Distribution patterns and indicator species of butterfly assemblages of wet meadows in southern Belgium

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ABSTRACT. Focal species are a valuable tool for proposing and evaluating management practices for biodiversity conservation. Assemblages of indicator species could be used to cover a wide range of habitats. We identified the main patterns of variation in butterfly assemblages on a diverse set of wet meadows in southern Belgium. We used multivariate techniques to identify the butterfly assemblages and the species that characterize these habitats. Three main assemblages were identified, based principally on the dominance of five butterfly species : *Brenthis ino, Clossiana selene, Lycaena helle, Lycaena hippothoe* and *Proclossiana eunomia*. These are indicator species of different habitats structured along a vegetation gradient. This gradient is partially determined by altitude and edaphic factors (base-richness, pH, fertility). We assume that focusing the conservation practices upon these species will promote the preservation of a wide range of organisms inhabiting the wet meadows.

KEY WORDS : butterflies, indicator species, multivariate analysis, wet meadows, biodiversity conservation.

INTRODUCTION

Almost every ecosystem on Earth is going through a strong anthropogenically induced disturbance biodiversity crisis, in which several thousands species are involved. Natural and semi-natural biotopes are suffering from high rates of modification and, as a consequence, the number of species threatened by extinction increases. In order to evaluate and monitor biodiversity as well as to implement conservation measures, the development and use of shortcuts is a necessity. Obviously, it is not viable to consider the development of a detailed study for each one of the endangered species. One possible approach is based on diversity measures, but it has several limitations like excluding particular taxonomic information about the communities (NOSS, 1990 ; DUFRÊNE & LEGENDRE, 1997). The criterion of representative diversity which is based on the recognition of assemblages of species that are typical for specific habitats seems more interesting (DUFRÊNE & LEGENDRE, 1997). A potentially useful tool is the single-species approach based on focal or surrogate species, which can indicate ecological change, patterns of richness or habitat type (NOSS, 1990 ; CARO & O'DOHERTY, 1999 ; FLEISHMAN et al., 2000). Focal species include umbrella, flagship and different kind of indicator species (MCGEOCH, 1998 ; SIMBERLOFF, 1998). Some of these species could be used as targets for conservation efforts, assuming their preservation may help to protect other species that share the same habitats. Thus, the use of surrogate species may be a valuable tool for conservation planning, allowing considerable reductions of time and funding costs. Moreover, the use of assemblages instead of single species as indicators enables the enlargement of the focus of the single-species approach to wider ecological situations (KREMEN, 1992). Assemblages of species, with determined species richness, level of dominance, and taxonomic composition, can characterize different habitats. The use of a small set of surrogate species allows the inclusion of a broader range of habitats. Using keystone species may be an interesting alternative to surrogate species (SIMBERLOFF, 1998). However, the effort and time demanded by this approach in the study of the community processes and the high rate of degradation of the biotic diversity, makes the use of other tools necessary (e.g., surrogate species) enabling conservation managers to develop faster responses to the anthropogenic disturbances. Hence, important research effort is needed to select appropriate surrogate species.

Lepidoptera have been proposed as surrogate species by several authors (KREMEN, 1992 ; BECCALONI & GASTON, 1995 ; FLEISHMAN et al., 2000). Several features of the butterflies make them good candidates for indicator, umbrella and/or flagship species (NEW, 1997 ; FLEISHMAN et al., 2000 ; MAES & VAN DYCK, 2001). They have a wide distribution, are relatively easy to sample and identify, and both as individuals and as species they show important numbers in different ecosystems (BLAIR, 1999 ; CARO & O'DOHERTY, 1999 ; RICKETTS et al., 2002). They are also strongly influenced by local weather and highly sensitive to environmental changes (SPITZER et al., 1997), besides being charismatic insects that could attract the public attention. Finally, some authors have identified patterns of co-variation between the abundance and/or the richness of Lepidoptera and those of other taxonomic groups (BLAIR, 1999 ; SWENGEL & SWENGEL, 1999). How-

ever, these relationships are highly dependent on the taxa and the spatial scales considered (RICKETTS et al., 2002).

Butterflies are extremely sensitive to changes in vegetation composition and structure, and different types of vegetation show different butterfly species composition. So, the butterfly assemblages may be used to characterize different habitats (ERHARDT, 1985). Plants are the essential source of nourishment of butterflies; some specific plant species provide the trophic resources for caterpillars, while others provide nectar for adults. The vegetation can also play an important role for butterfly survival offering particular structural elements for sun-basking or mating and determining certain suitable microclimates (DOVER et al., 1997). Therefore, it would be expected that butterflies respond more strongly to vegetation gradients than to edaphic gradients (SAWCHIK et al., 2003).

Human disturbance in Western Europe, including Belgium, has accelerated during the second half of the 20th century, resulting in a very high pressure on some particular ecosystems. An important factor in this relatively recent panorama has been changes in land use, i.e. the abandonment of traditional agricultural practices (MAES & VAN DYCK, 2001). Some of the most threatened ecosystems are the semi-natural wet meadows, which have been abandoned or modified by modern and intensive agricultural practices. Many of these biotopes are being invaded by shrubs or trees, while others suffer eutrophication, pollution or acidification. Moreover, these modifications lead to an increasing isolation of the remaining fragments. The result is that semi-natural humid grasslands are seriously threatened in Belgium (GOFFART et al., 2000).

The objectives of this study were : (1) to describe the main variation pattern in the butterfly fauna of a wide range of wet meadows; (2) to identify the indicator species of the different habitat types; (3) to explore the relationships between the butterfly assemblages and the vegetation and the soil properties of the sites.
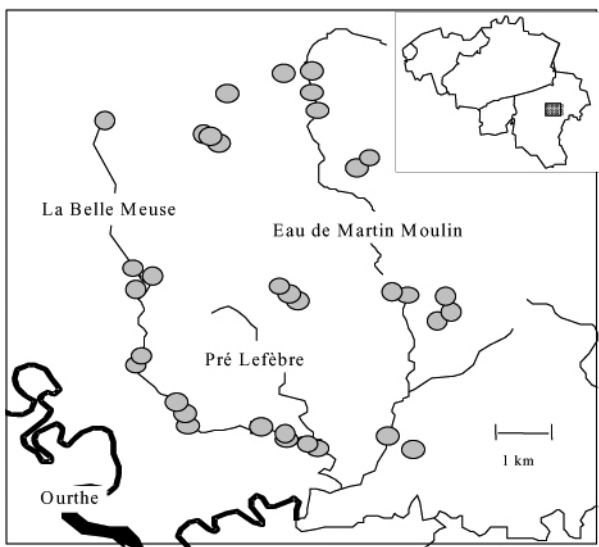

Fig. 1. – Study area in the Plateau des Tailles, Ardennes, Belgium.

METHODS

Study area

The studied area is the basin of the Martin-Moulin river located in the Plateau de Tailles (Fig. 1). This high plateau (max. height 654 m) is situated in the Ardennes biogeographical area, southern Belgium. Coniferous forests and pastures dominate the landscape. Immersed in this mixed forest-prairie matrix there are several semi-natural habitats, particularly a great diversity of wet meadows and mires. These meadows are biologically interesting habitats, displaying high levels of species richness and sustaining populations of many vulnerable species. Thirty-four sites were selected for the present work, all of them wet meadows. They account for all the main vegetation associations that may be found in this area.

Field measurements

The definition of the sites was based on homogeneous floristic composition. All these sites show relatively well-defined boundaries. We identified all the plant species present in a quadrate of 5 (5 meters randomly placed in each site. The size of the quadrate is considered convenient to describe meadow communities (KENT & COKER, 1992). Then, the size of the quadrate was progressively increased until no new species were identified. Percentage cover of plants was estimated visually. These vegetation surveys were made during the summer of 1996.

Within each site, soil samples were collected at five randomly located places. The soil was sampled at 0-20 cm depth. The samples were pooled, and the following edaphic variables measured : soil pH, organic matter (OM), soil moisture (SM), total N, concentrations of Ca, K, Mg and Na. We also determined water-table depth (WT) recorded in 3 locations at each site in the summer of 1997. Values of WT were averaged prior to data analysis. As OM and total N were highly correlated ($r > 0.9$) we dropped N from the analysis data set. The chemical analyses were carried out at "l'Unité d'Écologie des Prairies" (UCL/AGRO/ECOP/Belgium).

Butterflies (Rhopalocera : Papilionoidea) were recorded by visual observation along zigzag transects from May to August of the years 1994, 1995 and 1996. More precisely, in each site we walked at a regular step until the total area was visited. We registered all the individuals observed in a corridor of approximately 6 m of width. We sampled each site as many times as possible in sunny conditions (POLLARD, 1977; THOMAS, 1983). The sampling program covered the flying periods of most species and ensured that a minimum of 3 sampling visits was made for each species in each site and each year.

As a measure of relative abundance of the butterfly species we used the maximum number of observed individuals in each site (SMALLIDGE et al., 1996). This measure allows controlling sampling intensity and weather differences. Moreover, field experience in such habitats demonstrated that after three or four visits well distributed along the main activity period, the maximum does not change considerably. Indeed, very similar results were obtained with additional analyses using other measures of relative abundance (e.g. mean, median, and sum). The

data from the 3 years were pooled, so the maximum over the 3 years was used as a measure of abundance. Because all the sites were small (range from 0.1 to 2 ha; median = 0.775), they might be highly affected by stochastic effects. By pooling the data from different years, we integrate over annual weather effects on local abundance as well as extinction and rescue events generated by metapopulation dynamics on a network of habitat patches (HANSKI et al., 1995; FLEISHMAN et al., 1999).

Assemblage structure and indicator species identification

The structure of the butterfly species assemblage was studied by ordination and classification methods. The butterfly matrix details the distribution of 15 species in 34 sites and shows a 31% of zeros. As suggested by LEGENDRE & LEGENDRE (1998), rare species (< 1% of the total number recorded) were removed from the analysis. In general, these rare species were represented by single, vagrant individuals that probably do not concern local populations. This category also concerns migratory species as *Vanessa atalanta* and *Cynthia cardui*. Moreover, correspondence Analysis (CA) and classification techniques are sensitive to the occurrence of rare species, so these species may distort the analysis impeding a reliable description of the main patterns of variation (EZCURRA, 1987). A distance matrix was constructed using the Bray Curtis coefficient. Hierarchical agglomerative clustering was conducted on the distance matrix using the UPGMA method. We used the IndVal approach developed by DUFRÊNE & LEGENDRE (1997) to identify the indicator species of a site typology resulting from a clustering of the butterfly data set. For each step of the clustering process indicator species are revealed by an index (IV, i.e., Indicator Value) that combines an estimator of the species specificity and fidelity (MCGEOCH & CHOWN, 1998). With this kind of approach the indicator species are defined as the most characteristic species of a site cluster, found mostly in a site cluster of the typology (specificity) and present in the majority of the sites belonging to that cluster (fidelity). The significance of the IV index is assessed by a permutation approach (1000 permutations). Species with significant high IndVal values (> 70%) show strong habitat fidelity and specificity, so they may be considered as characteristic species of the assemblages (MCGEOCH et al., 2002.).

Ordination diagrams can be used to select a subset of the species as indicators (KREMEN, 1992). In order to identify the main information axes, ordination was carried out performing CA. Because CA ordination scores represent approximately optima localization of the species in the ordination plan (TER BRAAK & PRENTICE, 1988), the diagram could be used to find assemblages of characteristic species along the gradients (KREMEN, 1992).

Butterflies-habitat relationships

A first approach to interpret the ordination results with the aid of external data was to compute Spearman correlation coefficients (r_S) between CA ordination scores from the butterfly matrix and altitude (m). Additionally, we computed correlation coefficients between the scores of the CA axes and the diversity of the butterfly assemblages. In each site we computed species richness (S), Shannon diversity (H') and evenness (E). The Simpson's index (D) was used as a measure of dominance. All of these measures are widely used in ecology literature (KREBS, 1989; MAGURRAN, 1988).

In order to explore the relationships between the butterfly fauna and the habitat, defined by the edaphic factors and the plant assemblages, we used CO-inertia Analysis (COA). The choice of COA instead of canonical correspondence analysis (CCA) was based on the high number of variables compared to the number of sites. When the number of variables equals the number of sites CCA becomes a CA (TER BRAAK & PRENTICE, 1988; JONGMAN et al., 1995). COA is a symmetric ordination technique well adjusted to analyze data sets with many explaining variables and a relatively low number of sites (DOLÉDEC & CHESSEL, 1994). It is a symmetric method because it does not assume that one matrix is constituted by explaining variables and the other by explained variables. Indeed its use is well justified in this study because we are not attempting to explain the abundance of the butterfly species as a function of plant assemblages. We are merely interested in the associations between plants and butterflies without assuming a causal relationship between them. In a first step, faunal and environmental matrices are analyzed by standard ordination methods as CA or PCA. In a second step, COA maximizes the covariance between the first axes of variation of the two matrices.

We performed two COA's to link the CA ordination of butterfly data to vegetation and edaphic data. The first analysis (COA_1) linked the butterfly ordination to an ordination obtained by CA performed on the vegetation frequency data set. Because there was a high number of plant species recorded, and the fact that the great majority among them showed very low frequencies and cover values, we decided to remove rare species from the data set to gain in clarity and computation efficiency, and to reduce noise and redundancy. The vegetation matrix details the distribution of 40 species and shows 53% of zeros. The second analysis (COA_2) linked the butterfly ordination to a PCA performed on the correlation matrix of the edaphic data. The edaphic variables were log transformed prior to PCA. The signification of the co-inertia values was tested by a permutation procedure (1 000 permutations). Additionally, we performed another co-inertia analysis, COA_3, to explore the relationships between vegetation and edaphic factors.

CA was performed with the CANOCO software (TER BRAAK & SMILAUER, 1998). We used the R package (CASGRAIN & LEGENDRE, 1999) to compute correlation and distance coefficients and to perform cluster analysis. The version 2.0 of the program IndVal (DUFRÊNE & LEGENDRE, 1997) was used to identify the indicator species. COA was performed with ADE-4 (THIOULOUSE et al., 1997).

RESULTS

We identified a total of 30 butterfly species but only 15 species were retained for most of the analyses (Table 1). The matrix of butterfly abundance used in subsequent analyses is presented in Table 2. Hesperoidea were mostly represented by two species: *Thymelicus lineolus* and *T.*

sylvestris. For practical reasons, *Thymelicus* species were not retained for the analysis. These are two relatively common species that are not easily distinguishable in the field.

TABLE 1

Number of individuals, number of occupied sites and butterflies codes (used as labels in the figures).

Code	Species	No. individuals	No. sites
AGL	*Aglais urticae* (Linnaeus, 1758)	176	27
ANT	*Anthocaris cardamines* (Linnaeus, 1758)	50	21
APH	*Aphantopus hyperantus* (Linnaeus, 1758)	1,036	34
APO	*Aporia crataegi* (Linnaeus, 1758)	56	20
ARA	*Araschnia levana* (Linnaeus, 1758)	39	19
BRE	*Brenthis ino* (Rottemburg, 1775)	799	26
CLO	*Clossiana selene* ([Denis & Schiffermüller], 1775)	194	17
GON	*Gonepteryx rhamni* (Linnaeus, 1758)	39	21
INA	*Inachis io* (Linnaeus, 1758)	41	23
LHE	*Lycaena helle* ([Denis & Schiffermüller], 1775)	243	22
LHY	*Lycaena hippothoe* (Linnaeus, 1761)	102	18
MAN	*Maniola jurtina* (Linnaeus, 1758)	251	32
PNA	*Pieris napi* (Linnaeus, 1758)	120	29
PRA	*Pieris rapae* (Linnaeus, 1758)	29	20
PRO	*Proclossiana eunomia* (Esper, 1799)	221	22
	Argynnis aglaja (Linnaeus, 1758)	2	2
	Boloria aquilonaris (Stichel, 1908)	20	1
	Callophrys rubi (Linnaeus, 1758)	15	10
	Coenonympha arcania (Linnaeus, 1761)	5	4
	Coenonympha pamphilus (Linnaeus, 1758)	10	4
	Cynthia cardui (Linnaeus, 1758)	24	16
	Erebia medusa ([Denis & Schiffermüller], 1775)	4	4
	Lasiommata megera (Linnaeus, 1767)	2	2
	Melitaea diamina (Lang, 1789)	32	12
	Mellicta athalia (Rottenburg, 1775)	6	5
	Papilio machaon (Linnaeus, 1758)	2	2
	Pieris brassicae (Linnaeus, 1758)	15	11
	Polygonia c-album (Linnaeus, 1758)	3	1
	Polyommatus icarus (Rottenburg, 1775)	6	6
	Vanessa atalanta (Linnaeus, 1758)	14	12

TABLE 2

Matrix of butterfly abundance, recorded as the maximum number of individuals observed in each site.

AGL	ANT	APH	APO	ARA	BRE	CLO	GON	INA	LHE	LHI	MAN	PNA	PRA	PRO
6	0	15	5	2	28	0	3	1	9	2	6	5	1	3
4	0	17	0	2	28	0	1	0	0	0	3	2	0	0
6	1	42	2	0	54	0	1	1	15	0	13	3	1	6
0	0	53	1	1	84	0	0	1	7	0	14	2	2	15
3	0	14	0	1	42	0	1	1	6	0	7	1	1	2
20	1	32	10	0	13	0	0	3	25	2	24	0	0	12
0	2	20	2	3	30	0	0	2	25	0	4	4	1	8
19	2	40	1	2	58	0	1	2	3	0	23	2	1	18
2	5	40	2	4	75	0	0	2	0	0	20	7	2	0
0	0	15	0	1	10	0	2	3	7	0	0	3	1	8
5	0	25	3	0	6	3	1	0	1	1	10	2	0	2
4	1	25	7	3	19	1	5	2	8	4	12	3	0	2
2	1	20	3	2	2	4	3	2	25	2	5	2	1	17
5	0	30	0	0	0	21	1	1	0	7	3	13	2	0
13	0	36	2	0	37	0	0	1	4	0	7	2	0	0
15	5	35	3	6	10	0	2	7	16	0	6	7	1	10
2	4	29	1	1	5	1	2	0	0	0	5	5	1	0
3	2	30	4	1	0	20	5	2	1	0	4	5	0	5
0	1	30	1	0	20	0	0	0	2	0	5	1	2	3
1	0	40	1	0	0	9	1	0	0	0	8	0	0	0
15	5	30	3	0	17	5	2	1	5	8	10	7	2	10
15	5	20	2	1	8	10	0	0	2	7	6	7	1	8
5	1	50	0	0	30	4	0	0	0	10	5	1	0	0
1	2	46	0	2	2	2	1	2	9	6	5	3	1	7
0	3	33	0	2	0	6	0	1	29	4	1	4	1	35
3	2	32	0	0	0	27	0	0	0	13	6	7	0	0
1	0	15	0	0	0	53	1	1	0	10	0	1	3	0
2	0	25	1	0	10	0	1	2	38	0	2	2	0	12
11	0	64	2	1	92	0	3	1	0	1	12	0	0	0
7	0	43	0	0	0	1	0	0	0	2	8	0	0	5
0	1	30	0	2	25	0	1	1	5	3	4	4	0	17
1	2	20	0	2	74	0	0	0	0	0	4	10	0	0
0	1	10	0	0	0	17	0	0	0	0	5	2	0	1
5	3	30	0	0	20	10	1	1	1	15	7	5	3	16

We recorded a total of 138 plant species, but the great majority of them were only present in low abundance and frequency species. The 25 species considered for multivariate analysis are listed in Table 3.

TABLE 3

Number of occupied sites and plant codes (used as labels in the figures).

Code	Species	No. Sites
ACN	*Agrostis canina*	21
ACP	*Agrostis capillaris*	4
ANG	*Angelica sylvestris*	27
CNI	*Carex nigra*	10
CRO	*Carex rostrata*	5
CIR	*Cirsium palustre*	32
DES	*Deschampsia caespitosa*	21
FES	*Festuca rubra*	14
FIL	*Filipendula ulmaria*	24
GPA	*Galium palustris*	24
GTE	*Galeopsis tetrahit*	17
HLA	*Holcus lanatus*	25
HMO	*Holcus mollis*	6
JAC	*Juncus acutiflorus*	25
JEF	*Juncus effusus*	20
LOT	*Lotus pedunculatus*	25
LYS	*Lysimachia vulgaris*	13
POL	*Polygonum bistorta*	29
POA	*Poa trivialis*	18
RAN	*Ranunculus repens*	17
RUM	*Rumex acetosa*	24
SCI	*Scirpus sylvaticus*	14
URT	*Urtica dioica*	14
VAL	*Valeriana repens*	17
VIO	*Viola palustris*	18
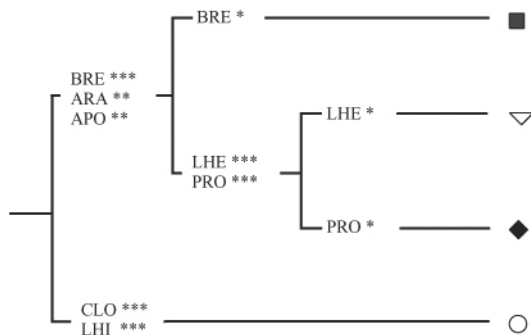
Fig. 2. – UPGMA clustering results and the indicator species proposed by the IndVal approach (IV). ***. Maximal and significant (at the 0.05 level), IV > 70%. **. Maximal and significant (at the 0.05 level), IV < 70%. *. Significant (at the 0.05 level) but not maximal IV.

The site typology obtained by UPGMA (Fig. 2) was very similar to that obtained using a *k*-means non-hierarchical partition method (SAWCHIK, 1999) so we concluded that the hierarchical pattern of the groups was robust (DUFRÊNE & LEGENDRE, 1997). First, the whole set of sites was split in two main groups separating lowland meadow sites from high altitude, acid and poor (oligotrophic) sites. At this step, IndVal identified only *B. ino* (IV = 89.7), *A. levana* (IV = 69.0) and *A. crataegi* (IV = 65.5) as having significant IndVal values for the group constituted by all the lowland meadow sites. The oligotrophic sites, represented by peat-bogs (rich in *Carex sp.* and *Sphagnum sp.*) and wet rush (*Juncus sp.*) meadows,

were characterized by *C. selene* (IV = 90.2) and *L. hippothoe* (IV = 76.8). The next level subdivided lowland meadows in one cluster of eutrophic sites and another cluster of mesotrophic meadows dominated by the association *Deschampsio cespitosae-Polygonetum bistortae*. The wet meadowsweet (*Filipendula ulmaria*) grasslands are distributed between these two clusters. Two butterfly species showed statistically significant IndVal values : *L. helle* (IV = 85.8) and *P. eunomia* (IV = 82.5). Only *B. ino* showed a significant, although not maximal, indicator value (IV = 71.0) for the cluster of eutrophic sites. Finally, the classification divides the cluster of bistort meadows in one group characterized by *L. helle* (53.4) and another group characterized by *P. eunomia* (48.4). These IV values were significant but not maximal, so the division at this level is less defined. This suggests subtle differences in the gradient of humid bistort grasslands from rush meadows to meadowsweet grasslands. After this division level the sum of IndVal values decreased, suggesting that the next divisions of the typology are not informative.
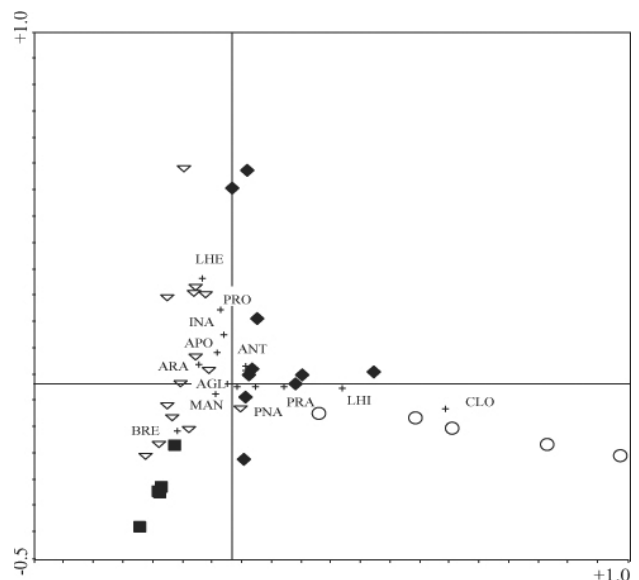
Fig. 3. – Ordination plot (2 first axes) of the Correspondence Analysis (CA) of the butterfly species matrix. The symbols correspond to the groups identified by the UPGMA algorithm.

The total variance explained by the first two CA axes was 64% (40.4% and 23.6% respectively). The two-dimension diagram obtained from CA ordination (Fig. 3) revealed the same species assemblage structure suggested by the clustering results. The species that mainly contribute to the species assemblage originality were *B. ino*, *C. selene*, *Inachis io*, *L. helle*, *L. hippothoe*, and *P. eunomia*. The first axis opposes *C. selene* to *B. ino*. *Clossiana selene* was present in high altitude, oligotrophic sites while *B. ino* was associated with lowland meadows. The second axis showed a rather slight arch effect, suggesting the prevalence of the main gradient. However, it is unlikely that this axis was simply an artifact and probably reflects a secondary gradient. This was confirmed by detrended correspondence analysis and principal coordinate analysis two-dimension ordinations (not shown),

which produced essentially the same spatial arrangement of the data revealed by CA.

The second axis opposes bistort meadows characterized by *L. helle* and *P. eunomia* to meadowsweet grasslands sites strongly associated with *B. ino*. The eutrophic sites dominated by *F. ulmaria* and *Urtica dioica* are located at one extreme of this axis, while the other meadow sites are characterized by the association of *Deschampsia cespitosa* and *Polygonum bistorta*. However, the slight arch effect suggested by this axis could also be interpreted in another way. The species repelled to the extreme of the parabola (*L. helle, P. eunomia*) would be typical of the middle of the gradient, whereas the species in the center of the parabola would be more widespread, generalist or ubiquitous species (LEBRETON & YOCCOZ, 1987; DUFRÊNE & LEGENDRE, 1997). No significant correlations were observed between species' frequencies among sites and species' CA scores. Therefore, we concluded that ordination results did not result from a sampling artifact (KREMEN, 1992). The first eigenvalues were different in magnitude so we concluded that the ordination was stable (OKSANEN & MINCHIN, 1997). The first CA axis showed a statistically significant correlation with altitude (r_S = 0.668). The second axis showed a signifi-

cantly positive correlation with butterfly diversity (r_S = 0.589). In spite of the fact that species richness, H' and E may be affected by sampling effort variations, in our case, however, the differences do not seem strong enough to have a great impact on these measures. This assertion is supported by the very low correlations showed between the diversity measures, the total number of individuals, and the area of the sites (Table 4).

TABLE 4

Rank correlation computed as Spearman coefficients between the site scores on the two first axis of the correspondence analysis, the patch area and the total number of individuals (N), and butterfly diversity (S = species richness; H' = Shannon index; E = evenness; D = Simpson index). *. Correlation is significant at the 0.05 level. **. Correlation is significant at the 0.01 level.

	Axis 1	Axis 2	Area	N
S	-0,117	+0.427 *	-0,103	+0,136
H'	+0,129	+0.589 **	-0,002	-0,053
E	+0,382	+0.524 **	+0,087	-0,160
D	-0,134	-0.619 **	-0,016	-0,092

TABLE 5

Summary of the co-inertia analyses : inertia and co-inertia values for the butterfly and environmental (CO1 : vegetation; CO2 : edaphic) matrices, and correlation values between the butterfly and environmental ordination axes.

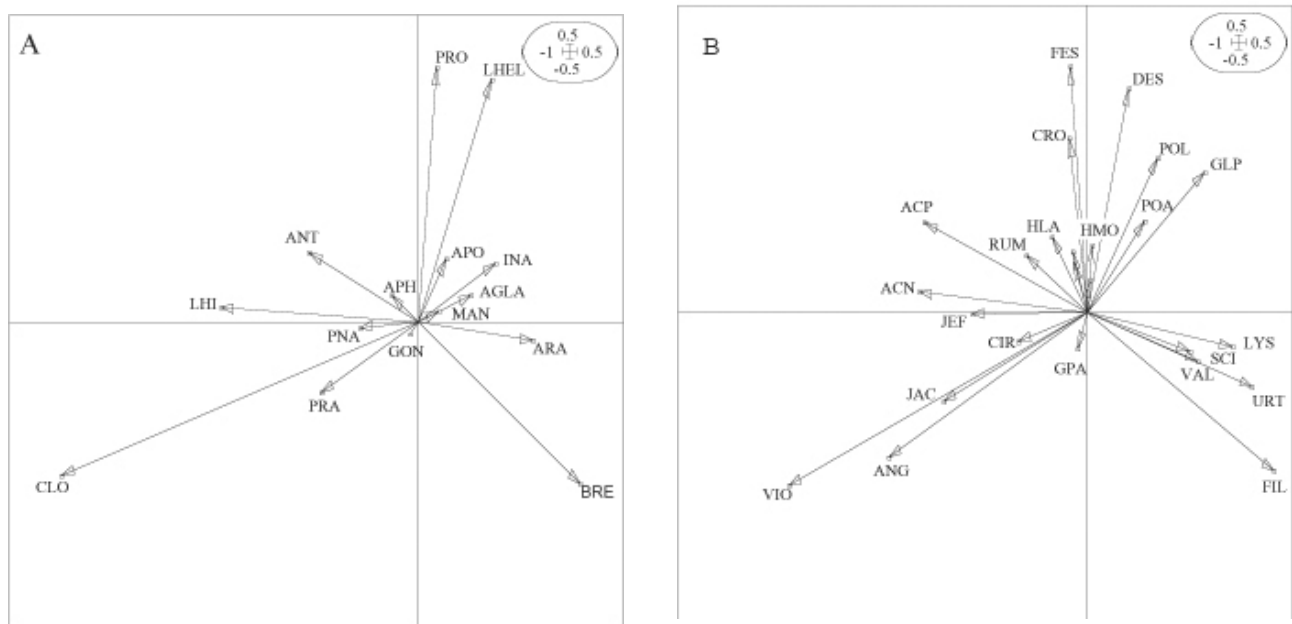| Analysis	Axis	Correlation	Inertia butterflies	Inertia environmental	Co-inertia butterlies	Co-inertia environmental
CO1	1	0,809	0,396	0,503	0,384	0,416
	2	0,818	0,215	0,413	0,204	0,305
CO2	1	0,631	0,396	3,551	0,355	1,944
	2	0,496	0,215	2,091	0,193	2,879

Fig. 4. – Results of the co-inertia analysis performed on the 15 butterfly species and the 25 plant species. (a) Position of butterfly species on the F1 x F2 co-inertia plane. (b) Position of plant species on the F1 x F2 co-inertia plane.
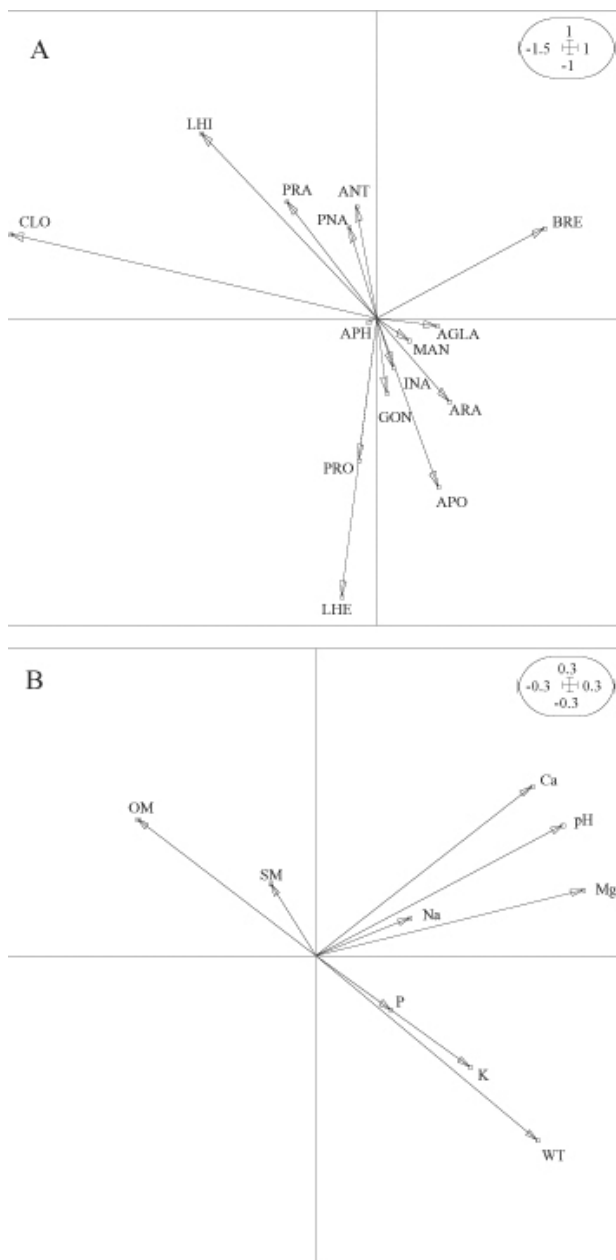
Fig. 5. – Results of the co-inertia analysis performed on the 15 butterfly species and the 9 edaphic variables. (a) Position of butterfly species on the F1 x F2 co-inertia plane. (b) Position of edaphic variables on the F1 x F2 co-inertia plane.

The main two axes of COA explain 55.81% and 22.14% of the common structure shared by the butterfly and the plant matrices. The co-inertia test was highly significant (p < 0.001). The inertia values for the two first axes of the CA, both for butterfly and plant species, are shown in Table 5. The correlation values between the butterfly and the plant ordination axes as well as the co-inertia values indicate that the structure described separately for butterfly and plant species data are relatively well captured by the first co-inertia plane. The ordination diagram (Fig. 4) shows almost the same pattern than the CA diagram, indicating that the structure of co-variation between butterfly and plant species succeeds in explaining the

main pattern of the distribution of the butterfly assemblages. At one extreme of the first axis we found *C. selene* associated with *V. palustris*, *A. sylvestris* and *J. acutiflorus*. *Lycaena hippothoe* was also located on this side of the diagram, mainly associated with *A. canina*, *J. effusus* and *A. capillaris*. *Brenthis ino* and *A. levana* were situated at the other end of this axis, associated to *F. ulmaria*, *U. dioica*, *V. repens* and *L. vulgaris*. In the middle of the axis, *L. helle* and *P. eunomia* occupied sites dominated by *D. cespitosa*, *F. rubra* and *P. bistorta*. In this study, COA succeeds in removing a strong arch effect seen on the main plane of the CA performed on the vegetation matrix (not shown).

The two main axes of the COA_2 explain 58.56% and 29.12% of the common structure shared by the butterfly and the edaphic matrices. The inertia values for edaphic factors were 3.551 and 2.091 respectively. The co-inertia test was significant (p = 0.022). The correlation values between the butterfly and the soil ordination axes as well as the co-inertia values are shown in Table 5. In the ordination diagram (Fig. 5), we visualize that *C. selene* and *L. hippothoe* were associated with acid and wet sites with accumulated OM and low levels of mineral bases. The butterfly species *A. crataegi* and *A. levana* were associated with the sites with the highest levels of K and P which are also less humid and with deeper WT than the previous sites cited. The less acidic sites with the highest levels in Ca and Mg were associated to *B. ino*, while *L. helle* and *P. eunomia* were preferentially situated in relatively dry sites with low levels in bases and OM. The ordination of edaphic factors obtained from COA_3 (not shown) showed a spatial configuration very similar to the ordination obtained from COA_2.

DISCUSSION

Methodological considerations

Multivariate techniques are highly useful tools for identifying sets of indicator species for different habitats along a gradient. Classical and canonical ordination methods allow the study of the relationships between species distributions and environmental gradients. Classification techniques are helpful for identifying discontinuities and to define the main habitat types. In particular, UPGMA combined with IndVal may provide a better alternative than TWINSPAN for classification and identification of indicator species (DUFRÊNE & LEGENDRE, 1997; LEE & MCDONALD, 1993). In short, these methods reflect local patterns of habitat heterogeneity indicating which assemblage could be considered as an appropriate indicator of these habitat types (KREMEN, 1992).

COA is a useful and robust tool for displaying species-habitat relationships because it allows the simultaneous and symmetric study of two data sets. The symmetric approach allows the connections to be made between tables with different number of environmental variables, taxa and/or sites. As an ordination technique, it also has other potential benefits, for example the removal of the arch effect (DOLÉDEC & CHESSEL, 1994). This approach may be also useful for defining and comparing biological assemblages indicative of different habitats or to evaluate the effects of disturbances on the habitats.

Vegetation gradients

Complex vegetation gradients partially reflect underlying edaphic gradients, i.e., nutrients, acidity and moisture gradients (OOSTERMEIJER & VAN SWAAY, 1998). The main direction of variation in edaphic factors is probably along the base-richness and acidity gradient. At one extreme of the gradient are located the acid, base-poor sites, at the other those rich in bases and showing neutral pH. This gradient may be very important in structuring the floristic composition of the sites (WHEELER & PROCTOR, 2000; ØKLAND et al., 2001), and hence in determining the composition of the butterfly fauna of these sites.

The analyses of edaphic factors show that two other gradients may be also of great relevance to the composition of the vegetation and fauna. One of these ecoclines is the fertility gradient, determined by the availability of P and K. This gradient seems to be associated with the other gradient determined by the summer water table depth and negatively correlated with the organic matter accumulation, soil moisture and altitude. However, this assertion should be treated cautiously because the variable WT usually shows a high spatial and temporal variability (WHEELER & PROCTOR, 2000).

Nevertheless, there is not a complete correspondence between vegetation and edaphic gradients. For example, floristically close associations may be found in habitats with considerable differences in edaphic characteristics. Hence, apart from the soil characteristics there are other factors that determine the vegetation in a place (e.g. topography, land use, history).

The information from the plant assemblages seems to be a better predictor of butterfly composition than edaphic factors (SAWCHIK et al., 2003). Vegetation integrates many ecological factors that may determine the distribution and abundance of butterfly species. Some plants represent essential resources for the survival and reproduction of butterflies. This is notably the case of the host plants, which represent the larval resources. Significant correlations have been detected between butterfly species and their respective host plants (SAWCHIK et al., 2003). Other plants may provide nectar sources for the adults as well as the conditions necessary for sun-basking or mating places. On the other hand, the presence of some plants can also correlate with peculiar ecological situations that are favourable to butterfly species, although this does not imply any causal relationship about their simultaneous presence. In summary, the important associations that exist between butterflies and plant assemblages make the former valuable indicators of habitat types.

Butterfly assemblages and indicator species

The butterfly assemblages are structured among a gradient from lowland eutrophic grasslands to high altitude oligotrophic bogs. Three main butterfly assemblages were identified, which showed characteristic sets of indicator species. Five butterfly species were identified as characteristic of the different assemblages : *B. ino, C. selene, L. helle, L. hippothoe*, and *P. eunomia*. As a result of the variation in their optima, the relative abundances of these five species changed gradually along the main ecocline.

Therefore, the composition of the assemblages changes principally according to the dominance structure of these species. The other butterfly species are in general more widespread, generalist or ubiquitous.

One assemblage is characterized by the strong abundance of *C. selene* and, in lesser extent by *L. hippothoe*. This assemblage is typical of oligotrophic sites (bogs and wet rush meadows). A second assemblage, essentially consisting of bistort meadows is characterized by high numbers of *L. helle* and *P. eunomia*. These were both the most diverse assemblages and showed a lesser dominant structure in distribution of species abundances. The third group, typified by meadowsweet grasslands is dominated by *B. ino*. The vegetation of these sites is dominated by tall herbs like *Angelica sylvestris*, *Cirsium palustris*, *Lysimachia vulgaris*, and *Valeriana repens*. These categories seem to be clearly structured along a vegetation gradient, showing various intermediate habitat types. In summary, one extreme of the gradient is represented by peat bogs, followed successively by oligotrophic rush meadows, bistort meadows, meadowsweet grasslands and highly eutrophic wet sites at the other extreme. The latter are represented by species-poor grasslands dominated by the common nettle (*U. dioica*) and with low biological value (i.e., low butterfly species richness and abundance). All along this gradient, the composition of butterfly assemblages' changes gradually from sites dominated by *C. selene* to sites dominates by *B. ino*.

Ecological breadths of the species vary from the more eurytopic ones, typical of high-level structure (e.g., *B. ino*), to stenotopic species characteristic of some well-defined groups (e.g., *C. selene, P. eunomia*). The presence of *B. ino* seems to be assured by the presence of the meadowsweet (*F. ulmaria*), its larva host-plant. This plant is well represented in different types of semi-natural meadows comprising eutrophic grasslands, bistort meadows, and wet peaty meadowsweet grasslands.

The ecological niches of the five indicator species are probably confined to wet grasslands and they are rarely observed elsewhere. On the other hand, field observations reveal that the butterflies identified as detector species (*A. levana* and *A. Crataegi*) also occupy drier grasslands. Detector species are defined by moderate levels of fidelity and specificity. Changes in abundance of these species may provide information on the direction of ecological change (MCGEOCH et al., 2002). In particular *A. levana*, which feeds principally on *U. dioica*, shows a higher tolerance than the other mentioned species (OOSTERMEIJER & VAN SWAAY, 1998). Although its indicator value is limited in this way, it may be an interesting detector species for identifying changes in the level of eutrophication of the habitats. However, detector species are also more prone to important inter-annual or spatial variations in their IndVal values (MCGEOCH et al., 2002). For this reason, future research should consider the validation of the results with independent data sets.

Our results suggest that the set of five species identified as indicators may constitute a useful tool for conservation purposes. Focusing the conservation efforts on the habitat requirements of these species, may be beneficial to protect a significant proportion of wet meadows. These five species are more or less specialized within distinct

sections of the main gradient and are indicators of particular habitats. Therefore, if we preserve and manage refuge sites for these species we are likely to be providing protection for other organisms living in the same biotopes. Concentrating management practices on these five butterfly species will also result in cost-effective administration of time and funding resources.

The five indicator species show features that make them ideal candidates for focal species. They form relatively large colonies in spite of being more or less threatened species (GOFFART et al., 1992). They may be assessed quickly with cheap and standard methods. Moreover, some of these species show narrow tolerances so they may be particularly sensitive to environmental changes (OOSTERMEIJER & VAN SWAAY, 1998). By using a multi-species approach we are covering a long gradient of environmental conditions. The five indicator species encompass al the range of the studied biotopes. All the sites showed the presence of at least one of these species. The simultaneous presence of many of these species may be an indicator habitat heterogeneity.

Indicator and umbrella species are not equivalent concepts, and may be interesting complementary tools for conservation practices (FLEISHMAN et al., 2000). However, some particular species may constitute indicator as well as umbrella species. For example, the 5 species identified as indicators have some characteristics that suggest they may be candidates to conform a suite of umbrella species. They are easily recognizable, show an intermediate degree of rarity, are moderately sensitive to human disturbance, and encompass a large range of habitats (FLEISHMAN et al., 2000; MAES, 2004). However, to be considered as umbrella species, they must show a high pattern of co-occurrence with many other typical species and that was not tested in the present study. *Proclossiana eunomia* and *L. helle* are probably the best candidates because they were associated to the second correspondence axis, which was significantly correlated with species richness and diversity. One particular advantage, in the case of *P. eunomia*, is the considerable knowledge about its life-history and ecology (BAGUETTE & NÈVE, 1994; BAGUETTE et al., 1998; NÈVE et al., 1996; PETIT et al., 2001; SCHTICKZELLE et al., 2002).

To conclude, because of the many advantages described above, we propose that *B. ino, C. selene, L. helle, L. hippothoe* and *P. eunomia* may be used as indicators of habitat types, and as surrogate species for conservation efforts. These species are habitat specialist of small size so they represent interesting tools at small spatial scales as those involved in the present article. The use of species assemblages as indicators may be considerably improved by extending the approach to organisms that are taxonomically and functionally different (MAES, 2004). Future research should be oriented to integrate over larger spatial scales by incorporating knowledge from other taxonomic groups such as birds.

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