Nestedness of habitat specialists within habitat generalists in a butterfly assemblage

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Abstract. 1. The habitat requirements of a species are the resources, conditions and space required for survival and reproduction. The habitat requirements of butterflies have been well studied, but the extent to which individuals within a species and between species utilise and share the habitat is poorly known.

2. In a butterfly assemblage in northern Italy, we found that adults from 30 species avoid deciduous high-density forests and their ecotones, and they were positively related to open areas and their ecotones. Besides these common features, five groups of species can be discriminated in relation to a gradient from open area to forest, and species within groups were not equally specialised, as observed from a bipartite network analysis. In particular, some species appeared to be specialised and others appeared to be generalist, suggesting a nested pattern of resource use, rather than a clustered pattern in which each species uses a different subset of habitat types.

3. The degree of variation in specialisation among species varied with the number of species falling in each group. Thus, an increased number of species, and thus possibly competition, is more likely to promote the co-occurrence of generalist and specialised species (nested patterns) rather than an increased niche segregation among species.

4. Ascertaining how species overlap their habitat use at a local scale can be relevant for conservation purposes, because specialised populations are potentially more susceptible to network distortions.

Key words. Butterfly, ecological network, habitat requirements, habitat specialisation, niche segregation.

Introduction

Butterflies (Lepidoptera) are a well-studied insect group both taxonomically and ecologically (e.g. Gilbert, 1984; Kocher & Williams, 2000; Ghazoul, 2002; Stefanescu *et al.*, 2004, 2011), particularly because they interact with

plants both as larval herbivores and adult pollinators (Borges *et al.*, 2003; Hardy *et al.*, 2007) and have different habitat requirements even at each life stage (New *et al.*, 1995; Samways & Lu, 2007; Dennis, 2010). In turn, because these interactions are important mechanisms of biological diversification (Leather, 1990; Basset, 1991a,b; Slansky, 1993), the abundance and distribution of butterflies within a particular area can give insights into the environment's quality (Kocher & Williams, 2000).

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These largely specialised butterfly–habitat associations are influenced by many physical and biological factors (Leps & Spitzer, 1990; Spitzer *et al.*, 1993), including temperature, humidity, light levels, rainfall patterns, local microclimate conditions, grassland type and host–plant species richness and distribution (Hill *et al.*, 2001; Meyer & Sisk, 2001; Collinge *et al.*, 2003; Menéndez *et al.*, 2007). All these variables define the *resource-based habitat* or *habitat requirements* of each butterfly species, i.e., the habitat characterised by resources and conditions required by organisms and their access to them (Dennis, 2010). Furthermore, competition among species, presence of invasive species and avoidance strategies to escape from natural enemies (enemy-free space) also affect species occurrence in a given area (Murphy, 2004; Dennis, 2010; Wagner & Van Driesche, 2010; Hanula & Horn, 2011).

While habitat requirements have been assessed for many butterfly species in detail, the degree to which species within an assemblage segregate their habitat use (i.e. how they are specialised in a subset of habitat types) has been less well studied.

The concepts of *habitat requirements* and *habitat specialisation* are strongly linked to the classical differences between the *pure* ecological niche based on the *physiological optimum* (Austin, 1980, 1985) (niche requirements) and the true and realised ecological niche (Hutchinson, 1957; Pulliam, 2000) (niche specialisation). Thus, while habitat requirements can be seen as a property of a species, habitat specialisation is a variable property that can vary with many interacting factors such as the presence of inter-specific competitors, the population density and thus the intra-specific competition and the body size of the individuals, among others (Chase & Leibold, 2003; Dennis *et al.*, 2003, 2011; Rosenfeld, 2003).

Consider, for example, two butterfly species occurring in the same area that use similar habitat types, e.g., open grass areas and ecotones, and thus require the same habitat conditions. The two species may either use both habitat types equally (generalism) or segregate the use of the two habitat types (specialisation). Species may in fact overlap to different degrees the relative use of each type of habitat (Julliard *et al.*, 2006; Poisot *et al.*, 2011), a pattern that may occur either because of an evolutionary trade-off between the ability of species to exploit a range of resources and their capability of using each one (Futuyma & Moreno, 1988), or because of factors such as inter-specific competition (Schroder & Rosenzweig, 1975). It is thus important to analyse the two concepts (habitat requirements and habitat specialisation) simultaneously in a given species assemblage.

Here, we analysed the relationship between habitat requirements and habitat specialisation in an assemblage of butterflies in a riverine natural park in northern Italy. The aim was to test whether butterfly species with similar habitat requirements overlap their habitat use or, alternatively, they segregate their habitat use despite having similar habitat requirements. To do this, we first defined the requirements, i.e., which habitat types positively or

negatively correlate with species abundances, and then used a network-based approach (Blüthgen *et al.*, 2006) to analyse the degree of habitat use overlap within groups of species with similar habitat requirements.

Materials and methods

Study area

The study was conducted in the Ticino Regional Park (Lombardy, northern Italy, approximately from 45°44'0" N to 45°10'0" N and from 8°38'0" E to 9°16'0" E, with an elevation of 50–200 m a.s.l.) in summer 2008–2009. The study area is characterised by a continental climate with cold winters and hot summers and covers about 91 000 hectares. The park represents one of the most important green hot spots of the Po river Plain, which is otherwise very anthropised.

Within the park, four sites were chosen for the sampling programme. The two southern sites (coded as 1 and 4) (Fig. 1) were generally characterised by a thick riparian oak forest with some agricultural areas (poplar plantations, transects 1 and 2). Site 1 in particular showed a high degree of habitat diversity, including a flooded forest with *Alnus glutinosa*, *Salix alba* and *Populus alba*. The two northern sites (coded as 2 and 3) (Fig. 1) include areas that have previously been intensively used as military areas, and they can be considered partially anthropised areas. Although some natural parts still persist, the presence of alien and invasive plant species is relevant. These two sites were mainly characterised by open areas with shrubs (*Calluna vulgaris*, *Crataegus monogyna*, *Rubus caesius*, *Cytisus scoparius*) and by a low density of trees of exotic species, such as *Robinia pseudoacacia*, *Ailanthus altissima* and *Prunus serotina*.

Species sampling procedure

Sampling was carried out from the end of June to the first days of September for 9 weeks/year. Sites 1 and 2 were sampled in 2008, while sites 3 and 4 were sampled in 2009. Surveys were conducted between 09:30 and 17:00 h. Every site contained three 500 m line transects ($n = 12$ plots), in which we followed established guidelines for butterfly monitoring to minimise observer bias (Pollard & Yates, 1993). Observations were carried out during systematic walking surveys (75 min per plot visit – standardised search time) only in favourable temperature conditions (temperature $>17^{\circ}\text{C}$) and in calm to light winds. Every transect walk was repeated twice a week, for a total of 216 transects and 108 walked kilometres.

In contrast to previous research on butterflies, this study was based on a precise georeference of every sampled individual leading to a database of butterfly positions characterised by a large sample ($n = 2924$) and a good level of accuracy (Breed & Severns, 2015). All the points

Fig. 1. Study area and sampling. (a) Location of the four plots (numbered 1–4) where species were sampled; (b) a plot in which the three transects performed are shown; (c) details of one transect showing the points recorded with the GPS for a single species of butterfly throughout the whole study.

were recorded by a GPS (Garmin 60 CSx) whose standard error is considered to be between 4 and 7 m in good weather conditions. We considered the average GPS error to be two to seven times lower than the minimum detectable linear dimension of the available land cover, which is more accurate (minimum cartographic unit: 0.16 ha, scale 1:10 000) than land cover data used in other studies of butterfly species distributions (minimum cartographic unit: 2.25 ha, scale 1:50 000, Gutiérrez *et al.*, 2010).

GIS analyses and definition of habitat types

All the recorded points were imported and overlaid under a GIS environment (ESRI Arc View 3.2a) with the database of land cover classes (hereafter: habitat classes) describing the whole Lombardy region, thereby including the 12 plots (DUSAF, *Destinazione Uso dei Suoli Agricoli e Forestali*, ERSAF 2008). In the sampled areas, eight habitats were recognised together with a further seven ecotones between these habitats for a total of 15 habitat classes (see Table 1). The detail level is equal to an information scale of 1:10 000. For each land use feature with a polygonal configuration and a measurable surface, the minimum dimensional threshold of representability corresponds to 0.16 ha, and the smallest detectable linear dimension of the polygon is 20 m (http://www.ersaf.lombardia.it/upload/ersaf/gestionedocumentale/WEB_Libro_-suolo_EN_784_11577.pdf).

All twelve 500 m transects were first divided into 300 sectors ($n = 25$ in each transect), each characterised by a length of 20 m and a width of 50 m, in order to consider the influence of habitat classes contiguous to the point of the supposed selection operated by every individual (Fig. 1). A double GIS spatial join application associated the field-sampled points with the sectors previously associated with habitat classes (Fig. 1). When more than one habitat class fell into the same sector, we considered it as a separate habitat class composed and identified by the two source–habitat classes. We then counted the abundance of sampled individuals of each species in each sector and clustered the sectors according to the habitat class they showed, thereby calculating abundances for each habitat type.

Habitat requirements of species

Data were prepared for analysis with the R package ‘vegan’ (version 2.0–5, The R Development Core Team 2011) to associate the different habitat preferences with each species and assess habitat requirements. The matrix had species as rows and line transects as columns. We calculated Morisita dissimilarity indices among species using the ‘vegdist’ function. Morisita is an index of patchiness, and it is used to calculate overlap among samples (Morisita, 1959). The dissimilarity matrix obtained was clustered specifying the ‘complete linkage’ method in the ‘hclust’ function in the ‘vegan’ package. We applied the

Table 1. Habitats in our study area in which butterfly species were sampled.

DUSAF code	Habitat description	Dominant plant species	Phytosociological classification
31111	Coppice deciduous forests at high and medium density	<i>Quercus robur</i> , <i>Carpinus betulus</i>	<i>Carpinion betuli</i>
31121	Coppice deciduous forests at low density	<i>Quercus robur</i> , <i>Carpinus betulus</i>	<i>Carpinion betuli</i>
3113	Riparian forest	<i>Alnus glutinosa</i> , <i>Salix alba</i> , <i>Populus alba</i>	<i>Salicion albae</i>
331	Beaches, dunes and stony riverbeds	<i>Bidens frondosus</i> , <i>Persicaria hydropiper</i> , <i>Ranunculus sceleratus</i>	<i>Bidention tripartitae</i>
324	Shrubland or grassland with scattered trees	<i>Calluna vulgaris</i> , <i>Cytisus scoparius</i> , <i>Molinia caerulea</i>	<i>Calluno-Genistion pilosae</i>
2311	Permanent grassland in the absence of shrubs and trees	<i>Arrhenatherum elatius</i> , <i>Dactylis glomerata</i> , <i>Holcus lanatus</i> , <i>Poa trivialis</i> , <i>Rumex acetosa</i> , <i>Achillea millefolium</i>	<i>Arrhenatherion elatioris</i>
2241	Poplar plantations	<i>Populus</i> spp.	
1412	Uncultivated green areas	<i>Artemisia vulgaris</i> , <i>Elytrigia repens</i> , <i>Melilotus officinalis</i> , <i>Solidago gigantea</i> , <i>Urtica dioica</i>	<i>Dauco carotae</i> – <i>Melilotion albi</i>

A further seven habitat classes, i.e., ecotones between pairs of these habitats (1412_31111, 2311_31121, 2311_3113, 31111_31121, 31111_324, 31121_3113, 3113_331), were also recorded.

‘cascadeKM’ function to the standardised square root indices and applied the Calinski–Harabasz criterion (Milligan & Cooper, 1985) to select the best partition inside the dendrogram obtained by the cluster, thereby dividing the whole species group into subgroups with a similar use of habitat types.

In order to understand whether environmental characteristics influenced the abundance of individuals, we applied a generalised linear mixed effects model (GLMM) for count data (Atkins *et al.*, 2012) for each of the previously selected groups. We ran zero-inflated GLMMs due to the huge amount of zeros in our data set. GLMMs were run by applying the AD Model Builder procedure using the ‘glmmADMB’ package in R (Skaug *et al.*, 2013), specifying the Poisson distribution inside the model (Bolker *et al.*, 2012). Before starting the analysis, we applied a conservative method to calculate significance for each factor included in the model based on the evaluation of the standard deviation calculated for each estimate multiplied by 2. Consequently, estimates were significant only if the interval enclosed by two times their standard deviations did not cross 0. According to Breslow (2004), generalised linear mixed models may yield biased estimates of the variance component when the mean number of counts per treatment combination is less than five, so we removed from the analysis all the species whose number of counted individuals was below this value. Land cover class classification and the year of the sample were inserted in the model as fixed effects and the plot as a random effect.

Habitat–species network

Even though network analysis has been previously applied to feeding interactions (Bosch *et al.*, 2009; Poulin,

2010; Junker *et al.*, 2012; Polidori *et al.*, 2013), it is also adequate for species–habitat webs within given areas when habitats are well characterised and thus represent discrete nodes of the webs. Ecologists continuously produce detailed classifications of habitat types based on sophisticated analyses of their components (e.g. soil, vegetation structure, climate), so that these classifications can be used as a basis for species–habitat webs.

Network indices based on information theory (Shannon entropy) were used to characterise our species–habitat webs in each area (2008 and 2009) and site (two in 2008 and two in 2009) (Dormann *et al.*, 2009). The nodes of each network are composed of butterfly species versus their habitat types, and link weights are the number of individuals collected in each habitat type.

Because we were interested in the degree of habitat segregation between species, we considered the degree of complementarity (or exclusiveness) of interactions, defined as the standardised index H_2' (Blüthgen *et al.*, 2006). This index characterises the specialisation and habitat partitioning (niche overlap) in the butterfly assemblage. We first calculated the two-dimensional Shannon index as:

$$H_2 = - \sum_{i=1}^I \sum_{j=1}^J \frac{a_{ij}}{m} \cdot \ln \frac{a_{ij}}{m}$$

where i is a butterfly species, I is the total number of individuals collected, j is one of J total habitat types, a_{ij} represents the number of records of the habitat type j frequented by the butterfly species i and m is the total number of habitat records associated with all butterfly species in a network.

We then standardised H_2 against its minimum ($H_{2\min}$) and maximum ($H_{2\max}$) possible value for the given habitat type frequencies (i.e. marginal totals in the $I \times J$ matrix fixed), as follows:

$$H'_2 = \frac{H_{2\max} - H_2}{H_{2\max} - H_{2\min}}$$

Consequently, H'_2 ranges from 0 for the most generalised to 1 for the most specialised case.

Each value of H'_2 was tested against a null model of random associations ($H_{2\text{ran}}$). A number of random permutations of the matrix were performed using Patefield's $r \times c$ randomisation algorithm (Blüthgen *et al.*, 2006) for which probability is derived (Fonseca & Ganade, 1996; Manly, 1997).

As a species-level measure of *partner diversity*, we used the Kullback–Leibler distance (Blüthgen *et al.*, 2006), which not only considers the diversity of partners but also their respective availability, thereby comparing the distribution of the interactions with each butterfly species (p'_j) to the overall habitat type availability (q_j); the index is defined as:

$$d_i = \sum_{j=1}^c (p'_{ij} \cdot \ln \frac{p'_{ij}}{q_j})$$

and then normalised as:

$$d'_i = \frac{d_i - d_{\min}}{d_{\max} - d_{\min}}$$

The mean values of d'_i between groups of species were compared using ANOVA. Linear relationships between indices and other parameters were tested with Spearman's rank correlation test.

Results

Overall habitat requirements of species

Overall, 30 species of butterflies were collected. The Calinski-Harabasz partition applied to the dissimilarity matrix (Fig. S1) suggested five subgroups of species based on their habitat requirements (Table 2, Fig. 2).

Following the GLMM results (Fig. 3), in the first group, we found a positive influence on the abundance of open areas (324, 1412) and a negative influence of natural dense deciduous forests (3111) and poplar plantations (2241) on the abundance. Ecotones between open and forest areas were also positively associated with abundance (3111_324, 1412_3111). Similarly, in the second group, we found a positive influence on the abundance of open heath land (324) and a negative influence on the abundance of poplar plantations and dense deciduous forests (3111). Many types of ecotones (3111_324, 2311_3113, 2311_31121) and riparian vegetation (3113) were also positively associated with the abundances of these species. Negative relationships between the abundance and open heath land (324) and its ecotone and natural dense deciduous forests (3111_324) were found in the third group, while the abundances of the species belonging to the

fourth group were negatively associated with open anthropised heath areas (1412) and positively associated with the ecotone between open green areas and sparse deciduous forests (2311_31121). In the fifth group, positive influences on the abundance of sparse deciduous forests (31121), open green areas (2311) and poplar plantations (2241) were found. In addition, species of this group were positively associated with some ecotones, particularly those including riparian vegetation (31121_3113, 2311_3113). The year of sampling also affected the abundance of the species of groups 1, 2 and 3 (Fig. 3).

Habitat–species network

Overall, the relationships between species and habitats were not random in the studied butterfly assemblage ($H'_2 = 0.302$, $P < 0.0001$). Among species, habitat specialisation (d'_i) varied from 0.043 (denoting a generalist species) to 0.60 (denoting a highly specialised species) with an average of 0.21 ± 0.127 , showing important variability.

The mean d'_i calculated for each of the five groups identified by the Calinski criterion differed marginally ($F = 2.9$, $df = 27$, $P = 0.04$), and was highest in group 1 (species strongly positively associated with open habitats) and more similar among the other four groups (Fig. 4). The weakness of this difference was clearly due to the large variance within groups, i.e., species with similar habitat requirements were not all equally specialised (Table 2, Fig. 2). In particular, within all groups but one (group 2), it seemed that one or two species were much more specialised than the others. In group 2, this subdivision was more difficult but the difference between the highest d'_i (species 1 and 22) and the lowest d'_i (species 6 and 9) was still large. The within-group variability in d'_i , when expressed with its coefficient of variation (CV), increased with the number of species included in the group (Spearman's correlation test, $\rho = 0.97$, $n = 5$, $P = 0.017$).

Discussion

We showed for the first time that high precision of handheld GPS georeferenced data can give detailed insights into how butterflies occupy possible suitable habitats based on their ecological needs, as previously shown for other animal groups (Lindeque & Lindeque, 1991; Kissling *et al.*, 2014; Breed & Severns, 2015).

We found a coherent picture and a general rule by which butterfly species seemed to occupy suitable habitats based on their species-specific ecological needs. In particular, species grouping seemed to be based on a continuum index of lightness, ranging from group 1 generally being influenced by open areas to group 5 being strongly related to closed-canopy forests. If we look at the estimates associated with forest habitats and open habitats in our model

Table 2. Species sampled, number of individuals collected (N), group assigned by the 'cascadeKM' analysis of the dendrogram based on the number of observations in the habitat classes and specialisation index (d'_i) for the species.

Code	Family	Species	N	Group	d'_i
1	Nymphalidae	<i>Apatura ilia</i> (Denis & Schiffermüller)	6	1	0.242
2	Nymphalidae	<i>Argynnis paphia</i> (Linnaeus)	18	2	0.200
3	Pieridae	<i>Pieris mannii</i> Mayer	59	4	0.112
4	Pieridae	<i>Pieris napi</i> Linnaeus	90	4	0.244
5	Pieridae	<i>Pieris rapae</i> Linnaeus	60	3	0.166
6	Nymphalidae	<i>Brenthis daphne</i> Bergsträsser	89	2	0.044
7	Hesperiidae	<i>Carcharodus alceae</i> (Esper)	3	5	–
8	Lycaenidae	<i>Celastrina argiolus</i> (Linnaeus)	70	1	0.166
9	Nymphalidae	<i>Coenonympha pamphilus</i> (Linnaeus)	53	2	0.077
10	Pieridae	<i>Colias crocea</i> (Geoffroy)	20	5	0.171
11	Lycaenidae	<i>Cupido (Everes) argiades</i> (Pallas)	52	3	0.369
12	Pieridae	<i>Gonepteryx rhamni</i> (Linnaeus)	31	5	0.112
13	Hesperiidae	<i>Hesperia comma</i> (Linnaeus)	2	5	–
14	Hesperiidae	<i>Heteropterus morpheus</i> (Pallas)	62	3	0.168
15	Nymphalidae	<i>Aglais io</i> (Linnaeus)	82	3	0.387
16	Papilionidae	<i>Ipheclides podalirius</i> (Linnaeus)	224	1	0.281
17	Nymphalidae	<i>Issoria lathonia</i> (Linnaeus)	54	5	0.130
18	Lycaenidae	<i>Lycaena phlaeas</i> (Linnaeus)	26	5	0.197
19	Lycaenidae	<i>Lycaena tityrus</i> (Poda)	45	5	0.198
20	Nymphalidae	<i>Maniola jurtina</i> (Linnaeus)	166	2	0.155
21	Nymphalidae	<i>Melanargia galathaea</i> (Linnaeus)	68	5	0.162
22	Nymphalidae	<i>Minois dryas</i> (Scopoli)	895	2	0.215
23	Nymphalidae	<i>Hipparchia statilinus</i> (Hufnagel)	192	1	0.603
24	Hesperiidae	<i>Ochlodes sylvanus</i> (Bremer & Grey)	60	5	0.135
25	Nymphalidae	<i>Pararge aegeria</i> (Linnaeus)	165	5	0.525
26	Lycaenidae	<i>Plebejus argus</i> (Linnaeus)	13	5	0.050
27	Nymphalidae	<i>Polygonia c-album</i> (Linnaeus)	52	5	0.239
28	Lycaenidae	<i>Polyommatus icarus</i> (Rottemburg)	10	5	0.200
29	Hesperiidae	<i>Pyrgus malvae</i> (Linnaeus)	9	5	0.131
30	Nymphalidae	<i>Vanessa atalanta</i> (Linnaeus)	46	5	0.217

d'_i was not calculated in cases of small sample size (denoted by '–') (see text for details).

(Fig. 3), we can see that they changed sign while proceeding from group 1 to group 5. In particular, forests are negatively related to the abundance of the sampled individuals in the first group, but they increase their positive influence while moving to group 5; the opposite occurs for the above-mentioned general open dry areas. Furthermore, all the species, regardless of the group identity, seemed to avoid natural dense deciduous forests and associated ecotones. These are closed and shaded habitats, and luminosity was shown to be very important for butterflies, determining minimum and maximum flight conditions (Douwes, 1976) as well as flight duration (Shreeve, 1984). For example, in a restoration experiment of ponderosa pine forests in North America, butterfly species richness and abundance were up to three times greater in restoration treatment units where host plant and nectar plant species richness were similar to control units, but insolation (light intensity) was significantly greater (Waltz & Covington, 2004).

This differential importance of habitat light intensity in structuring our studied butterfly assemblage is confirmed by a comparison of our results with previous information on the habitat characteristics for the studied species,

which revealed a general congruence. All the three species of the first group are reported to inhabit bushy places, and dry and hot areas, and are usually associated with forest ecotones and clearing margins (Table S1). Species of the second group showed a negative relationship with the presence of forest areas, and a positive association with open natural heath areas. Furthermore, species of group 2 seemed to be more influenced by ecotones than species of group 1, with grassland representing the key habitat type. We also found a positive influence of riparian vegetation on individual abundance, probably because most of the individuals belonging to group 2 were sampled in a site characterised by a single line of riparian vegetation and large open areas, thereby representing once again a sort of structural ecotone inside an open area. According to literature, many species of group 2 are defined as ecotone species (Table S1). Groups 3 and 4, on the other hand, which were negatively associated with open heath land and its ecotones, confirmed the decreasing importance of open areas while moving from group 1 to group 5. Indeed, species of group 5 were clearly associated with poplar plantations and sparse deciduous forests. Many species in group 5 were previously reported as

Fig. 2. Cluster dendrogram (above) obtained by applying Morisita dissimilarity indices based on the dissimilarity index matrix. The five group partitions determined by the Calinski criterion are shown by dotted lines. Below, there are the values of habitat specialisation (d_i') for each species included in the five groups (codes for species above the bars, see Table 2 for species names). Note that for a few species (e.g. eight), the sample size was too small to perform the network analysis (see text for details).

being somehow linked to a forest environment (Table S1), but some species are reported to be associated with very different habitats (from open grassland to forests) (Table S1). Additionally, the significant effect of the year in some groups showed a temporal shift in species abundance, suggesting population fluctuations depending on change in abiotic or biotic conditions.

The degree of specialisation was not similar among species within groups in our studied assemblage. For example, *Hipparchia statilinus* was much more specialised than the other species of group 1, while *Minois dryas* frequented many more coppice deciduous forests and their ecotones than the other species of group 2. Two species of group 3 (*Everes argiades* and *Aglais io*), which in literature are reported as generalist species in many habitats including flowery bushy places, grassy banks, forest clearings, flowering meadows, forest edges and sheltered rocky gullies (Table S1), were very specialised in our study. Even the two closely related species (both from the genus *Pieris*) comprising group 4 differed greatly in specialisation.

Because the index of specialisation d_i' increases with the level of specialisation, the positive correlation between the variance of d_i' and the number of species across groups means that an increased number of species are more likely to promote the co-occurrence of generalist and specialised species (nested patterns) rather than an increased niche segregation among species (a clustered pattern). Thus, possible increased competition (i.e. a higher number of species sharing the same habitat requirements) would not increase habitat segregation, while a few highly specialised species would use a subset of habitats exploited by more generalist species. This consideration is consistent with previous network-based studies, which showed nestedness common in both mutualistic (e.g. plant–pollinator) networks (Bascompte *et al.*, 2003) and agonistic (e.g. host–parasite) networks (Joppa *et al.*, 2010) (although compartmentalisation is also observed in agonistic webs, e.g. Prado & Lewinsohn, 2004). Bastolla *et al.* (2009) showed that the process by which a new species enters a community will likely lead to a nested pattern of interactions, because the new species tries to minimise its competitive

Fig. 3. Estimated parameters derived from GLMM. Significant influences of the parameters on the species abundance are shown by grey highlighting. The estimates (round black points) and their standard deviations multiplied by 2 (bold black lines enclosed by vertical segments) are provided. Standard deviation segments were cut when their values crossed -3 or $+3$ in each graph. Continuous bold lines without vertical segments are used when the model was not able to estimate reliable parameters. Significant influences of the parameters on the species abundance are shown by grey highlighting. Digits refer to habitat and ecotone codes as in Table 1.

Fig. 4. Box-and-whisker diagrams showing medians (horizontal lines within boxes), means (+), 1st and 3rd quartile (top and bottom lines of the boxes) and maximum and minimum values (O) of habitat specialization (d_i') of the species included in the five groups determined by the Calinski criterion. Ends of the whiskers represent the lowest datum still within $1.5 \times$ interquartile range of the lower quartile and the highest datum still within $1.5 \times$ interquartile range of the upper quartile.

load, an action that forces it to interact with the most generalist species. This could also be applied to habitats: When a new species arrives in a given area, it will first select sites with certain characteristics (requirements) and then reduce the overlap with competing species, largely using only a subset of the habitats used by generalist species.

Conclusion

Our results stress the importance of considering both the habitat requirements of species and the specialisation in habitat use by each species when analysing butterfly assemblages. Indeed, many species studied here, previously known as generalists, were found to be locally specialised in our analysis. This is particularly important in the light of butterfly conservation, because the more specialised species may also be those with higher susceptibility to network distortions and thus with higher fragility to co-extinction (Blüthgen, 2010). This would provide elements for deciding protection priorities for the species at local scales.

Although none of the sampled species are listed as threatened in the European Red List of Lepidoptera (with the exception of *Hipparchia statilinus*, which is listed as Near Threatened, Van Swaay *et al.*, 2010), five species (*Coenonympha pamphilus*, *Lycaena phlaeas*, *Maniola jurtina*, *Ochlodes sylvanus* and *Polyommatus icarus*) are reported as being declined in Europe since 1990 (Van Swaay *et al.*, 2013). These species may become locally vulnerable through persistent increased habitat specialisation associated with habitat disturbance; therefore, their ecology should be finely monitored in order to adopt adequate conservation plans.

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Supporting Information

Additional Supporting Information may be found in the online version of this article under the DOI reference: doi: 10.1111/icad.12193:

Fig. S1. Calinski criterion applied to the dissimilarity matrix. The number of possible group partitions is shown on the y -axis and the values obtained by the Calinski–Harabasz algorithm are shown on the x -axis.

Table S1. Previous published information on the habitat requirements of the studied butterfly species.

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