targeted management in the region, however the species continues to decline and since 2001 has disappeared from 11 of the 17 sites in the region which are monitored by the U.K. Butterfly Monitoring Scheme (Blomfield, 2021; Botham et al., 2020).

Here we focus on one of the principal sites for *B. euphrosyne* and *B. selene* in the Morecambe Bay Limestones network. As immature stages of butterflies are relatively immobile, maternal oviposition choices largely determine the environmental conditions under which larvae develop and is therefore a key determinant of growth and survival (Gripenberg et al., 2010; Janz, 2005). Therefore, we report female oviposition choice as an indicator of larval habitat preference. We use Mark Release Recapture techniques to define the distribution of these species at the site, and we used detailed micro-habitat assessment at oviposition and control sites to compare the oviposition requirements and to infer differences in oviposition site selection between the two species. We hypothesise that 1) Gravid females of both species select habitat with high larval food plant availability, 2) Interspecific differences in breeding habitat selection are not caused by phenological changes in vegetation, but by selection of different microhabitats by the two species, and 3) Differences in breeding habitat selection are driven by different temperature preferences, with *B. euphrosyne* exhibiting thermophily to a greater extent than *B. selene*.

Materials and Methods

Study site

The study site was Warton Crag Nature Reserve (2.7799°W 54.1484°N) in the north-west of England. The reserve is one of the principal sites for *Viola*-feeding fritillaries in the Morecambe Bay Limestones area. *Viola riviniana* (common dog-violet) is the most abundant and widespread *Viola* species in the areas occupied by *Boloria* spp., although *V. reichenbachia*, *V. odorata* and *V. hirsuta* occur in small numbers elsewhere on the site. The site has three sections (Figure S1), managed independently by the Arnside and Silverdale Area of Outstanding Natural Beauty (AONB), the Royal Society for the Protection of Birds (RSPB) and the Wildlife Trust (WT) respectively. Each section has a UKBMS butterfly transect that have been operational since 1989 (AONB), 1990 (RSPB) and 1992 (WT). The site covers 86 ha and comprises secondary woodland dominated by ash and hazel, limestone grassland, limestone outcrops and scree, scrub and bracken beds. *Boloria* spp. are widespread at the site occupying the open habitats and woodland clearings/rides.

Mark Release Recapture

The mark-release-recapture study took place through May and June in two consecutive years (2016 and 2017). Sampling encompassed all open areas of the site and the entire site was covered in a day when weather conditions permitted, and a different starting point was used each day. When weather prevented the coverage of the entire site in a day, the following day’s sampling began with areas not covered in the previous visit. Where fritillaries were seen, they were caught with a net and unmarked individuals were marked through the net on the underside of the hindwings with a unique identification mark using a fine water-insoluble marker pen. Individuals were kept in the net for the minimum time possible during marking and released immediately after at the site of capture. The mark-release-recapture study involved one main marker/recorder with some assistance in both years and effort was approximately equal over the two years. For each capture/recapture the GPS location (Garmin Etrex 20x, accuracy c.3 m), unique identification number, date and time of capture, and sex of the butterfly were recorded.

Oviposition observations

Female oviposition choices were used to define optimal larval habitat for each species (Thomas et al., 2001). Oviposition observations were made throughout the flight periods in the same areas used for the mark-release-recapture study, in which gravid females displaying characteristic searching behaviour were followed until they oviposited. When inspecting a plant, a female typically walks around on plant leaves or litter whilst moving antennae and lowering her abdomen. Eggs are deposited on the food plant or on other live or dead plants in the vicinity of *Viola* spp. (Eeles, 2019). An oviposition event was recorded when the female was observed to curl its abdomen and deposit an egg. Rejected sites were also marked (2017 only) if the female exhibited antennal movement and abdomen lowering, but no egg was released. To ensure that the females were indeed gravid and displaying oviposition behaviour, rejected sites were only considered if the same female oviposited during the same observation period. Oviposition and rejected points were marked, coordinates using a GPS, and in 2017 only, five replicate temperature measurements were taken immediately on the plant/litter surface at the marked points using an IR thermometer (GoolRC, 0.1°C resolution, accuracy 1.5%). Ambient air temperature was also recorded at the same time at waist height in the shade (Thlevel, 0.1°C resolution, accuracy 1.0%).

For each oviposition point a local control point was identified as a random point within a 5 m radius of the oviposition point (restricted to areas of similar habitat). Microhabitat data were recorded for each oviposition, rejected and local control point as soon as possible after the oviposition event. In 2017, in addition to the local control points, flight-area control points were identified to give an indication of general vegetation during the flight season of each species. Control points were selected in each of the three UKBMS transect routes, 10 in May and 10 in June to represent the oviposition period of each species. Transect sections where *Boloria* spp. are consistently recorded were selected and quadrats were placed at random positions along the transect route several paces from the path to ensure the vegetation was not disturbed. This recording regime meant that three separate control sets were available for oviposition points: *rejected points* - representing sites investigated but rejected by egg-laying females; *local controls* – representing the local area (5 m radius) in which oviposition behaviour was occurring; and *flight-area controls* – representing the broader area occupied by each species during their flight periods.

For all points, microhabitat was sampled by centring a 1 m^2 quadrat on the marked point and recording visual estimates of percentage cover of bare ground, bracken, live grass, total live vegetation, *Viola* spp., litter/thrash. In addition, sward height was recorded as the mean resting height of five drop-disc (30 cm diameter; 230 g) samples taken at the corners and centre of the quadrat. Litter depth was recorded as the mean of five measurements of the highest dead plant material at the same points as the drop-disc measurements. Finally, violet density was recorded as the number of individual *Viola* spp. plants within the quadrat.

Data analysis

All statistical analyses were done using R Statistical Software V4.1.2 (R Core Team, 2021). For the mark-release-recapture study, capture histories of each marked individual were based on half-week sampling periods to ensure that all the sampling area had been covered in each period. Population estimates were calculated using a log-linear Jolly-Seber model calculated assuming an open population in the package RCapture (Baillargeon and Rivest, 2007). Euclidean distances between the two most distant capture points for each recaptured individual were calculated in ArcMap 10.4 (ESRI Inc. 2016).

To show how habitat differed between oviposition points and respective local control quadrats of the two species, a NMDS analysis using Bray-Curtis dissimilarity across the recorded habitat variables was undertaken in the Vegan (Oksanen et al., 2020) package in R Statistical Software V4.1.2 (R Core

Team, 2021). The significance of factors (oviposition versus control points) was assessed using the *envfit* function.

To test for habitat differences between the oviposition points of each species and between the oviposition points and local control quadrats for each species, each habitat variable was tested using a mixed-effects model in the Lme4 package (Bates et al., 2015), with quadrat type as the explanatory variable and year as the random variable. Percentage cover variables were logit transformed and sward height, litter depth and number of violets were log transformed prior to analysis to improve model fit. In all cases, model simplification was done by deletion of terms from the full model with the term explaining the least amount of deviance in the model deleted at each step until only significant terms remained (Crawley, 2007). Model parameters were then extracted from the minimum adequate model. For the 2017 data, where habitat data were available for points rejected for oviposition in addition to local and flight-area controls for each species, linear models were constructed and a priori contrasts used to test for: (1) *oviposition habitat* choice of females of each species i.e. differences in habitat variables between the sites in which female butterflies were active (oviposition sites, rejected sites and local controls) *vs.* concurrent flight-area controls; (*2) species differences in oviposition sites, B. euphrosyne* oviposition site *vs. B. selene* oviposition site; and (3) *oviposition site selection:* oviposition sites vs. rejected sites for each species. In this way, it was possible to show how the species differed in the habitat chosen by gravid females in terms of fine-scale oviposition site choice, but also to show which of these effects were a result of real habitat preferences rather than phenological changes in vegetation due to the differences in flight periods.

Results

In the mark-release-recapture study, *Boloria euphrosyne* were recorded from 5 - 30 May in 2016, and 2 - 26 May in 2017. *B. selene* flight period occurred later, but overlapped with *B. euphrosyne*: 23 May - 27 June in 2016 and 25 May - 26 June in 2017. This resulted in 9 and 8 half-weekly sampling period for *B. euphrosyne* in 2016 and 2017, respectively; and 10 and 12 sampling periods for *B. selene* in 2016 and 2107, respectively. The mark-release-recapture study resulted in estimates of population size of 332 (± 90 SE) and 366 (± 33 SE) for *B. selene* in 2016 and 2017 respectively, and much smaller estimates of 65 (± 6 SE) and 87 (± 4 SE) for *B. euphrosyne*. For both species more males were marked than females across the two years, particularly for *B. selene* (Table S1). As population estimates for *B. euphrosyne* are similar for both sexes, this likely reflects differences in detectability due to their contrasting behaviours (Adamski, 2004). Paucity of female recaptures for *B. selene* prevented female population estimates (Table S1). Though there was some spatial overlap in the distribution of captures of the two species, and both were largely restricted to open areas and woodland clearings, the majority of the *B. euphrosyne* captures were to the south of the site, principally on a south-facing slope, while *B. selene* occupied higher sections to the centre of the site (Figure 1). Both species occurred to the north of the site where the terrain is relatively flat. By contrast, oviposition points were largely spatially segregated and restricted to small areas within the ranges of the two species (Figure 1). The largest maximum distance recorded between captures for individual females was 373 m for *B. selene* and 421 m for *B. euphrosyne,* and for males 660 m and 381 m respectively, though long-distance movements outside the study area would not have been recorded (the maximum distance possible between occupied areas of the site is approximately 950 m). Both sexes in both species exhibited skewed distribution with the majority of individuals recorded to move relatively short distances (overall median = 113 m). While there was no difference in distance moved between females of the two species, on average males of *B. selene* moved longer distances than those of *B. euphrosyne* (Table 1). In *B. euphrosyne,* there was no difference between males and females in the maximum distance moved by individual butterflies, whereas there was a marginally significant difference between males and females of *B. selene,* though these comparisons lack power due to the relatively small number of female recaptures (Table 1).

In total, 17 (from 15 females) and 27 (26 females) oviposition points were recorded for *B. selene* in 2016 and 2017, respectively, compared with 19 (18 females) and 16 (15 females) for *B. euphrosyne* in the same years. Oviposition events were distributed throughout the flight periods and, for the majority of females, only a single oviposition event was recorded: for *B. selene* two oviposition sites were recorded from two females in 2017 and from one female in 2016; for *B. euphrosyne*, one female contributed two oviposition sites in 2017 and three females contributed two oviposition sites in 2016. NMDS analysis (Figure 2) of the combined quadrat data from both years showed significant separation in oviposition habitat characteristics between the species (local control and oviposition points combined; $r^2 = 0.22$; $p < 0.001$) with *B. selene* points characterised by higher cover of live vegetation and grass in particular, while *B. euphrosyne* points showed higher cover of bare ground, plant litter and bracken. For each species there was also a shift consistent with higher *Viola* spp. density and lower sward height and litter depth for oviposition sites relative to their respective local controls (a concurrent randomly located point within 5 m radius of the oviposition site), though there remains overlap in the centroid confidence intervals for each species indicating that these shifts are not statistically significant. These differences in habitat characteristics between species are associated with both spatial and temporal separation of the two species with *B. euphrosyne* oviposition events being recorded on average 29 days earlier than *B. selene* in both 2016 and 2017, and oviposition occurring in different locations at the site (Figure 1).

The oviposition habitat differences between the species suggested by the NMDS analysis were confirmed by general linear models of the 2017 data. *B. euphrosyne* oviposition sites were shown to have lower grass ($t_{151} = 5.1$; $P < 0.001$; est = -1.32 [logit %]) and, more generally, lower live vegetation cover ($t_{151} = 5.1$; $P < 0.001$; est = -0.6 [logit %]), and shorter sward height ($t_{151} = 3.9$; $P < 0.001$; est = -0.53 [log cm]) than *B. selene* oviposition sites (Figure 3 A-C). There were also differences in cover and depth of litter with *B. euphrosyne* oviposition sites having higher litter cover ($t_{151} = 4.4$; $P < 0.001$; est = 1.75 [logit %]), but lower litter depth ($t_{151} = 3.0$; $P = 0.003$; est = -0.40 [log cm]) than *B. selene* oviposition sites. As with the NMDS analysis, these differences reflect both the earlier stage of vegetation development during the *B. euphrosyne* flight period and differences in habitat selection between the two species. Comparisons of the flight-area controls of the two species show higher sward height (t_{151} = 3.4; $P < 0.001$; est = 0.43 [log cm]) and live vegetation cover (t_{151} = 0.34; P = 0.003; est = 0.34 [logit %]), but lower bare-ground cover (t_{151} = 2.5; P = 0.026; est = -0.63 [logit %]; for *B. selene* compared with *B. euphrosyne*, which likely result largely from phenological vegetation change, though *B. selene* flight areas were also characterised by lower litter cover (t_{151} = 3.7; $P < 0.001$; est = -1.31 [logit %]), and lower bracken cover (t_{151} = 3.5; $P < 0.001$; est = -1.34 [logit %]) , which are contrary to the phenological trend and therefore likely reflect habitat selection. Although it is difficult to partition vegetation phenology from habitat choice, the comparison of vegetation characteristics in areas used by gravid females with the vegetation at the site during the flight season of each species helps us to identify which habitat variables drive female oviposition independent of seasonal changes in vegetation.

Comparison between areas occupied by egg-laying female *B. euphrosyne* (defined as oviposition sites, rejected sites and local controls) with the flight-area controls showed that *oviposition habitat choice* of *B. euphrosyne* favoured areas that had relatively low cover of grass (t_{151} = 5.1; $P < 0.001$; est = -3.3 [logit %]), live vegetation (t_{151} = 4.0; $P < 0.001$; est = -1.3 [logit %]) , shorter sward height (t_{151} = 2.8; $P < 0.005$; est = -1.0 [log cm]), and higher *Viola* spp. cover (t_{151} = 2.9; P = 0.005; est = 1.7 [logit %]) and density (t_{151} = 2.4; P = 0.003; est = 2.4 [log no. m^{-2}]) compared with flight-area controls. This shows that gravid *B. euphrosyne* females occurred in sparsely vegetated areas in the landscape with relatively high cover of the larval food plant (Figure 3 C-D). Within these areas, *oviposition site selection* favoured sites with relatively high *Viola* spp. cover compared with rejected sites (t_{151} = 2.8; P = 0.005; est = 0.8 [logit %]), though there was no significant effect of *Viola* spp. density.

By contrast, *B. selene* females did not occupy areas where the vegetation differed significantly from the general vegetation in the site during the flight season of the species (flight-area controls), other than in the higher cover of *Viola* spp. (t_{151} = 2.1; P = 0.035; est = 1.0 [logit %]), which was the only significant variable for this analysis. For *B. selene,* comparisons of oviposition sites with rejected sites showed that oviposition sites have higher bare ground cover (t_{151} = 2.4; P = 0.016; est = 0.9 [logit %]), although much lower that *B. euphrosyne* oviposition sites, and higher *Viola* spp. cover (t_{151} = 2.1; P = 0.036; est = 0.6 [logit %]) and density (t_{151} = 2.3; P = 0.022; est = 0.8 [log no. m^{-2}]) than rejected sites. Bracken cover was not a statistically significant explanatory variable for either species.

Analysis of the temperature data recorded at the time of oviposition revealed that response to microhabitat temperature may also be a key difference in oviposition behaviour of the two species. The mean plant-surface temperature at oviposition sites was significantly higher for *B. euphrosyne* (31.6 °C ± 1.2 SE) than *B. selene* (24.3 °C ± 1.3 SE; $F_{1,41}$= 14.8; P < 0.001), despite the ambient temperature being significantly higher for *B. selene* than *B. euphrosyne* ($F_{1,41}$= 9.1; P = 0.004): 15.6 °C (± 1.2 SE) compared with 13.7 °C (± 1.2 SE). This suggests that *B. euphrosyne* females actively located areas of locally high temperature to oviposit. This is confirmed by the relationship between ambient air temperature and the plant surface temperature at oviposition sites. There was a significant interaction between ambient temperature and butterfly species in determining the plant-surface temperature at oviposition sites ($F_{1,39}$= 12.7; P < 0.001). While *B. euphrosyne* was able to locate very warm sites to oviposit whatever the ambient temperature, even selecting warmer sites on cool days, this was not the case for *B. selene*, which showed a steeply positive relationship between ambient temperature and the plant surface temperature at the oviposition site (Figure 4).

Discussion

The mark-release-recapture data reveal that although peak densities of the two species occur on different parts of the site, with *B. euphrosyne* occurring predominantly on the sloping south-facing areas of the site and *B. selene* occurring at the higher elevations, there was considerable spatial overlap in the distribution of captures. However, the sites chosen for oviposition, and thereby larval development, were largely distinct. Consistent with previous reports (Eeles, 2019, Thomas and

Lewington, 2014), *B. selene* was associated with more vegetated areas, while *B. euphrosyne* occupied, principally, sparsely vegetated early successional habitat, often where scrub had been cleared in the previous two or three years. Females of the two species displayed similar movement distributions, with the majority of females moving less than approximately 100 m, but with a small number of individuals of both species moving 350 m or more. Although the restricted area of the mark-release-recapture study means that very long-distance movements (> 950 m) would not have been recorded, it is likely that these are very infrequent. Our data suggest that both species have similar limited capacity for colonisation of favourable habitat confirming what has been reported elsewhere for *B. selene* (Ellis et al., 2011; Barnett and Warren, 1995a) and *B. euphrosyne* (Blomfield et al., 2023). Although overall movement distances were similar in the two species, the species did differ with respect to sex differences in movement. There was no significant difference in the distance moved by male and female *B. euphrosyne*, with the longest movements made by individual females, a pattern similar to that recently reported at Whitbarrow, another site in the north-west of England (Blomfield, 2021). However, male *B. selene* moved greater distances than females, a pattern not observed in previous studies (Ellis et al., 2011). It is possible that increased propensity of females to move longer distances is an adaptive response to fragmentation in *B. euphosyne*, which is more of a habitat specialist than *B. selene* (Barnett and Warren, 1995b). Recent evidence suggests that females of this species may have adapted to recent habitat fragmentation by changed wing morphology consistent with greater dispersal ability (Blomfield, 2021).

The differences in habitat use shown in the adult capture data were reflected by the oviposition data, which show spatial segregation of oviposition in the two species (Figure 1) and significant differences in oviposition habitat characteristics. In *B. euphrosyne,* gravid females predominantly occurred in more sparsely vegetated areas than *B. selene*, ovipositing in sites with less vegetation cover, lower sward height and with shallower litter depth, but higher litter cover than *B. selene*. Oviposition habitat preferences of *B. selene* confirmed those reported in previous studies in similar habitats in north-east of England (Ellis et al., 2011), though average vegetation height was greater, litter cover slightly lower and violet cover considerably lower in our study. Studies of oviposition habitat of both species in south-west England showed similar interspecific habitat differences with *B. euphrosyne* favouring warmer microsites with more open vegetation structure and occupied by smaller violets compared with *B. selene* (Randle 2009). Despite the reported importance of bracken for violet-feeding fritillaries and the importance of bracken beds as habitat (Randle, 2009), we found no significant effect of bracken cover on oviposition choices for either species. The reported link with bracken likely arises from the importance of limited bracken litter in providing a warm microsite for larval basking in spring (Barnett and Warren, 1995a). Although we found no evidence of bracken importance *per se*, we did find that both species were affected by the cover and/or depth of plant litter, consistent with the reported requirement of *B. euphrosyne* for warmer microsites for larval development, with a shallower but more extensive coverage of plant litter.

The inter-specific differences in oviposition habitat were, in part, a function of phenology, due to *B. selene* flying approximately a month later in the season. Comparisons of the flight area controls quadrats recorded during the flight period of each species show greater vegetation cover and height, and lower bare-ground cover for *B. selene* compared with *B. euphrosyne*, consistent with increased seasonal vegetation growth. However, lower bracken and litter cover in *B. selene* compared with *B. euphrosyne* flight areas are contrary to the direction of vegetation development suggesting species differences in habitat preference. This is confirmed by the comparisons of female locations with the flight-area control quadrats that were surveyed at the same time, which showed that female *B. euphrosyne* were active in relatively sparsely vegetated areas with relatively low grass and live vegetation cover and lower sward height compared to their flight-area controls. Cover and density of the larval food plant was also higher in the areas in which females were active, although neither litter cover nor litter depth differed from the flight-area controls. This is in contrast with *B. selene* females, which were less selective, being recorded in habitat conditions that only differed from their flight-area controls in terms of the cover of *Viola* spp.

Comparisons of female activity areas with flight-area controls reveal the broad scale preferences of females for habitat, while comparisons of oviposition sites with rejected sites allowed us to determine which micro-habitat variables determine the fine-scale preferences for oviposition. For *B. euphrosyne*, females chose to oviposit in places with higher host-plant cover compared with rejected sites, while *B. selene* selected sites for oviposition with higher cover and density of host-plant, but also with relatively high bare-ground cover than rejected sites.

For both species, habitat preference appears to depend on a combination of vegetation and litter structure and the availability of the larval host plants. The temperature data allow further interpretation of the likely importance of vegetation and litter structure in oviposition decisions. Strategies with respect to temperature contrast markedly between the two species. Females of *B. euphrosyne* selected microsites with high plant-surface temperatures irrespective of ambient temperature, a pattern which contrasted strongly with *B. selene*, in which the plant-surface temperature of selected sites increased with ambient temperature. Local vegetation structure is known to have a strong effect on micro-climate (Suggitt et al., 2011) and it is likely that the observed selectivity of *B. euphrosyne* for sparsely-vegetated areas with extensive but relatively shallow litter cover reflects selection for warm microsites. The thermophilous species, *Hesperia comma*, has also shown to have the ability to select warmer microhabitats when ambient temperature is low (Davies et al., 2006), and vegetation and topographic effects on microclimate have been shown to drive habitat use more than regional temperatures (Lawson et al., 2014). Assuming that oviposition choices in butterflies are optimal in terms of offspring survival (Salgado et al., 2020), we can infer that *B. euphrosyne* larval performance is more temperature sensitive than *B. selene*. This is consistent with the fact that *B. selene* occupies a wider variety of habitats, including wetter sites, than *B. euphrosyne* in the U.K. (Eeles, 2019), though *B. euphrosyne* is reported to occupy raised bogs in northern Europe (Eliason 2005). At the cool parts of species ranges, spring larval development is highly dependent on temperature and warmest microclimates are found in short vegetation on dry substrates that warm up quickly in sunny conditions (WallisDeVries, 2006, WallisDeVries and van Swaay, 2006).

The link between vegetation structure and microclimate explains the paradox that *B. euphrosyne*, a thermophilous species, is declining in the U.K. while climate is warming. It is likely that this is an indirect effect mediated by microclimatic cooling caused by enhanced plant productivity in warmer winter conditions. Such microclimate warming has been highlighted as a key factor for species dependent on warm spring conditions for larval development (WallisDeVries and van Swaay, 2006)**.** It is also likely that the winter warming effect is facilitated by nitrogen deposition, which is known to drive reductions in plant species richness in U.K. semi-natural habitats, largely as a result of increased productivity (Maskell et al., 2010), and in grasslands results in reduced cover of forbs and increased cover of grasses (Stevens et al., 2006). Consistent with a microclimate cooling impact, there is little evidence that *B. euphrosyne* has performed better in warmer locations. In fact, evidence suggests that in the U.K., this species becomes more habitat specific in sites with warmer winter temperatures (Oliver et al., 2009). Scottish populations of populations of *B. euphrosyne* (and *B. selene*) show increases in abundance, and lower rates of distribution decline than English populations (Fox et al., 2022), which may be explained by relatively low rates of nitrogen deposition and the cooler climate.

The data presented here suggest that the continuing decline of *B. euphrosyne* in Morecambe Bay, and other *Viola*-feeding fritillaries such as *Argynnis adippe*, may be a response to microclimatic cooling due to changing vegetation cover. Evidence from our study site suggests that there was a significant increase in vegetation height and cover, and decreased litter and bare ground cover between 2004 and 2016 in the southern part of the site which hosts the bulk of the *B. euphrosyne* population (Ellis et al. 2019). There was also evidence that these changes had occurred more generally across the Morecambe Bay Limestones area (Ellis et al., 2019). However, the same study reported a decrease in *Viola* spp. abundance across much of the site, leading to the possibility that increased vegetation cover and perhaps sensitivity of *Viola riviniana* to nitrogen deposition (Payne et al., 2020), is also having a negative effect on the availability of the larval host plant. Although *B. selene* was shown not to select for warm microsites for oviposition, and is likely therefore to be less sensitive to cool temperatures during larval development, it is notable that the most recent data also show this species to be in severe decline in the U.K. (Fox et al., 2022). Given the importance of host plant availability in habitat selection and oviposition choices of both species, declines in violet density and distribution may also be implicated in their decline. The data suggest that population persistence in these species, in particular *B. euphrosyne,* may rely on the availability of sites which remain sparsely vegetated as climate warms. Both species traditionally occurred in managed woodland habitats, and re-establishment of woodland management that ensures small scale heterogeneity in regrowth age, is likely to mitigate their population declines. Habitat management techniques should also be developed that restrict spring-time vegetation development, while promoting persistence of violets, to ensure the availability of suitable oviposition sites for both species.

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Table 1. Median maximum Euclidean distance (m) and IQR between captures of individual butterflies in 2016 and 2017. Mann Whitney U test statistics and corresponding p values are presented for sex comparisons within species, and for species comparisons for each sex. Statistically significant comparisons are highlighted in bold text.

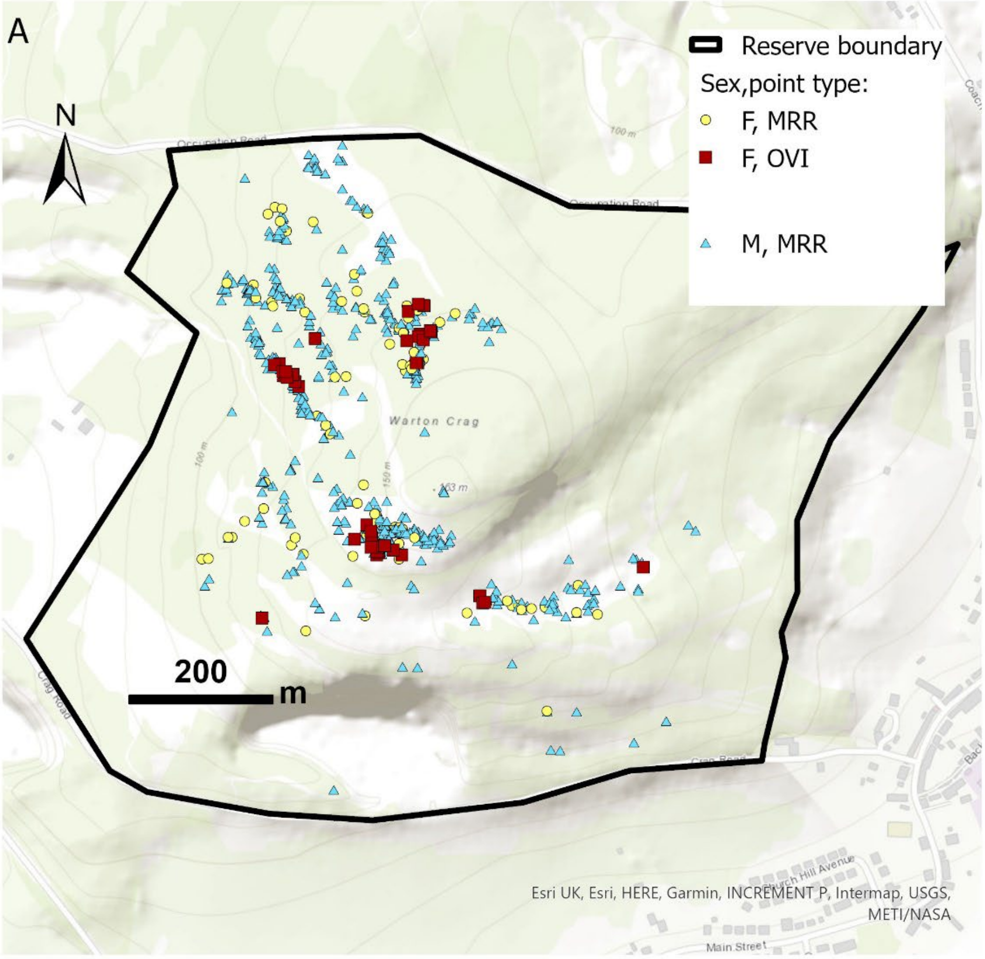
	B. euphrosyne	*B. selene*	Species comparison
♀	107 (45 – 174), n=27	65 (30 – 174), n=18	U = 271; P = 0.524
♂	97 (63 – 127), n=67	130 (66 – 213), n=132	**U = 5494; P = 0.005**
Sex comparison	U = 840; P = 0.590	U = 1482; P = 0.090	

FIGURE 1 Capture points (blue triangles = male; yellow circles = female) and oviposition points (red squares) for A) *Boloria selene* and B) *B. euphrosyne* at Warton Crag nature reserve during mark-release-recapture and oviposition studies. The black perimeter shows the extent of the reserve and study area. Data are for 2016 and 2017 seasons combined.

FIGURE 2 NMDS based on habitat variables measured at oviposition locations (egg) and local control locations (con) for *Boloria euphrosyne* (Be) and *B. selene* (Bs)(Stress = 0.168). Ellipses represent 95% confidence intervals around the centroids for the point types. Those intrinsic variables with a significant ($P < 0.05$) influence are represented with arrows: Viola no. = violet plant density; live vegetation cover (live); graminoid cover (grass); sward height; litter depth; bare-ground cover (bare); litter cover (litter).

FIGURE 3 Microhabitat data recorded from 1 m^2 quadrats at oviposition points (egg), rejected points (rejected), local controls (5 m) and flight-area controls (control) for *Boloria euphrosyne* (Be, left side of each chart) and *B. selene* (Bs, right side of each chart). Bars and boxed indicate medians and IQR, dashed lines extend to minimum and maximum values and open circles denote outliers. Panels present: % grass cover (A); % live vegetation cover (B); sward height (C); % litter cover (D); litter depth (E); % bare-ground cover (F); % violet (*Viola* spp.) cover (G); violet (*Viola* spp.) plant density (H); and % cover of bracken (I).

FIGURE 4 The relationship between plant surface temperature and ambient temperature at oviposition sites for *Boloria euphrosyne* (red) and *B. selene* (blue) in 2017. Shaded regions represent 95% confidence intervals.



Esri UK, Esri, HERE, Garmin, INCREMENT P, Intermap, USGS, METI/NASA

Fig. 1A

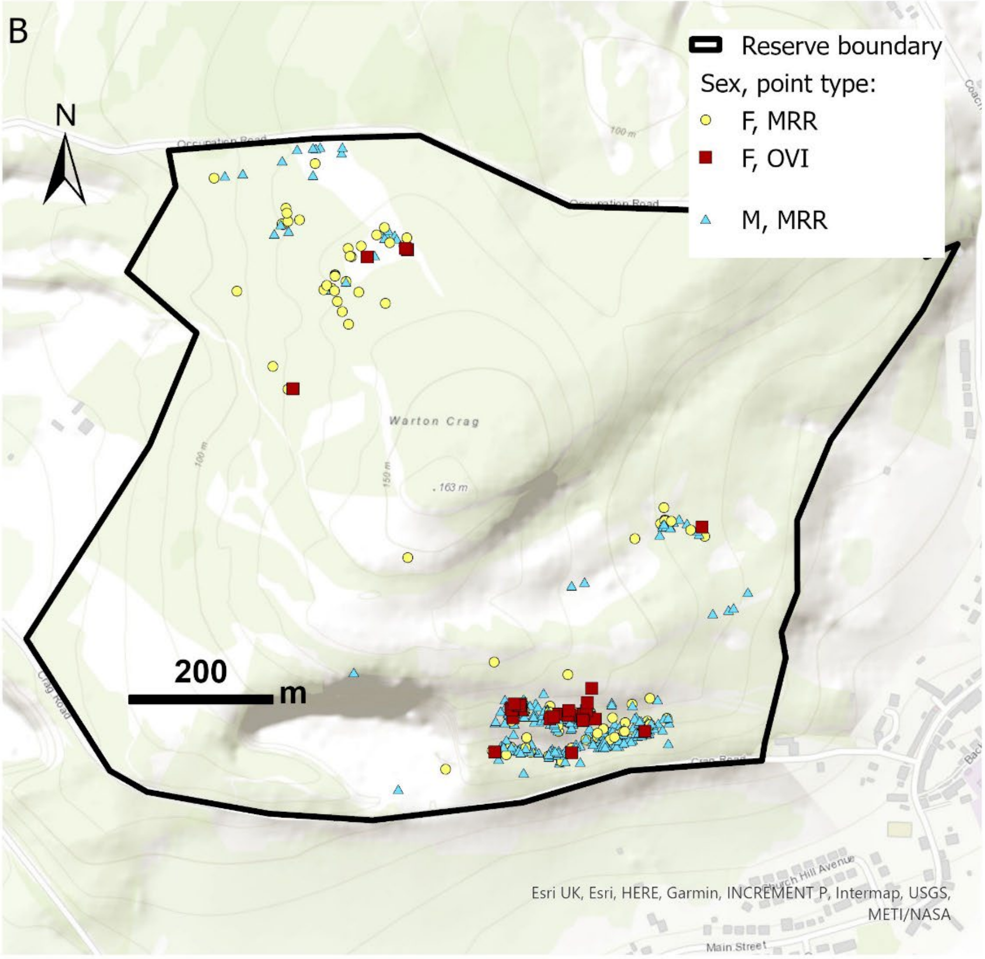
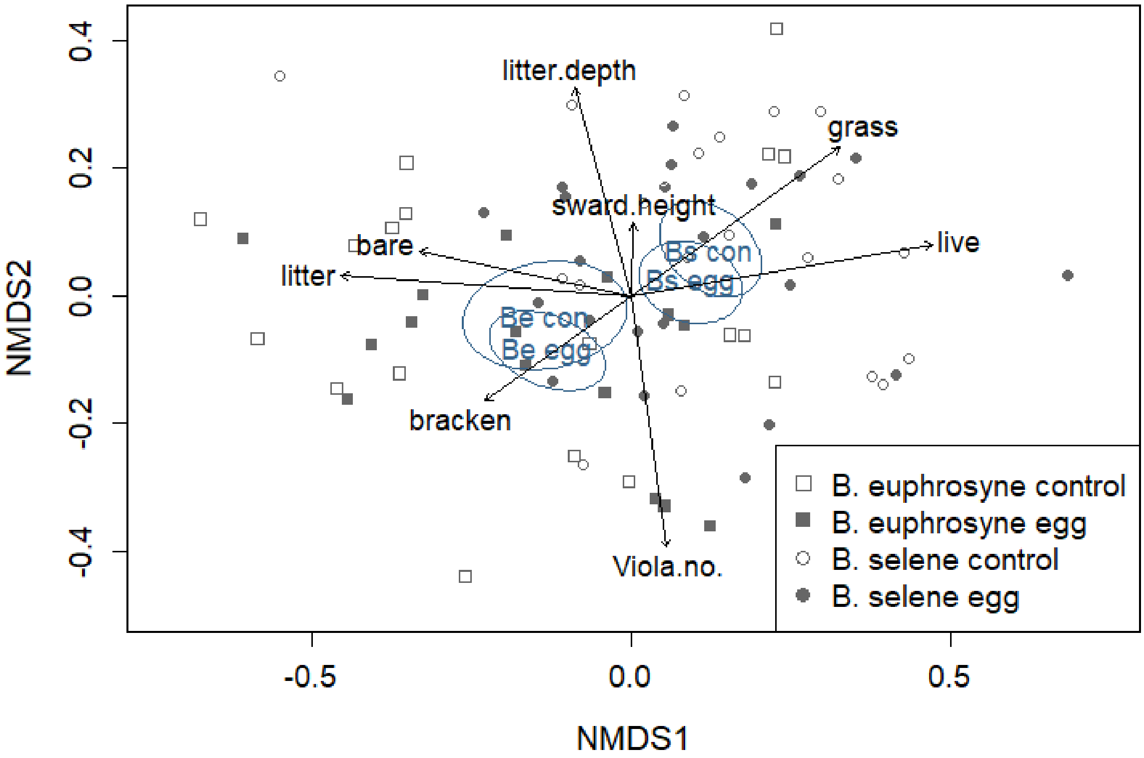

B
N
Reserve boundary
Sex, point type:
F, MRR
F, OVI
M, MRR
200 m
Warton Crag
Occupation Road
Crag Road
Church Hill Avenue
Main Street
Esri UK, Esri, HERE, Garmin, INCREMENT P, Intermap, USGS, METI/NASA

Fig. 1B



0.4
0.2
0.0
-0.2
-0.4
NMDS2
-0.5
0.0
0.5
NMDS1
litter.depth
grass
sward.height
live
bare
litter
Bs con
Bs egg
Be con
Be egg
bracken
Viola.no.
□ B. euphrosyne control
■ B. euphrosyne egg
○ B. selene control
● B. selene egg

Fig. 2

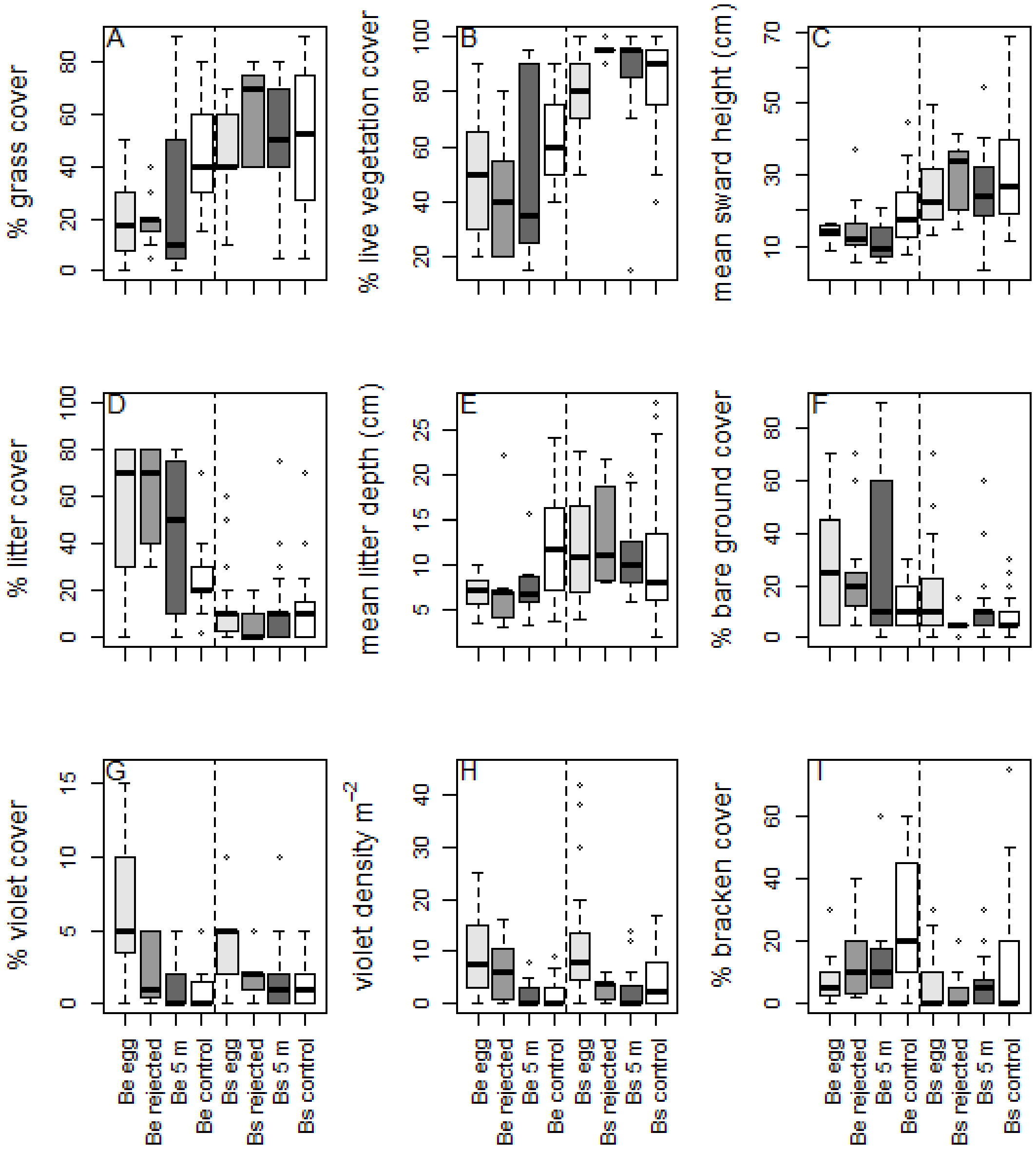
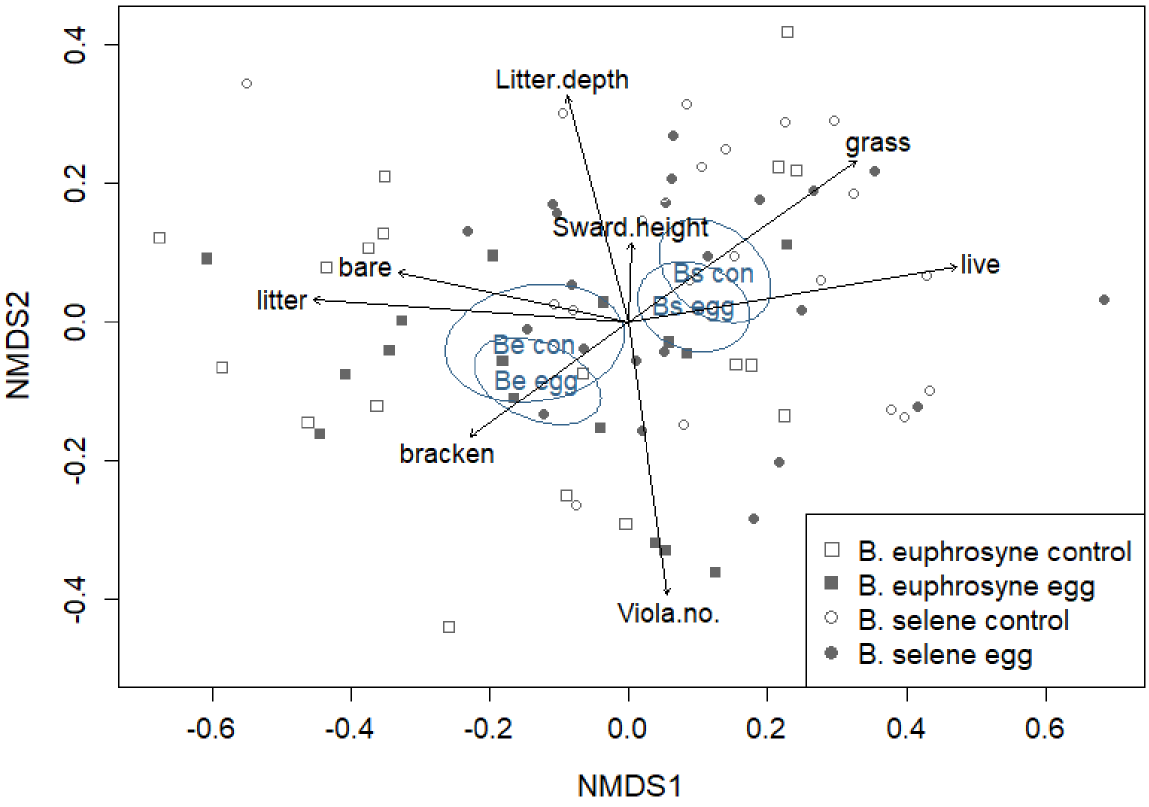

A
% grass cover
0
20
40
60
80
B
% live vegetation cover
20
40
60
80
100
C
mean sward height (cm)
10
30
50
70
D
% litter cover
0
20
40
60
80
100
E
mean litter depth (cm)
5
10
15
20
25
F
% bare ground cover
0
20
40
60
80
G
% violet cover
0
5
10
15
H
violet density m^{-2}
0
10
20
30
40
I
% bracken cover
0
20
40
60
Be egg
Be rejected
Be 5 m
Be control
Bs egg
Bs rejected
Bs 5 m
Bs control

Fig. 3



Litter.depth
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bracken
Viola.no.
□ B. euphrosyne control
■ B. euphrosyne egg
○ B. selene control
● B. selene egg
NMDS2
NMDS1
0.4
0.2
0.0
-0.2
-0.4
-0.6
-0.4
-0.2
0.0
0.2
0.4
0.6

635

636 Fig. 4

Supplementary Material: Contrasting responses to microhabitat and temperature determine breeding habitat differentiation between two *Viola*-feeding butterflies

Andrew Wilby, Lydia Atkinson Grubb, Jessica Burrows, Rosa Menéndez

FIGURE S1. The location of the study site in Lancashire, north-west England. The site is split into three management units each with a UKBMS transect: the Wildlife Trust for Lancashire, Manchester and North Merseyside reserve, the Royal Society for the Protection of Birds reserve (RSPB), and a Local Nature Reserve (LNR) managed by the Arnside and Silverdale Area of Outstanding Natural Beauty team.

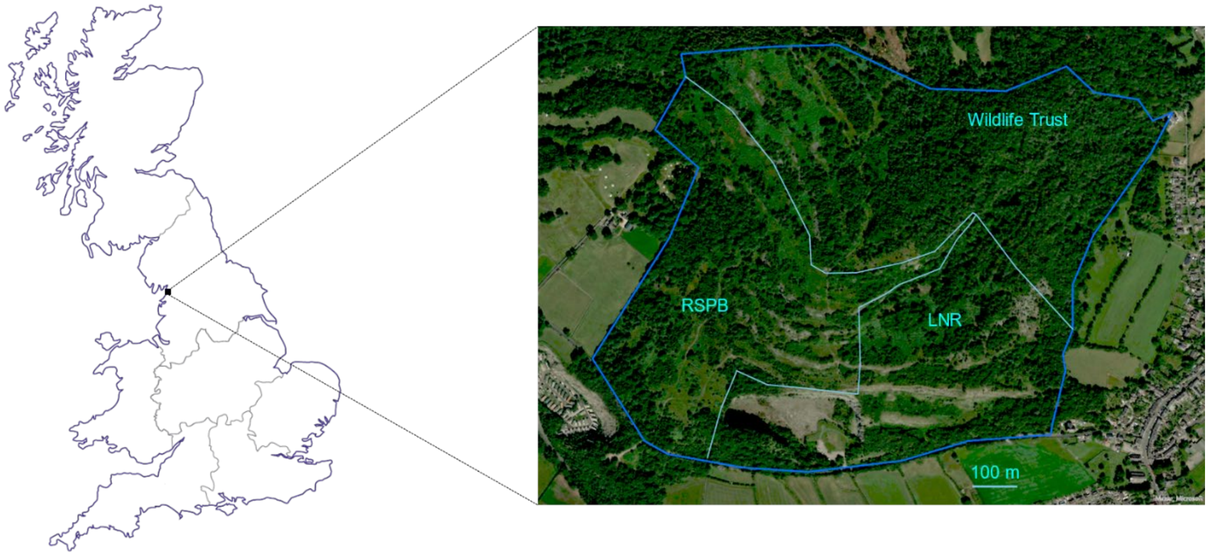

Table S1. Mark-release-recapture data for *B. euyphrosyne* and *B. selene* over the study years 2016 and 2017. Population estimates result from a log-linear Jolly-Seber model calculated assuming an open population. Numbers of captures and recaptures were too small to produce estimates for female *B. selene* in both years.

			Number Marked	% recaptured	Total captures & recaptures	Population estimate (SE)
B. euphrosyne	2016	♀	24	70	42	33(5)
		♂	27	82	53	32(3)
		total	51	78	95	65(6)
	2017	♀	28	36	28	42(9)
		♂	52	83	63	55(2)
		total	80	66	91	87(4)
B. selene	2016	♀	33	24	31	?
		♂	131	33	149	261(45)
		total	164	31	180	330(60)
	2017	♀	40	23	49	?
		♂	152	39	239	260(27)
		total	192	32	288	366(33)