ORIGINAL PAPER

Plant invasion alters movement behaviour in endangered butterflies but not their morphology or genetic variability

Joanna Kajzer-Bonk[1] · Ewa B. Śliwińska[2] · Kamila Malik[1,3]

Abstract

Invasions of alien plants often result in biodiversity loss and may impact the biology of native species. However, the effects of biological invasions on the behavioural responses of native species have rarely been investigated. We studied how the alteration of habitat due to the invasion of alien goldenrod (*Solidago* spp) affects a native butterfly, the scarce large blue *Phengaris teleius*, which is a flagship species for grassland biodiversity conservation. To better understand immediate responses in flight behaviour (daily movements, resting, and dispersal) to a new habitat, we performed observations of experimentally translocated butterflies of two origins (invaded vs. non-invaded habitats) to four different environments: invaded habitat, non-invaded habitat, invaded matrix, non-invaded matrix. Moreover, we tested whether the level of invasion may be related to the variation in morphological traits associated with flight (wing size, body mass) and genetic variability. Flight behaviour was affected by the high goldenrod cover and the sex of the butterflies, regardless of the butterflies' origin. In the habitat and matrix invaded by goldenrod, the butterflies tended to display dispersal behaviour more often compared to the non-invaded ones. Flight distances were longest in the matrix with goldenrod and resting time was longest in habitats invaded by goldenrod. Analysis of morphological traits as well as eight microsatellite loci did not reveal significant differences in morphology or genetic variation among the populations studied.

Implications for insect conservation

Our findings give important insights into immediate behavioural responses within invaded sites and may reflect the plasticity of the species to the changing environment. It indicates an urgent need to properly manage existing habitats as well as to create at least steppingstone habitats as a chance of survival for poor dispersers.

Keywords Biological invasions · Biodiversity conservation · Invertebrates · Flight behaviour · *Maculinea* · Phenotypic plasticity

✉ Joanna Kajzer-Bonk
   joanna.kajzer.bonk@gmail.com

1   Institute of Zoology and Biomedical Research, Faculty of Biology, Jagiellonian University, Gronostajowa 9 Street, Kraków 30-387, Poland

2   Institute of Nature Conservation, Polish Academy of Sciences, Al. Adama Mickiewicza 33, Kraków 31- 120, Poland

3   Institute of Biology, Biotechnology and Environmental Protection, Faculty of Natural Sciences, University of Silesia in Katowice, Katowice, Poland

Introduction

Biological invasions induce fundamental changes in native environments by decreasing their functions and diversity at the species, genetic and ecosystem levels (Crooks 2002). Invasions happen more frequently in disturbed, fragmented and destroyed habitats, which may exacerbate habitat deterioration (Marvier et al. 2004). Invasions also trigger cascading effects among species from different trophic levels (Moroń et al. 2009; Reif et al. 2016; Simao et al. 2010) and cause declines in ecosystem services provision (Walsh et al. 2016). The consequences of species loss due to invasion may be shifted in time, as the abundance and richness of native invertebrates decreases non-linearly (Moroń et al. 2019). The majority of research focuses on exploring

invasions from the invader's perspective, e.g., traits and behavioural flexibility of a successful invader (Sol et al. 2002), pathways, mechanisms and circumstances promoting invasion success (Nentwig 2007) and impact on native population dynamics (Carrol and Fox 2008).

Apart from the effects on demographic trends of native biodiversity, little is known about the mechanistic framework of the impact of invasions on changes in animal behaviour (Stewart et al. 2021). Invasive plants may drive behavioural changes due to changes in: (1) physical structure of the habitat and abiotic conditions, (2) distribution, quality and availability of food resources, water or construction material and (3) plant-animal communication (chemical, visual, structural or even acoustic signals emitted from plants to animals). Behavioural responses refer to a broad spectrum of shifts in foraging, predator avoidance, movement, reproduction, communication and construction of nests / traps. The effects of invasive plants on movement behaviour are particularly poorly described (Stewart et al. 2021). Plant invasions may decrease the overall suitable habitat due to changes in microclimate, vegetation structure, constriction of access to diverse nectar and pollen food through the displacement of native plants, and blooming during a limited time window (Trigos-Peral et al. 2018), and therefore lead to habitat fragmentation. Habitat fragmentation modifies dispersive behaviour with a tendency to decreased dispersal frequency in more fragmented habitats (Schtickzelle et al. 2006), but if it occurs, it covers further distances (Nowicki et al. 2014; Cheptou et al. 2017). Also, small scale habitat fragmentation causes lower visitation rates and reduction of pollen dispersal among flowers in bumblebees (Goverde et al. 2002) and may cause more aggressive behaviour among male butterflies (Merckx & Van Dyck 2005). Invaded plants may change the environment structurally, making it less accessible to food resources and leading to avoidance (Holsman et al. 2010) or, alternatively, support movements through it (Cronin and Haynes 2004). However, there is still an urgent need to quantify the effects of invasion-related fragmentation on behavioural plasticity or potentially adaptive species responses (Cheptou et al. 2017), and knowledge of the mechanism of changes in behaviour is crucial for developing effective conservation tools (Stewart et al. 2021).

In response to invasion, animals may change behaviour in different ways including behavioural plasticity, nongenetic intergenerational mechanisms or natural selection (Stewart et al. 2021). Behavioural changes are constrained by physiological, morphological, sensory, cognitive traits, life span and genetic variation, and trade-offs between costly behaviour and other traits create a degree of adaptation to novel habitats (Bonte et al. 2012). Deteriorated environments may cause changes in butterfly flight morphology (Van Dyck & Matthyssen 1999), change the species' metabolism and enhance dispersal capacities (Lebeau et al. 2016; Vanden Broeck et al. 2017) or promote sedentary behaviour, in extreme cases, leading to wing degeneration (Adamski and Witkowski 1999). Moderate levels of habitat fragmentation may lead to more dispersive phenotypes, but above a certain threshold, the opposite pattern may arise (Bonte et al. 2012). More dispersive individuals may have lower body mass compared to more sedentary ones (Hill et al. 1999). Additionally, landscapes with different levels of habitat fragmentation may generate developmental plasticity of morphological traits (Merckx and Van Dyck 2006). Changes in microclimate, i.e., temperature, may affect development time (Serruys and Van Dyck 2014). Moreover, reduced availability of food during the larval period may result in poorer condition of the offspring (wing length and body weight) and lower fitness (reduced survival of adults) (Boggs and Freeman 2005).

Native species may possess a certain level of behavioural plasticity that may help them to live in invaded habitats. Furthermore, changes in movement patterns may affect genetic variation and immigration rates, and the genetic basis of movements may depend on the environment (Saastamoinen et al. 2018). Still, little is known about the modifying effects of plant invasions on behavioural plasticity, and adaptative genetic changes in native animal species are difficult to show (Lambret et al. 2020). Molecular studies are restricted to exploring possible hybridization and introgression effects between alien and native species from related taxons (Largiadèr 2007; Carrol and Fox 2008). Flight behaviour may modify the expression of many genes, and flight metabolic rate is correlated with heterozygosity (Kvist et al. 2015) but may lead to the opposite effects depending on gender (Niitepõld et al. 2011).

Invasion of alien plants may change species interactions, which is especially well visible in plant-herbivore systems, with effects that may be positive (Rodríguez et al. 2019) or negative (Heleno et al. 2009; Moroń et al. 2009) for populations of native herbivores. Several studies focus on interactions in related native and invasive species i.e., competitive or aggressive behaviour (Phillips and Suarez 2012). It seems that more flexible native generalists have a higher probability of remaining in disturbed dynamic environments (Clavel et al. 2011). The situation may be more complicated if the invasion affects the hosts of specialist parasites, leading to the coextinction of both organisms (Koh et al. 2004; Kajzer-Bonk et al. 2016a). More specifically, there is a lack of studies looking at invader-native species interactions at a behavioural level i.e., testing if the presence of a plant invader modifies the behaviour of native arthropods. For example, we need to know if the presence of plant invaders alters the behaviour of native arthropods and if those

changes, for instance to the movement patterns of arthropods, are plastic or genetic. In the latter case, changes in movement might alter the genetic structure of populations.

In this study, we examined the impact of goldenrod *Solidago* spp. (Asteraceae) invasion on movement behaviour, morphological traits and genetics of the scarce large blue *Phengaris teleius* butterfly. This species is a good indicator of terrestrial biodiversity hot spots, constituting an umbrella species for grassland biodiversity (Thomas and Settele 2004). The narrow niche, high ecological demands and complex life cycle (obligatorily demanding both the presence of the food plant, the great burnet *Sanguisorba officinalis*, and host ants from the genus *Myrmica*; Tartally et al. 2019) make the scarce large blue an indicator of diversity across different taxa. The habitats of the focal species are clearly restricted to meadow patches with food plant and host ant (Nowicki et al. 2005). Nowadays, these habitats are under heavy anthropogenic pressure (Kajzer-Bonk and Nowicki 2022, 2023), due to changes in agricultural practices, drainages, succession, invasion of American goldenrods, decreasing numbers and abundance of host ant species, and changing ant species composition (Kajzer-Bonk et al. 2016a). During most of the flight season of the adult butterflies (second half of June, July and early August), the goldenrod does not bloom and limits access to nectar plants by displacing them and increasing the internal fragmentation of resources within the habitat patch. On the other hand, the tall stems may allow the great burnet to compete with goldenrod and to bloom in invaded meadows (Kajzer-Bonk et al. 2016a).

The aim of this study was to test whether the presence of goldenrod in habitat patches causes changes in (1) movement behaviour (making butterflies more sedentary or imposing dispersal flight and whether potential responses differ between sexes), (2) morphological traits and (3) genetic variation of scarce large blue butterflies. As we occasionally observed flying and resting butterflies during our previous studies (Kajzer-Bonk et al. 2013, 2016b), we expected that butterflies in invaded habitats have a tendency to display more sedentary behaviour and a higher frequency of dispersal flights due to structural changes in the habitat by goldenrod. To test whether there is behavioural plasticity to invaded plant cover, we planned an experiment with translocations of individuals of two origins (non-invaded vs. invaded habitats) to non-invaded and invaded habitats, as well as non-invaded and invaded matrix (habitats between patches). Additionally, as invaded habitats are more isolated, have less available food and have changed microhabitat conditions, we expected that this may affect the wing length and body weight of the butterflies regardless of sex. We also wanted to know whether potential changes

in movement behaviour may change the genetic structure of local populations.

Methods

Study area and species

We conducted the study in 2018 in a complex of semi-natural meadows in the southwestern part of Kraków, Poland. The complex includes several dozen patches, constituting a habitat for a large metapopulation of *Phengaris teleius* butterflies (Nowicki et al. 2007; Kajzer-Bonk et al. 2016b). Endangered scarce large blue butterflies are protected by the EU and Polish law, and part of their habitats has been included in the Natura 2000 network (Kajzer-Bonk and Nowicki 2022). Habitat patches within the studied metapopulation are located close to each other (mean ± SE: 127 ± 16 m, Kajzer-Bonk et al. 2016b; Fig. 1) and ensure good connectivity as they are within the mean covered dispersal distances of the species (80–480 m, Nowicki et al. 2007). The existing local habitat patches are predominantly occupied, and the local population sizes oscillate between several hundred to several thousand individuals (Kajzer-Bonk and Nowicki 2023).

The meadow complex, once managed by extensive mowing/grazing, has been largely abandoned for the last 20 years (Skórka et al. 2007). This has resulted in the succession and expansion of reeds and invasive North American goldenrods (*Solidago gigantea* and *S. canadensis*, Asteraceae, Fig. 1), which is one of the major threats in this area. Goldenrods were introduced to Europe as ornamental plants in the 19th century and due to their colonizing and competing abilities and they gradually change microclimatic conditions. Due to a lack of natural enemies, they also displace the native vegetation (Weber 1998). Characteristic homogenous goldenrod patches affect various animal taxa (Fenesi et al. 2015; Kajzer-Bonk et al. 2016a), and the local climatic niche promotes their further expansion (Weber 2001).

Field study

The study was conducted in habitat patches ranging from 0.1 to 26.5 ha and constituting part of the complex meadow system described above (Fig. 1). To assess the possible effects of goldenrod cover on *P. teleius* movement behaviour, two types of habitats of origin were chosen: (1) non-invaded control habitat (three replicates, with native plant species) and (2) invaded habitat (three replicates, with both native plant species and goldenrod covering more than 40% of a patch). 40% goldenrod cover was selected because the decline in butterfly abundances may be observed from

Fig. 1 Map of the study area in Kraków, Poland

non-invaded habitat patches
invaded habitat patches
non-invaded matrix
invaded matrix
other habitat patches present in 2018
additional samples for genetic analyses

0   500 1000 m
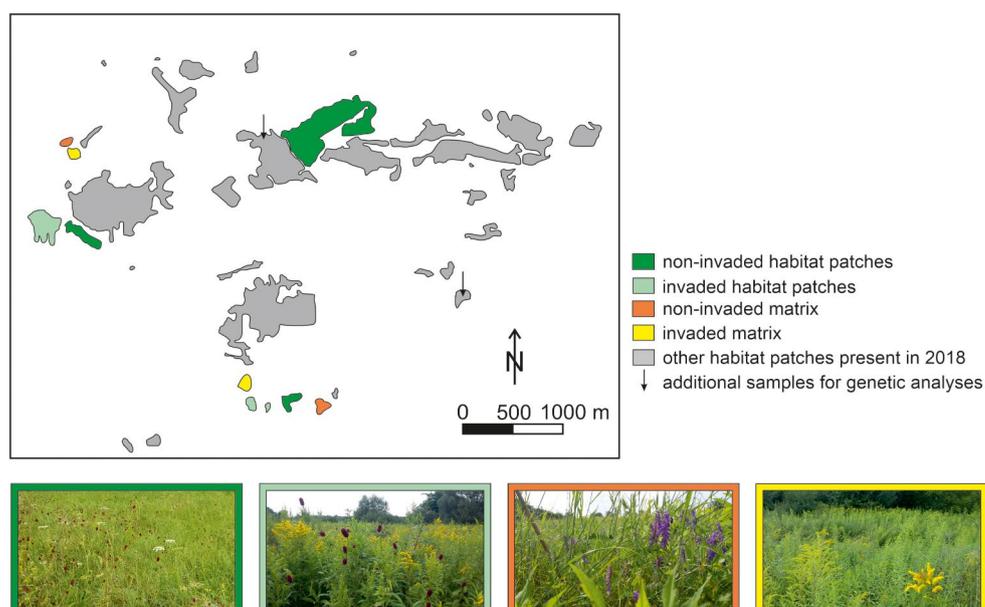
this threshold (Moroń et al. 2019). We used aerial photos taken by a drone in the first half of September during the peak of goldenrod flowering to estimate goldenrod cover with ImageJ software (Abramoff et al. 2004). We estimated habitat quality as the cover of the food plant *S. officinalis* and host ant availability from the *Myrmica* genus in each habitat patch. On each habitat patch, we used three to four randomly selected 5×5 m plots to assess *S. officinalis* abundance using the Braun-Blanquet Cover-Abundance Scale in six categories (see Kajzer-Bonk et al. 2016b for more details). To assess ant density in each habitat patch we used 20 randomly selected traps on 50 m transects except for one small patch where we used 10 traps, according to the method described by Sielezniew (2012). There were no differences in availability of limiting resources between the control and the invaded habitat patches which may modify butterfly behaviour (food plant cover: Mann-Whitney U test, $U = 40$, $N_{invaded} = 11$, $N_{non-invaded} = 12$, $P = 0.115$; host ant availability: Mann-Whitney U test, $U = 1360$, $N_{invaded} = 50$, $N_{non-invaded} = 60$, $P = 0.334$). Additionally, vegetation height within the invaded matrix sites was significantly higher compared to the non-invaded ones (mean $\pm$ SE: 1.02 ± 0.20 and 0.54 ± 0.24, respectively, Mann-Whitney U test: $U = 4211$, $N_{invaded} = 81$, $N_{non-invaded} = 158$, $P < 0.001$).

We conducted behavioural observations of adult butterflies from 4th July to 6th August 2018. We captured butterflies from two habitats of origin (non-invaded control vs. invaded, three replications per habitat) and experimentally moved them to one of the four release habitats, which included: (1) non-invaded control habitat (three sites, with native foodplants); (2) invaded habitat (three sites, with native foodplants and goldenrod covering more than 40% of the patch); (3) non-invaded matrix (two sites, without

foodplants and without goldenrod); (4) invaded matrix (two sites, without foodplants and with domination of goldenrod, more than 40% cover). The capture site of each individual was never used as the release site, to avoid potential biases which may stem from releasing butterflies in familiar patches.

Butterflies were treated according to the procedure described by Skórka et al. (2013), including: marking with a non-toxic marker, putting them into small paper bags, storing and transportation in a cooler box at 10 °C (for 0.5 h), gentle releasing and observation. We conducted observations between 9:00 and 16:00 in conditions which are optimal for butterflies (sunny day, warm weather with a minimum temperature of 20 °C, a maximum wind force of 3 on the Beaufort Scale). On each observation day, we randomized the order of surveys in two types of habitat patches to avoid time effects on changes in dispersal (Plazio et al. 2020a). Once we released a butterfly, we tracked it, keeping a distance of 5 m so as not to disturb its behaviour, and we observed and classified each behaviour: (1) type of flight (routine vs. dispersal movement); (2) flight length (m); (3) turning angle (°); (4) resting time (s). Routine movements are characterised by a chaotic trajectory, whereas dispersal movement is straight, fast and long-distance (Kareiva and Shigesada 1983; Schtickzelle and Baguette 2003). Butterflies may switch from a routine to a dispersal flight or vice-versa, hence the flight type was recorded separately for each flight bout. We matched resting points with numbered bamboo sticks to further measure flight length and turning angle as a measure of flight directionality (Goverde et al. 2002). The observations were standardised and included six distances of routine movements and seven resting points per individual, hence the duration of an observation bout

varied in time. We decided to merge all non-flying behaviour as "resting time" (sitting, feeding, walking on a plant) because of the high homogeneity of observed behaviours (mainly sitting). Oviposition behaviour was excluded from the analyses as it did not occur in the matrix (Skórka et al. 2013). Next, we captured butterflies to make the following morphological measurements: (1) forewing length to the nearest 0.1 mm using a Vernier calliper and (2) weighing in a small paper bag with a TOMOPOL S-50 balance with a precision of 0.005 g. The parameters obtained allowed us to estimate the wing loading index (mg/mm) which is indicative for dispersal abilities (Kalarus et al. 2013). Next, we took a tiny part of butterfly hindwing (i.e., a wingclip) with the non-invasive method described by Hamm et al. (2010) for further genetic analyses. At the end, we released the individually marked butterflies into their habitat of origin. Overall, we conducted observations of at least 15 individuals for each release site.

To exclude the possible effects of the experimental manipulations on butterfly behaviour, we additionally provided analogous observations for 49 individuals without previous catching, cooling, transportation and release in the habitats of origin. There were no differences in behaviour between the manipulated and unmanipulated butterflies (see Table S1 and Fig. S1 for more details).

Genetic analyses

We extracted DNA from all wingclips using the QIAGEN Multiplex DNA kit and arrayed them in 96-well plates for genotyping. We amplified the isolated DNA via polymerase chain reaction (PCR) at a total of eight polymorphic microsatellite loci (See Table S2, Zeisset et al. 2005). We performed amplification and genotyping of microsatellite loci according to the method described by Schuelke (2000). The fluorescently labelled PCR products were separated using the Applied Biosystems 3130xl Genetic Analyzer. Next, we performed an analysis of frequency in GenMapper.

Statistical analyses

We used a generalized linear mixed model (GLMM) with binomial distribution to analyse the probability of taking routine (0) or dispersal (1) flights. Mixed modelling was used in regard of the nested scheme of our study: meadow ID was set as the random factor in all models. The fixed effects were: habitat of origin (non-invaded vs. invaded), release site (habitat with / without goldenrod, matrix with / without goldenrod), sex and two-way interactions (sex * habitat of origin; sex * release site). Additionally, due to the fact that the dataset contained measures on the same individuals, individual ID was added as an additional random

factor. To analyse the distances covered between two resting points and the resting time of the butterflies moved in the experiment (proportion of time resting relative to the total observation duration) we used a GLMM with a Gaussian distribution. The fixed and random factors were the same as in the previous procedure. To compare the distribution of turning angles in different habitat types we used Watson-William's F-Test. The angles were categorized in 12 classes, each of 30°. Analyses were performed in R (R Core Team 2022) using package 'lme4' for GLMM models (Bates et al. 2020) and in the Oriana software for estimates of the angles' distribution (Kovach 2011). We used a backward selection, excluding interaction terms of fixed factors from the final models if non-significant and then used post-hoc Tukey tests.

Next, a generalized linear mixed model (GLMM) with a Gaussian error variance was used for the analysis of factors influencing morphological traits represented as the wing loading index. The wing loading index was $\log_{10}$ transformed to obtain a normal distribution. Sex, habitat of origin (non-invaded vs. invaded) and their interaction were the fixed effects.

Eight polymorphic microsatellite loci were used to analyse genetic diversity in the eight studied subpopulations. For each local population, the percentage of polymorphic loci, Shannon information index (I), observed heterozygosity (Ho), unbiased expected heterozygosity (uHe) and Fixation index (F) were calculated. An AMOVA test was used to check potential differences in genetic variability between butterflies from invaded and non-invaded meadows. This test was performed based on 999 random permutations. Next, a Principal Coordinate Analysis (PCoA) was performed to visualize the components of total genetic variation (within and between local populations) based on the matrix of genetic distances. A Discriminant Analysis for Principal Components (DAPC) was used to consider relationships among the local populations studied and the probabilities of individual memberships within sampled localizations. Genetic analyses were performed in the GenAIEx 6.5 software (Peakall and Smouse 2012) and the package 'adegenet' in R (Jombart 2008).

Results

Butterflies' behaviour and morphology

In total, we experimentally transferred 74 butterflies (40 and 34 from non-invaded and invaded habitats, respectively; Table S3) to four different habitat types. Our experiment revealed modified flight behaviour of butterflies in habitats

with goldenrod cover (Table 1a, Table S4a). The modified behaviour did not depend on butterfly origin (Table 1).

Altogether, 35 (47.3%) of the butterflies started dispersal flight (Table S4B), with a significantly higher number (31) in habitats and matrix with goldenrod (42% of total number of flights) compared to four in habitats and matrix without goldenrod (5% of total number of flights) (Table 1a, Fig. 2a). The probability of dispersal flight was significantly higher in males than females (Table 1a, Fig. 2a). Males flew for the longest distances and these flights occurred in

Table 1 Results of general linear mixed models (GLMMs) on the behaviour and morphology of butterflies. Explanation of fixed factors: 'Meadow type' (non-invaded control vs. invaded habitat), 'Habitat of origin' means the origin of the experimentally moved butterfly (non-invaded control vs. invaded habitat), 'Release site' – the destination (non-invaded control habitat / invaded habitat / non-invaded matrix / invaded matrix). *P* values estimated for an effect (not for particular levels). Significant results are bolded

Fixed effects	Estimate	SE	95% CI	t	P
(a) Effect on flight type					
Habitat of origin	0.397	0.584	1.144	-4.249	0.497
Release site (non-invaded habitat*)					**< 0.001**
Release site (invaded habitat)	2.609	1.227	2.404	2.532	
Release site (invaded matrix)	5.587	1.565	3.067	3.570	
Release site (non-invaded matrix)	1.109	1.262	2.473	0.878	
Sex	1.564	0.618	1.210	2.532	**0.011**
(b) Effect on flight length					
Habitat of origin	0.017	0.480	0.940	-0.256	0.972
Release site (non-invaded habitat*)					**0.024**
Release site (invaded habitat)	-0.104	0.275	0.539	-0.379	
Release site (invaded matrix)	1.089	0.382	0.749	2.847	
Release site (non-invaded matrix)	0.180	0.343	0.672	0.526	
Sex	0.667	0.300	0.587	2.226	**0.026**
(c) Effect on rest time					
Habitat of origin	-0.017	0.065	0.127	-0.265	0.791
Release site (non-invaded habitat*)					**0.002**
Release site (invaded habitat)	0.035	0.055	0.108	0.630	
Release site (invaded matrix)	0.267	0.070	0.137	3.804	
Release site (non-invaded matrix)	0.022	0.046	0.090	0.480	
Sex	0.0001	0.041	0.081	0.003	0.998
(d) Effect on wing loading index					
Meadow type	0.031	0.024	0.048	1.281	0.200
Sex	-0.145	0.015	0.029	-9.902	**< 0.001**

*non-invaded habitat: category '0'

non-invaded habitats and in matrix patches with goldenrod, whereas females in habitats (regardless of goldenrod cover) moved over shorter distances (Table 1b, Fig. 2b). Resting time was on average longer in females than males, regardless of meadow type (Table 1c, Fig. 2c). There were no significant differences in the distribution of turning angles among different habitat types (Watson-Williams test: $F_{3,164}$ = 1.97, $P = 0.12$). The analysis of the angle distributions revealed that turning angles were clustered around 0° in non-invaded habitats (concentration coefficient = 0.993, mean ± SE = -11.4° ± 10.8°), invaded habitats (concentration coefficient = 0.894, mean ± SE = 7.8° ± 15.8°) and non-invaded matrix (concentration coefficient = 0.808, mean ± SE = -2.8° ± 13.6°), but not in the matrix with goldenrod, where the distribution of angles was much more stochastic (concentration coefficient = 0, mean = -103.2°) (See Fig. S2 in Supplementary material). The distribution of turning angles was similar in both sexes with a higher concentration in males (concentration coefficient = 0.993, mean ± SE = 2.3° ± 13.00°) than in females (concentration coefficient = 0.782, mean ± SE = -9.1° ± 9.6°; Watson U test: $U = 0.888$, $df_1 = 44$, $df_2 = 124$, $P < 0.005$).

Overall, we measured and weighed 137 individuals (83 and 54 from non-invaded and invaded habitats, respectively; Table S3c). Females were significantly larger and heavier than males (Table S4C) and revealed a significantly higher wing loading index than males (Table 1d, Fig. S3), but there was no effect of goldenrod cover on this morphological trait (Table 1d).

Genetic diversity

In total, we analysed 142 wingclips: 97 and 45 from non-invaded and invaded meadows, respectively (Table S5). The eight studied microsatellite loci of *P. teleius* were polymorphic in the vast majority of the local populations studied (Table S6). A total of 68 alleles were detected, ranging from three (*Macu 3* and *Macu 7*) to 15 (*Macu 16*) per locus (Table S6). Population genetic diversity indices did not differ between non-invaded and invaded habitats (Mann-Whitney U tests: $N_{non-invaded} = 5$, $N_{invaded} = 3$; Shannon index: $U = 5$, $P = 0.571$, observed heterozygosity: $U = 6$, $P = 0.765$, unbiased expected heterozygosity: $U = 2$, $P = 0.147$, and Fixation index: $U = 7$, $P = 1.0$, Table S7).

The genetic differentiation calculated among local populations (F_{st} indices) was low (Table S8). The AMOVA analysis revealed no differences in genetic diversity among populations differing in goldenrod cover nor generally among subpopulations and a substantial portion of variance was found within individuals (Table S9). The first three axes of the PCoA explained 16.84, 11.01 and 9.33% of the variation respectively, and revealed no patterns of genetic

Fig. 2 Mean (±SE) ratio dispersal movement (**a**), covered distances (**b**) and resting time (**c**) in *Phengaris teleius* females (white bars) and males (blue bars) in four different habitat types. Tukey *post hoc* test results are marked with letters, with different letters indicating significant differences
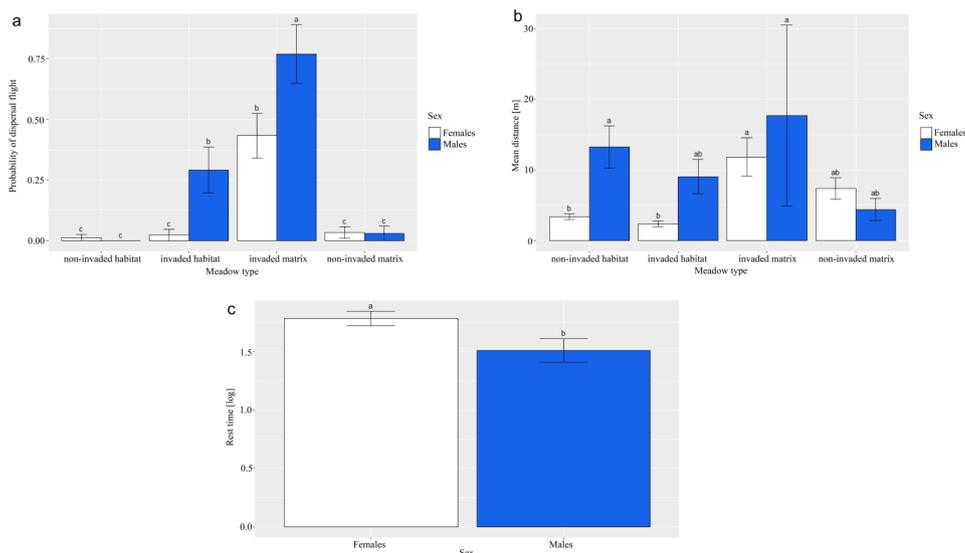
Discussion

This study revealed that invasion of goldenrod in wet meadows modifies the behaviour of scarce large blue butterflies. The butterflies in habitat patches and matrix invaded by goldenrod started dispersal movement more frequently. Butterflies in the invaded matrix covered the longest distances on average. The modifying effect of a matrix with goldenrod may stem from the lack of access to their food plants which provide nectar for adults and host plants for egg-laying. These results are concordant with the results of Skórka et al. (2013) who revealed that the movement distances in the matrix were three times longer than in patch interiors. Invaded habitats may reduce individual fitness through wasting time and energy on long fast flights. The possible effects on population demography should be explored in further studies.

Movement patterns may stem from resource distribution but also from the structure of the landscape (Schultz et al. 2012). It is known that open vs. forest matrix type differentiates the probability of migration with a tendency of lower migration rate but further distances in the more impermeable matrix (Nowicki et al. 2014). Invaded meadows may act as an intermediary between high-contrasting forest matrix and low-contrasting meadow matrix. Our study shows that vegetation which is twice as high within the invaded matrix compared to the non-invaded one leads to higher and faster dispersal movement than in non-invaded

sites. Also, the concentration of flight angles in the invaded matrix is more stochastic which suggests less directed movements. The proximity of habitat patches within the metapopulation enables dispersal and ensures high patch occupancy (Kajzer-Bonk et al. 2016b). Hence, it is not clear whether increasing goldenrod cover may disturb demography. Further study should include intensive Capture – Mark – Recapture methods and meadow systems with patches differing in permeability to answer this question.

Our study revealed behavioural differences between the sexes. Females covered shorter distances more frequently than males. This may be connected to inter-sexual morphological differences (Skórka et al. 2013). The bigger and heavier females carrying eggs may reveal limited dispersal (Gilchrist 1990; Konvička and Kuras 1999; Plazio et al. 2020b). This result is concordant with Schultz et al. (2012) but opposite to Skórka et al. (2013). As increasing isolation may particularly prevent migration of *Phengaris* females (Bonelli et al. 2013), goldenrod cover may have similar additional effects in abandoned meadows. Another probable reason for the discrepancies may stem from different dispersal behaviour as the season progresses, with an increasing emigration rate of females in the second half of the season to reduce intraspecific competition of caterpillars (Plazio et al. 2020a). Our study covered the whole flight period to avoid differences derived from different moments in the flight in season. Behavioural differences also applied to resting time and direction of flight and are consistent with previous results on *Phengaris teleius* (Skórka et al. 2013).

Altered costs of dispersal related to habitat fragmentation caused by goldenrod invasion were not confirmed in our morphological or genetic results. The lack of differences in morphological traits between butterflies from meadows differing in goldenrod cover may be the result of the relatively short period since goldenrod encroachment (Skórka

differentiation in local populations (Table S10). DAPC revealed no differentiation in genetic structure between the local populations studied but was slightly clustered in populations located most eastward (P37) and westward (P1) (Fig. S4).

et al. 2007) and the lack of time for a reaction highlighted in changes in morphological traits. Differences in morphological traits may happen even in neighbouring patches if habitats are contrasting (Kalarus et al. 2013). Some studies have revealed that even increased isolation does not correlate with changes in morphological traits (*Plebejus argus*, Thomas et al. 1998; *Pararge aegeria*, Merckx and Van Dyck 2006). Additionally, low variation in mobility-related traits in a fragmented landscape may explain the lack of detected differences in poor dispersers (Ducatez et al. 2014). In the case of *Phengaris* butterflies, their ecology and highly specialised lifestyle may support the lack of changes in morphological traits: host ant nests which remained present in meadows invaded by goldenrod (Kajzer-Bonk et al. 2016a) may presumably be temporally stable and safe microhabitats ensuring food provisioning regardless of goldenrod invasion. Even if the habitat becomes unsuitable, limited food may not affect morphological traits (Boggs and Freeman 2005). Also, the genetic structure of the local populations studied showed similar observed heterozygosity (mean Ho = 0.69) and similar genetic variability. These results are consistent with previous studies which revealed that the structure of this population more resembled an intra-population spatial fragmentation model than a metapopulation model (Śliwińska 2010). Gene flow in *Phengaris teleius* populations may be negatively affected by habitat fragmentation (Pecsenye et al. 2007). The system of habitats we studied is still quite well connected and local populations are numerous (Nowicki et al. 2007; Kajzer-Bonk et al. 2016b), ensuring a sufficient amount of gene flow. However, especially in the last decade, the metapopulation studied has been exposed to increasing anthropogenic pressure (Kajzer-Bonk and Nowicki 2022, 2023). Hence, further differentiation in genetic structure is possible as indicated by two clusters of the most spatially isolated local populations (Fig. S4). Also, modern genetic methods as more accurate and precise SNPs should be implemented in future analyses (Puckett and Eggert 2016).

Conclusions

Our study provides insight into immediate movement-related behavioural responses within invaded sites and may reflect the plasticity of the species to the changing environmental conditions. Further work is needed to evaluate whether behavioural changes translate into reduced individual fitness and ultimately alter population dynamics. To date, *Phengaris* butterflies as flagship and umbrella species of semi-natural meadows have revealed resistance to flood (Kajzer-Bonk et al. 2013) and fire (Nowicki et al. 2015) but not to habitat loss (Wynhoff 1998; Munguira and Martin

1999). This study indicates a new aspect of the urgent need for active protection of semi-natural meadows, since goldenrod invasion causes habitat loss and modifies flight behaviour. Financial resources should be directed towards eliminating goldenrod using valid, effective methods (van Swaay et al. 2010).

Declarations

References

Abramoff MD, Magalhaes PJ, Ram SJ (2004) Image processing with ImageJ. Biophotonics Int 11:36–42

Adamski P, Witkowski Z (1999) Wing deformation in an isolated carpathian population of *Parnassius apollo* (Papilionidae: Parnassinae). Nota Lepidopterologica 22:67–73

Bates D, Maechler M, Bolker B, Walker S (2020) lme4:Linear mixed-effects models using Eigen and S4. R package version 1.1–23. http://CRAN.R-project.org/package=lme4

Boggs CL, Freeman KD (2005) Larval food limitation in butterflies: effects on adult resourceallocation and fitness. Oecologia 144:353–361. https://doi.org/10.1007/s00442-005-0076-6

Bonelli S, Vrabec V, Witek M, Barbero F, Patricelli D, Nowicki P (2013) Selection on dispersal in isolated butterfly metapopulations. Popul Ecol 55:469–478. https://doi.org/10.1007/s10144-013-0377-2

Bonte D, Van Dyck H, Bullock JM, Coulon A, Delgado M, Gibbs M, Lehouck V, Matthysen E, Mustin K, Saastamoinen M, Schtickzelle N, Stevens VM, Vandewoestijne S, Baguette M, Barton K, Benton TG, Chaput-Bardy A, Clobert J, Dytham C, Hovestadt T, Meier CM, Palmer SCF, Turlure C, Travis JMJ (2012) Costs of dispersal. Biol Rev 87:290–312. https://doi.org/10.1111/j.1469-185X.2011.00201.x

Carrol SP, Fox CW (2008) The next communities: evolution and integration of invasive species. In: Conservation Biology: Evolution in Action. Edited by: Carrol SP, Fox CW. Oxford University Press

Cheptou P-O, Hargreaves AL, Bonte D, Jacquemyn H (2017) Adaptation to fragmentation: evolutionary dynamics driven by human influences. Phil Trans R Soc B 372:20160037. https://doi.org/10.1098/rstb.2016.0037

Clavel J, Julliard R, Devictor V (2011) Worldwide decline of specialist species: toward a global functional homogenization? Front Ecol Environ 9:222–228. https://doi.org/10.1890/080216

Cronin JT, Haynes KJ (2004) An invasive plant promotes unstable host-parasitoid patch dynamics. Ecology 85:2772–2782. https://doi.org/10.1890/04-0303

Crooks JA (2002) Characterizing ecosystem-level consequences of biological invasions: the role of ecosystem engineers. Oikos 97:153–166. https://doi.org/10.1034/j.1600-0706.2002.970201.x

Ducatez S, Humeau A, Congretel M, Fréville H, Baguette M (2014) Butterfly species differing in mobility show different structures of dispersal-related syndromes in the same fragmented landscape. Ecography 37:378–389. https://doi.org/10.1111/j.1600-0587.2013.00365.x

Fenesi A, Vágási CI, Beldean M, Földesi R, Kolcsár L, Shapiro JT, Török E, Kovács-Hostyánszki A (2015) *Solidago canadensis* impacts on native plant and pollinator communities in differentaged old fields. Basic Appl Ecol 16:335–346. https://doi.org/10.1016/j.baae.2015.03.003

Gilchrist GW (1990) The consequences of sexual dimorphism in body size for butterfly flight and thermoregulation. Funct Ecol 4:475–487. https://doi.org/10.2307/2389315

Goverde M, Schweizer B, Baur B, Erhardt A (2002) Small-scale habitat fragmentation effects on pollinator behaviour: experimental evidence from the bumblebee *Bombus veteranus* on calcareous grasslands. Biol Conserv 104:293–299. https://doi.org/10.1016/S0006-3207(01)00194-X

Hamm CA, Aggarwal D, Landis DA (2010) Evaluating the impact of non-lethal DNA sampling on two butterflies, *Vanessa cardui* and *Satyrodes eurydice*. J Insect Conserv 14:11–18. https://doi.org/10.1007/s10841-009-9219-0

Heleno RH, Ceia RS, Ramos JA, Memmott J (2009) Effects of alien plants on insect abundance and biomass: a food-web Approach. Conserv Biol 23:410–419. https://doi.org/10.1111/j.1523-1739.2008.01129.x

Hill JK, Thomas CD, Lewis OT (1999) Flight morphology in fragmented populations of a rare British butterfly, *Hesperia comma*. Biol Conserv 87:277–283. https://doi.org/10.1016/S0006-3207(98)00091-3

Holsman KK, McDonald PS, Barreyro PA, Armstrong DA (2010) Restoration through eradication? Removal of an invasive bioengineer restores some habitat function for a native predator. Ecol Appl 20:2249–2262. https://doi.org/10.1890/09-1168.1

Jombart T (2008) Adegenet: a R package for the multivariate analysis of genetic markers. Bioinformatics 24:1403–1405. https://doi.org/10.1093/bioinformatics/btn129

Kajzer-Bonk J, Nowicki P (2022) Butterflies in trouble: the effectiveness of Natura 2000 network in preventing habitat loss and population declines of endangered species in urban area. Ecol Indic 108518. https://doi.org/10.1016/j.ecolind.2021.108518

Kajzer-Bonk J, Nowicki P (2023) Vanishing meadows – quantitative analysis of factors driving population declines of endangered butterflies. Biol Conserv 282:110050. https://doi.org/10.1016/j.biocon.2023.110050

Kajzer-Bonk J, Nowicki P, Bonk M, Skórka P, Witek M, Woyciechowski M (2013) Local populations of endangered *Maculinea* (*Phengaris*) butterflies are flood resistant. J Insect Conserv 17:1105–1112. https://doi.org/10.1007/s10841-013-9591-7

Kajzer-Bonk J, Szpiłyk D, Woyciechowski M (2016a) Invasive goldenrods affect abundance and diversity of grassland ant communities (Hymenoptera: Formicidae). J Insect Conserv 20:99–105. https://doi.org/10.1007/s10841-016-9843-4

Kajzer-Bonk J, Skórka P, Nowicki P, Bonk M, Król W, Szpiłyk D, Woyciechowski M (2016b) Relative contribution of matrix structure, patch resources and management to the local densities of two large blue butterfly species. PLoS ONE 11:e0168679. https://doi.org/10.1371/journal.pone.0168679

Kalarus K, Skórka P, Halecki W, Jirak A, Kajzer-Bonk J, Nowicki P (2013) Within-patch mobility and flight morphology reflect resource use and dispersal potential in the dryad butterfly *Minois dryas*. J Insect Conserv 17:1221–1228. https://depot.ceon.pl/handle/123456789/3173

Kareiva PM, Shigesada N (1983) Analyzing insect movement as a correlated random walk. Oecologia 56:234–238. https://doi.org/10.1007/BF00379695

Koh LP, Dunn RR, Sodhi NS, Colwell RK, Proctor HC, Smith VS (2004) Species coextinctions and the biodiversity crisis. Science 305:1632–1634. https://doi.org/10.1126/science.1101101

Konvička M, Kuras T (1999) Population structure, behaviour and selection of oviposition sites of an endangered butterfly, *Parnassius mnemosyne*, in Litovelské Pomoravíl. Czech Republic. J Insect Conserv 3:211–223. https://doi.org/10.1023/A:1009641618795

Kovach WL (2011) Oriana – Circular Statistics for Windows,ver. 4. Kovach Computing Services, Pentraeth, Wales, U.K. https://www.kovcomp.co.uk/oriana/oriana4man.pdf

Kvist J, Mattila ALK, Somervuo P, Ahola V, Koskinen P, Paulin L, Salmela L, Fountain T, Rastas P, Ruokolainen A, Taipale M, Holm L, Auvinen P, Lehtonen R, Frilander MJ, Hanski I (2015) Flight-induced changes in gene expression in the Glanville Fritillary butterfly. Mol Ecol 24:4886–4900. https://doi.org/10.1111/mec.13359

Lambret MR, Brans KI, Des Roches S, Donihue CM, Diamond SE (2020) Adaptive evolution in cities: Progress and misconceptions. TREE 36:239–257. https://doi.org/10.1016/j.tree.2020.11.002

Largiadèr CR (2007) Hybridization and Introgression between Native and alien species. In: Nentwig W (ed) Biological invasions. Ecological studies (analysis and synthesis), vol 193. Springer, Berlin, Heidelberg. https://doi.org/10.1007/978-3-540-36920-2_16

Lebeau J, Wesselingh RA, Van Dyck H (2016) Nectar resource limitation affects butterfly flight performance and metabolism differently in intensive and extensive agricultural landscapes. *Proc. R. Soc. B* 283:20160455. https://doi.org/10.1098/rspb.2016.0455

Marvier M, Kareiva P, Neubert MG (2004) Habitat destruction, fragmentation, and disturbance promote invasion by habitat generalists in a multispecies metapopulation. Risk Anal 24:869–878. https://doi.org/10.1111/j.0272-4332.2004.00485.x

Merckx T, Van Dyck H (2005) Mate location behaviour of the butterfly *pararge aegeria* in woodland and fragmented

landscapes. Anim Behav 70:411–416. https://doi.org/10.1016/j.anbehav.2004.12.005

Merckx T, Van Dyck H (2006) Landscape structure and phenotypic plasticity in flight morphology in the butterfly *pararge aegeria*. Oikos 113:226–232. https://doi.org/10.1111/j.2006.0030-1299.14501.x

Moroń D, Lenda M, Skórka P, Szentgyörgyi H, Settele J, Woyciechowski M (2009) Wild pollinator communities are negatively affected by invasion of alien goldenrods in grassland landscapes. Biol Conserv 142:1322–1332. https://doi.org/10.1016/j.biocon.2008.12.036

Moroń D, Skórka P, Lenda M, Kajzer-Bonk J, Mielczarek Ł, Rożej-Pabijan E, Wantuch M (2019) Linear and non-linear effects of goldenrod invasions on native pollinator and plant populations. Biol Invasions 21:947–960. https://doi.org/10.1007/s10530-018-1874-1

Munguira ML, Martin J (1999) Action plan for *Maculinea* butterflies in Europe. Nat Environ 97

Nentwig W (ed) 2007. Biological invasions. Ecological studies (analysis and synthesis), vol 193. Springer, Berlin, Heidelberg. https://doi.org/10.1007/978-3-540-36920-2_16

Niitepõld K, Mattila ALK, Harrison PJ, Hanski I (2011) Flight metabolic rate has contrasting effects on dispersal in the two sexes of the Glanville Fritillary butterfly. Oecologia 165:847–854. https://doi.org/10.1007/s00442-010-1886-8

Nowicki P, Witek M, Skórka P, Settele J, Woyciechowski M (2005) Population ecology of the endangered butterflies *Maculinea teleius* and *M. Nausithous* and the implications for conservation. Popul Ecol 47:193–202. https://doi.org/10.1007/s10144-005-0222-3

Nowicki P, Pepkowska A, Kudlek J, Skórka P, Witek M, Settele J, Woyciechowski M (2007) From metapopulation theory to conservation recommendations: lessons from spatial occurrence and abundance patterns of *Maculinea* butterflies. Biol Conserv 140:119–129. https://doi.org/10.1016/j.biocon.2007.08.001

Nowicki P, Vrabec V, Binzenhöfer B, Feil J, Zakšek B, Hovestadt T, Settele J (2014) Butterfly dispersal in inhospitable matrix: rare, risky, but long-distance. Landsc Ecol 29:401–412. https://doi.org/10.1007/s10980-013-9971-0

Nowicki P, Marczyk J, Kajzer-Bonk J (2015) Metapopulations of endangered *Maculinea* butterflies are resilient to large-scale. Ecohydrology 8:398–405. https://doi.org/10.1002/eco.1484

Peakall R, Smouse PE (2012) GenAlEx 6.5: genetic analysis in Excel. Population genetic software for teaching and research—an update. Bioinformatics 28:2537–2539. https://doi.org/10.1093/bioinformatics/bts460

Pecsenye K, Bereczki J, Tihanyi B, Toth A, Peregovits L, Varga Z (2007) Genetic differentiation among *Maculinea* species (Lepidoptera: Lycaenidae) in eastern Central Europe. Biol J Linn Soc 91:11–21. https://doi.org/10.1111/j.1095-8312.2007.00781.x

Phillips BL, Suarez AV (2012) The role of behavioural variation in the invasion of new areas. Chapter 14. In: Candolin U, Wong BBM (eds) Behavioural responses to a changing world: mechanisms and consequences. Oxford University Press, pp 190–200

Plazio E, Margot T, Nowicki P (2020a) Intersexual differences in density-dependent dispersal and their evolutionary drivers. J Evol Biol 33:1495–1506. https://doi.org/10.1111/jeb.13688

Plazio E, Bubová T, Vrabec V, Nowicki P (2020b) Sex-biased topography effects on butterfly dispersal. Mov Ecol 8:50. https://doi.org/10.1186/s40462-020-00234-6

Puckett EE, Eggert LS (2016) Comparison of SNP and microsatellite genotyping panels for spatial assignment of individuals to natal range: a case study using the American black bear (*Ursus americanus*). Biol Conserv 193:86–93. https://doi.org/10.1016/j.biocon.2015.11.020

R Core Team (2022) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. R version 4.2.1

Reif J, Hanzelka J, Kadlec T, Štrobl M, Hejda M (2016) Conservation implications of cascading effects among groups of organisms: the alien tree *Robinia pseudacacia* in the Czech Republic as a case study. Biol Conserv 198:50–59. https://doi.org/10.1016/j.biocon.2016.04.003

Rodríguez J, Thompson V, Rubido-Bará M, Cordero-Rivera A, González L (2019) Herbivore accumulation on invasive alien plants increases the distribution range of generalist herbivorous insects and supports proliferation of non-native insect pests. Biol Invasions 21:1511–1527. https://doi.org/10.1007/s10530-019-01913-1

Saastamoinen M, Bocedi G, Cote J, Legrand D, Guillaume F, Wheat CW, Fronhofer EA, Garcia C, Henry R, Husby A, Baguette M, Bonte D, Coulon A, Kokko H, Matthysen E, Niitepõld K, Nonaka E, Stevens VM, Travis JMJ, Donohue K, Bullock JM, del Mar Delgado M (2018) Genetics of dispersal. Biol Rev 93:574–599. https://doi.org/10.1111/brv.12356

Schtickzelle N, Baguette M (2003) Behavioural responses to habitat patch boundaries restrict dispersal and generate emigration–patch area relationships in fragmented landscapes. J Anim Ecol 72:533–545. https://doi.org/10.1046/j.1365-2656.2003.00723.x

Schtickzelle N, Gwenaelle M, Baguette M (2006) Dispersal depression with habitat fragmentation in the bog fritillary butterfly. Ecology 87:1057–1065. https://www.jstor.org/stable/20069035

Schuelke M (2000) An economic method for the fluorecscent labeling of PCR fragments. Nat Biotechnol 18:233–234. https://doi.org/10.1038/72708

Schultz CB, Franco AMA, Crone EE (2012) Response of butterflies to structural and resource boundaries. J Anim Ecol 81:724–734. https://doi.org/10.1111/j.1365-2656.2011.01947.x

Serruys M, Van Dyck H (2014) Development, survival, and phenotypic plasticity in anthropogenic landscapes: trade-offs between offspring quantity and quality in the nettle-feeding peacock butterfly. Oecologia 176:379–387. https://doi.org/10.1007/s00442-014-3016-5

Sielezniew M (2012) Modraszek telejus *Phengaris* (*Maculinea*) *teleius* In: Makomaska-Juchiewicz M, Baran P (red.) 2012. Monitoring gatunków zwierząt. Przewodnik metodyczny. Część II. GIOŚ, Warszawa, pp. 199–218

Simao MCM, Flory SL, Rudgers JA (2010) Experimental plant invasion reduces arthropod abundance and richness across multiple trophic levels. Oikos 119:1553–1562. https://doi.org/10.1111/j.1600-0706.2010.18382.x

Skórka P, Settele J, Woyciechowski M (2007) Effects of management cessation on grassland butterflies in southern Poland. Agr Ecosyst Environ 121:319–324. https://doi.org/10.1016/j.agee.2006.11.001

Skórka P, Nowicki P, Lenda M, Witek M, Śliwińska EB, Settele J, Woyciechowski M (2013) Different flight behaviour of the endangered scarce large blue butterfly *Phengaris teleius* (Lepidoptera: Lycaenidae) within and outside its habitat patches. Landsc Ecol 28:533–546. https://doi.org/10.1007/s10980-013-9855-3

Śliwińska EB (2010) Genetic structure of the myrmecophilous butterfly *Maculinea teleius* (Lepidoptera: Lycaenidae) population. PhD thesis. Kraków

Sol D, Timmermans S, Lefebvre L (2002) Behavioural flexibility and invasion success in birds. Anim Behav 63:495–502. https://doi.org/10.1006/anbe.2001.1953

Stewart PS, Hill RA, Stephens PA, Whittingham MJ, Dawson W (2021) Impacts of invasive plants on animal behaviour. Ecol Lett 24:891–907. https://doi.org/10.1111/ele.13687

Tartally A, Thomas JA, Anton C, Balletto E, Barbero F, Bonelli S, Bräu M, Casacci LP, Csősz S, Czekes Z, Dolek M, Dziekańska I, Elmes G, Fürst MA, Glinka U, Hochberg ME, Höttinger H, Hula V, Maes D, Munguira ML, Musche M, Nielsen PS, Nowicki P, Oliveira PS, Peregovits L, Ritter S, Schlick-Steiner BC, Settele J,

Sielezniew M, Simcox DJ, Stankiewicz AM, Steiner FM, Švitra G, Ugelvig LV, Van Dyck H, Varga Z, Witek M, Woyciechowski M, Wynhoff I, Nash DR (2019) Patterns of host use by brood-parasitic *Maculinea* butterflies across Europe. Phil Trans R Soc B 374:20180202. https://doi.org/10.1098/rstb.2018.0202

Thomas JA, Settele J (2004) Butterfly mimics of ants. Nature 432:283–284. https://doi.org/10.1038/432283a

Thomas CD, Hill JK, Lewis OT (1998) Evolutionary consequences of habitat fragmentation in a localized butterfly. J Anim Ecol 67:485–497. https://doi.org/10.1046/j.1365-2656.1998.00213.x

Trigos-Peral G, Casacci LP, Ślipiński P, Grześ IM, Moroń D, Babik H, Witek M (2018) Ant communities and *Solidago* plant invasion: environmental properties and food sources. Entomol Sci 21:270–278. https://doi.org/10.1111/ens.12304

Van Dyck H, Matthysen E (1999) Habitat fragmentation and insect flight: a changing 'design' in a changing landscape? TREE 14:172–174. https://doi.org/10.1016/S0169-5347(99)01610-9

Van Swaay CAM, Collins S, Dusej G, Maes D, Munguira ML, Rakosy L, Ryrholm N, Šašid M, Settele J, Thomas J, Verovnik R, Verstrael T, Warren MS, Wiemers M, Wynhoff I (2010) Do's and don'ts for butterflies of the habitats Directive. Report VS2010.037. Butterfly Conservation Europe & De Vlinderstichting

Vanden Broeck A, Maes D, Kelager A, Wynhoff I, WallisDeVries MF, Nash DR, Oostermeijer JGB, Van Dyck H, Mergeay J (2017) Gene flow and effective population sizes of the butterfly *Maculinea alcon* in a highly fragmented, anthropogenic landscape. Biol Conserv 209:89–97. https://doi.org/10.1016/j.biocon.2017.02.001

Walsh JR, Carpente SR, Vander Zanden MJ (2016) Invasive species triggers a massive loss of ecosystem services through a trophic cascade. PNAS 113:4081–4085. https://doi.org/10.1073/pnas.1600366113

Weber E (1998) The dynamics of plant invasions: a case study of three exotic goldenrod species (*Solidago* L.) in Europe. J Biogeogr 25:147–154. http://www.jstor.org/stable/2846283

Weber E (2001) Current and potential ranges of three exotic goldenrods (*Solidago*) in Europe. Conserv Biol 15:122–128. https://doi.org/10.1111/j.1523-1739.2001.99424.x

Wynhoff I (1998) The recent distribution of the European *Maculinea* species. J Insect Conserv 2:15–27. https://doi.org/10.1023/A:1009636605309

Zeisset I, Damm Als T, Settele J, Boomsma J (2005) Microsatellite markers for the large blue butterflies *Maculinea Nausithous* and *Maculinea alcon* (Lepidoptera: Lycaenidae) and their amplification in other *Maculinea* species. Mol Ecol Notes 5:165–168. https://doi.org/10.1111/j.1471-8286.2004.00872.x