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Title: Mobility and oviposition site-selection in *Zerynthia cassandra* (Lepidoptera, Papilionidae): implications for its conservation.

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Abstract

The adults' mobility and oviposition preferences of *Zerynthia cassandra* have been studied for the first time, with the aim of integrating auto-ecological information into recommendations for the habitat's management of this species. Results of our mark-release-recapture study have highlighted that *Z. cassandra* is a strictly sedentary species, since detected movements only occurred over very short distances (≤ 200 m) and mainly within the species' reproductive habitat (i.e. around *Aristolochia rotunda* stands), with males moving further than females. Our study shows that the main oviposition habitat of *Z. cassandra* is found where *A. rotunda* plants are growing in large stands; sites where plants growing in half to full sun and mostly oriented to the South are preferred. The distance of deposited eggs from the plants' roots was narrowly correlated with the plants' length. Eggs were deposited singly, mainly on the underside of leaflets. Management strategies necessary for improving the most important habitat features for the conservation of this species are suggested.

Keywords: Butterfly conservation, *Zerynthia cassandra*, oviposition, *Aristolochia rotunda*

Introduction

Causes for the widespread decline of many European butterfly species are primarily recognised in habitat degradation and loss (New 1997; Fox 2012; Maes & Van Dyck 2001; van Swaay et al. 2010). At least in principle, however, any perturbation of the environment can negatively affect species' survival and may be at the core of many extinction processes. As Samways (2007) suggests, strategies for insect conservation must be planned at regional scale, to reduce locally negative impacts. Stenotopic and univoltine butterfly species are particularly threatened worldwide by habitat destruction and climate change, most particularly at the edges of their range (Hoyle & James 2005; Bonelli et al. 2011). Habitat changes have even stronger negative effects on species with low dispersal ability, including many terrestrial invertebrates (Thomas et al. 2004) such as the Papilionid species of the genus *Zerynthia*.

Zerynthia polyxena and *Zerynthia cassandra* are among the potentially most vulnerable butterflies in the Mediterranean area. *Z. polyxena* is a strictly European species, ranging from Southern France to the Urals, Italy and the Balkans (Kudrna et al. 2011). In Italy, however, two separate species have been demonstrated to exist. Their species-level separation was initially proposed by Dapporto (2010) on the basis of genitalic characters and more recently confirmed by genetic data (Zinetti et al. 2013). In Italy, *Z. polyxena* occurs in the North of the Country, mainly in the northern plains of the Po river valley and the surrounding foothills of the Alps. The other species, known as *Z. cassandra*, occupies most of the Peninsula, starting from the northern Tyrrhenian divide and as far South as Calabria, as well as in Sicily. Since they are almost indistinguishable in external morphology, the two species remained lumped under *Z. polyxena* for a long time. Since *Z. cassandra* was separated by *Z. polyxena* (a EU Habitats Directive species) responsibility for the conservation of this species has become a matter of particular importance for Italy, although not yet for the European Union (Maes et al. 2013).

However, characteristics of the life history and ecology of these species are not well understood and they can probably differ in such characters as habitat selection, oviposition behaviour and dispersal ability. As concerns ecological characteristics, adults of *Z. polyxena* require a sub-nemoral habitat and only spend a relatively short part of the day in open herbaceous areas. Nothing is known, at the moment, as concerns *Z. cassandra*.

The present paper was designed to gain information on some population traits of *Z. cassandra* deemed particularly useful for planning the conservation of this endemic species. More in detail, our objectives were: (i) to obtain data on adult mobility, by investigating whether *Z. cassandra* adults moved all through the landscape matrix, (ii) to investigate the habitat factors and larval food-plant characteristics positively influencing the choice of *Z. cassandra* females in oviposition-site selection and (iii) to consider implications of results from this analysis for the conservation of the species. Gaining this information will be a first step for developing a specific action plan for the conservation of *Z. cassandra* and will provide useful guidelines for the management of its habitat.

Material and Methods

The study species

Similarly to *Z. polyxena*, *Z. cassandra* is single brooded and the flight period of adults spans from late February, in Sicily, to the beginning of June, depending on altitude and latitude (Verity 1947; our data), with hibernation diapause in the pupal stage. For an adult female, total fecundity is about 50/60 eggs (personal observations). During the flight period, which lasts around 15 days, females lay on the *Aristolochia* leaves. In Italy, larvae generally feed on *A. rotunda* or *A. pallida*, which always grow in small scattered stands within semi-natural ecotonal grasslands, between (0) 300 and 900 m.

At least 15 populations of *Z. cassandra* are known for having become extinct in Italy during the past 50 years (Bonelli et al. 2011), generally as a consequence of habitat loss and/or demographic stochasticity, since this species typically occurs only at low-densities. Populations of all *Zerynthia* species are restricted to micro-habitats where their larval food plants (*Aristolochia* spp.) grow, and their restricted, spots-like distribution, is probably related to host plants' distribution, even though *Zerynthia* populations are generally much rarer than those of their food-plants. Adults of *Z. polyxena* do not move over great distances and seldom fly far from their reproductive areas in search of suitable host plants. In a population from Slovenia, the maximum recorded flight distance was 400 m (Çelik 2012).

The study sites

Two disjunct areas were used in Italy as study sites for the work presented here (Fig.1). The "Capanne di Marcarolo" Regional Park (SCI IT 1180026) and the "Laghi di Conversano e Gravina di Monsignore" Regional Park (SCI IT 9120006). The two sites were chosen for their position at the extremity of the distribution range of the species, as well as because of logistic reasons. These two areas contain large and persistent populations of *Z. cassandra*. We investigated egg-laying behaviour at both sites, but we carried out also mark-release-recapture only at "Laghi di Conversano".

The "Laghi di Conversano e Gravina di Monsignore" Regional Park is in Apulia, in the Southeast of the Italian peninsula. The Natural Park area consists of a set of ten karst ponds (*dolines*) located in a fragmented agricultural matrix (Altini et al. 2007). Populations of *Z. cassandra* are here under threat from habitat loss by anthropogenic disturbance and spread of vineyards. We analysed the egg-laying behaviour and the adults' movement of *Z. cassandra* in one of these dolines, i.e the "Chienna lake" (48°58'37" N, 17°04'21" E), which is surrounded by orchards and vineyards. For the mobility study, we divided the site into 7 arbitrary plots from 0.68 to 3.32 ha in size. Distances between occupied plots ranged from 110 to 477 meters.
The oviposition study was conducted at 3 sites a) Site 1: a grassland area of 96 m^2 located in plot C, near a Mediterranean pond and with a 25% canopy cover (40° 58' 17" N, 17° 04' 24" E).
b) Site 2: a 170 m^2 area within plot D (40° 68' 17" N, 17° 04' 30" E) with less than 10% canopy cover.
c) Site 3: an area of 78 m^2 in plot G, with 10% canopy cover (40° 58' 41" N, 17° 04' 47" E).

The Regional Park of "Capanne di Marcarolo" is located in the North-west of Italy. Our field study was conducted at three sites, where the butterfly apparently reached its locally highest densities

a) Site 1: Woodland edge. (44°36'22" N 8°46'01" E): consisting of a 128 m^2 mesophilous meadow bordered by a Black locust (*Robinia pseudoacacia*) grove and with 10% canopy cover.
b) Site 2: Riparian grassland (44°36'21" N 8°45'58" E): having a 50 m^2 grassland with sparse alder (*Alnus glutinosa*) shrub and hygrophilous vegetation Canopy cover was 25% ;
c) Site 3: Dry grassland (44°38′ N 8°51′E): a 170 m^2 semi-natural grassland with 5% canopy cover.

Egg-laying studies

We thoroughly investigated our sites to locate egg-laying areas and to record details of food-plant's characteristics, eggs' location and surrounding vegetation/habitat. In April/May 2010, we exhaustively assessed the distribution of *Aristolochia* plants and *Zerynthia* eggs at "Capanne di Marcarolo" and in April 2013 at "Laghi di Conversano". *Z. cassandra* occurred in small and often isolated populations, so that individually following females and observing their egg-laying behaviour was not the appropriate field method, in this case. To avoid any bias towards expected habitat characteristics, we used intensive search method. Some adults were still flying during the survey period, but the season was nearing its end, so that we conducted egg census shortly after the butterflies had completed oviposition.
Observations were aimed at determining eggs' distribution on each host-plant. *Z. cassandra* eggs are not morphologically different from those of *Z. polyxena*, but since they are characteristic in shape, size and colour they can be unequivocally identified even when hatched, since egg shells remain on the leaves for at least two weeks. To avoid recounting the same plant twice during the survey, we marked each plant with a flag-bearing stick.
The characteristics of *Z. cassandra* oviposition habitat were recorded at the landscape, patch and plant level. At landscape level, the numbers of eggs and of *A. rotunda* plants were assessed at all study sites. At patch level, the geographical orientation (the direction the slope faces with respect to the sun, or "aspect"), number of plants (1 plant, small stand with 2-5 plants, large stand with > 5 plants) and sun exposure (exposure to sunlight reaching the spot during a sunny day) were collected.

At plant level, habitat parameters were recorded in 1 x 1 m sample quadrats having the plant in the centre. Measurements of plant height, height of the surrounding vegetation, vegetation coverage (estimated in 5% units) and distance from the nearest tree were recorded in each quadrat. Finally, the number of eggs observed on each plant, the height of each egg above the ground and its position on the plant were registered. For data analysis we calculated the difference between the egg's position in height and the average vegetation height, as a proxy for the "prominence" of the host plant. Positive values show positive prominence of the eggs-bearing plants. These parameters were then compared between occupied and unoccupied host plants.

Butterfly mobility

From 10 to 20 April 2013, a mark-release-recapture study (MRR) was conducted at the "Laghi di Conversano" Regional Park (40°58' N, 17°04' E, see Fig. 2) within the peak flight periods of *Z. cassandra* adults. Since the population occurring at this general area is patchily distributed and our target species occurred at a number of patches, we conducted a preliminary study (Altini & Tarasco 2011), to assess which patch contained the highest population density. All the areas were walked during weather conditions suitable for adults' flight, and three to six people participated in marking and capturing, summing up to 50 person-days in total. Occasional visits were also made to apparently unsuitable areas, to check for the occurrence of adults that might be moving outside their usual habitats. An attempt was made to capture every observed individual and since adults of *Z. cassandra* are not difficult to net, most sightings resulted in captures. On each day we carried out three capture sessions, each 30 minutes long, between 10:00 and 14:00.

On each survey event, butterflies were captured, individually marked and immediately released. Larval food plants density was estimated by counting the number of *A. rotunda* plants per plot. Correlation between butterfly abundance and food plant density was determined at each plot. For each capture or recapture, the location (using a hand-held GPS device, min. accuracy 3 m), date, time, individual's number and sex were recorded.

Statistical analysis

Adults' mobility was estimated separately for the two sexes, as the straight-line measurement of the distance between consecutive captures.

The summation of all single distances was taken to represent the minimum cumulative distance travelled by each individual. The maximum distance between any two observations of each individual was also recorded. The operational sex ratio was defined as the ratio between the number of estimated males and the total estimated number of females. To test if the total number of males and females fitted the expected 1:1 ratio, a chi-squared test was performed. At each plot, correlations between the following parameters were computed: number of marked males, number of marked females, total marked specimens, recaptured butterflies, plot area and *A. rotunda* densities. A non-parametric Mann-Whitney U test was used to analyse intersexual differences in mobility, to test association between sexes and moved distances. All statistical analyses were performed on SPSS 21.

Data collected during MRR surveys were analysed by Cormack-Jolly-Seber type constrained models (Schwarz & Arnason 1996; Schwarz & Seber 1999) using MARK 5.1 program (White & Burnham 1999).

For the egg-laying study, statistical analyses were performed on *R-2.9.0 (R development core team).* Since it was impossible to determinate if eggs on a plant belonged to one or more females, each eggs-bearing host plant was treated as a single sample in our data set, regardless of the number of batches it carried.

In those cases when data were normally distributed (Komogorov-Smirnov test) and variances were homogenous (Levene test), parameters for occupied and unoccupied plants were compared using *t*-tests. Otherwise, the Mann-Whitney U test was used. Data from all sites were merged for evaluation of oviposition preferences at landscape levels. To define the oviposition preferences at the landscape and patch levels, the comparison of absolute frequencies for categorical variables, between occupied and unoccupied plants was assessed using Likelihood ratio statistic, to establish if environmental variables differed between eggs-bearing and unoccupied *Aristolochia* plants, at both the landscape and the patch level. Standardized residuals were used to define significant contributors to the overall Chi square value.

We used a generalized linear mixed-effects model to recognise those parameters possessing the highest explanatory power for oviposition sites selectivity. The variable "egg presence" was set as a random factor to examine the relationship between the occurrence of oviposition and habitat variables. The best model was assessed using the Akaike information criterion (AIC; cf. Zuur et al. 2009). Using a multi-model inference, we examined the AICc values for all possible models with all different combination of the explanatory variables mentioned above. Owing to the large number of candidate models, we restricted model averaging to models for which D AICc < 4 compared with model with the lowest AICc.

Statistical analyses were performed on SPSS 21 *and R-2.9.0 (R development core team).* Multimodel inference analyses were performed using 'MuMIn' package (Barton 2011) for R.

Results

Egg laying habitat

To evaluate how eggs' occurrence and abundance were affected by environmental variables, we surveyed 275 *A. rotunda* plants potentially available for *Z. cassandra* oviposition. At landscape level, eggs were found at all surveyed sites, but host-plant quantity differed between patches (site 1: N = 95, site 2: N = 141, site 3: N = 39; Table 1b, c, d). Of 275 potential host plants for *Z. cassandra*, 120 were selected for oviposition and 153 eggs were found (site 1: 47, site 2: 40, site 3: 66) (Fig. 3). At patch level, the occupied and unoccupied plants differed significantly in all measured landscape and patch parameters (Table 1a) except for the "Aspect" parameter: eggs were predominantly laid on plants growing in large stands (68.5%), and in half or full sun (83.1%), whereas single plants or plants growing in small stands (2-5 plants) (31%), or in full shadow (16%) were generally avoided and played minor roles in eggs deposition. < The great majority (> 70%) of occupied plants were found on south or south-east facing slopes. More exactly, females preferentially oviposited in south (44.9%) or south-east facing sites (29.1%), while they avoided northern orientation (Fig. 4) respect south and south-east facing sites ($\chi 2 = 29.930$; d.f. = 1; $p < 0.001$). > At site 2, eggs were laid in full shadow (45%), but females completely avoided plants in full shadow at sites 1 and 3, except in one case at site 1.

At plant level, the distribution of occupied and unoccupied plants was best explained by the combination of prominence (difference in height with respect to the surrounding vegetation), number of plants per stand and exposure to sun. The occupied and the unoccupied plants also differed in height (Mann-Whitney U-test: $N = 275$; $Z = 3.468$; $P = 0.001$) and the more prominent *A. rotunda* plants were significantly preferred $P < 0.05$ by t-test (Fig. 5).

The distributions of host plants and egg-deposition heights were more or less bell-shaped (Fig. 6) and eggs were in significantly higher number (N: 65 = 62%) than expected on plants 21-40 cm high. More than a half of the egg-bearing plants were 21-60 cm high (min: 17 cm, max 75 cm) (Figure 7).

The vast majority of eggs (N: 136 = 88.88%) were laid on the underside (abaxial) surface of the leaves, while a small fraction were laid on the upper surface (N: 15 = 9.8%), or even more rarely (N: 2 = 1.3%) on flower buds (χ^2 =199,991 df = 2, $P < 0.001$). Usually one egg per plant was found. Eggs, in fact, were laid mainly singly (N: 56 = 63%), sometimes in pairs (N: 16 = 18%), or rarely in small batches of 3 (N: 7 = 8%), 4 (N: 7 = 8%), 5 (N: 2 = 2%) or 6 (N: 1 = 1%) eggs. The vegetation cover was over 70% in all plots and areas. Most of the eggs (40%) were observed in plots with grass coverage between 80 and 90%.

GLM analysis showed that the likelihood of an *A. rotunda* plant being selected for oviposition was positively correlated with the distance from the nearest tree (Tab. 2). The likelihood of a site being accepted as oviposition habitat increased with host plant presence and with southerly orientation. Most egg-bearing *A. rotunda* plants were found in areas with no tree cover.

To evaluate eggs' occurrence at "Laghi di Conversano", we surveyed 82 *A. rotunda* plants potentially available for *Z. cassandra* oviposition. Eggs were found at all surveyed sites, but host-plant quantity differed between patches (site 1: N = 21, site 2: N = 21, site 3: N = 40). Of 82 potential host plants, 15 (18%) were selected for oviposition (site 1: 7, site 2: 7, site 3: 25). Eggs were predominantly laid on plants growing in large stands (N= 296; 82%), whereas single plants or plants growing in small stands were avoided, and in half (24.3%) or full sun (75.6%). The great majority of occupied plants were found on dry stone wall surfaces (58.5%) and plants on dry stone wall bore more eggs (N = 28, 73.6%). Females preferentially oviposited in south- (66.6%), or east facing sites, but the latter bore more eggs (N = 23; 59%).

The great majority (> 70%) of occupied plants were found on south facing slopes. Eggs were laid mainly singly (N: 67 = 43%). The maximum number of eggs per plant was 6 at Marcarolo and 14 at Conversano. Probably the smaller number of plants on the site of Conversano induces individuals to lay a larger number of eggs on a single plant. At Marcarolo the chosen plants grew in very thick vegetation cover (> 80%), whereas in southern Italy plants carrying more eggs were found on bare soil. Most sites with egg-bearing *A. rotunda* plants were found in areas with no tree cover.

Mobility

In total 34 individual were marked (23 males and 11 females) and 14 (41%) of them were recaptured at least once (Tab. 3). Based on these data, we estimated a total population size of 116 (± 19) individuals, with 79 (± 11) males and 37 females (± 6). No individuals were captured in nearby plots, showing no exchange between populations. The sex ratio of captures was male biased (χ^2 = 4.235, d.f. = 1, P = 0.039) and males were recaptured more often than females (χ^2 = 4.083, d.f. = 1, P = 0.043). Grouped for sexes, distances between captures did not markedly vary and ranged from 8.8 to 96.7 m. The longest detected movement between successive captures was 110.63 m. Nevertheless, most (75%) of the movements were within 60 m from the release spot. Residence time provides a rough estimate of maximum adult life span. The maximum recorded time between the first and the last capture was 6 days and was similar for males and females.

Discussion

Egg laying habitat

In this study we investigated the oviposition microhabitat of *Z. cassandra*. Characteristics of the oviposition site play an important role in determining habitat suitability since, according to several studies (e.g. Rausher 1983; Janz 2002) the choice of the deposition site is generally not random and is structured according to various maternal behaviours. Probably the most interesting result of our eggs-laying study is that irrespectively of a lower food-plants density, females lay in the more open areas ($\chi^2 = 7.098$, d.f. = 2, P = 0.028) at both study sites.(Fig. 3).

The habitat requirements by *Z. cassandra* for egg laying are best explained by a combination of presence in small stands of *Aristolochia* plants growing in medium sun conditions, with no other preference for any type of vegetation structure and/or feature of host plant quality. Although *Aristolochia* plants preferentially tend to colonise ecotonal areas and are generally less abundant in full sunlight, *Z. cassandra* prefers the more open habitats. This is probably a consequence of the fact that *Zerynthia* caterpillars are chemically protected ectotherms in no need of concealing in the shadow, while in the early Spring, when adults are flying, weather is surely more unpredictable than in other seasons. *Aristolochia* plants growing the in the shadow potentially represent ecological traps for strictly sedentary larvae. This is in contrast with data from the Hungarian population of *Z. polyxena* recorded by Batary et al. (2008). Considering that *Z. cassandra* occurs in Mediterranean areas, we would expected the opposite result. The majority of eggs, however, were placed on the abaxial surface of the leaflets, which may be explained by the fact that eggs need sufficient humidity to avoid desiccation (Anthes et al. 2008), as well as detection by predators or parasites. Eggs and young larvae, in fact, are not as chemically protected as the older larvae and adults (Albanese et al. 2008). Elevated ambient temperatures appear to be important for many butterfly species because they may increase rates of larval development, decrease mortality (McKay 1991), improve females' fecundity by increasing the time available for egg-laying, and therefore generally increase egg-laying rates (Davies et al 2006). The significant relationship between landscape, patch and plant parameters of *Z. cassandra* oviposition habitat showed that, as is common for butterflies in general (Dennis 2010), selection of oviposition sites is determined by characteristics operating at different hierarchical levels, reflecting their importance in the process of egg-laying site selection. A sufficient amount of food is essential for larval survival, in particular for species having "sedentary" caterpillars. Visual attraction is an important factor when searching for a suitable host plant (Porter 1992). For oviposition, females frequently choose the most conspicuous host plants (Porter 1992; Garcia-Barros & Fartmann 2009). Compared to plants growing in shadier areas, shoots that grow higher than their surrounding vegetation are also less shaded and offer better microclimatic conditions for a quick development of eggs and larvae (Kuer & Fartmann 2005). Furthermore, *Z. cassandra* females laid their eggs on the intermediate parts of plants probably also due to better food quality for the larvae, and this may be a direct consequence of female oviposition choice. Upper plant parts generally have lower amounts of alcaloids, as well as nitrogen contents (Agerbirk et al. 2010), compared to lower parts. Between occupied and unoccupied host plants, plants' height was the most important discriminating variable and larger plants bore more eggs, while Dennis (1996) showed that in *Allancastria* (or *Zerynthia*) *cretica*, larger food plants bore more eggs.

At least in some cases, laying eggs on the higher parts of the plant may provide some advantage. In *Z. rumina*, Jordano & Gomariz (1994) found that the freshly hatched larvae consumed the younger and softer leaves of the food plant (*A. pistolochia*). This could be related with the lower concentration of defence chemicals (Batary 2008). Early instars of Lepidopteran larvae are known to be sensitive to environmental and chemical changes (Zalucki et al. 2002). Some larvae are known to shelter inside the flower buds during their first instar, when they are less mobile and more prone to suffer from environmental stress (Pinto et al. 2011) and can thereby increase larval survival and growth. Anthes et al. (2003) noted a similar behaviour in *Euphydryas aurinia*. They suggested that this strategy eliminated the risks of predation and of exposure to adverse weather conditions, associated with moving along the host plant or to another plant.

Mobility

No movement of adult individuals was detected away from their breeding areas, which reveals that *Z. cassandra* is strongly sedentary and has relatively closed population structure. Similarly to many other butterfly species, *Z. cassandra* is strongly dependent upon particular microhabitats, both at the larval and at the imaginal stages. Due to strong human influence, suitable habitats have become increasingly fragmented, thereby restricting gene flow among populations as well as chances for a successful re-colonization of the remaining but isolated patches. Fitness benefits of intermediate-distance dispersal will therefore become strongly reduced, which will finally impose strong selection against dispersal (Bonelli et al. 2013).

Regional extinctions, in such a situation, represent a very likely scenario, perhaps the most likely at least in many cases.

Conclusion and implications for conservation

Protecting *Z. cassandra* populations requires that areas containing suitable nectar plants are also protected. In the northern parts of the species' range, the main problem is in the abandonment of rural areas. Thus, it is vital to maintain the few remaining meadows, by promoting cyclical grass cutting or light cattle grazing, as well as to create new grasslands, wherever possible. In the study area, which is part of the EU NATURA 2000 network, appropriate agro-environmental schemes have already been used to allocate the necessary funding for the maintenance of traditional land management, within the framework of the regional Rural Development Program (RDP). Grazing or grass cutting, whenever implemented in the end of June or in late summer, does not affect the survival of *Z. cassandra* pupae, which shelter at the base of their (unpalatable) host plant.

In Apulia, in contrast, the "tendone"-type vineyards have become largely dominant since their plastic covering is particularly suitable to protect the (mainly table-consumed) grapes from excessively high summer temperatures, strong winds and frequent hail. This particular cultivation type, however, is highly and negatively impacting on *Z. cassandra* populations, since adults are unable to fly across or over the vines. In our study area, as a consequence, butterfly populations tend to become increasingly fragmented by the still spreading vineyards. Mobility data obtained in our study are alarming, and urgent action is needed. To counteract the currently heavy fragmentation, suitable habitats should be created within the framework of current agro environmental schemes, to ensure host plant stands connectivity.

In South Italy *Aristolochia* plants that grow on dry stone walls will act as stepping stones. Dry-stone walls are important landscape elements in this area, but their importance has only been recognised in their aesthetic and cultural dimension. Current promotion policies officially aimed at preserving dry stone walls need to be better implemented to prevent any further loss of this irreplaceable asset, at communitarian level.

In general, schemes based on the application of the integrated organic production rules financed by RDPs will be less impacting for butterfly populations occurring in cultivated areas and particularly in vineyards. These include the insertion of buffer stripes between the fields, the (cyclical) abandonment of some mown areas and the encouragement of spontaneous re-vegetation in the alleyways and areas around crops. In the current economical crisis, reaching a trade-off compromise between agro-industrial needs and biodiversity conservation may locally generate important revenues, both by guaranteeing sustainability and by preserving the touristic attractiveness of landscapes (Lasanta et al 2001).

We also agree with Thomas et al. (1992) and with Maes et al. (2004), that installing a stepping stones system of suitable habitat patches is the most efficient way to restore a healthy meta-population structure, which surely works much better than ‘generalistic’ corridors in enhancing the conservation status of many invertebrates.

In the case of butterflies, each ontogenetic instar requires its own specific resources. In *Z. cassandra*, however, resources are spatially overlapping, since the adults’ habitat closely matches that of the immature stages, at least insofar as suitable nectar sources are maintained. The way to a successful management is in keeping the sites open and free from invading scrub. In both study sites, *Z. cassandra* is distributed mainly in the meadows and along the surrounding hedges. Managing marginal lands to preserve their biodiversity values and traditional farming systems, with mowing once or twice a year, could contribute to species persistence in a fragmented landscape. An optimum mowing heights for this species could range from 5 to 15 cm.

Conservation efforts are generally focused on maintaining species *in situ*, with considerable debate about the possible merits of reintroductions. Natural recolonisation of suitable habitats will be a slow process, provided that recolonisation rates will not match extinction rates. So we suggest that at least in some case the colonisations of some particularly suitable patches should be artificially encouraged. A well-connected network of suitable habitats ought, however, to be established well before any reintroduction scheme is implemented.

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References

Agerbirk N, Chew FS, Olsen CE, Jørgensen K (2010) Leaf and floral parts feeding by orange tip butterfly larvae depends on larval position but not on glucosinolate profile or nitrogen level. Journal of Chemical Ecology 36: 1335-1345.

Albanese G, Vickery PD, Sievert PR (2008) Microhabitat use by larvae and females of a rare barrens butterfly, frosted elfin (*Callophrys irus).* Journal of Insect Conservation 12: 603-615.

Altini E, Tarasco E (2011) Struttura di comunità di lepidotteri ropaloceri in diversi habitat della Riserva Naturale Regionale Orientata "Laghi di Conversano e Gravina di Monsignore" (Bari). Atti XXIII Congresso Nazionale Italiano di Entomologia, Genova 13-16 giugno 2011.

Altini E, Tarasco E, Gallo M, Triggiani O (2007) I lepidotteri diurni della Riserva naturale regionale orientata dei "Laghi di Conversano e Gravina di Monsignore" e note di biologia di *Zerynthia polyxena* (Lepidoptera, Papilionidae). Atti XXI Congresso Nazionale Italiano di Entomologia, Campobasso 11-16 giugno 2007: 74.

Anthes N, Fartmann T, Hermann G (2008) The Duke of Burgundy butterfly and its dukedom: larval niche variation in *Hamearis lucina* across Central Europe. Journal of Insect Conservation 12: 3–14.

Anthes N, Fartmann T, Hermann G, Kaule G (2003) Combining larval habitat quality and metapopulation structure – the key for successful management of pre-alpine *Euphydrias aurinia* colonies. Journal of Insect Conservation 7: 175-185.

Barton K (2011) MuMIn: Multi-model inference. R package version 1.0.0. http://CRAN.R-project.org/package=MuMIn

Batáry P, Örvössy N, Kőrösi Á, Peregovits L (2008) Egg distribution of the Southern Festoon (*Zerynthia polyxena)* (Lepidoptera, Papilionidae). Acta Zoologica Academiae Scientiarum Hungaricae 54: 401-410.

Bonelli S, Cerrato C, Loglisci N, Balletto E (2011) Population extinctions in the Italian diurnal Lepidoptera: an analysis of possible causes. Journal of Insect Conservation 15: 879–890.

Bonelli S, Vrabec V, Witek M, Barbero F, Patricelli D, Nowicki P (2013) Selection on dispersal in isolated butterfly metapopulations. Population Ecology 55: 469-478.

Çelik T (2012) Adult demography, spatial distribution and movements of *Zerynthia polyxena* (Lepidoptera: Papilionidae) in a dense network of permanent habitats, European Journal of Entomology 109: 217–227.

Dapporto L (2010) Speciation in Mediterranean refugia and post-glacial expansion of *Zerynthia polyxena* (Lepidotera, Papilionidae). Journal of Zoological Systematics and Evolutionary Research 48: 229-237.

Davies ZG, Wilson RJ, Coles S, Thomas CD (2006) Changing habitat associations of a thermally constrained species, the silver-spotted skipper butterfly, in response to climate warming. Journal of Animal Ecology 75: 247–256.

Dennis RLH (1996) Oviposition in *Zerynthia cretica* (Rebel, 1904): loading on leaves, shoots and plant patches (Lepidoptera, Papilionidae). Nota lepidopterologica 18: 3-15.

Dennis RLH (2010) A resource-based habitat view for conservation. Wiley-Blackwell, Oxford.

Fox R (2012) The decline of moths in Great Britain: a review of possible causes. Insect Conservation and Diversity 6: 5–19.

Garcia Barros E & Fartmann T (2009) Butterfly oviposition: sites, behaviour, modes. In: Ecology of Butterflies in Europe, J. Settele, T. Shreeve, M. Konvicka & H. Van Dyck Eds. Cambridge University Press.

Hoyle M, James M (2005) Global warming, human population pressure, and viability of the world's smallest butterfly. Conservation Biology 19: 1113–1124.

Janz N (2002) Evolutionary ecology of oviposition strategies. In M. Hilker, & T. Meiners (eds.), Chemoecology of insect eggs and egg deposition.

Jordano D, Gomariz G (1994) Variation in phenology and nutritional quality between host plants and its effect on larval performance in a specialist butterfly, *Zerynthia rumina*. Entomologia Experimentalis et Applicata 71: 271 - 277.

Lasanta T, Arnáez J, Oserín M, & Ortigosa LM (2001). Marginal lands and erosion in terraced fields in the Mediterranean mountains: a case study in the Camero Viejo (Northwestern Iberian System, Spain). Mountain Research and Development 21: 69-76.

Krämer B, Kämpf I, Enderle J, Poniatowski D & Fartmann T (2012) Microhabitat selection in a grassland butterfly: a trade-off between microclimate and food availability. Journal of Insect Conservation 16: 857- 865.

Kudrna O, Harpke A, Lux K, Pennerstorfer J, Schweiger O, Settele J, Wiemers M (2011) Distribution Atlas of butterflies in the Europe. – Gesellschaft fur Schmetterlingschutz, Halle, Germany. 576 pp.

Küer A & Fartmann T (2005) Prominent shoots are preferred: microhabitat preferences of *Maculinea alcon* (Denis & Schiffermüller 1775) in Northern Germany (Lycaenidae). Nota Lepidopterologica 27: 309-319.

Maes D, Vanreusel W, Talloen W, Van Dyck H (2004) Functional conservation units for the endangered Alcon Blue butterfly *Maculinea alcon* in Belgium (Lepidoptera: Lycaenidae). Biological Conservation 120: 229-241.

Maes D & van Dyck H (2001) Butterfly diversity loss in Flandres (north Belgium): Europes worst case scenario? Biological Conservation 99: 263–276.

Maes D, Collins S, Munguira ML, Šasic M, Settele J, van Swaay C, Verovnik R, Warren M., Wiemers M, Wynhoff I (2013) Not the right time to amend the Annexes of the European Habitats Directive. Conservation Letters 6: 468-469.

McKay HV (1991) Egg-laying requirements of woodland butterflies; brimstones (*Gonepteryx rhamni)* and alder buckthorn (*Frangula alnus).* Journal of Applied Ecology 28:731-74.

New TR (1997) Are Lepidoptera an effective 'umbrella group' for biodiversity conservation? Journal of Insect Conservation 1: 5–12.

Pinto CF, Urzúa A, Niemeyer HM (2011) Sequestration of aristolochic acids from meridic diets by larvae of *Battus polydamas archidamas* (papilionidae: troidini). European Journal of Entomology 108*:* 41-45.

Porter K (1992) Eggs and egg-laying. In: The Ecology of Butterflies in Britain. Oxford University Press, Dennis R.L.H. (ed.), Oxford, pp. 46–72.

R Development Core Team (2006) R: a Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0, URL http://www.R-project.org.

Rauscher M (1983) Alteration of oviposition behavior by *Battus philenor* butterflies in response to variation in host-plant density. Ecology 64:1028-1034.

Samways MJ (2007) Insect Conservation: a synthetic management approach. Annual Review of Entomology 52: 465–87.

Schwarz CJ & Arnason AN (1996) A general methodology for the analysis of capture-recapture experiments in open populations. Biometrics 53: 860-873.

Schwarz CJ & Seber GA (1999) Estimating animal abundance: review III. Statistical Science 14: 427-456.

Thomas CD, Cameron A, Green RE, Bakkenes M, Beaumont LJ, Collingham YC, Erasmus BFN, Ferreira de Siqueira M, Grainger A, Hannah L, Hughes L, Huntley B, van Jaarsveld AS, Midgley GF, Miles L, Ortega-Huerta MA, Townsend Peterson A, Phillips OL, Williams SE (2004) Extinction risk from climate change. Nature 427: 145-148.

Thomas CD, Thomas JA, Warren MS (1992) Distributions of occupied and vacant butterfly habitats in fragmented landscapes. Oecologia 92: 563-567.

Van Swaay CAM, Cuttelod A, Collins S, Maes D, Munguira Lopez M, Šasic M, Settele J, Verovnik R, Verstrael T, Warren M, Wiemers M, Wynhof I (2010) European Red List of Butterfies. Publications Office of the European Union, Luxembourg, 46 pp.

Verity R (1947) Le Farfalle Diurne D' Italia 3. Divisione Papilonida. Famiglie Papilionidae e Pieridae. Marzocco, Firenze.

White GC & Burnham KP 1999 (1999). Program MARK: survival estimation from populations of marked animals. Bird study, 46: 120-S139.

Zalucki MP, Clarke AR, Malcolm SB (2002) Ecology and behaviour of first instar larval Lepidoptera. Annual Review of Entomology 47: 361-393.

Zinetti F, Dapporto L, Vovlas A, Chelazzi G, Bonelli S, Balletto E, Ciofi C (2013) When the rule becomes the exception. No evidence of gene flow between two *Zerynthia* cryptic butterflies suggests the emergence of a new model group. PLoS One 8(6):e65746.

Zuur A, Ieno EN, Walker N, Saveliev AA., Smith GM (2009) Mixed effects models and extensions in ecology with R. New York: Springer.

Captions

Figure 1. Location of our study sites. Different habitat types are represented with

dots: white for woodland edge, black for riparian grassland, gray for dry grassland.

Figure 2. Schematic map of the population of Z. *cassandra* investigated at Conversano S-E Italy. Areas in grey represent the investigated (a-g) habitat patches.

Figure 3. Number of available food plants and number of eggs observed in the three habitat types.

Figure 4. Polarplot of the geographical orientation (in %) of *A. rotunda* plants, either unoccupied (black line, N = 189), or occupied (grey line, N = 89) by *Zerynthia cassandra* eggs.

Figure 5. Prominence of unoccupied and occupied plants. Prominence was calculated as the difference between the host plant's and the turf's height. The dotted line indicates turf height.

Figure 6. Distribution of plant and oviposition height in NW Italy.

Figure 7. Differences in height between plants receiving or not receiving eggs, vs plant height.

Table 1. Absolute (N) and relative (%) frequencies of landscape and patch parameters of foodplants (Aristolochia *rotunda),* either occupied, or unoccupied by *Zerynthia cassandra* eggs in NW Italy. Likelihood ratio statistics (LR) are shown for comparisons of absolute frequencies between occupied and unoccupied plants (a-d).

Table 2. GLMM statistic: relationship between probability of occurrence (binomial response variable: presence [N = 89 occupied plants] or of absence [N = 186 unoccupied plants] of *A. rotunda,* in relation to environmental parameters (predictor variables: host plant height, height of surrounding vegetation, distance from the nearest tree and vegetation cover).

Table 3. Summary of the MRR data.