Original article

Congruence among vascular plants and butterflies in the evaluation of grassland restoration success

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\textbf{A B S T R A C T}

Using surrogate taxa in the monitoring of restoration success is strongly advocated but currently under debate. Are butterfly and vascular plant communities good surrogates for each other in monitoring the restoration of calcareous grassland grassland? The research was conducted in a small area in Tuscany (central Italy), which was the focus of a restoration project. We sampled vascular plants using 35 plots (1 m\textsuperscript{2}) and butterflies using 20 transects (100 m in length) belonging to four treatment types: control scrubland, restored ex-arable, restored grassland and reference grassland. A mixed model nested ANOVA showed that reference grassland, 3 years after restoration, had the highest number of vascular plant species and the highest number of butterfly species. The different treatments were found to be distinct from a compositional point of view: pRDA showed that the “treatment” factor, independent of spatial variables, accounted for 22.1 and 21.9% of the total variance in the plant community and butterfly community, respectively ($p < 0.01$). The butterfly community was significantly correlated with the entire plant community data set and with the phanerophyte community (i.e. shrubs); larval host plants were significantly correlated with the composition of non-migratory butterflies. The results indicated that butterfly and vascular plant communities were congruent, suggesting the use of community composition of different taxa as a valuable tool for the evaluation of management success.

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1. \textbf{Introduction}

Cross-taxon congruence exists when spatial patterns of species richness, species composition or rarity coincide for two or more taxonomic groups (Lamoreux et al., 2006). One of the principal challenges in conservation biology is to identify efficient surrogate species, or groups of species, in the measuring and monitoring of restoration programs (Pharo et al., 1999; Wolters et al., 2006), but little work has been done to evaluate their effectiveness in restoration matters (Anand et al., 2005). Restoration intervention has mainly focused on the restoration of habitat, defined as vegetation types, regardless of the requirements of other taxa (Adam, 2001). Since native vegetation is important for many species, many

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authors have equated ‘habitat’ with ‘native vegetation’, although this classification may be appropriate in some situations (Terborgh et al., 2001) but misleading in others (Dennis et al., 2006; Vanreusel and Van Dyck, 2007). Following this trend, the assessment of restoration management has been dominated by studies on plant communities (Young, 2000), but butterflies have also been used recently to evaluate the success of restoration programs (Huntzinger, 2003; Waltz and Covington, 2004; Pöyry et al., 2005; Shepherd and Debinski, 2005; Lomov et al., 2006; Öckinger et al., 2006). Moreover, few studies integrate both floral and faunal components in the evaluation of restoration success (e.g. Shepherd and Debinski, 2005; Öckinger et al., 2006; Vogel et al., 2007) despite the fact that this integration has been strongly advocated (Vessby et al., 2002; WallisDeVries et al., 2002; Öckinger et al., 2006).

Butterflies and vascular plants are considered two of the most appropriate taxa for environmental evaluation (Sætersdal et al., 2003; Uehara-Prado et al., 2007); both are generally easily identified in the field and taxonomically well described. Butterfly and vascular plants are also considered good surrogates for each other: butterfly diversity may provide a surrogate measure of plant diversity because they are directly dependent on plants as a result of coevolution (Ehrlich and Raven, 1964; Pearman and Weber, 2007), and vascular plant diversity is considered a good surrogate of invertebrate diversity in general (Panzer and Swartz, 1998; Sætersdal et al., 2003). However, insects may respond more rapidly than plants to changes provoked by restoration management, because of their short life-cycles and sensitivity to small-scale environmental conditions (Mortimer et al., 1998); in fact, insects respond faster than plants to environmental changes and are considered to be better indicators (Mortimer et al., 1998;Öckinger et al., 2006). Based on these findings, we can assume a more detectable response to restoration management from butterflies than from vascular plants.

Analyses based on community composition are more sensitive to management actions than diversity indices (Pueyo et al., 2006) and are also considered superior in the evaluation of cross-taxon congruency (Su et al., 2004; Lovell et al., 2007) and for setting conservation planning targets (Fleishman et al., 2006). It is therefore preferable to use analyses focusing on compositional data, i.e. the presence and abundance of species, to integrate the data on species richness (Pueyo et al., 2006; Maccherini et al., 2007).

Calcareous grasslands can be considered a suitable example to illustrate an integrated approach for conservation, since they: (i) host high plant and invertebrate richness; (ii) generally need some form of management to maintain their status; and (iii) represent a severely endangered habitat of high conservation interest (WallisDeVries et al., 2002). Restoration intervention on semi-natural grasslands is also well documented in the literature (Muller et al., 1998; Ruiz-Jaen and Aide, 2005; Maccherini et al., 2007). In particular, in semi-natural grasslands, plant species richness is not always correlated with the species richness of other taxa (see Öckinger et al., 2006) and the same management actions are not always effective for the restoration of both plants and invertebrate fauna (Mortimer et al., 1998; WallisDeVries et al., 2002). The increasing need to test community similarity as a measure of cross-taxon congruence at the local scale has also become evident (Kati et al., 2004; Su et al., 2004), since conservation actions (e.g. management and restoration ecology programs) are mainly performed at this scale.

A multi-taxon approach to monitoring can be applied to define successful proxies and to detect the unexpected (positive and negative) consequences of intervention. For these reasons, we focused our analysis on both plant and butterfly community composition at the local scale, addressing the question: following restoration intervention, do butterfly and vascular plant communities converge toward a defined reference community, showing a similar successional path, or do they respond differently?

2. Methods

2.1. Study area

The research was conducted near the summit of Monte Labbro (42° 49' 13" N, 11° 31' 33" E), a prevalently calcareous massif on the Uccellina-Monte Amiata ridge, in Tuscany, central Italy. The study area covers about 17 ha, ranging in altitude from 1089 to 1193 m a.s.l. and is part of a Natura 2000 network site (SCI, “Monte Labbro and Upper Albegna Valley”). Mean annual rainfall in the area is 1002 – 1469 mm and the average annual temperature is 12.1 – 12.5 °C. The summit of Monte Labbro has been grazed for centuries, mostly by sheep, and human traces in the area date back to the Bronze Age. After the 1960s the grazing pressure decreased and arable land was abandoned. A restoration project was launched in 1999 with the aim of preserving the calcareous grassland (Maccherini et al., 2007), including the cutting of shrubs on overgrown grassland.

2.2. Restoration practices, plant and butterfly sampling

To test the effects of restoration practices via an analysis of the species diversity, we used a sampling design with replicated patches of habitat to take into account natural spatial variability. Between 2000/2001, laborers manually cut shrubs on selected patches of overgrown grassland and scrubland in ex-arable areas, for a total area of about 8 ha. Shrub clearing was not repeated. For more information on the restoration treatments, see Maccherini et al. (2007). The unmanaged scrubland was taken as a “control” to assess the changes of the restored ex-arable and restored grassland, as they represented “sites similar to the site being restored prior to restoration”. For reference, we adopted the interspersed patches of semi-natural calcareous grasslands recognized in the study area, in order to avoid the bias of changing spatial context and the environmental distance from the site to be restored. In summary, plots were divided into four treatment types, namely: control scrubland (two patches, mean 0.94 ha); restored ex-arable (two patches, mean 1.66 ha); restored grassland (three patches, mean 1.10 ha); reference grassland (three patches, mean 1.57 ha).

The plant data set was taken from Maccherini et al. (2007) and comprised 36 plots (excluding four plots representing patches not sampled for butterflies from the original data set) of 1 x 1 m square belonging to the four treatment types:
control scrubland (5 plots), restored ex-arable (6 plots), restored grassland (13 plots) and reference grassland (12 plots). All the vascular plants growing in each plot were recorded in late June 2003 and their cover estimated using a point-quadrant method (Moore and Chapman, 1986) with a density of 100 pins/m². Species present in a plot but not touched by any pins were recorded with an arbitrary cover of 0.1%. The nomenclature of vascular plant species follows Pignatti (1982), and Bechi (1998) for Cerastium arvense ssp. arvense var. etruscum.

To sample butterflies, five 100 m long transects were randomly selected for each treatment type and sampling was undertaken 2.5 m either side of the transect lines (Pollard and Yates, 1993) in June, July and August 2003; transects were at least 30 m from each other. All butterflies were identified to species level in the field (with the exception of a few species identified in the laboratory: genus Plebejus and Lycaenidae) and recorded. The taxonomic nomenclature and indication of larval host plants follows Balletto et al. (2007); the selection of non-migratory species was according to Balletto and Kudrna (1985), while for the red-listed butterfly species we followed Fabiano et al. (2001), van Swaay and Warren (1999) and Balletto et al. (2007). Butterfly abundance data (number of individuals per sample) and species from the three sampling dates for each site were pooled to obtain a composite of the whole assemblage.

2.3 Data analysis

Plant, butterfly and red-listed butterfly species richness, as well as butterfly abundance per plot, were analysed using a mixed model nested ANOVA (GLM) to test for the effect of treatment (fixed factor with four levels, i.e. control scrubland, restored ex-arable and reference grassland) and patch (random factor nested within treatment). Whenever ANOVA detected significant differences for the treatment factor, independent comparisons were also used (Tukey’ Unequal N HSD). We performed Spearman’s rank correlation test to determine any correlations between the phanerophyte cover (i.e. tree and scrub) and butterfly species richness. ANOVA and correlation analysis were performed using NCSS 2007 (Hintze, 2008).

The differences between treatments in the species composition of plant and butterfly communities were analyzed using partial redundancy analysis (pRDA; Borcard et al., 1992). Our species data had relatively low beta diversity. With such data, it is preferable to use multivariate methods based on linear assumptions (Lepš and Šmilauer, 2003), i.e. principal component analysis (PCA) and its constrained equivalent redundancy analysis (RDA).

The presence of spatial autocorrelation between observations in plant communities and butterfly communities could lead to overestimation of the effect of the treatment factor. To account for any spatial associations in the distribution of butterfly and plant species, we created a geographical location data set that contained the longitude and latitude of each survey plot. Such coordinated data acts as a synthetic variable for any underlying processes that may have generated spatial structure (Borcard et al., 1992), and may be statistically controlled for (i.e. partialled out) in subsequent analyses.

Variation partitioning for RDA using two sets of predictors, i.e. treatment types (included in the analysis as four dummy variables) and spatial variables, is based on three canonical analyses. The first analysis uses both predictors, whereas the second only uses treatment variables and the last one only spatial variables. The remaining fractions of the partitioning can be obtained by subtraction of: (i) the unique fraction of variation explained by the treatment variables; (ii) the unique fraction of variation explained by the spatial variables; (iii) the common fraction of variation shared by the treatment variables and spatial variables; and (iv) the residual fraction of variation not explained by treatment types or spatial variables.

To investigate patterns of cross-taxon congruence between the plant and butterfly communities, we first analysed the community data sets separately by indirect gradient analysis, then we correlated the first axes from these analyses by calculating pairwise correlations (Hájek et al., 2002; Ter Braak and Shaffer, 2004). To investigate the major gradients in the species data for plant and butterfly data sets, principal component analysis (PCA) was performed for each of the following groups: composition of vascular plants, composition of butterflies, composition of non-migratory butterflies and composition of their larval host plants. Possible covariation between the main gradients (first PCA axis) was tested by Pearson product-moment correlation for: (a) the complete butterfly vs. complete plant data set; and (b) the non-migratory butterflies vs. their host plants. For each patch sampled for butterflies, we calculated the mean cover value of vascular plant species and the mean cover value of larval host plants for all the vegetation plots belonging to the patch. In order to perform multivariate analysis, the abundance of plant and butterfly species was transformed according to a logarithmic function [ln(x + 1)]. The significance of the multivariate analyses was tested by Monte Carlo permutation tests (199 permutations). Multivariate analyses were performed using the CANOCO for Windows 4.5 software package (Ter Braak and Šmilauer, 2002).

3. Results

A total of 136 species of vascular plants and 59 species of butterflies (821 individuals) were recorded; 44 species were non-migratory butterflies and 31 plant species were larval host plants. The nested ANOVA model showed that the treatment factor was significant for plant and butterfly species richness (Table 1). The effect of restoration treatments on plant species richness differed only when comparing control scrubland vs. the other treatments (Fig. 1a), while butterfly species richness was different for control, restored and reference treatments (Fig. 1b); numbers of red-listed butterflies (Argynnis pandora, Carcharodus lautherae, Glaucoptysche alexis, Maculinea arion, Melanargia arge, Pyrgus sidae, Thymelicus acteon and Zerynthia polyxena) were not significant different among the treatments (Fig. 1c).

Correlation tests showed that the phanerophyte cover showed a negative correlation (p < 0.05) with the species richness of butterflies, i.e. the total number of butterfly
species ($r = -0.94$) and the total number of red-listed butterfly species ($r = -0.85$).

In the plant community treatment variables, independent of spatial variables, accounted for 22.1% of the total variance and were statistically significant ($p < 0.01$); pure spatial variables, independent of treatment variables, accounted for 5% of the total variance and were not statistically significant. Almost 22% of the total variance explainable by treatment variables (28.3%) was confounded with spatial variables. In the butterfly community treatment variables, independent of spatial variables, accounted for 21.77% of the total variance and were statistically significant ($p < 0.01$); pure spatial variables, independent of treatment variables, accounted for 7.9% of the total variance and were not statistically significant. Approximately 56% of the total variance explainable by treatment variables (49.77%) was confounded with spatial variables.

Regarding multivariate analyses, the redundancy analysis ordination diagrams showed the differences between treatments in the species composition of plant and butterfly communities (Figs. 2 and 3). The first two ordination axes explained 13 and 17% of the total species variability for the vascular plant data set and butterfly data set, respectively. *Anthyllis vulneraria* ssp. *praepropera*, *Centaurea deusta* and *Sedum album* showed higher cover in reference grassland; *Crepis neglecta*, *Cynosurus echinatus*, *Phleum pretense* and *Plantago lanceolata* were correlated with restored grassland. The species *P. spinosa*, *Galium album*, *Picris hieracioides*, *Rubus ulmifolius*, *Trifolium pratense* and *Dianthus sylvestris* characterized restored ex-arable in the vascular plants data set.

The butterfly species associated with reference grasslands were *Chazara briseis*, *Hipparchia semele*, *M. arge*, *Polyommatus cisoron*, *Pseudophilotes bato*, *Thymelicus lineola* and *Thymelicus sylvestris*. In contrast, only *Aricia agestis*, *Callophrys rubi*, *Lycæides abetonicus* and *Zerynthia polyxena* were more frequent on restored grassland. The species *P. spinosa*, *Galium album*, *Picris hieracioides*, *Rubus ulmifolius*, *Trifolium pratense* and *Dianthus sylvestris* characterized restored ex-arable in the vascular plants data set.

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**Table 1 – Summary of results of the mixed model nested ANOVA for species richness using a generalized linear model (GLM).**

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<th>df</th>
<th>MS</th>
<th>F</th>
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<td></td>
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</tr>
<tr>
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<td>331.383</td>
<td>146.20***</td>
</tr>
<tr>
<td></td>
<td>Patch (Treatment)</td>
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<td>0.416 ns</td>
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<td>5.446</td>
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<td>6.20 ns</td>
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<td></td>
<td>Residual</td>
<td>13</td>
<td>1.1769</td>
<td></td>
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</table>

*p < 0.05; **p < 0.001.

**4. Discussion**

Data from the current survey show that the two restored treatment types (i.e. restored ex-arable and restored grassland) appear different from reference grassland respect to butterfly species richness and community composition. The habitat specialist butterflies of calcareous grasslands (see Balletto and Kudrna, 1985; Fabiano et al., 2001; van Swaay,
Fig. 2 – Biplot based on partial redundancy analysis of vascular plant species abundance, using treatment variables independent of spatial variables. Only species that show a minimum fit to the model of 12% or more are displayed. Species arrows pointing in approximately the same direction as the triangles, representing dummy treatment variables, indicate a high positive correlation with that restoration treatment and arrows pointing in opposite directions indicate high negative correlation. Longer species arrows indicate variables that provide more certainty about the predicted correlations. Species are coded as follow: Ant vül: Anthyllis vulneraria ssp. praepropera; Bro ere: Bromus erectus; Car nut: Carduus nutans; Cen deu: Centaurea deusta; Cre neg: Crepis neglecta; Cyn cri: Cynosurus cristatus; Dia syl: Dianthus sylvestris; Fes bre: Festuca brevipila; Phi amb: Phleum ambiguum; Phi pra: Phleum pratense; Pla lan: Plantago lanceolata; Pic hie: Picris hieracioides; Pru sp: Prunus spinosa; Rub ulm: Rubus ulmifolius; Sed alb: Sedum album; Tri inc: Trifolium incarnatum; Tri pra: Trifolium pratense.

Fig. 3 – Biplot based on partial redundancy analysis of butterfly species abundance using treatment variables independent of spatial variables. Only species that show a minimum fit to the model of 20% or more are displayed. Species arrows pointing in approximately the same direction as the triangles, representing dummy treatment variables, indicate a high positive correlation with that restoration treatment and arrows pointing in opposite directions indicate high negative correlation. Longer species arrows indicate variables that provide more certainty about the predicted correlations. Species are coded as follow: Ari age: Aricia agestis; Cal rub: Callophrys rubi; Cha bri: Chazara briseis; Coe arc: Coenonympha arcania; Cya sem: Cyaniris semiarigus; Gla ale: Glaucopsyche alexis; Hip sem: Hipparchia semele; Iss lat: Issoria lathonia; Lyc abe: Lycaenidae abetonicus; Man jur: Maniola jurtina; Mel arg: Melanargia arge; Pol cor: Polymatmous coridon; Pol ica: Polymatmous icarus; Psy bat: Pseudophilotes baton; Psy arm: Pyrgus armoricanus; Psy cec: Pyronia cecilia; Thy lin: Thymelicus lineola; Thy syl: Thymelicus sylvestris; Zer pol: Zerynthia polyxena.

2002; van Swaay and Warren, 2006; Balletto et al., 2007) were exclusively related to the remnant grasslands (i.e. reference treatment type) that hosted the highest number of butterfly species and the highest abundance of individuals, in accordance with the study carried out in central Iowa, USA (Shepherd and Debinski, 2005). The butterflies A. agestis, C. rubi, G. alexis, P. icarus and Z. polyxena that characterized the restored treatment type are considered in Italy as sub-nemoral species (Balletto and Kudrna, 1985; Balletto et al., 2007), even if A. agestis is classified as a species of calcareous grasslands in the rest of Europe (van Swaay, 2002; van Swaay and Warren, 2006). Calcareous grasslands overgrown by P. spinosa (control scrubland) showed the lowest butterfly richness, as also observed in a descriptive observation of the same study area (Favilli et al., 2004), and on other calcareous grasslands of Central and Northern Europe (Balmer and Erhardt, 2000; Vessby et al., 2002). Based on these findings, there was evidence that, 3 years after the launch of the restoration actions, the cutting regime had not achieved the aim of restoring butterfly community composition, as reported for plant community restoration in the Monte Labbro area (Maccherini et al., 2007).

In the literature, the effectiveness of the restoration of overgrown grassland appears to be habitat dependent (see Woodcock et al., 2005). In our case, the plant community structure (in terms of phanerophyte coverage) was negatively correlated with butterfly richness and the abundance of individuals; red-listed butterflies in particular, such as Carcharodus lavatherae, Maculinea arion, Melanargia arge, Pyrgus idae and Zerynthia polyxena, were collected exclusively on reference grassland. This is consistent with observations.
by Balmer and Erhardt (2000) and Öckinger et al. (2006), that an increase of phanerophyte cover has a negative effect on both the species richness and abundance of butterflies. It is noteworthy that Söderström et al. (2001) affirm that in Swedish semi-natural grasslands the number, cover and volume of trees and shrubs seem to have a positive effect on butterflies, providing food resources over season, suitable nesting substrate and favorable microclimate; it suggests that the collateral effects on other taxa of shrub removal, which is usually performed to promote vascular plant conservation (Maccherini et al., 2007), merit careful consideration.

Restored plant and butterfly assemblages differed from the reference one, as they are characterized by distinct groups of species, but when we considered species richness alone these differences were not confirmed for vascular plants: reference grassland and restored treatments showed the same high number of species. This was also observed by subtracting the spatial autocorrelation among plots and considering that the patches belonging to different treatments were small and very close to each other. Highly significant correlations between the main gradients (first PCA axis) for complete butterfly vs. complete plant data sets, and for non-migratory butterflies and their larval host plants, demonstrated a certain degree of co-variation between vascular plant composition and butterfly community composition, corroborating cross-taxon congruency. Part of the association of butterflies with vascular plants probably arises from the observation that butterflies are positively associated with nectar availability, with preferences for specific plant species (Stefanescu et al., 2005), and vegetation structure conditions. The results of our study suggest that the turnover in butterfly species composition can be correlated with the turnover in vascular plant species composition. We confirmed that classical species richness analyses should be integrated with analyses based on the entire community composition, since the latter were more efficient for evaluating restoration effectiveness in either taxon.

5. Management implications

General guidelines for biodiversity conservation encourage habitat heterogeneity and the maintenance of a mosaic of different successional stages as instruments for the conservation of plant and butterfly species (Pöyry et al., 2005). Contrariwise, in our study butterfly communities responded differently to heterogeneity: butterflies seemed to clearly distinguish among contiguous and physiognomically similar grasslands demonstrating that they are particularly demanding regarding habitat selection, preferring reference grasslands and avoiding areas in successional transition such as restored grasslands. Restoration intervention aimed at restoring plant communities indirectly influenced the butterfly communities; it is therefore important to bear in mind that any restoration intervention might be magnified in a positive or negative (see Söderström et al., 2001) way, following different patterns in space and time for different taxa.

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