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**Evaluation of agro-pastoral activities on insect  
biodiversity and conservation in northern Italy rangelands**

Ph.D. Dissertation

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# 1. INTRODUCTION

## *Evolution of agro-pastoral activities and land use in the Alps*

The origins of pastoralism in the Italian Alps are placed around the IV millennium BC, however, at local scale, pastoral activities started also some centuries later depending on the different mountainous contexts, while agriculture was already practiced in the middle mountains. In Piedmont region, starting from the V millennium BC, we had the first forest managements with the fire and the use of the high ground caves along tracks that conducted to mountain pastures as livestock shelters (Maggi 2004). From the Bronze Age the phenomenon has intensified, even at highest altitude, as confirmed by pollen analyses of core drilling in mountain peat bogs (Della Casa 2001).

We can estimate that for over 6000 years humans shaped and modified the mountain environment for and with the help of herds. The pastures are in fact the result both of logging works implemented by man to recover forage resources and the direct pressure of grazing by browsing actions, trampling and organic fertilization (Gusmeroli et al. 2005).

Throughout the 20th century the strong human population decline in the Italian Alps was linked to the collapse of mountain socio-economy (Pasakarmis and Maliene 2009, García-Ruiz and Lana-Renault 2011, Lasanta et al. 2015), associated with the development of industrial economy in lowlands and valley bottoms. We see a decline in traditional agro-pastoral systems also in the French and in some areas of the Swiss Alps (Mottet et al. 2006, Gellrich et al. 2007, Fassio et al. 2014). Furthermore, the negative image of the hard work and lifestyle in mountain areas, has certainly contributed to the escape from mountains and valleys of young generations, as well as preventing their potential re-colonization (Fassio et al. 2014). Moreover, the costs of agriculture and the low potential yield of mountain lands, have made economically uncompetitive the alpine areas respect to lowland areas (MacDonald et al. 2000, Gellrich et al. 2007). Because of that, family groups moved towards the valley bottoms, with a progressive abandonment of traditional agro-pastoral practices, that when it includes a complete elimination of grazing, determines a natural evolution of grasslands into forests (MacDonald et al. 2000).

As highlighted by the study of Fassio and colleagues (2014) conducted in the North-West Alps, in some valleys of Piedmont region in particular, between 1914 and 2013, the families who used the Alps for pastoralism were subjected to a strong decline, dropping from 210 to

18 between 1914 and 2013. The decline concerned also the livestock, with the loss of almost 50% of reared animals. In particular, we observed a noticeable reduction in herds of goats and flocks of sheep, which were the first animals of mountain pastoralism (Marzatico 2007).

The mountain land abandonment is not a linear phenomenon that affects in the same way all the areas, because it depends on local topography, type of ecosystem and climatic conditions (Targetti et al. 2010). Nevertheless, it is now a widespread phenomenon in all mountains across the Europe (Tesser et al. 2007, Lasanta et al. 2015, Zethof et al. 2016). The results of some studies revealed that there are two pattern of abandonment: one at medium altitude and another in the higher mountain areas. Despite intuitively the traditional human activities in high mountain areas are too hard to be preserved, Hinojosa et al. (2016) show that the strongest abandonment occurs in the middle mountains, and they called this phenomenon "mountain-effect". In this work, they suggest that grassland abandonment is caused by a combination of both local and regional/global factors. They demonstrated the positive effect in preserving grassland of transhumance practices and the beneficial effect of land use regulation issued by national parks, mainly concerning high mountain areas. Conversely, at medium altitude villages were progressively depopulated due to the attractiveness of comforts of near urban centres that allure the rural people leaving strenuous lifestyle of mountain rural areas. This result in a desertion of villages, which become groups of second homes used only for vacations, and a consequent abandonment of meadows and pastures management, with a negative effect on grassland conservation.

Indeed, associated with the abandonment of the most inaccessible or less profitable pastures, an over-exploitation of the remaining land frequently occurs (MacDonald et al. 2000, Tasser & Tappeiner 2002, Mottet et al. 2006). Cattle grazing, for example, has an important effect on the structure and composition of the vegetation in grasslands (Olf & Ritchie 1998). Morris (2000) has classified the three main effects of grazing animal upon the sward: defoliation, treading and manuring. Overgrazing in grasslands determines manure accumulation and make a substrate more compact, which results in a grassy layer depletion and the nitrophilous species increase. In addition, the selection made by livestock grazing causes a progressive increase in the dominance of unpalatable species (Edwards & Crawley 1999, Bonato et al. 2005), reducing the floristic diversity and simplifying the vegetation structure. Instead, a mild pasture takes place directly on the dominant species favouring an increase of plant diversity, and limits the growth of grass encouraging the seed germination thank to more light availability (Olf & Ritchie 1998, Osem et al. 2002).

### *Land use and climate change*

The climate change is the second main driving force that affects biodiversity structure and community of present-day, but in the coming decades, is expected to become at least equally important to land use changes (Bennet and Saunders 2010, Lovejoy 2010, Dawson et al. 2011). The interaction between climate change and habitat loss will deeply influence future extinction rates and the communities composition throughout the world, however, few studies integrated the two phenomena (Warren et al. 2001, Clavero et al. 2011). This is probably due to the difficulties in disentangling the pure or combined effect of the two processes. (Mantyka-Pringle et al. 2012, Oliver and Morecroft 2014). Indeed, some researches showed that land use and climate changes effects on species assemblages vary according to the different local habitats, and thus, the responses are often difficult to extend to the whole population (Kampichler et al. 2012).

Certainly the effect of climate change can be seen more clearly in environments characterized by extreme temperatures, such as mountain areas (Brunetti et al. 2009, Acquavotta et al. 2014). But in these environments, as I described in a previous paragraphs, the recent changes in land use may have a confounding effect, thus, to understand direct impact of global warming is very difficult. According to a recent study on timberline made by Feurdean and colleagues (2016), the tree line will further shift upwards stimulated by climate warming but according to different pattern of changes according to the different local land use dynamics.

Anyway, there are some studies that reported the pure effect of climate change on assemblage and species range distribution. Parmesan and Yole (2003) demonstrated that climate warming directly affect the shifts of species' spring phenology, latitudinal distributions and abundances, whereas other studies showed the climate warming effect on the increase in species richness and on the range expansion of common and generalist species (Thomas 2010) which often replaced more specialized species. Gaüzère et al. (2015) suggested that variations in temperature seems to be responsible of functional community homogenization while Taylor et al. (2016) found that a noticeable temperature increase in North Sweden, favoured the colonization of new sites by warm-dwelling at the expenses of cold-dwelling species. Moreover, the low detectability of rare and vulnerable species increase their threaten status because of the difficulties in detecting climate impacts on them.

A long-term study (about 200 year) on butterfly communities in Germany, pointed out three frequent phenomena under the climate change: loss in the species number, changes in beta-diversity and a gradual transformation from a species assemblage hosting many habitat

specialist species to a community dominated by few habitat generalist species (Habel et al. 2016). These negative trends were probably due to a combination of large-global and small-scale effects, such as climate change and land use intensification.

Thus, long-term studies about temperature variation and community responses could be a central topic to understand how climate changes affect biodiversity.

### ***Common Agricultural Policy (CAP)***

Several international institutions (UNESCO, The European Landscape Convention, the Institute for European Environmental Policy, IEEP, the European Union through the CAP, etc.) have applied measures to conserve traditional landscapes. The aim is the harmonious integration of environmental, social and economic factors in space and time (Kizos et al., 2010; Agnoletti, 2014) for a sustainable development.

The European Agricultural Fund for Rural Development (EAFRD) represents about 23% of total budget that the European Union allocates to the Common Agricultural Policy for the period 2007-2013. Only 2.56% (447.000.0000 euros) of these resources was directly dedicated for the conservation of agro-ecosystem biodiversity.

In Italy, the CAP policy is based on two main pillars: on the one hand, the system of direct payments and market regulations, by the other side, supporting measures for rural development. The purpose of rural development measures is to maintain the vitality of the countryside through investment programs, modernization and support for the activities in rural areas. Among the most interesting measures, we could find the interventions for balancing territorial development through specific payments dedicated to less favoured areas, young farmers/herders and small farms. Recently, the CAP policies increased funding in eco-sustainable actions. Due to the rising problems of land abandonment and overexploitation, policy measures like CAP, are even more important, in order to mitigate these processes and to support traditional agriculture, pastoralism and mountain society.

The RDP measures are good tools to sustain mountain rural activities but reports about the effectiveness of these actions are scarce. Thus, it is impossible to assess the quality of the measures and to improve the successive CAP.

Indeed, agricultural policies have not always positive effects on territories. García-Ruiz and Lana-Renault (2011 ) for the western European countries, highlighted that at the end of the 20th century (between 1988 and 2008), CAP supporting *set-aside* land retirements favoured

the land abandonment paying farmers that stopped cultivating at least the 20% of their cropland to avoid surplus production.

For these reasons, it is crucial to monitor the effects of RDP actions using specific indicators, in order to drive efficiently the future agricultural policies.

### ***Mountain grassland biodiversity and bio-indicators***

The land abandonment and the forest re-colonization could also have a positive impact on the environment. For example, the soil stabilization (Tasser et al. 2003), the natural re-growing of ancient forest formations and the initially increase of biodiversity (Skorka et al. 2007; Marini et al. 2009a), probably due to a higher environmental heterogeneity. Anyway, this is a short-temporary process. Indeed, on a long-time scale, the forest regrowth leads to a reduction of the species richness and diversity with a dominance of forest "shadow-lover" species.

Changes in agro-pastoral practices are now causing the fragmentation of most arthropod communities (Benton et al. 2003, Tschanrtke et al 2005, Stefanescu et al.2009; Molina et al. 2014). For example, the loss of suitable habitats caused by changes in land abandonment, with the consequent invasion of shrubs and trees, is one of the main threat for several butterfly species (van Swaay et al. 2012). Another adverse factor is the irreversible loss of *cultivar* and traditional farming methods, mostly transmitted from father to son within households (Fassio et al. 2014) representing a perfect symbiosis between man and nature.

The European Alps are characterised by a great diversity of habitats and species thank to a natural gradient of temperature, landscape heterogeneity, topography and climate (Fischer et al. 2008). Also human land use shaped biodiversity, especially in the grassland habitats below the treeline. In this changing environment, several animal groups benefit from these human activities, evolving and adapting themselves to new habitats. However, nowadays they are threaten by changes in land use caused by socio-economic transformations. Many previous cited studies have confirmed the importance of traditional pastoral and farming practices in preserving the diversity both of vertebrate and invertebrate taxa. In particular, many invertebrate groups have been supported by these human agro-pastoral activities. Dung beetles, stimulated by the presence of grazing livestock, butterflies, grasshoppers, and true bugs, affected by the structure and the composition of grassland vegetation (Marini et al.2009; van Swaay et al. 2010; Korosi et al. 2012, Tocco et al. 2012). Some vertebrates, such as birds

or bats, were favoured by the maintenance of meadows and by the livestock presence too (Laiolo et al. 2004, Ambrosini et al. 2011, Obrist et al. 2011).

Several studies have shown that some insect groups are good indicators of environmental status because they respond readily to changes both at landscape and locale scale (Viterbi et al. 2013, Pateman et al. 2016). The strict ecological relationship of insects to the habitats where they occur, such as alpine grasslands, makes them excellent bio-indicators that can be used to assess habitat alterations. Frequently in mountain environments, such as high-altitude pastures, we found animal species adapted to extreme climates and oligotrophic soils, that are often rare or localized, glacial relicts or endemic.

Concluding, it is fundamental to assess the ecological requirements of the taxa chosen as bio-indicators to understand how they could respond to the adverse environmental and climatic factors and thus preserve them and their habitats with *ad hoc* management plans.



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## 2. RESEARCH AIMS

European mountains have a long history of human presence and exploitation: during the last centuries, agro-pastoral activities determined a lowering of the treeline ecotone and shaped community composition of plants and animals, creating peculiar semi-natural habitats, on which high levels of biodiversity depend on. Currently, two contrasting phenomena are occurring in the rural areas: the abandonment of traditional farming systems in most of the areas and the overexploitation of some remaining pastures.

We identified two different approaches to study these events by using insects as bio-indicators. The first one is a multi-taxonomic approach, aiming to assess the effects of grazing management at local scale, by investigating the community composition and structure of four macro-invertebrates groups (Coleoptera Carabidae, Coleoptera Scarabeidae, Lepidoptera ropalocera, Orthoptera). The second approach is single species approach, aiming to study the population dynamics, habitat preferences and dispersal ability of a target butterfly, *Euphydryas aurinia* (included in Annex II of Habitats Directive), in two areas of different biogeographical regions, inside and outside the Alps, to define specific management plans designed for the species conservation.

### **3. SINGLE SPECIES APPROACH**

#### **3.1 Dispersal and connectivity effects at different altitudes in the *Euphydryas aurinia* complex**

Casacci L.P., Cerrato C., Barbero F., Bosso L., Ghidotti S., Paveto M., Pesce M., Plazio E., Panizza G., Balletto E., Viterbi R., Bonelli S.

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#### **INTRODUCTION**

Populations of the *Euphydryas aurinia* complex range from all across Europe and temperate Asia to as far East as Yakutia. Because of this very broad range, *E. aurinia* is not at risk of becoming extinct (Least Concern, IUCN 2013 regional assessment - van Swaay et al. 2010), but this taxon is known for having declined in most European countries, and is reported as extinct in the Netherlands. Declines of over 30% in range or population size have been reported from Germany, Latvia, Luxembourg, the Republic of Ireland, Slovakia and Ukraine while smaller declines of 6-30% are known for having occurred in fifteen other European countries (Asher et al. 2001). *E. aurinia* is, accordingly, listed in the Annex II of the Habitats Directive (HD).

In butterflies, the study of the flight behaviour and use of the environmental matrix is crucial for management planning, particularly in more or less fragmented landscapes, and butterflies are currently among the most well-known model organisms for dispersal studies (Stevens et al. 2010a, b).

Dispersal abilities are assumed to be fixed traits in most metapopulation studies and especially by the classic formulation of this theory (Travis and French 2000; Goodwin 2003; Bowler and Benton 2005), where individual movements between populations are modelled as a function of these fixed dispersal traits, on the one hand, and of extremely variable spatial structures, on the other.

The configuration of habitat patches can also positively or negatively act as a selective pressure on dispersal abilities. Habitat fragmentation, for instance, is known to affect dispersal within systems of populations, by reducing both the emigration rate as well as the

length of movements (Schtickzelle et al. 2006). Various studies have pointed out differences among species and populations, as well as between sexes (e.g. Fric et al. 2010; Stevens et al. 2010b; Bonelli et al. 2013). Other differences are known to occur in butterflies' propensity to move, as well as in more general flight patterns.

*E. aurinia* is a complex of generally allopatric taxa characterised by more or less distinct external morphologies and strongly different ecological requirements. As is the case for American *E. editha*, many subspecies have been described (see Catalogue of life, under *Eurodryas*), but at the moment no genetic evidence is available to validate them (e.g. Zimmermann et al. 1999, 2000; Descimon and Mallett 2009; Sinama et al. 2011; Mikheyev et al. 2013) and few studies are currently under way at European level to investigate these differences. As concerns Italy, we have at least three Evolutionarily (and Ecologically) Significant Units (ESUs, see Casacci et al. 2013) which occupy all three of the Italian biogeographical regions (Balletto et al 2007; Balletto et al. 2014b) and have been listed as separate species in the Check List of the Italian butterflies (Balletto and Cassulo 1995; Balletto et al. 2014a). More in detail, *E. (a.) aurinia* (Rottemburg, 1775) occurs in the wet meadows in the Po Plains, within the 'Continental Region', *E. (a.) glaciegenita* (Verity, 1928) is restricted to the 'Alpine Region' around and above 2000 meters, while *E. (a.) provincialis* (Boisduval, 1828) typically occurs in the dry calcareous grasslands and maquis of the 'Mediterranean Region' (Fig. 1).

According to the most recent assessment (under HD Art. 17) the conservation status of the continental *E. aurinia* populations is as "Bad" as in central and northern Europe, since suitable habitat has become strongly reduced and fragmented. In Italy, at least 12 populations have become extinct because of habitat destruction (Bonelli et al. 2011). On the contrary, the conservation status of the Mediterranean and Alpine populations is "Favourable" and we have no records of any population having become lost.

In this work we focused on two of the three Italian taxa, i.e the Alpine and the Mediterranean populations, since they are the only ones which may potentially guarantee the long-term survival of the *E. aurinia* complex in Italy. Both are threatened by land abandonment and/or land use intensification, two threats which can still be reversed if and when more appropriate forms of land use are implemented.

At a local level, recent conservation schemes, such as the "Environmental Sensitive Areas Agro-environmental Schemes, Rural Development Practices" promoted by the EU agricultural policy, have become crucial for the long term conservation of grassland butterflies. Working within this protocol, farmers are expected to adopt environmentally



friendly agricultural practices, such as low intensity grazing regimes. Conservationists, however, will be able to advise on how these opportunities may be translated into practice only after we will be able to understand how distances are perceived by individual butterflies, as well as how barriers are identified, and how all this affects adults' movements within the environmental matrix. In other words, we need to understand the effect of fragmentation and connectivity on movements within the patch systems.

*In general* we wish to contribute to investigations into whether in a metapopulation system emigration propensity is species-specific or may be variable depending on different environmental pressures.

In synthesis, we focused our work on adult dispersal, in order to obtain crucial information on butterflies' behaviour, in the expectation that our results could be directly converted into conservation practice, at various scales.

## METHODS

### *Study system*

The three Italian taxa of the *E. aurinia* complex have similar life cycles and all are single brooded. Females lay eggs in large batches, while larvae are gregarious and overwinter in smaller or larger webs, close to the ground. *E. (a.) aurinia*'s food plant is *Succisa pratensis*, that of *E. (a.) provincialis* (Mediterranean) is either *Cephalaria leucantha* or *Knautia arvensis* (all three are Dipsacaceae), while larvae of *E. (a.) glaciegenita* (Alpine) feed on *Gentiana acaulis* (Gentianaceae). We will only mention for completeness that Spanish *E. (a.) beckeri* (Herrich-Schäffer, [1851]) feeds on shrubs or bushes of yet another family such as *Lonicera implexa* (Loniceraceae see Singer and Wee 2005; Peñuelas et al. 2006; Stefanescu et al. 2006), a plant that is never consumed by Italian populations, even though it is generally abundant in many habitats where *E. (a.) provincialis* is common. Matters, however, are not always as clear-cut, since for instance in Lithuania some populations of *E. (a.) aurinia* live on *G. cruciata* (Švitra and Sielzeniev 2010), while in Belgium larvae feed on *S. pratensis*, in the wet meadows, whereas on chalky grasslands they exploit other food plants, such as *K. arvensis* or *Scabiosa columbaria* (Schtickzelle et al. 2005).

Interestingly enough, each group of the Italian *E. aurinia* complex is also locally linked to a specific habitat included in the Annex I of the Habitats Directive, i.e. *E. (a.) aurinia* to Habitat 6410, *Molinia* meadows on calcareous, peaty or clayey-silt-laden soils - *Molinion caeruleae*; *E. (a.) provincialis* to Habitat 6210, Semi-natural dry grasslands and scrubland

facies on calcareous substrates - *Festuco-Brometalia*; *E. (a.) glaciegenita* mainly to Habitat 4060, Alpine and Boreal heaths. In particular, the first two taxa are good candidates for becoming “typical species” (according to the definition of Habitats Directive).

### *Study sites*

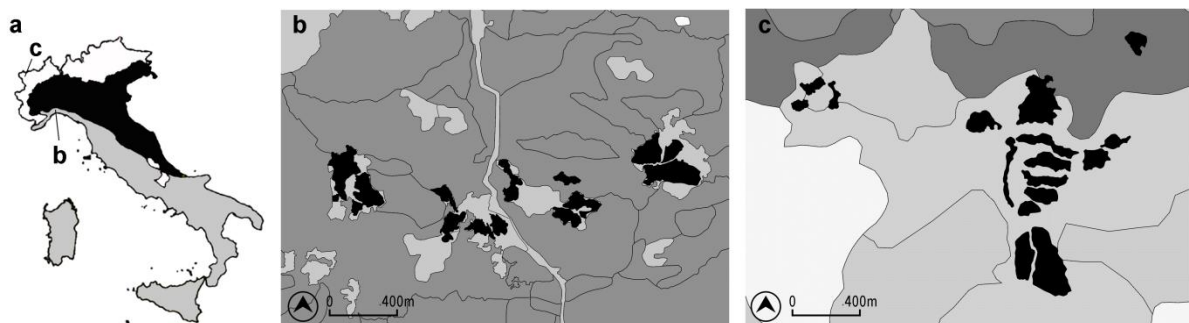
During 2013, two populations of *E. aurinia*, one from the Alps and one located in the Mediterranean biogeographical region, in the northern Apennine (Fig. 1 and Table 1), were studied with a shared protocol. Both populations were relatively numerous and inhabited large semi-natural grasslands in fairly good ecological conditions. The two areas are protected within the NATURA 2000 network and the two Parks where they occur (see below) are actively involved in ensuring the long-term conservation of open habitats within the framework of the Rural Development Programme (Measure 323 - Conservation and upgrading of the rural heritage).

The *Mediterranean* population is located within the "Capanne di Marcarolo" Natural Park, in the northern (Ligurian) Apennines, at around 700 m in altitude. It occurs in a fragmented semi-natural grassland (Habitat 6210 - HD Annex I), of the so-called *Arrhenatherion elatioris* and *Brometalia erecti* type, where vegetation is dominated by a mixture of mesophilous and xerophilous plants, such as *Knautia arvensis* (*E. a. provincialis*'s food plant), *Poa pratensis*, *Sanguisorba officinalis*, *Achillea millefolium*, *Silene vulgaris*, *Trisetum flavescens*, *Daucus carota*, *Leucanthemum vulgare*, *Lolium perenne*, *Lotus corniculatus*, *Taraxacum officinale* and *Centaurea nigrescens*. In this area, the vegetation is particularly rich because of the geographical position, climate and geology, which results in a mixture of Mediterranean and Alpine species. Most of the meadows occur on south facing slopes and are surrounded by forest and shrubs, while northern slopes are covered in woods or sparse trees. The site is partially fragmented because of the abandonment of traditional hay cutting and is locally invaded by shrubs and small trees.

The *Alpine* population lies in the Cogne Valley (Bardoney area, ca. 2200 m), within the Gran Paradiso National Park, in the north-western Italian Alps. The population occurs above the timberline in an open and apparently continuous Alpine grassland. At its lower altitudinal boundary the site is limited by a coniferous woodland (mainly with *Larix decidua* and *Pinus cembra*), and is irregularly fragmented by rocks and screes, as well as by Alpine heaths (Habitat 4060 - HD Annex I). This Alpine grassland is characterised by a mixture of mesophilous, meso-xerophilous, and hygrophilous habitats. In particular, the central part of

the site is composed by wet meadows and buried marshes growing on acidic soils. The larval food plant, *Gentiana acaulis*, is widespread and abundant, mainly at the border between the meso-xerophilous and the more hygrophilous habitats. The area is currently managed by extensive grazing, which maintains floristic heterogeneity and diversity.

For both sites, potential habitat patches around the areas where butterflies were known to occur were mapped on the basis of a rough vegetational survey and of aerial pictures. Patches were defined as distinct when separated by obvious barriers (e.g. woods, roads), or when land cover features changed consistently. The patch configuration was confirmed at the end of the study (Hanski et al. 2000) by superimposing the GPS fixes of captures on the initially selected patches (see *sampling* section for details). Information on the investigated populations is summarised in Table 1.



**Fig. 1.** a) Distribution of the three *Euphydryas aurinia* subspecies and location of the two study sites in Italy. White: *E. (a.) glaciegenita*; black: *E. (a.) aurinia*; grey: *E. (a.) provincialis*. b) Network of habitat patches for the Mediterranean (Marcarolo site) and c) the Alpine (Bardoney site) *E. aurinia* populations. Black areas: habitat patches; dark grey: bushes; grey: woodland; light grey: grassland; white: rock and screes.

**Table 1.** Characteristics of the investigated metapopulations of *E. aurinia*.

	<i>E. (a.) provincialis</i>	<i>E. (a.) glaciegenita</i>
Site	Marcarolo	Bardoney
Biogeographical Region	Mediterranean	Alpine
Altitude	650-750 m	2100-2300 m
Location (WGS84)	8°46'16" E - 44°33'49" N	7°25'42" E - 45°34'56" N
Protected area	"Capanne di Marcarolo" Natural Park	"Gran Paradiso" National Park
SCI <sup>a</sup> code	IT 1180026	IT1201000
Number of patches	15	16
Total area of patches (ha)	20.34	28.15
Habitat patch areas (min–max) (ha)	0.62–2.44	0.49–5.78
Habitat patch areas (mean–CV) (ha)	1.35–0.39	1.76–0.84
Inter-patch distances (min–max) (m)	105–1984	88–2014
Habitat type	Dry grassland	Alpine grassland
Marked individuals	1499 (509 ♀; 990 ♂)	1684 (532 ♀; 1151 ♂)
Recaptured individuals	764 (229 ♀; 535 ♂)	483 (108 ♀; 375 ♂)
Intra-patch recapture events	870 (252 ♀; 618 ♂)	523 (108 ♀; 415 ♂)
Inter-patches recapture events	571 (132 ♀; 439 ♂)	161 (20 ♀; 141 ♂)

<sup>a</sup> SCI: Site of Community Importance (EU Habitat Directive 92/43/EEC)

### ***Population Sampling***

We performed a mark-release-recapture (MRR) study, designed to analyse dispersal ability by Virtual Migration Model. Sampling of the Mediterranean population was carried out from 27 May to 22 June, 2013 while the study of the Alpine population was conducted from 14 July to 14 August, 2013 by following the same protocol. All patches were visited daily, weather permitting, and all observed butterflies were captured and permanently marked with an individual number on the ventral surface of the hind wings. For each (re)capture, we recorded the individual code, date, patch location and sex along with the GPS position. After marking, butterflies were immediately released at the location of their capture.

### ***The Virtual Migration Model***

We analysed MRR data by means of the virtual migration model (program VM2, see <http://www.helsinki.fi/science/metapop/>), to investigate the movement patterns of *E. aurinia* metapopulations and estimate their parameters of survival and migration. The Virtual Migration Model has been described in detail by Hanski et al. (2000), Petit et al. (2001), Wahlberg et al. (2002), and we only briefly summarize its main assumptions. The model assumes that individuals staying in habitat patches experience a certain dispersal-independent and constant mortality  $\mu_p$ . Emigration rate from a natal patch ( $\varepsilon_j$ ) depends on area ( $A_j$ ):

$$\varepsilon_j = \eta A_j^{\zeta_{em}}$$

where  $\eta$  defines emigration propensity (here expressed as daily emigration rate from a 1-ha patch), while  $\zeta_{em}$  represents the emigration scaling with patch area. Survival of dispersing individuals ( $\varphi_{mj}$ ) is a sigmoid function of their natal patch connectivity ( $S_j$ ):

$$\varphi_{mj} = \frac{S_j^2}{\lambda + S_j^2}$$

The square root of the scaling parameter  $\lambda$  represents the connectivity level up to which half of the dispersers successfully reach other patches. Patch connectivity is measured as:

$$S_j = \sum_{k \neq j} \exp(-\alpha d_{jk}) A_k^{\zeta_{im}}$$

with  $d_{jk}$  being the Euclidean distance between patches,  $A_k$  refers to the target patch area and finally  $\alpha$  and  $\zeta_{im}$  are respectively scaling distance-dependence of dispersal and immigration probability. Successful dispersers are distributed among target patches proportionally to their contributions to the natal patch connectivity. The VM model allows to estimate all six parameters ( $\mu_p$ ,  $\eta$ ,  $\zeta_{em}$ ,  $\lambda$ ,  $\alpha$ ,  $\zeta_{im}$ ) as well as their 95% confidence intervals. Parameter estimates

for the two populations were produced separately for each sex. We considered VM model parameter estimates to differ significantly if the confidence intervals of one parameter did not include the parameter estimate of the other one, and vice versa (as suggested by Wahlberg et al. 2002).

### ***Patch Quality***

The quality of each habitat patch was estimated at 86 randomly distributed 5x5 m quadrats, in the case of the Alpine site, and at 30 5x5 m quadrats for the Mediterranean site. For each quadrat we measured the number and height of food plants (either *Gentiana acaulis*, or *Knautia arvensis*) and the height of the surrounding vegetation. Using Braun-Blanquet's method (Braun-Blanquet 1932), we measured the percent cover of the vegetational and physical components of the environment, i.e. of nectar sources, graminaceous plants, grass litter or moss, rocks, bushes or larch trees, and bare soil. For each parameter, the mean values were calculated and used for further analysis.

To evaluate the effect of host plant and nectar plant density (taken as indicators of patch quality), as well as their influence on *E. aurinia* male and female density, on scaling of immigration and emigration, we replaced patch area with an “effective” area, by following the approach described by Rabasa et al. (2007). “Effective” areas were calculated by multiplying the area of each patch by the number of host or nectar plants, or by the number of female or male butterflies, and dividing this value by the median value of each parameter calculated on all the patches.

Factors affecting dispersal were also analysed by generalized linear models (GLM) with binomial response and logit link function, by the software R 2.15.0 (R Core Team 2012). The total fraction of individuals that moved to a “recipient” patch (immigrants) and the total fraction of individuals moving from a “source” patch (emigrants) were set as the dependent variables. The models were performed separately for males and females of both populations, to test for the effect of average male and female densities, food plant cover and height and their interaction, cover of the other vegetational components, patch area and connectivity ( $S_j$ ). We used the *dredge* function of the R package MuMIn (Barton 2013) to automatically construct all possible models based on the set of explanatory variables in the full model, including the null model, as well as to identify minimum-adequate models by using the Akaike Information Criterion ( $AIC_c$ ) for model evaluation (Burnham and Anderson 2004). Finally, only models with  $\Delta(AIC_c) < 2$  were retained.

## RESULTS

### *Population dynamics*

In total, 1684 individuals (532 females, 1151 males, 1 undetermined) of the Alpine population (hereafter ALP) and 1499 (509 females and 990 males) individuals of the Mediterranean population (hereafter MED) were marked. 483 (28.7%) butterflies were recaptured at least once (108 females, 20.3%, and 375 males, 32.6%) in the Alps, while from the Mediterranean population 764 (51.0%) butterflies were recaptured (229 females, 45.0%, and 535 males, 54.0%). The sex ratio was around 2:1 in favour of males in both populations (MED: 1.94 males per female; ALP: 2.16 males per female). In both populations we recaptured a higher proportion of males than of females (ALP:  $\chi^2=26.21$ ,  $df=1$ ,  $p<0.001$ ,  $n=1683$ ; MED:  $\chi^2=10.66$ ,  $df=1$ ,  $p=0.001$ ,  $n=1499$ ).

The capture probability estimated in the VM model was higher for the Mediterranean population ( $0.214\pm 0.001$  males;  $0.207\pm 0.003$  females) than for the Alpine one ( $0.125\pm 0.004$  males;  $0.084\pm 0.001$  females).

### *Dispersal behaviour*

Figure 2 shows the parameters' estimated values from the VM model and their 95% confidence intervals. The probability of daily mortality within a patch ( $\mu$ ) ranged from 0.079 to 0.161. The Mediterranean population had lower within-patch daily mortality (12% for females and 8% for males) than the Alpine population (16% for females and 14% for males) but estimates were significantly different only for males as confidence intervals did not overlap (Fig. 2a). This parameter allows to calculate the average life span for both sexes and both populations. In the Alpine population, lifespan values were similar for males (mean from VMM, 6.2 days; maximum from field data, 19 days) and females (mean, 5.7 days; max, 18 days). In the Mediterranean population, values were higher, in particular for males, with an average survival of 12.2 days (max, 23 days), while females survived on average 7.7 days (max, 20 days).

Adults' propensity to move was significantly different between populations, as indicated by the ( $\eta$ ) parameter (Fig. 2b). The Mediterranean population was more prone to disperse (around 21% for males and 18% for females), while butterflies of the Alpine population showed a reduced tendency to migrate. The males had half migration rate values per 1 hectare of habitat patch (10%) and females showed an even lower value of migration propensity (5%) with respect to the Mediterranean individuals.

The parameter  $\alpha$ , describing the effect of distance on daily movements, was similar for males and females, but varied between populations (Fig. 2c). Values obtained for the Alpine population ( $\alpha \approx 6.5$ ) were almost double of those of the Mediterranean population ( $\alpha \approx 3.5$ ). The average range of daily dispersal distances (calculated using the parameter  $\alpha$ ) were about 150 meters for the Alpine population and 270 m for the Mediterranean form. Male and female mortalities during dispersal of both populations were not found to be significant (confidence intervals overlap) (Fig. 2d).

### ***Movements in the patch networks***

Intra-patch movements distance was significantly different between populations and sexes (Kruskal-Wallis,  $\chi^2=9.118$ ,  $df=3$ ,  $p\text{-value}=0.028$ ) and its mean values ranged from 17 m for the Mediterranean females to 32 m for the Alpine males (Fig. 3a). Males and females did not differ in their daily intra-patch distances (MED: Mann-Whitney,  $W=889$ ,  $p\text{-value}=0.476$ ; ALP: Mann-Whitney,  $W=10375$ ,  $p\text{-value} = 0.119$ ). Distances travelled by males within the same patch were slightly but significantly higher for the Alpine population (MW,  $W=10048$ ,  $p\text{-value}=0.045$ ), but no difference was observed for females (MW,  $W=1536$ ,  $p\text{-value}=0.183$ ). Also inter-patch movements were significantly different between populations and sexes (KW,  $\chi^2=13.658$ ,  $df=3$ ,  $p\text{-value}=0.003$ ; Fig. 3). Males and females of the Mediterranean population moved similar distances when dispersing to another patch (MW,  $W=1157$ ,  $p\text{-value}=0.862$ ). Also in the Alps, movements were not significantly different between sexes (MW,  $W=22$ ,  $p\text{-value}=0.471$ ), but this may be due to the few events of inter-patch dispersal recorded for females. We found a significant difference between males of the two populations. Males of the Mediterranean population tended to move for longer distances than those of the Alpine one (ALP: 132.8 m; MED: 309.0 m; MW,  $W=316$ ,  $p\text{-value}=0.0005$ ) but the same was not true for females (ALP: 208.9 m; MED: 300.5 m; MW,  $W=15$ ,  $p\text{-value}=0.381$ ).

### ***Factors influencing dispersal***

As concerns patch area, scaling parameters for emigration ( $\zeta_{em}$ ) and immigration ( $\zeta_{im}$ ) differed between populations (Fig. 4). In the Mediterranean population they equalled zero for both sexes, thereby indicating that there was no effect of patch area on emigration and/or immigration (Fig. 4a, b).

In the Alpine system, on the contrary, patch area affected both emigration and immigration, although in the case of females, the observed values presented large confidence intervals (Fig 4c, d). The combination of negative effect of patch area on emigration (-0.40) and its positive

effect on immigration (0.80) indicates that large patches discourage emigration and promote immigration, in particular in the case of males (Fig. 4c, d).

When patch areas were modified to account for patch quality, calculated separately as cover in food plant and nectar sources, the emigration scaling coefficient estimated for females of the Mediterranean population differed from zero when area was corrected for nectar sources. Thus, a higher abundance of nectar sources can dampen the females' tendency to emigrate (Fig. 4a). On the opposite, it was the cover in food plants (gentians) that reduced females' emigration, in the Alpine population (Fig. 4c).

Interestingly, the immigration scaling coefficient had positive value when area was corrected for the number of specimens, both of the same and of opposite sex (Fig. 4b, d). This is true in all the cases, but in particular for males of the Mediterranean population, when area is corrected for female numbers ( $\zeta_{im}$  reached the highest value, 1.2 – Fig. 4b), thereby indicating that high female densities strongly promoted male immigration.

In Fig. 5, the daily probability of emigration was plotted against patch area. The values of daily probability of emigration were higher for the Mediterranean population and males were more likely to emigrate than females. In the Mediterranean population the probability of emigration was not affected by patch area in any of the two sexes. On the contrary, in the Alpine system, males and females were more likely to emigrate from small patches, while migration probability decreased in the case of larger patches. Comparing patches of similar size, however, the daily emigration rate was around 10% for the Alpine males and 20% for the Mediterranean ones (patch area  $\approx 0.5$  ha). In the largest patch of the Alpine system (5-6 ha), the daily emigration rate went down to as far as 5.6% for males and to 2.6% in females.

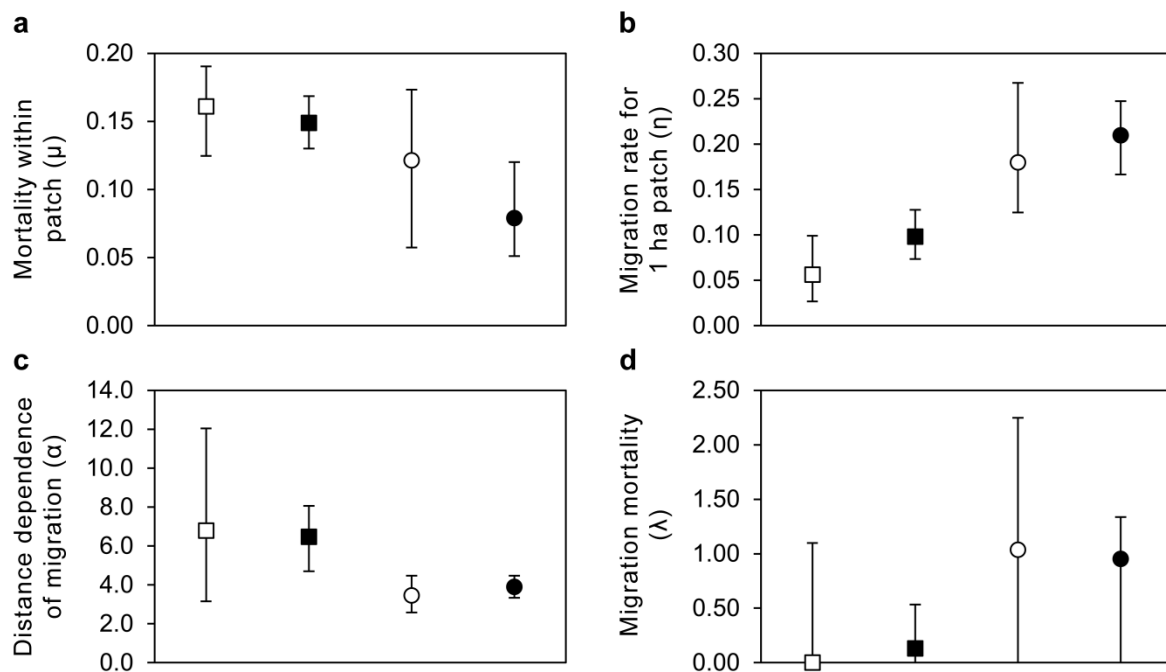
We obtained from 1 to 3 appropriate models ( $\Delta AIC < 2$ ; Table 2) for males of both populations. In the case of Mediterranean females, however, the fraction of immigrant and emigrant individuals was too low to allow for any statistical comparison. In both systems the fraction of immigrating males was positively affected by the density of males in the “recipient” patch, but it was also influenced by the patch area in the Mediterranean system, and by the grass height in the Alps. Female density was a significant predictor of emigrating males in both populations, indicating that low numbers of females at the “source” patch encourage males to disperse. Patch connectivity also entered the best appropriate models, suggesting that males tend to disperse more successfully from “source” to “recipient” patches having high connectivity values (Table 2).

Connectivity ( $S_j$ ), together with patch area, also influences female migration in the Mediterranean system, although parameter estimates were not significant. The fraction of

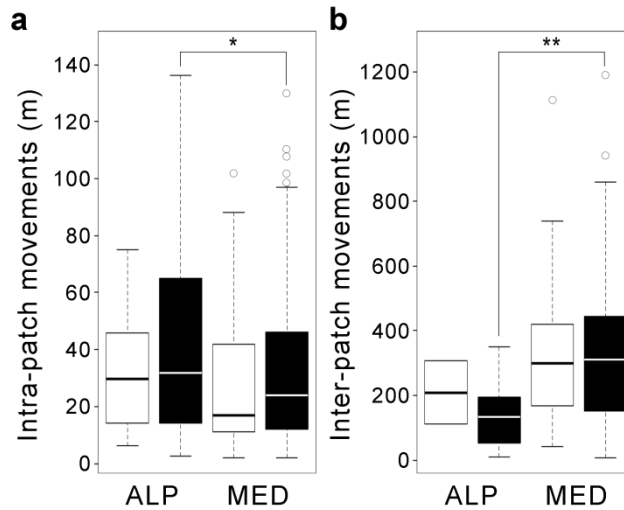


immigrating females was positively affected by the density of nectar sources and by the grass height at the “recipient” patches (Table 2).

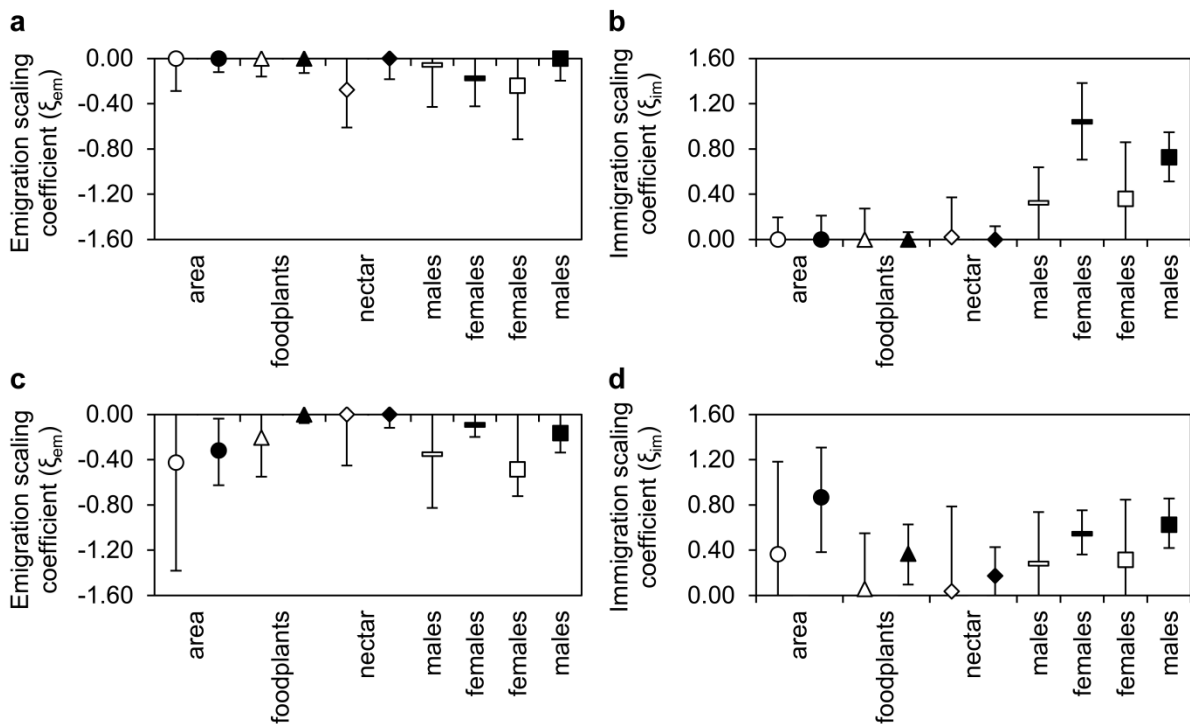
Estimated daily mortalities during migration are shown as a function of connectivity ( $S_j$ ) in Fig. 6, for both populations. For the Alpine system, patch connectivity ranged from 1.18 to -2.88 and the estimated daily mortality rate during migration was less than 10%. In the case of the most isolated patch (connectivity value  $< -2$ ), however, migration mortality drastically increased beyond 60%. This pattern was evident for males, but not for females, whose migration mortality values were equal to zero across the whole patch system, probably because of too few recapture data. When grouping together 3 habitat patches where the lowest number of adult females was recaptured, the values of migration mortality differed from zero (dotted squares and curves – Fig. 6). Although the connectivity values for the Mediterranean patch system ranged from 0.21 to 0.98, the slope of the curve describing the relationship between migration mortality and connectivity was steeper than the one observed for the Alpine system, for both males and females. At this connectivity interval ( $0 < S_j < 1$ ) the Mediterranean population showed estimated daily mortality ranging from 13% to 35% while the estimates for the Alpine system hardly exceeded 1%.



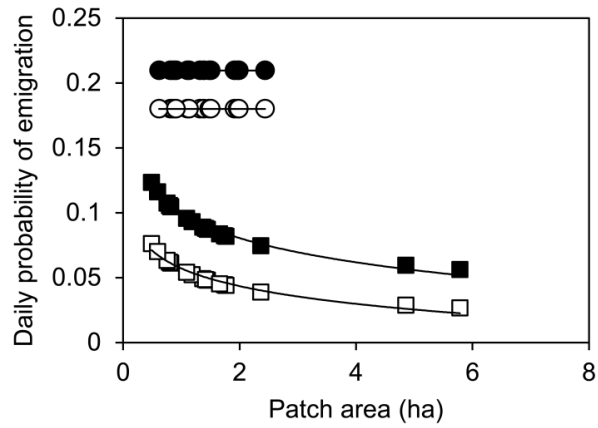
**Fig. 2.** Parameters estimated by Virtual Migration Model plotted with their confidence intervals (95%) for the Alpine (squares) and the Mediterranean (circles) populations of *E. aurinia*. Values were calculated separately for females (white) and males (black). Bars represent 95% confidence intervals. a) Mortality within patch ( $\mu$ ). b) Migration rate for 1 hectare patch ( $\eta$ ). c) Distance dependence of migration ( $\alpha$ ). d) Migration mortality ( $\lambda$ ).



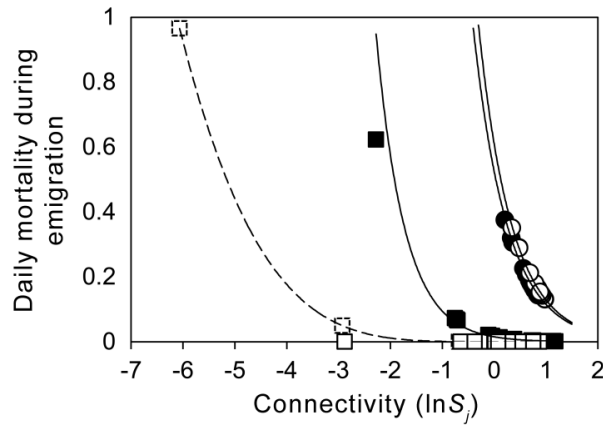
**Fig. 3.** Distance of daily movements within (a) and between (b) patches recorded in the Alpine (ALP) and Mediterranean (MED) populations, calculated separately for females (white) and males (black). The boxes show the median, first and third quartile, the whiskers the minimum and maximum values. Outliers are plotted as open circles. Comparisons were significant only for males. \* $p < 0.05$  \*\* $p < 0.01$ .



**Fig. 4.** Virtual Migration Model estimated values of Emigration and Immigration scaling coefficients and their confidence limits (95%) calculated on the basis of real area (area), and on “effective” areas. The latter was calculated by considering either the cover of i) food plant or ii) nectar sources, and the number of specimens of either iii) the same sex or the iv) opposite sex. Each coefficient was estimated separately for females (white) and for males (black). a), b) show data for the Mediterranean population; c), d) for the Alpine population.



**Fig. 5.** Daily probability of emigration from a given patch, based on the estimates of provided by VM model for females (white) and males (black) of the Alpine (squares) and Mediterranean (circles) population of *E. aurinia*.



**Fig. 6.** Relationship between daily mortality during emigration and connectivity, based on the estimated values of parameters generated by VM model, for females (white) and males (black) of the Alpine (squares) and Mediterranean (circles) populations of *E. aurinia*. Dotted curve and squares were obtained for females of the Alpine population, grouping together 3 habitat patches where few recaptures of adult females occurred.

**Table 2.** Best generalized linear models, explaining the fraction of immigrating and emigrating males and females in our two populations.

	Sex	Model number	Intercept	N of individuals		Patch features		Vegetation			AIC <sub>c</sub>	
				Males	Females	Area (m <sup>2</sup> )	Connectivity (S <sub>j</sub> )	Nectar sources (%)	Grass cover (%)	Grass height (cm)		
<i>E. a. provincialis</i>												
	Fraction of Immigrants	♂	1	-11.820 ± 1.649**	0.646 ± 0.090**	0.600 ± 0.160**					98.6	
	Fraction of Emigrants	♂	1	-0.782 ± 0.478			0.861 ± 0.392*				89.2	
			2	-1.530 ± 0.250**			0.558 ± 0.345				89.4	
			3	-0.691 ± 0.475		-0.114 ± 0.120					91.2	
	Fraction of Immigrants	♀	1	-19.227 ± 5.052**					0.488 ± 0.217*		3.954 ± 1.344**	70.3
			2	-15.430 ± 4.890**							3.431 ± 1.362	72.2
	Fraction of Emigrants	♀	1	-0.232 ± 0.483**				0.558 ± 0.563				76.4
			2	-4.131 ± 2.628			0.274 ± 0.280					76.4
	<i>E. a. glaciogentia</i>	Fraction of Immigrants	♂	1	-7.495 ± 1.074**	0.293 ± 0.103**						1.029 ± 0.421*
Fraction of Emigrants		♂	1	16.079 ± 3.577**					-0.607 ± 0.206**	-1.933 ± 0.538**		81.8
			2	3.263 ± 1.660*		-0.223 ± 0.109*	-0.492 ± 0.191*	0.474 ± 0.155**				83.8

The predictor variables entering the initial models were males and females of both populations, to test for the effect of average male and female densities, cover and height of food plants and their interaction; cover of the vegetational components; patch area and connectivity (S<sub>j</sub>). Coefficient estimates ± standard errors and AIC<sub>c</sub> values of each best (ΔAIC < 2) model are shown.

## DISCUSSION

The aim of this work was to study the metapopulation dynamics of two *E. aurinia* populations occurring in two different environmental frameworks. In choosing the two populations we ensured that i) both of them included a considerable number of individuals, ii) both were spatially organised in a system of linked patches and iii) the habitats occupied by each population was in relatively suitable conservation conditions.

### *Population dynamics*

In both populations the sex-ratio is strongly male-biased, a fact that can negatively influence their effective population size (Brook et al. 2008). This pattern, however, is commonly observed in MRR studies carried out on *Euphydryas aurinia* in many European countries (e.g. Belgium: Schitzkelle et al. 2005; the Czech Republic: Fric et al. 2010; Finland: Wahlberg et al. 2002; N. China: Wang et al. 2004). As shown by VMM estimates, expressed as a higher catchability, our results may be partially explained by the stronger detectability of males, which makes them easier to net than females, especially in the Alpine population. Males are also longer-lived than females, particularly at our Mediterranean site, which may increase the probability that each of them is caught at least once in its lifetime. Another explanation for this unbalanced sex ratio may be a consequence of males' mate-searching behaviour, which in this case, as in most Melitaeini, is described as an alternating mixture of perching and patrolling (see Wahlberg 2000). In both populations we observed patrolling, but in the Alpine site this behaviour is apparently mixed with a lek-like assembly strategy (see Wickman and Rutowski 1999), where male aggregations allow for their higher probability of detection and capture. At the same time, however, a highly biased sex-ratio towards males is apparent and cannot be justified by different catchability alone.

### *Dispersal behaviour*

Virtual Migration parameters have shown strong differences between populations as well as between sexes. In our study system males are more prone to move than females and we observed a higher emigration rate ( $\eta$ ) for the Mediterranean population. In contrast, adults of the Alpine population are more sedentary and consequently show higher within patch mortality ( $\mu$ ). In our study, differences between populations are stronger than between sexes, a finding that contrasts with results obtained on several other Melitaeini, where differences between males and females are generally stronger than between species (see Fric et al. 2010).

Other works have observed that dispersal parameters differ between metapopulations located in landscapes characterised by different networks of patches and different matrix composition, in a variety of butterflies (e.g. Schtickzelle et al. 2006; Turlure et al. 2011; Bonelli et al. 2013; Nowicki et al. 2014), which clearly shows that dispersal propensity is highly context-dependent (Baguette and Van Dyck 2007).

Fragmented landscapes usually imply lower emigration rates (e.g. Schtickzelle et al. 2006; Nowicki et al. 2014), and a stronger and inverse influence of patch area on emigration propensity (Mennechez et al. 2004; Schtickzelle et al. 2006). This is apparently in contrast with our results, since we observed higher propensity to move in the Mediterranean system where, although the open areas are fragmented in a wooded matrix, dispersal parameters suggest a higher degree of landscape connectivity. Consequently, as already suggested by other authors (e.g. Baguette and Van Dyck 2007) it is necessary to consider landscape connectivity not only in its *structural* component (spatial configuration of habitat patches), but mainly in its *functional* counterpart (how the behaviour of organisms is actually affected by landscape structure).

Dispersal rate, in butterflies and more in general in insects, can be positively influenced by temperature. Warm weather promotes dispersal (e.g., Dennis and Bardell 1996; Walters et al 2006; Mitikka et al. 2008) and dispersal can be influenced by weather fluctuation, with hotter years characterised by higher dispersal rates (e.g. Franzen and Nillson 2012). In any case, the relationship between temperature, flight and dispersal propensity is not always straightforward and clear (e.g, Matter et al. 2011), because many other factors, both climate related or not, are also involved.

Differences between our two populations could be partially explained in the light of different climatic conditions, but not directly by temperature. Individuals of the Alpine population are adapted to low temperature and can fly also in days with suboptimal weather conditions (own observations). Similarly to what observed by Junker et al. (2010), our Alpine population is characterised by low mobility, which may be seen as an adaptation to the alpine environment and climate. As these authors underline, such behaviour could prevent accidental drift events due to strong wind. Indeed, many high-altitude species living in open environment show lower dispersal capacity than species living in forest clearings (see Junker et al. 2010 and references therein).

In our Alpine system, patches are more isolated than in the Mediterranean one, where emigration is more risky in terms of daily mortality. The degree of connectivity of the system

needed to ensure the long term survival of a metapopulation should, therefore, be different in the two systems.

According to Travis et al. (2012), selection should operate at two parallel levels and should have favoured both the ability to reach a new patch and the propensity to leave the old patch, even though movement towards a new patch is necessarily more risky and energetically expensive. This is certainly true in a hostile matrix where only highly skilled or 'lucky' individuals are able to find new suitable sites. In our Mediterranean population, adults have occupied until recently a vast and very well preserved area, where patches were not very fragmented and were always rich in nectar sources as well as in larval food plants. With the abandonment of traditional agricultural practices, the system became progressively more fragmented because of forest expansion, and movements between patches have become more risky. In other words, the recent emergence of wooded barriers may have caught the adults of our Mediterranean population unprepared. Despite occurring in a still relatively good habitat, populations having these characteristics are therefore particularly at risk in case forest re-colonization will continue to advance.

Even if males are more prone to move between patches, particularly if these are well connected or close to each other, the mean distance that they can cover is not significantly larger than the distance covered by females, and is in general small. The Mediterranean population not only shows higher propensity to move, but adults fly longer distances than those from the Alpine one (calculated daily) when moving to a different patch ( $\alpha$  parameter of VM). On the other hand, adults of the Alpine population move longer distances in intra-patch movements but this difference may be partly explained by the larger patch sizes in the Alpine system.

According to Hanski et al. (2000), size area strongly influences movements. In our Alpine system where patches present stronger differences in size, it is clear that butterflies are more prone to move from the small patches to the bigger patches, and small patches receive fewer immigrating adults. However, in the Mediterranean system, where the landscape is a matrix of more similarly sized patches in a homogeneous context created by an anthropic tradition of land use, immigration and emigration are not driven by patch size. Recalculating the VM parameters according to Rabasa et al. (2007), we have been able to evidence that in both systems movements are driven by a density factor. In the Mediterranean population this factor actually is the only one that explains the immigration and emigration scaling coefficient.

In particular, the immigration scaling coefficient ( $\zeta_{im}$ ) is positively influenced by higher numbers of specimens both of the same and opposite sex. This type of immigration may be considered a kind of Allee effect (Greene and Stamps 2001; Altwegg et al. 2013), which occurs when species show conspecific attraction. High densities of conspecifics may suggest good habitat quality, as well as an opportunity for finding possible mates (Greene and Stamps 2001; Altwegg et al. 2013).

### ***Comparing European *Euphydryas aurinia* populations***

Since Hanski (2000) proposed the modelling approach known as Virtual Migration Model to estimate crucial parameters in metapopulation systems, measures of migration became comparable. In the last decade these parameters were estimated in different butterfly populations, some of which belong to the *Euphydryas aurinia* complex (Wahlberg et al. 2002; Wang et al. 2004; Schtickzelle et al. 2005; Fric et al. 2010). Comparing propensity to migrate, as expressed by the ( $\eta$ ) parameter, we observe both a general pattern as well as visible differences among populations (Fig. 7). In general, females are more sedentary than males, with strong differences among populations.

The *E. aurinia* populations studied in N China, in the Czech Republic and at our two Italian sites show higher propensity to move than those from Finland and Belgium. Observing the system of patches where our *E. aurinia* populations occur, we notice that differences in emigration propensities cannot be explained by distances between patches.

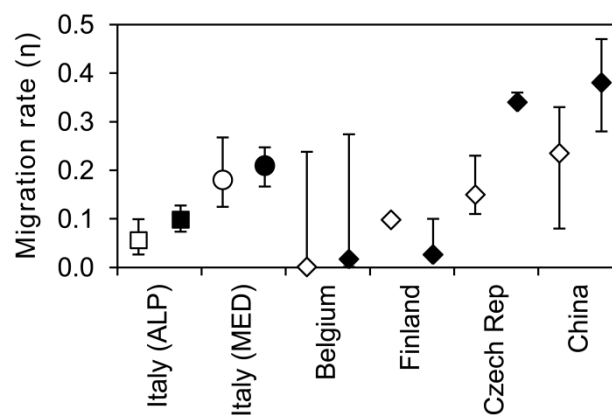
All the analyzed metapopulations are organized in groups of patches quite close to each other (see legend of Fig. 7 for patch system descriptions of each *E. aurinia* population). The only exception is the population studied in Finland by Wahlberg et al. (2002), which occupies a network of more isolated patches, constituted by 12 patches, within 1.5x2 km. In general we can observe that emigration propensity ( $\eta$ ) is stronger in bigger metapopulations occupying a system of more numerous patches, and even when patches are reciprocally not very close. *E. aurinia* populations from Finland and Belgium show the lowest  $\eta$  values (i.e. the parameter describing propensity to move). Both populations are rather (Finland) or very small (Belgium) and have been studied in a system of 11-12 patches. In contrast, the highest values of migration propensity are shown in the large populations from China and the Czech Republic, which were studied in a system of more than 30 well connected patches. The two Italian



populations, both quite numerous and each occupying a system of more than 15 patches, show intermediate values.

One can speculate that a bad conservation status and a small number of individuals may depress populations and make individuals slightly more mobile, even in a system of closed patches. This might be the condition for many European populations of *E. aurinia*.

In this case, management should be aimed at improving the size of suitable habitat and consequentially also population size (see Anthes et al. 2003). On the other hand, strong habitat fragmentation may depress propensity to move, as in the case of the Finnish population where distances among patches are more than triple those in the other sites. In this case the probability to reach a new suitable patch is low and mortality during migration ( $\lambda$ ) is above zero (Wahlberg et al. 2002).



**Fig. 7.** Estimated values of VM model emigration propensity ( $\eta$ ), as reported in literature for *E. aurinia* populations of different Countries. Belgium (Schtickzelle et al. 2005): 11 patches within 0.3x1 km; Finland (Wahlberg et al. 2002): 12 patches, within 1.5x2 km; Czech Republic (Fric et al. 2010): 30 patches within 2x1.5 km; China (Wang et al. 2004): 38 patches, within 3x3 km. The Italian data referred to our research in the Alpine (ALP) 16 patches, within 3x1.5 km and in the Mediterranean (MED) sites, 15 patches, within 2x0.6 km. Values of females (white) and males (black) are reported separately.

## CONCLUSION

As in the case of several other systems (e.g. *Maculinea arion*, see Bonelli et al. 2013), dispersal propensity varies depending on environmental pressures. Not only the propensity to move differs between species, populations and sexes, but consequences of individual movement, in terms of mortality during migration, differ depending on context. This is not

only interesting *per se* but fundamental while making concrete management decisions, always keeping in mind necessities of “*actual functional connectivity*” (see Calabrese and Fagan 2004).

Of course we cannot always simplify habitats in order to destroy or avoid barriers. What we can do is to try and obtain *permeable barriers* that allow grassland specialist species to penetrate. Where habitat restoration is planned and resources for long term species conservation are available we suggest that a *population-centred approach rather than a species centred approach* is pursued (see Casacci et al. 2013).

In this work we observed that not only butterflies are sensitive to fragmentation and can be differently prone to leave their native patch, but the same level of isolation among patches can play different roles in different landscape contexts. Consequences of these findings need to be addressed in any management program.

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## **3.2 Multiscale habitat requirements of the *Euphydryas aurinia* complex: a conservation perspective**

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### **INTRODUCTION**

Human activities have shaped the European landscapes for thousands of years (Williams 2003). The management activities implemented for agricultural and pastoral practices have increasingly transformed large expanses of forest into grasslands, and have thereby indirectly benefited many groups of invertebrates, such as Scarabaeidae (Tocco et al. 2013), Orthoptera (Marini et al. 2009b) or Lepidoptera Rhopalocera (van Swaay et al. 2012). A number of previous studies confirmed the importance of traditional pastoral and farming practices in preserving also the diversity of some vertebrate taxa, such as birds and bats, that directly or indirectly can benefit from the conservation of open areas and meadows (Laiolo et al. 2004; Ambrosini et al. 2011; Obrist et al. 2011; Lasanta et al. 2015).

In current times, however, economic growth and globalisation are concurring in modifying the habits and customs of farmers, leading to a shift from traditional agricultural practices to more intensive systems (especially in lowlands), or involving the abandonment of the less profitable or too small agricultural plots and the symmetric overexploitation of the remaining areas (MacDonald et al. 2000; Schenk and Bätzing 2003; Mottet et al. 2006). Although in some cases the abandonment could initially favour certain species (Skorka et al. 2007; Marini et al. 2009a), the progressive re-colonization by the forest slowly but steadily leads to the complete disappearance of open habitats. Therefore changes in agro-pastoral practices are now causing the fragmentation of most arthropod communities (Benton et al. 2003; Tschanrtke et al. 2005; Stefanescu et al. 2009; Molina et al. 2014).

In particular, the loss of suitable habitats in relation to changes in land use, and land abandonment followed by the invasion of shrubs and trees, are the first cause of threat for many butterfly species (van Swaay et al. 2012). Butterflies are among the major recipients of the human action on grasslands, since they represent a very large proportion of species breed in these habitats. Most grasslands, indeed, are not characterized by climax vegetation and are only maintained by traditional practices such as low intensity livestock grazing and mowing (Baldock et al. 1994; van Swaay and Warren 1999).

The Marsh fritillary (*Euphydryas aurinia*) is an internationally protected species. It has been included in Annex II of the Bern Convention on the Conservation of European Wildlife since 1979, and in the Annex II of the EU Habitats Directive since 1992. *E. aurinia* is widespread in Europe and, although not at risk of imminent extinction at the continental level (Least Concern, IUCN 2013 regional assessment - van Swaay et al. 2012), it is known for having declined in most European countries. The species is reported as extinct in the Netherlands (Asher et al. 2001), while declines of over 30% either in range or population size have been documented from Germany, Latvia, Luxembourg, the Republic of Ireland, Slovakia and Ukraine. Smaller declines of 6-30% are known for having occurred in Austria, Belarus, Belgium, the Czech Republic, Finland, France, Hungary, Lithuania, Poland, Portugal, Serbia, Slovenia, Spain, Switzerland and the United Kingdom (Asher et al. 2001). The European *E. aurinia* complex includes a series of taxa, each showing distinct morphological and eco-ethological features, whose intricate phylogenetic history seems to be the result of intraspecific differentiation processes shaped by glaciations (Junker et al. 2015; Korb et al. 2016). At least three Evolutionarily Significant Units (ESUs, Casacci et al. 2014) occur in Italy and have been provisionally treated as distinct species in the Check List of the Italian butterflies (Balletto et al. 2014a): *E. (a.) aurinia* (Rottemburg, 1775) inhabiting the Continental Region (*sensu* European Union), *E. (a.) provincialis* (Boisduval, 1828) in the Mediterranean Region and *E. (a.) glaciegenita* (Verity, 1928) in the Alps (Fig.1). All of them



occupy distinct habitats protected at European level (Habitat 6410, *Molinia* meadows; Habitat 6210, semi-natural dry grasslands on calcareous substrates; Habitat 4060, Alpine and boreal heaths, respectively) and exploit distinctive food plants. *E. (a.) aurinia* caterpillars feed on *Succisa pratensis* (Dipsacaceae), *E. (a.) glaciegenita* larvae on *Gentiana acaulis* (Gentianaceae), and *E. (a.) provincialis* uses either *Cephalaria leucantha* or *Knautia arvensis* (both *Dipsacaceae*).

The three Italian taxa of the *E. aurinia* complex (Balletto et al. 2014a) are univoltine and have a similar life cycle. Females lay eggs in large batches; each female may lay more than one batch, with subsequently decreasing number of eggs per clutch (Porter 1992). After hatching, larvae weave a nest between food plant leaves, feeding on them, and before pupation, larvae of all taxa go through a solitary phase.

According to the national checklist the 3 Italian entities of the *E. aurinia* complex were also separately evaluated into the recently published "Red List of Italian Butterflies" (Balletto et al. 2015). The *E. (a.) aurinia* is ranked as "Vulnerable" (VU), mainly as a consequence of habitat destruction, which caused the extinction of at least 12 populations in recent times (Bonelli et al. 2011). The conservation status of the "Mediterranean" *E. (a.) provincialis* and of the "Alpine" *E. (a.) glaciegenita* is assessed as "Least Concern" (LC).

In the present study, we compared the resource-based distribution of one population of *E. (a.) glaciegenita* inhabiting a site in the NW Alps (2,100–2,300 m) and one of *E. (a.) provincialis* occurring in the Mediterranean biogeographical region (ca. 700 m) whose dispersal abilities were compared in a previous research (Casacci et al. 2015). Both populations are threatened by land use changes, which modify both the structural and the functional connectivity of habitat patches and habitat quality affecting migration rates (Casacci et al. 20015).

Gaining full understanding of the requirements of all life stages, in relation to resource distribution, is essential to develop the most appropriate conservation strategies, because butterflies are highly selective to both the quality and the spatial distribution of habitats

(Porter 1984; Munguira et al. 1997; Lewis and Hurford 1997). Recent studies on *E. (a.) aurinia* remarked the necessity to analyse both the macro- and the microhabitat exploited by butterflies throughout their life cycle, in terms of features of the habitat patches as well as the abundance and quality of resources that larvae need for growing (Tjørnløv et al. 2015; Janovsky et al. 2016).

In this framework, we analysed the resource use at three different scales. First, to understand which parameters affect adult male and female distributions at *landscape* scale, we separately analysed individual densities of both the sexes as function of patch characteristics. Second, in order to test the degree of adults' aggregation at *patch* scale, we examined the distribution of individuals within the patches, describing the pattern of local habitat use by the species. Finally, aiming to identify the features affecting the oviposition behaviour, we analysed the characteristics of the *microhabitat* colonised by first instar larvae.

## **METHODS**

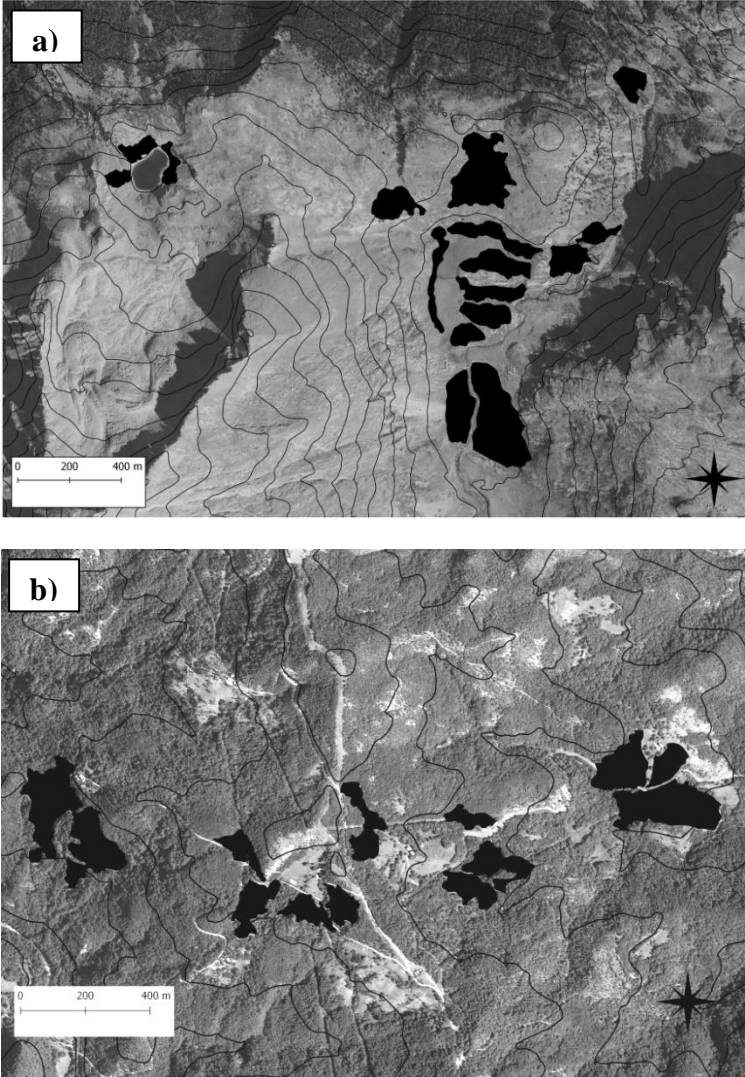
### ***Study sites***

During summer 2013, the same protocol was used to study two populations of the Marsh fritillary, one located in the Western Alps and one in the Mediterranean biogeographical region, in the northern Apennine (Table 1). Both areas are included in sites protected by the NATURA 2000 network. The first Site of Community Importance (SCI IT1201000; E 7°19'; N 45°32') is also part of the "Gran Paradiso" National Park (Alpine population), whilst the second (SCI IT1180026; E 8°47'; N 44°34') falls within the "Capanne di Marcarolo" Natural Park (Mediterranean population). The two populations inhabit large semi-natural grasslands, managed by extensive cattle grazing or traditional mowing. It is noteworthy that the preservation and management of the open habitats is one of the conservation priorities at both protected areas.

The Alpine population, identified as *E. (a.) glaciegenita*, inhabits the north-western Italian Alps, in the Bardoney valley, at about 2,200 m of altitude (Cogne, Aosta Valley Region). It occurs above the timberline in open alpine grasslands, having different degrees of soil humidity and acidity. At its lower altitudinal boundary, the site is limited by a coniferous woodland (*Larix decidua*, *Pinus cembra*) and is fragmented by screes and heaths. In particular, the central part of the site is covered by wet meadows and buried marshes growing on acidic soils. We observed floral species adapted to different levels of soil moisture, such as *Pinguicula leptoceras*, *Sphagnum* sp., *Nigritella nigra*, *Arnica montana*, *Pulsatilla alpina*, *Anthyllis vulneraria*, *Trifolium alpinum* and *Myosotis alpestris*. The larval food plant, *Gentiana acaulis*, is widespread and abundant. The area is currently managed by an extensive cattle grazing, which maintains floristic heterogeneity and the structural diversity of vegetation.

The Mediterranean population, identified as *E. (a.) provincialis*, occurs in the northern (Ligurian) Apennines at around 700 m of altitude, at the southern boundary of the Piedmont Region. It inhabits historically managed grasslands (Habitat 6210 and 6510 - Habitats Directive, Annex I), in particular of *Molinio-Arrhenatheretea* and *Festuco-Brometea* types. Geology, climate and geographical location have shaped the particularly rich vegetation which includes both Mediterranean and Alpine species such as *Achillea millefolium*, *Agrostis tenuis*, *Arrhenatherum elatius*, *Bunium bulbocastanum*, *Centaurea nigrescens*, *Daucus carota*, *Filipendula vulgaris*, *Galium verum*, *Knautia arvensis* (*E. (a.) provincialis*'s food plant), *Lathyrus pratensis*, *Linum bienne*, *Rhinanthus alectorolophus*, *Sanguisorba officinalis*, *Trifolium pratense*, *Trifolium ochroleucon*, *Thymus pulegioides*. Grassland composition reflects the historical land use of the site and the occurrence of some species (oats and rye) denotes a past cereal cultivation. We found most of the meadows on south-facing slopes immersed in a matrix of forest and shrubs, while the northern slopes are covered in forest or

sparse trees. The forest re-colonization, following the abandonment of traditional hay cutting practices, produced a habitat fragmentation at local scale.



**Fig. 1. a)** Alpine ("Gran Paradiso" National Park), **b)** Mediterranean ("Capanne di Marcarolo" Natural Park) and patch systems identified in black. Orthophotographs showing land cover and level curves with 50 m interval bands are represented on the background.

**Table 1.** The *Euphydryas aurinia* study sites.

	<i>E. (a.) provincialis</i>	<i>E. (a.) glaciegenita</i>
<b>Site</b>	Marcarolo	Bardoney
<b>Altitude</b>	650-750 m	2100-2300 m
<b>Habitat type</b>	Dry grassland	Alpine grassland
<b>Protected area</b>	"Capanne di Marcarolo" Natural Park	"Gran Paradiso" National Park
<b>Biogeographical Region</b>	Mediterranean	Alpine
<b>Number of patches</b>	15	16
<b>Total area of patches</b>	20.24	28.15
<b>Patch area (min-max)</b>	0.62-2.44	0.49-5.78
<b>Marked individuals</b>	1,499 (509 ♀; 990 ♂)	1,684 (532 ♀; 1,151 ♂)
<b>Number of larval webs (occupied vs unoccupied host plants)</b>	57 vs 61	161 vs 109

### *Sampling protocol*

#### *Landscape and patch scales*

#### Adult survey

At both sites, potential habitat patches were mapped on the basis of some preliminary visit and examination of aerial photographs. Patches were defined as a basically homogeneous meadows and were considered as distinct if separated by obvious barriers or discontinuities (in our study: forests, roads, screes, pit bogs and streams), or when the characteristics of the land cover changed noticeably (Fig. 1).

We collected data on adults' movements by Mark-Release-Recapture method (MRR) throughout the flight period of the year 2013. Data on adult dispersal abilities are reported in Casacci et al. (2015). Adults of *E. (a.) provincialis* flew from 27 May to 22 June, while those of *E. (a.) glaciegenita* occurred from 14 July to 14 August. We visited all patches every day (except on rainy days), approximately from 10 am to 5 pm. All observed butterflies were captured and marked individually and permanently on the ventral surface of the hind wings. For each capture or recapture event, we recorded the individual code, date, patch location and

sex, as well as the GPS point. After marking, butterflies were immediately released at the same location of their capture.

#### Landscape environmental variables

For each patch we characterized the vegetation cover at randomly distributed squares (5x5 m), in proportional number to patch size, for a total of 86 plots at the Alpine site and 30 at the Mediterranean site. We divided each 5x5 m square into smaller 25 squares (1x1 m) and within each of them we measured at sight the percentages of vegetation cover and physical variables, i.e. nectar sources, food plants, grasses, litter or mosses, rocks, low bushes, and bare soil. To characterize each habitat patch, we used the overall mean for each variable evaluated into each small square. In addition, for each patch we also considered the management type (grazed and/or mowed, abandoned) during all life stages of butterflies.

For each patch we also obtained data on terrain slope and solar radiation derived from a Digital Elevation Model (DEM), a raster with a spatial resolution of 10x10 m cells (Tarquini et al. 2012), analysed with the ArcGis 10.3 software (ESRI 2014). More in particular, we used the "Area Solar Radiation" tool, which permits to analyse and map the solar radiation over a given geographic area and for a specific time period. In our case, we used cumulated solar radiation, expressed as watt/hours per square meter (WH/m<sup>2</sup>), for the entire flight period of each population (see the previous paragraph for the specific flight periods). We characterized each patch according to the terrain slope and solar radiation using the mean values of all raster cells laying within the patch border.

#### Patch environmental variables

At this scale of analysis, we superimposed a grid of 10x10 m over both the study areas and we obtained solar radiation and distance from the patches' margins for all the cells (100 m<sup>2</sup>) located within the patches. Solar radiation was calculated as explained in the previous

paragraph with the "Area Solar Radiation" tool of the ArcGis software (ESRI 2014). The distance from the patches' margins was calculated as the distance between the centre of each cell and the nearest point in the patch's margin, successively classified as 10-m increasing distance belt.

### *Microhabitat scale*

#### Larval survey and environmental variables

We sampled first instar larvae within fixed squares (plots), each of 0.25 m<sup>2</sup>, randomly chosen within the habitat patches and investigated microhabitat characteristics around each food plant occurring in the plot, by taking note of whether the plant hosted or not a larval nest. Overall, we sampled 270 plots for the *E. (a) glaciegenita* population, and 118 plots for *E. (a) provincialis*.

We took note, with a visual evaluation, of some microhabitat variables, i.e. percentage of vegetation cover (larval host plant, nectar sources, grasses, low shrubs) and height of herbaceous layer, as well as some proxy variables of micro-climate features (bare ground percentage, organic materials, moss and rocks as proxies for temperature and humidity).

### ***Data Analysis***

All the following statistical analyses, for each survey scale, were performed for both populations separately.

### *Landscape scale*

To understand which environmental variables affect male and female density in each patch, we analyzed them by generalized linear models (GLMs) with negative binomial response, using the landscape environmental variables as covariates. The area of each patch was log-transformed and included as an offset into each GLM, to take into account the patch size

effect. To make all explanatory variables comparable and for verifying their independence, we scaled them (Becker et al. 1988) and used only uncorrelated variables (*Pearson correlation*  $r < |0.7|$ ; Dormann et al. 2013) for further analysis. Being the coverage of graminaceous plants highly correlated with those of nectar sources (*Pearson correlation*  $r = -0.941$ ,  $p < 0.001$ ), the first variable was discarded from subsequent analyses.

The most parsimonious nested model was selected by using Akaike Information Criterion (AIC) (Burnham and Anderson 2004), implemented by the stepAIC function in the "MASS" package (Venables and Ripley 2002), using R 3.2.2 (R Core Team 2015). To test which variables affected the two populations at landscape scale, and if females and males had different habitat requirements, the models were run for each sex, separately.

#### *Patch scale*

At the patch scale analysis, we investigated the distribution of individuals within the habitat patches, identifying the patterns of aggregation, the overlap between males and females, and the habitat use by each sex. With QGIS 2.14.2 "Essen" software (2016), we measured the spatial distribution of each sex and determined if it was regularly dispersed, randomly dispersed, or clustered, by means of the Nearest Neighbour Index (NNI), which uses the distance between each point and its closest neighbour (Clark and Evans 1954). We only focused on patches where at least 20 individuals were observed and used the *p*-values to determine the probability of falsely rejecting the null hypothesis that individuals were randomly distributed, as well as *Z*-scores to test statistical significance as a measure of the standard deviation associated with a normal distribution.

To analyse the probabilistic co-occurrence of males and females, we superimposed a grid of 10x10 m over both study areas and evaluated the presence of males and females in each square. Following Veech (2013), we compared the observed and expected co-occurrence, calculated as the product of the probability of presence of each sex multiplied by the number



of 10x10 m squares. Using the R package "*cooccur*" (Griffith et al. 2016), we applied a probabilistic model which employs combinatorics, to evaluate if the observed co-occurrence was equal (random association), significantly larger (positive association), or smaller (negative association) than expected. We calculated such values for the study area taken as a whole, as well as for each patch separately.

To analyse the adult distribution within patches, we used the same 10x10 m grid superimposed over the study areas, and analysed adults presence/absence as a function of solar radiation and distance to the patches' margins (first and second order effect). To assess the role of these variables, we analysed adult presence/absence by generalized mixed-effect models (GLMMs), with a binomial response, using "*lme4*" package (Bates et al. 2015) and including the "patch" as random effect variable. The best models were selected by using Akaike Information Criterion corrected for small samples (AICc). Analyses were performed for sexes pooled together, and for males and females, separately.

We calculated the  $R^2$ s with "*MuMIn*" package (Barton 2016), which for GLMM can be categorized as *conditional* or *marginal*. The *marginal*  $R^2$  represents the variance explained by the fixed factors, whereas *conditional*  $R^2$  could be interpreted as the variance explained by both the fixed and the random factors.

**Table 2.** *Landscape scale.* The best generalized linear models, selected by stepAIC procedure for each population (ALP: Alpine; MED: Mediterranean) and sex. For each model and selected variable, the coefficient ( $\pm$  standard error) is shown. As a measure of goodness of fit, the adjusted D-squared ( $D^2_{adj}$ ) is also shown. \* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$ .

	<i>males ALP</i>	<i>females ALP</i>	<i>males MED</i>	<i>females MED</i>
<b>(Intercept)</b>	-5.800 ( $\pm$ 0.179)***	-7.728 ( $\pm$ 0.334)***	-5.747 ( $\pm$ 0.314)***	-5.819 ( $\pm$ 0.308)***
<b>Nectar sources</b>	0.437 ( $\pm$ 0.192)*		0.495 ( $\pm$ 0.155)**	0.473 ( $\pm$ 0.167)**
<b>Grass height</b>		-0.434 ( $\pm$ 0.168)**		
<b>Shrubs</b>	0.584 ( $\pm$ 0.209)**			
<b>Organic materials</b>		0.934 ( $\pm$ 0.185)***		
<b>Rocks</b>	-0.290 ( $\pm$ 0.208)	-0.351 ( $\pm$ 0.143)*		
<b>Solar radiation</b>	0.333 ( $\pm$ 0.212)	0.876 ( $\pm$ 0.156)***	-0.342 ( $\pm$ 0.153)*	
<b>Slope</b>	0.391 ( $\pm$ 0.196)*	0.643 ( $\pm$ 0.180)***		
<b>Grazing</b>		1.512 ( $\pm$ 0.392)***	1.670 ( $\pm$ 0.441)***	0.668 ( $\pm$ 407)
<b>Mowing</b>			-0.842 ( $\pm$ 0.399)*	-1.042 ( $\pm$ 0.410)*
<b>Grazing/Mowing</b>			0.835 ( $\pm$ 0.439)	0.102 ( $\pm$ 0.450)
<b><math>D^2_{adj}</math></b>	<b>0.494</b>	0.761	0.708	0.544

### *Microhabitat scale*

To identify which micro-habitat characteristics could drive the oviposition behaviour, we analysed the nest presence/absence by generalized linear models (GLMs), with a binomial response, and not with generalized mixed-effect models, despite that data are aggregated per patch, because of the very low contribution of the random variable to the models (Appendix S1).

Models to be compared with AIC were chosen by "Model Selection" approach (Johnson and Omland 2004), which generates biological hypotheses as candidate models, including the null model. As a measure of model goodness of fit, we calculated the adjusted  $D^2$  ( $D^2_{adj}$ ), which takes into account the number of observations and the number of model parameters (Guisan and Zimmermann 2000).

## RESULTS

In total, we captured and marked 1,499 individuals (990 males and 509 females) within the Mediterranean population (hereinafter MED) and 1,684 individuals (1,151 males, 532 females and 1 indeterminate) within the Alpine population (ALP).

### *Landscape scale*

We compared male and female density for the two study areas separately. In both populations we observed a positive correlation between the number of males and females per patch (ALP, *Pearson correlation*  $r = 0.905$ ,  $p < 0.001$ ; MED, *Pearson correlation*  $r = 0.958$ ,  $p < 0.001$ ), although in each patch males were always in greater numbers than females (ALP, *Wilcoxon test*,  $V = 132$ ,  $p < 0.001$ ; MED, *Wilcoxon test*,  $V = 119$ ,  $p < 0.001$ ).

We found that patch size was important for the Alpine population and for both sexes a positive correlation was found (*Spearman test*, males  $\rho = 0.588$ ,  $p = 0.019$ ; females  $\rho = 0.648$ ,  $p = 0.007$ ), while for the Mediterranean population we did not find any significant relationship between the number of individuals and the patch size (*Spearman test*, males  $\rho = -0.439$ ,  $p = 0.103$ ; females  $\rho = 0.446$ ,  $p = 0.097$ ).

The best generalized linear model suggested that vegetation structure affected the two populations in different ways (Tab. 2). The percentage of nectar sources had a positive influence on both populations (except for alpine females), but the percentage of low bushes (*Vaccinium uliginosum*, *Juniperus communis*, *Rhododendron ferrugineum*) was important only for alpine males. Alpine females preferred short grasses and larger percentage of litter and mosses (organic material). Terrain slope and rock cover play a role exclusively in the alpine habitat: slope had positive effect on the density of both sexes, whereas rocks had negative effect. Solar radiation had an opposite effect on the two populations: positive in the Alpine area and negative in the Mediterranean site. Finally, management type (grazing and/or mowing) affected the two populations in a dissimilar way. Interestingly, the percentage of the

larval host plants (*Gentiana acaulis* and *Knautia arvensis*) was not selected during the model selection process (Tab. 2).

#### *Patch scale*

The degree of aggregation was low and similar between the two populations for females: the NNI presented significant  $p$ -values ( $< 0.05$ ) only in 3 patches of each area (ALP  $mean = 0.620 \pm 0.007$ ; MED  $mean = 0.647 \pm 0.098$ ). This is probably a consequence of the low number of females captured in the patches (Appendix S2). Only alpine males showed a stronger aggregation pattern (*Mann-Whitney test* U, W = 4,  $p < 0.001$ ; ALP  $mean = 0.416 \pm 0.036$ ; MED  $mean = 0.735 \pm 0.039$ ).

We observed a high level of spatial overlap between males and females in both populations (Appendix S3). In the Mediterranean population, the observed occurrence was always higher than expected, while in the Alpine population, we observed only one case in which the observed value was not significantly higher than expected (Table S3.2), probably because of a lower number of adults captured.

The analysis of habitat use by the adults showed that in both populations individuals reached their highest probability of presence at a specified distance from the patch margin (second order effect, Tab. 3; Appendix S4), which can be quantified in 20 m for the Mediterranean and 43 m for the Alpine population. On the opposite, solar radiation had a contrasting effect on the two populations, i.e. positive in the Alpine and negative in the Mediterranean population (Tab. 3; Appendix S4). These results were consistent for each population as a whole as well as considering males and females separately (Appendix S3).

**Table 3.** *Patch scale.* Results of the GLM analysis of habitat use as a function of patch characteristics. In both populations we observed a second order effect of the distance from the margin (*distance*). Solar radiation (*sol rad*) had positive effect at high altitude and negative effect at low altitude. No second-order effect of solar radiation was observed. ns: not significant. \*\*\*: p-value<0.001.  $r^2_m$ : fixed effect r-squared.  $r^2_c$ : random and fixed effect r-squared.

	intercept	distance	distance <sup>2</sup>	sol rad	sol rad <sup>2</sup>	r <sup>2</sup> m	r <sup>2</sup> c
Mediterranean population	-0.095	0.912***	-0.627***	-0.354***	ns	15.12	36.6
Alpine population	-1.230	0.839***	-0.286***	0.663***	ns	16.57	37.17

### *Microhabitat scale*

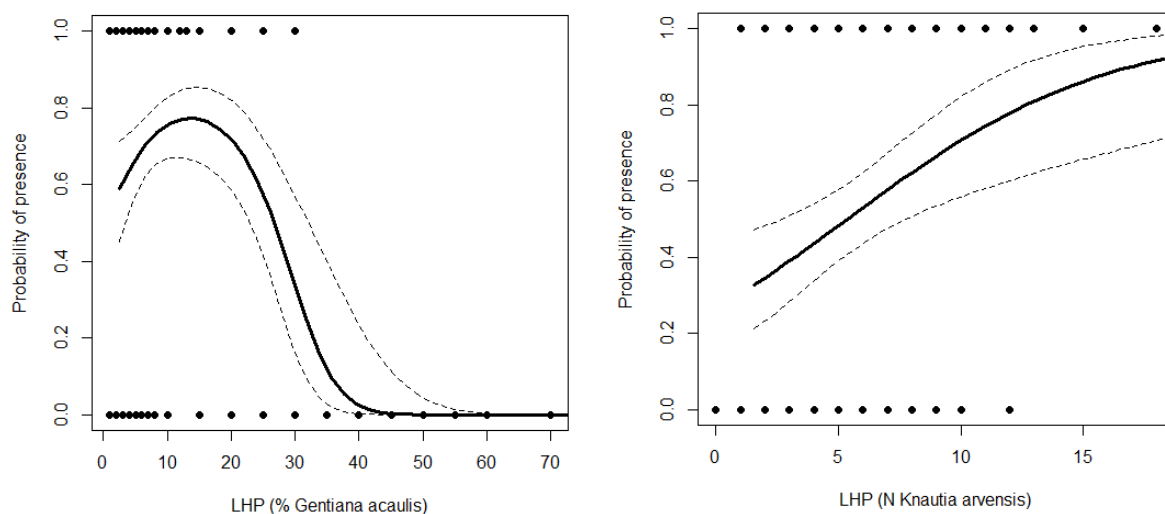
We compared microhabitats around host plants either occupied or not occupied by a larval nest. For the *E. (a.) glaciegenita* population, we found 161 occupied and 109 unoccupied host plants (270 in total), whereas for *E. (a.) provincialis*, we found 57 occupied and 61 unoccupied host plants (118 in total).

For both populations, host plants played a fundamental role in driving oviposition behaviour, but in different ways. For *E. (a.) provincialis*, the best model ( $D^2_{adj} = 0.069$ ) included only the number of *Knautia arvensis* plants as important variable, which resulted greater in microhabitats around occupied plants (Nest absence: *mean* = 4.741 ± 0.384; Nest presence: *mean* = 6.871 ± 0.483; Tab.4; Fig. 2).

In contrast, the choice of the oviposition site by females of the Alpine population was very peculiar and accurate. As expected, the host plants chosen for egg-laying were surrounded by a considerable number of other plants of *Gentiana acaulis*, but at the same time we identified an optimum value above which a further increase in the percentage of larval host plant was negatively selected by the females (Fig. 2). The selected best model included the host plant (second and first order), grass and bare ground percentages ( $D^2_{adj} = 0.215$ ), the latter with a positive effect (Tab. 4; Fig. 2).

**Table 4.** *Microhabitat scale.* The best selected model in each population confirms the importance of host plant. For each population and selected variable, the coefficient ( $\pm$  standard error) is shown. As a measure of goodness of fit, the adjusted D-squared ( $D^2_{adj}$ ) is also shown. \*\* $p < 0.01$ , \*\*\* $p < 0.001$ .

Mediterranean population	(Intercept)	0.089 ( $\pm$ 0.192)
	<i>Knautia arvensis</i>	0.673 ( $\pm$ 0.214)**
	$D^2_{adj}$	0.068
Alpine population	(Intercept)	1.334 ( $\pm$ 0.261)***
	<i>Gentiana acaulis</i> (second order)	-1.218 ( $\pm$ 0.388)**
	<i>Gentiana acaulis</i> (first order)	0.104 ( $\pm$ 0.291)
	Grass	0.666 ( $\pm$ 0.182)***
	Bare ground	0.260 ( $\pm$ 0.167)
	$D^2_{adj}$	0.215



**Fig. 2.** *Microhabitat scale.* The two graphs represent the probability of larval nest presence in the two populations in relation to their specific larval food plants. For the Alpine population (a) we use the percentage of *Gentiana acaulis* while for the Mediterranean system (b) the number of *Knautia arvensis* plants.

## DISCUSSION

Results of this comparative study showed that adults of the two populations of the *E. aurinia* complex, despite their similar life cycle, and besides having different dispersal potentials and flight behaviour (Casacci et al. 2015), also used different criteria to select patches for their

routine movements (landscape and patch scales) and oviposition behaviour (microhabitat scale).

### ***Landscape scale***

Several studies have already proved the importance of host plant abundance and distribution in defining population density and extinction/colonization rates, in butterflies (Wahlberg et al. 2002; Anthes et al. 2003). At *landscape scale* the two populations of the *E. aurinia* complex showed different habitat and food plant requirements, which do not constitute a limiting factor in defining adults' density between patches, probably because the two plants are abundant and evenly distributed.

According to recent evidence, microhabitat structure is central for *E. aurinia*, especially for larval development and (perhaps as a consequence) in driving oviposition behaviour (Janovsky et al. 2016). In our Alpine population we found that rocks, terrain slope and solar radiation played an important role in defining density of both males and females within patches. Moreover, low bushes and nectar sources affected the abundance of males while, for females the grass height, the percentage of organic materials (litter and mosses) and the grazing regime resulted more important.

It is evident that males' density was more closely related to the resources necessary to obtain immediate energy for patrolling and looking for a partner (Settele et al. 2009), while for females, the selection of patches having good characteristics for oviposition, such as the presence of low grasses (which is in turn a result of an extensive and low-intensity grazing), was more important. Moreover, butterflies that inhabit mountain areas, resulted affected by topo-climatic factors which are very variable (Illan et al. 2010). Thus, it was not surprising that patches receiving more solar radiation showed higher population density. The effect of the slope (see Tab. 2) is consistent with the behaviour shown by these butterflies. Indeed, in the study area there are several peat bogs and marshes and adults showed an interest in

inhabiting the steeper and less wet areas, which are, generally, also richer in flowers (Weiss et al. 1988).

For the Mediterranean population, agro-pastoral activities in the study area are critical in determining the presence and density of butterflies. Grazing or an alternation of mowing and grazing activities provided significantly better conditions than the total absence of management. It is already known that grazing, if well managed, can promote plant diversity, because it increases the soil nutrients and decreases competition for light by lowering the grass height (Bakker et al. 2006), and consequently the availability of nectar sources. Mowing has instead a negative effect on butterfly population density, because, if practiced during the wrong periods, has the potential to destroy nests or to eliminate the required nectar sources for the adults (Hula et al. 2004). Indeed, nectar sources resulted important to predict adult density in both sexes.

Males tend to avoid open patches with high levels of solar, probably to become less visible to potential predators. Generally speaking, the *Melitaeini* are known for their aposematic colours, which should reflect their unpalatable characteristics, deriving from their food plants' secondary metabolites (usually iridoid glycosides). Yet, *Gentiana acaulis* (*Gentianaceae*) and *Knautia arvensis* (*Dipsacaceae*) contain only secoiridoid compounds, which are not sequestered by larvae and are produced *de novo* (Bowers & Williams 1995, Zimmerman et al. 2000, Wahlberg 2001). A recent study, however, has shown that these chemicals are not sufficient to defend *E. aurinia* from predation by *Parus major* (Tesařová et al. 2013), therefore males could select less sunny areas, where they are more camouflaged with the background.

### ***Patch scale***

We found different levels of aggregation of butterflies, with significantly stronger aggregation patterns in males of *E. (a.) glaciegenita* with respect to *E. (a.) provincialis*, whereas female



distribution appeared similar for both populations. As already observed in others species (Lederhouse 1982; Knapton 1985), the mating behaviour of alpine males could be interpreted as a lekking behaviour, where the non-overlapping male territories are patrolled and resources (i.e. nectar sources, food plants) actively defended.

However, in both populations, the male and female home-range showed a significant overlap, highlighting a common habitat use within patches, also confirmed by the consistent results in the analysis of habitat use as a function of distance from the margin and solar radiation.

In both the Mediterranean and the Alpine population, we observed an edge-distribution, with individuals of both sexes presenting a higher probability of presence at a certain distance from the patch margins. This effect was clearly more pronounced in the Mediterranean population, where proximity to the patch edge represented an important feature in determining butterfly abundance. Such a pattern has been observed in many butterfly species having different ecological requirements, although a common and clear explanation is still lacking (Dennis 2010). In some nymphalids, however, mate-location sites are often represented by the bright edges of forests (Dennis 2004), which at the same time provide shelter and create an optimal micro-climate. This could be also the case for the Mediterranean population of *E. (a.) provincialis*, where "margins" are represented by sparsely wooded areas. Consequently, margins can represent an easier escape from predation and a shelter from the more exposed central open areas (Dennis 2010). Both taxa were described as "subnemoral" by Balletto and Kudrna (1985) to highlight adults' propensity to fly in the vicinity of bushes, also perhaps for thermoregulatory reasons in the hot Mediterranean area.

The negative effect of solar radiation on the adults' probability of presence can also be interpreted in the light of predatory pressure: as previously said, *E. aurinia* s.l. has not enough chemical defence against predators and camouflage in a mixed sunny and shady background can be advantageous (Tesařová et al. 2013).

For the Alpine population, the most important feature in driving butterfly distribution inside patches was represented, in contrast, by a positive effect of solar radiation. A higher solar radiation, positively and significantly influenced the probability of presence of both sexes of *E. (a.) glaciegenita*. This pattern can be clearly interpreted as thermoregulatory. Indeed, the solar heat is important in increasing thorax temperature and allowing flight activities, with all other related behaviours (e.g. Dennis 1993; Dennis 2010), for populations located at higher altitude and in a much cooler microclimate (e.g. Buckley and Kingsolver 2012; Cerrato et al. 2016).

### ***Microhabitat scale***

"Optimal oviposition theory" (Mayhew 1997) predicts that females will choose food plants capable of maximising larval performance and consequentially their own fitness. Accordingly, food plant density was the most important predictor of the presence/absence of larval webs for both of the populations in our study areas, but the modality of female choice seems to be different. *E. (a.) provincialis* selected as egg-laying locations those sites with the highest possible number of food plants, probably because females are visually attracted by a good number of plants to prevent larval starvation during the first life stages and in the post-hibernation phase, as was observed also by other authors for *E. (a.) aurinia* (Anthes et al. 2003; Konvikca et al. 2003; Tjørnløv et al. 2015). We did not observe the same behaviour in *E. (a.) glaciegenita* whose females carried out a careful selection based on the "optimal" density of *Gentiana acaulis* at the deposition site. Their avoidance of a too low density of food plants is easy to explain, considering the scarce mobility of pre-hibernating larvae. The females lay eggs exactly where they can ensure enough food resources to early immature life stages. The avoidance of a too high percentage of food plants is more difficult to explain. A study conducted on *Pararge aegeria* by Gibbs et al. (2004) demonstrated that a too high larval density during the growth period (post-hibernating phase for *E. aurinia*) caused a

decline in the pupal size of both sexes and that was more costly for females than for males because it reduced female fertility. So females could spend more time to select the best place where laying their eggs and guarantee maximum growth for their female offspring. This could explain why *E. (a.) provincialis* females choose the greatest possible amount of plants in the nest's neighbourhood whilst *E. (a.) glaciegenita* oviposition choices are subject to a threshold level.

Perhaps females can avoid a too high food plant density also by using visual or chemical signals, such as the oviposition-detering pheromones left by other females or hormones secreted by the host in response to oviposition (Dempster 1992; Bruinsma et al. 2007; Little et al. 2007). Moreover, many larval webs close together could produce more chemical signals and be more readily detected by parasitoids, as reported for large larval webs; nevertheless in a good quality environment with a continuous host plant distribution, which is reflected in a high density of larval web, the population can benefit from a low parasitism pressure (Klapwijk and Lewis 2014).

For *E. (a.) glaciegenita* sward height and percentage of bare soil resulted also important, because they can contribute to control the microclimate, humidity and temperature, and permit larval thermoregulation and basking behaviour (Porter 1982; Anthes et al. 2003).

Gregarious larvae are forced to share food, requiring large plants or patches with high densities of host plant (Davies and Gilbert 1985). Also plant connections are important in the choice of the best oviposition place (Cain et al. 1985), since in this way larvae can avoid some plant defence mechanisms, such as growth arrest, moving easily to a nearby plant. *Gentiana acaulis* has a cluster distribution into the patches and plants are often well-connected to each other, while plants of *Knautia arvensis* are generally rather isolated and a single plant is not enough to feed a larval web.

## CONCLUSION

Summing up the results of this work and of a previous one (Casacci et al. 2015), we can say that, similarly to many other butterfly species, the long-term survival of these populations depends on the management of pastoral and agricultural activities.

In this framework, in accordance with the Habitats Directive, it is necessary to keep alive traditional agriculture and pastoralism, also by the way of Common Agricultural Policies measures, dedicated to the preservation of traditional practices in marginal or mountainous areas.

The Mediterranean population, as in many other sites of this biogeographical region, heavily suffers from land abandonment. The farming procedures that have allowed this and other similar populations to survive until our times have a very uncertain future in these areas. Poor lands have been cultivated and grazed until the beginning of this century, but today very few families of farmer are resident and may guarantee the persistence of grazing herds in these meadows, which are increasingly constricted by the advance of forest.

This paper shows that grazing should be preferred to a generalised mowing, but only if managed with low cattle numbers. Nevertheless, low intensity grazing or mown avoiding the first part of July (to respect larval development) represent both correct management systems.

In order to optimize chances of success we ought to provide incentives for the existing practices, without trying to upset the traditions of the few remaining resident households. It is also necessary to attract new young farmers to secure the future of this and many other species. Only if farmers remain, we can ensure the persistence of the sources of nectar as well as of the larval food plants crucial for the survival of *E. aurinia*. Maintaining open areas linked to traditional agricultural activities carried out by individual households has produced a fragmented landscape with patches of suitable habitat surrounded in a forest matrix. On the one hand, this type of management has almost created a series of barriers between patches

that a more appropriate management will have to break down by creating corridors. On the other hand, however, the preference shown by adults for the areas not too far from the forest margins indicates that a series of suitable fragmented patches may represent an ideal solution. Therefore it is still desirable to maintain habitat heterogeneity.

Also in the Alpine system, habitat heterogeneity has to be guaranteed. Indeed, a mosaic landscape, characterised by high density of nectar sources, shrubs coverage and larval host plants, alternating within more xeric mild slopes and wetter, flatter areas, represents the optimal habitat for *E. (a.) glaciegenita*. Such a mosaic can be partially assured by the natural topography of the mountain ecosystems, but in any case, even here, human intervention has to be controlled and managed. We observed that low intensity grazing, such as the one carried out in our study area, favoured a higher density of females, also creating a micro-habitat characterised by lower grass height and higher percentage of bare ground. Yet, if not properly controlled, grazing would have negative effects on habitat heterogeneity, by reducing in particular the diversity and abundance of nectar sources, but also by damaging the soil conditions necessary for the persistence of the larval host plant, *Gentiana acaulis*.

Finally, by taking into account their different ecologies and requirements, we confirm that *E. (a.) provincialis* and *E. (a.) glaciegenita* represent different Evolutionarily and Ecologically Significant Units (ESU), and that distinct management plans are needed to conserve all the taxa of the *E. aurinia* complex in Italy.

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## SUPPLEMENTARY MATERIALS

### Appendix S1 - *Microhabitat scale*: Microhabitat models

**Table S1.1** Alpine population: GLM model selection.

	(Int)	<i>Gentiana acaulis</i>	<i>Gentiana acaulis</i> (second order)	grass height	grass %	nectar sources %	organic materials %	lichens %	rocks %	bare ground %	shrubs %	df	logLik	AICc	delta	weight	D <sup>2</sup> <sub>adj</sub>
<b>mod3</b>	<b>1.318</b>	<b>0.420</b>	<b>-1.210</b>	<b>-0.238</b>	<b>0.996</b>	<b>0.382</b>	<b>0.398</b>	<b>-0.077</b>	<b>0.017</b>	<b>0.533</b>		<b>10</b>	<b>-128.26</b>	<b>277.4</b>	<b>0.00</b>	<b>0.959</b>	<b>0.234</b>
mod1	1.272	-0.104	-1.238									3	-138.83	283.8	6.33	0.041	0.170
mod2	0.540	-0.915										2	-147.77	299.6	22.16	0.000	0.117
mod5	0.623			-0.407	0.993		0.382	-0.057	0.077	0.433	-0.041	8	-143.90	304.4	26.95	0.000	0.140
mod6	0.615			-0.419	0.789		0.342				-0.097	5	-147.91	306.1	28.64	0.000	0.116
mod8	0.603			-0.457	0.762							3	-150.88	307.9	30.43	0.000	0.099
mod0	0.532											1	-167.39	336.8	59.36	0.000	0.000
mod4	0.532					0.012						2	-167.38	338.8	61.38	0.000	0.000
mod7	0.536							-0.127	-0.097	0.118		4	-166.29	340.7	63.30	0.000	0.007
<b>mod3sing</b>	<b>1.334</b>	<b>0.104</b>	<b>-1.218</b>		<b>0.667</b>					<b>0.260</b>		<b>5</b>		<b>272.8</b>			<b>0.215</b>

**Table S1.2** Mediterranean population: GLM model selection.

	(Int)	<i>Knautia arvensis</i>	nectar sources %	organic materials %	bare ground %	grass height	grass %	df	logLik	AICc	delta	weight	D <sup>2</sup> <sub>adj</sub>
mod1	0.089	0.673						2	-77.39	158.9	0.00	0.716	0.069
mod5	0.089	0.669	-0.059					3	-77.34	160.9	2.01	0.262	0.069
mod8	0.091	0.672	-0.172	0.216	0.014	0.177	-0.214	7	-76.40	167.8	8.92	0.008	0.081
mod0	0.067							1	-83.11	168.3	9.38	0.007	0
mod2	0.067		-0.108					2	-82.94	170.0	11.10	0.003	0.002
mod6	0.067					0.246	-0.026	3	-82.23	170.7	11.78	0.002	0.01
mod3	0.066			0.032	-0.14			3	-82.79	171.8	12.91	0.001	0.004
mod7	0.067			0.122	-0.009	0.27		4	-82.02	172.4	13.51	0.001	0.013
mod4	0.068		-0.323	0.082	-0.056	0.32	-0.26	6	-81.22	175.2	16.31	0	0.023

**Table S1.3** Alpine population: GLMM model selection.

	(Int)	<i>Gentiana acaulis</i>	<i>Gentiana acaulis</i> (second order)	grass height	grass %	flowers %	organic materials %	lichens %	rocks %	bare ground %	shrubs %	df	logLik	AICc	delta	weight	R <sup>2</sup> <sub>m</sub>	R <sup>2</sup> <sub>c</sub>
mod3_mix	1.324	0.473	-1.221	-0.234	1.055	0.416	0.414	0.036	0.565			10	-128.38	277.7	0.00	0.975	0.734	0.734
mod1_mix	0.958	-0.172	-1.193									4	-138.40	285.0	7.30	0.025	0.696	0.726
mod2_mix	0.131	-0.973										3	-146.22	298.5	20.87	0.000	0.192	0.331
mod5_mix	0.069			-0.335	1.040		0.444	0.071	0.526	-0.045	0.190	9	-141.58	301.9	24.22	0.000	0.206	0.392
mod6_mix	0.313			-0.359	0.758		0.382			-0.124		6	-146.98	306.3	28.64	0.000	0.177	0.252
mod8_mix	0.405			-0.385	0.741							4	-150.36	308.9	31.21	0.000	0.151	0.191
mod0_mix	0.192											2	-164.56	333.2	55.49	0.000	0.000	0.102
mod4_mix	0.180					-0.063						3	-164.46	335.0	57.35	0.000	0.001	0.108
mod7_mix	0.104							-0.182	0.169		0.052	5	-162.92	336.1	58.42	0.000	0.017	0.160

**Table S1.4** Mediterranean population: GLMM model selection.

	(Int)	<i>Knautia arvensis</i>	flowers %	organic materials %	bare ground %	grass height	grass %	df	logLik	AICc	delta	weight	R <sup>2</sup> m	R <sup>2</sup> c
<b>mod1_mix</b>	<b>0.089</b>	<b>0.673</b>						<b>3</b>	<b>-77.39</b>	<b>161.0</b>	<b>0.00</b>	<b>0.720</b>	<b>0.121</b>	<b>0.121</b>
mod5_mix	0.089	0.669	-0.059					4	-77.34	163.0	2.05	0.259	0.123	0.123
mod8_mix	0.091	0.672	-0.172	0.216	0.014	0.177	-0.214	8	-76.40	170.1	9.11	0.008	0.143	0.143
mod0_mix								2	-83.11	170.3	9.34	0.007	0.000	0.000
mod2_mix	0.067		-0.108					3	-82.94	172.1	11.10	0.003	0.004	0.004
mod6_mix	0.067					0.246	-0.026	4	-82.23	172.8	11.82	0.002	0.018	0.018
mod3_mix	0.066			0.032	-0.140			4	-82.79	173.9	12.94	0.001	0.007	0.007
mod7_mix	0.067			0.122	-0.009	0.270		5	-82.02	174.6	13.58	0.001	0.022	0.022
mod4_mix	0.068		-0.323	0.082	-0.056	0.320	-0.260	7	-81.22	177.4	16.46	0.000	0.039	0.039

## Appendix S2 - Patch scale: Nearest Neighbour Index (NNI)

**Table S2.1** Mediterranean population: Nearest Neighbour Index (NNI). Males and female densities were calculated as the number of individuals divided per area (hectares). If NNI is equal to 1, the adults distribution is random, while if NNI is equal to 0, the distribution is clustered. Grey cells indicate significant NNIs while the not coloured one correspond to not significant results (these last p-values were not reported but indicated as "-"). Where the number of individuals per patch was lower than 20, we did not compute the index (empty cells).

### *E. (a.) provincialis*

Patch	area (he)	male density	NNI	z	p	female density	NNI	z	p
A1	1.11	65.75	0.55	-18.54	< 0.001	36.94	0.51	-4.96	< 0.001
A2	1.92	12.04	0.88	-0.29	< 0.001	7.85	1.02	0.02	-
A3	2.44	8.61	0.90	-0.22	-	6.56	0.61	-1.33	-
B1	0.90	93.26	0.82	-39.96	< 0.001	48.31	0.78	-2.37	0.017
B2	0.86	124.42	0.66	-15.63	< 0.001	69.77	0.94	-0.65	-
B3	1.39	64.75	0.64	-13.15	< 0.001	25.90	0.65	-2.34	0.0198
B4	0.82	85.19	0.92	-2.91	0.004	41.98	0.86	-1.04	-
B5	1.32	41.67	0.77	-2.04	0.041	19.70	1.05	0.21	-
C1	0.62	112.90	0.75	-16.35	< 0.001	62.90	0.91	-0.47	-
C2	1.48	120.95	0.76	-14.76	< 0.001	45.95	0.81	-1.18	-
C3	1.14	103.51	0.83	-5.99	< 0.001	46.49	0.79	-1.14	-
D1	1.97	17.26	0.51	-4.19	< 0.001	9.65			
D2	1.50	31.33	0.73	-6.23	< 0.001	26.67			
D3	1.99	7.04	0.95	-0.04	-	3.52			

**Table S2.2** Alpine population: Nearest Neighbour Index (NNI). Males and female densities were calculated as the number of individuals divided per area (hectares). If NNI is equal to 1, the adults distribution is random, while if NNI is equal to 0, the distribution is clustered. Grey cells indicate significant NNIs while the not coloured one correspond to not significant results (these last p-values were not reported but indicated as "-"). Where the number of individuals per patch was lower than 20, we did not compute the index (empty cells).

<i>E. (a.) glaciegenita</i>									
Patch	area (he)	male density	NNI	z	p	female density	NNI	z	p
BardSAlt	1.45	77.31	0.48	-13.03	< 0.001	36.58	0.61	-3.68	< 0.001
BardSPr	0.80	97.84	0.58	-4.13	< 0.001	46.41	0.74	-1.23	-
BardE	1.74	117.21	0.43	-35.70	< 0.001	40.79	0.63	-3.35	< 0.001
BardF	1.77	22.60	0.44	-2.65	0.008	11.86	0.93	-0.06	-
BardG	1.37	70.73	0.57	-8.55	< 0.001	47.39	0.85	-1.62	-
BardPonte	5.79	28.69	0.46	-7.71	< 0.001	9.33	0.86	-0.33	-
LoieC	1.66	63.22	0.35	-8.46	< 0.001	24.69	0.76	-0.47	-
LoieD	4.86	48.37	0.38	-31.28	< 0.001	29.64	0.63	-5.79	< 0.001
BardH	1.18	28.71	0.40	-2.24	0.026	10.98	-	-	-
LoieA1	0.49	38.82	0.14	-12.32	< 0.001	2.04	-	-	-
LoieA2	0.76	35.69	0.34	-6.32	< 0.001	13.22	-	-	-



### Appendix S3 - Patch scale: Pattern of co-occurrence

**Table S3.1** Spatial overlap between males and females in the Mediterranean population. "All" indicates all the patch considered together, while subsequent codes refer to each patch in which the expected occurrence is higher than 1.

Obs cooc: observed co-occurrence. Prob cooc: probability of co-occurrence. Exp cooc: expected co-occurrence. P lt: probability of a observed co-occurrence lower than expected. P gt: probability of a observed co-occurrence greater than expected.

Patch	Male	Female	Obs cooc	Prob cooc	Exp cooc	P lt	P gt
All	747	414	302	0.050	124.9	1.000	0.000
A1	43	29	24	0.069	9.3	1.000	0.000
A2	36	19	10	0.013	3	0.999	0.000
A3	35	17	7	0.007	2.1	0.999	0.002
B1	58	42	32	0.178	20.8	1.000	0.000
B2	51	47	33	0.209	22.4	0.999	0.000
B3	63	32	24	0.062	11.2	1.000	0.000
B4	61	40	34	0.235	23.9	1.000	0.000
B5	45	40	22	0.065	10.8	1.000	0.000
C1	50	25	22	0.181	15.1	0.999	0.001
C2	100	48	39	0.136	25.5	1.000	0.000
C3	74	41	35	0.162	22.1	1.000	0.000
D1	33	10	6	0.006	1.5	0.999	0.001
D2	63	16	11	0.032	5.7	0.999	0.005

**Table S3.2** Spatial overlap between males and females in the Alpine population. "All" indicates all the patch considered together, while subsequent codes refer to each patch in which the expected occurrence is higher than 1.

Obs cooc: observed co-occurrence. Prob cooc: probability of co-occurrence. Exp cooc: expected co-occurrence. P lt: probability of a observed co-occurrence lower than expected. P gt: probability of a observed co-occurrence greater than expected.

Patch	Male	Female	Obs cooc	Prob cooc	Exp cooc	P lt	P gt
All	606	426	250	0.024	78.900	1.000	0.000
BardE	90	50	34	0.096	20.700	1.000	0.000
BardF	27	19	12	0.012	2.400	1.000	0.000
BardG	54	51	36	0.093	16.000	1.000	0.000
BardH	22	11	4	0.009	1.500	0.992	0.045
BardPonte	97	49	20	0.012	7.600	1.000	0.000
BardSAlt	58	41	29	0.084	14.200	1.000	0.000
BardSPr	36	28	16	0.101	10.100	0.998	0.006
LoieA2	22	10	3	0.024	2.300	0.828	0.420
LoieC	42	31	22	0.035	6.800	1.000	0.000
LoieD	119	115	68	0.047	25.400	1.000	0.000

## Appendix S4 - Patch scale: Habitat use

**Table S4.1** Model selection table for the whole Alpine population. The second best model present also a second-order effect of solar radiation, but the coefficient is not significant (standard error=0.052,  $p=0.654$ ).

c10: distance to the margin, classified as 10 m increase distance belt. c10<sup>2</sup>: distance to the margin, classified as 10 m increase distance belt (second order effect). rad: solar radiation. rad<sup>2</sup>: solar radiation (second order effect).

	(Int)	c10	c10 <sup>2</sup>	rad	rad <sup>2</sup>	Df	logLik	AICc	delta	weight
<b>modc2r</b>	<b>-1.230</b>	<b>0.839</b>	<b>-0.286</b>	<b>0.663</b>		<b>5</b>	<b>-1604.866</b>	<b>3219.7</b>	<b>0.00</b>	<b>0.712</b>
<b>modcr2</b>	<b>-1.251</b>	<b>0.839</b>	<b>-0.287</b>	<b>0.675</b>	<b>0.023</b>	<b>6</b>	<b>-1604.768</b>	<b>3221.6</b>	<b>1.81</b>	<b>0.288</b>
modc2	-1.240	0.915	-0.300			4	-1638.460	3284.9	65.18	0
modcr	-1.472	0.381		0.686		4	-1644.338	3296.7	76.94	0
modr1	-1.542			0.775		3	-1677.485	3361.0	141.23	0
modr2	-1.567			0.789	0.028	4	-1677.331	3362.7	142.92	0
modc1	-1.484	0.444				3	-1681.904	3369.8	150.07	0
mod	-1.556					2	-1729.135	3462.3	242.52	0

**Table S4.2** Model selection table for the females in the Alpine population. The second best model present also a second-order effect of solar radiation, but the coefficient is not significant (standard error =0.067,  $p=0.316$ ).

c10: distance to the margin, classified as 10 m increase distance belt. c10<sup>2</sup>: distance to the margin, classified as 10 m increase distance belt (second order effect). rad: solar radiation. rad<sup>2</sup>: solar radiation (second order effect).

	(Int)	c10	c10 <sup>2</sup>	rad	rad <sup>2</sup>	df	logLik	AICc	delta	weight
<b>modc2r</b>	<b>-2.153</b>	<b>0.832</b>	<b>-0.300</b>	<b>0.713</b>		<b>5</b>	<b>-1129.13</b>	<b>2268.3</b>	<b>0.00</b>	<b>0.634</b>
<b>modcr2</b>	<b>-2.214</b>	<b>0.831</b>	<b>-0.299</b>	<b>0.731</b>	<b>0.067</b>	<b>6</b>	<b>-1128.67</b>	<b>2269.4</b>	<b>1.09</b>	<b>0.366</b>
modc2	-2.134	0.917	-0.322			4	-1150.58	2309.2	40.89	0
modcr	-2.391	0.354		0.757		4	-1155.33	2318.7	50.39	0
modr1	-2.450			0.837		3	-1174.29	2354.6	86.31	0
modr2	-2.510			0.854	0.068	4	-1173.81	2355.6	87.35	0
modc1	-2.371	0.415				3	-1180.72	2367.4	99.16	0
mod	-2.426					2	-1207.71	2419.4	151.14	0

**Table S4.3** Model selection table for the males in the Alpine population. The second best model present also a second-order effect of solar radiation, but the coefficient is not significant (standard error =0.057, p=0.442).

c10: distance to the margin, classified as 10 m increase distance belt. c10<sup>2</sup>: distance to the margin, classified as 10 m increase distance belt (second order effect). rad: solar radiation. rad<sup>2</sup>: solar radiation (second order effect).

	(Int)	c10	c10 <sup>2</sup>	rad	rad <sup>2</sup>	df	logLik	AICc	delta	weight
<b>modc2r</b>	<b>-1.587</b>	<b>0.832</b>	<b>-0.317</b>	<b>0.686</b>		<b>5</b>	<b>-1421.57</b>	<b>2853.2</b>	<b>0.00</b>	<b>0.673</b>
<b>modcr2</b>	<b>-1.627</b>	<b>0.831</b>	<b>-0.316</b>	<b>0.707</b>	<b>0.044</b>	<b>6</b>	<b>-1421.29</b>	<b>2854.6</b>	<b>1.44</b>	<b>0.327</b>
modc2	-1.587	0.912	-0.334			4	-1451.29	2910.6	57.43	0
modcr	-1.845	0.335		0.716		4	-1460.38	2928.8	75.62	0
modr1	-1.905			0.795		3	-1482.30	2970.6	117.44	0
modr2	-1.949			0.818	0.048	4	-1481.93	2971.9	118.70	0
modc1	-1.845	0.400				3	-1494.33	2994.7	141.50	0
mod	-1.906					2	-1527.13	3058.3	205.11	0

**Table S4.4** Model selection table for the whole Mediterranean population. The second best model present also a second-order effect of solar radiation, but the coefficient is not significant (standard error =0.037, p=0.476).

c10: distance to the margin, classified as 10 m increase distance belt. c10<sup>2</sup>: distance to the margin, classified as 10 m increase distance belt (second order effect). rad: solar radiation. rad<sup>2</sup>: solar radiation (second order effect).

	(Int)	c10	c10 <sup>2</sup>	rad	rad <sup>2</sup>	df	logLik	AICc	delta	weight
<b>modc2r</b>	<b>-0.09481</b>	<b>0.912</b>	<b>-0.627</b>	<b>-0.354</b>		<b>5</b>	<b>-1360.02</b>	<b>2730.1</b>	<b>0.00</b>	<b>0.679</b>
<b>modcr2</b>	<b>-0.1238</b>	<b>0.915</b>	<b>-0.627</b>	<b>-0.311</b>	<b>0.026</b>	<b>6</b>	<b>-1359.77</b>	<b>2731.6</b>	<b>1.50</b>	<b>0.321</b>
modc2	-0.114	0.893	-0.614			4	-1380.07	2768.2	38.08	0
modcr	-0.634	0.438		-0.335		4	-1438.21	2884.4	154.37	0
modc1	-0.644	0.426				3	-1457.56	2921.1	191.06	0
modr1	-0.636			-0.315		3	-1476.38	2958.8	228.69	0
modr2	-0.631			-0.322	-0.004	4	-1476.37	2960.8	230.68	0
mod	-0.643					2	-1494.34	2992.7	262.62	0

**Table S4.5** Model selection table for the females in the Mediterranean population. The second best model present also a second-order effect of solar radiation, but the coefficient is not significant (standard error =0.043, p=0.281).

c10: distance to the margin, classified as 10 m increase distance belt. c10<sup>2</sup>: distance to the margin, classified as 10 m increase distance belt (second order effect). rad: solar radiation. rad<sup>2</sup>: solar radiation (second order effect).

	(Int)	c10	c10 <sup>2</sup>	rad	rad <sup>2</sup>	df	logLik	AICc	delta	weight
<b>modc2r</b>	<b>-1.282</b>	<b>0.807</b>	<b>-0.719</b>	<b>-0.418</b>		<b>5</b>	<b>-947.02</b>	<b>1904.1</b>	<b>0.00</b>	<b>0.604</b>
<b>modcr2</b>	<b>-1.231</b>	<b>0.799</b>	<b>-0.724</b>	<b>-0.499</b>	<b>-0.046</b>	<b>6</b>	<b>-946.43</b>	<b>1904.9</b>	<b>0.84</b>	<b>0.396</b>
modc2	-1.284	0.787	-0.690			4	-966.85	1941.7	37.66	0
modcr	-1.822	0.405		-0.384		4	-993.32	1994.7	90.60	0
modc1	-1.811	0.397				3	-1011.33	2028.7	124.61	0
modr2	-1.736			-0.475	-0.059	4	-1011.12	2030.3	126.21	0
modr1	-1.798			-0.369		3	-1012.25	2030.5	126.44	0
mod	-1.784					2	-1029.90	2063.8	159.74	0

**Table S4.6** Model selection table for the males in the Mediterranean population. The second best model present also a second-order effect of solar radiation, but the coefficient is not significant (standard error =0.037, p=0.283).

c10: distance to the margin, classified as 10 m increase distance belt. c10<sup>2</sup>: distance to the margin, classified as 10 m increase distance belt (second order effect). rad: solar radiation. rad<sup>2</sup>: solar radiation (second order effect).

	(Int)	c10	c10 <sup>2</sup>	rad	rad <sup>2</sup>	df	logLik	AICc	delta	weight
<b>modc2r</b>	<b>-0.364</b>	<b>0.900</b>	<b>-0.610</b>	<b>-0.312</b>		<b>5</b>	<b>-1302.10</b>	<b>2614.2</b>	<b>0.00</b>	<b>0.606</b>
<b>modcr2</b>	<b>-0.408</b>	<b>0.906</b>	<b>-0.610</b>	<b>-0.245</b>	<b>0.041</b>	<b>6</b>	<b>-1301.52</b>	<b>2615.1</b>	<b>0.86</b>	<b>0.394</b>
modc2	-0.378	0.887	-0.600			4	-1317.33	2642.7	28.46	0
modcr	-0.875	0.439		-0.295		4	-1371.55	2751.1	136.90	0
modc1	-0.881	0.431				3	-1386.28	2778.6	164.35	0
modr1	-0.868			-0.280		3	-1407.64	2821.3	207.07	0
modr2	-0.877			-0.267	0.008	4	-1407.61	2823.2	209.02	0
mod	-0.873					2	-1421.56	2847.1	232.91	0



## **4. MULTITAXA APPROACH**

### **4.1 Grazing effects on insect communities in alpine pasture: a multitaxa approach**

#### **INTRODUCTION**

Due to their sensitivity to climatic and environmental variability (Huber et al. 2005; Beniston 2006) mountains are among the most vulnerable, fragile and threatened ecosystems (Dirnböck et al. 2003; Pauchard et al. 2009; Loarie et al. 2009). Thanks to the impressive variety of habitats and climatic conditions along narrow spatial scales, mountains are considered as biodiversity “hotspot” containing heterogeneous biological communities with several different species showing different degree of specialization (Dirnböck et al. 2011; Fernandes et al. 2016). In this framework, the Alps represent a centre of biodiversity for whole the European continent, combining a complex physical and biological evolution with a long history of human presence and exploitation (Chemini and Rizzoli 2003). Human activities on the Alps, started thousand years ago (Bätzing 2015) and pastoralism was one of the first rural activities made by humans on the alpine chain (Maggi 2004), even at highest altitude (Della Casa 1999). Throughout the 20th century the strong human population decline in the Italian Alps led to a collapse of mountain socio-economy (Lasanta et al. 2015). Indeed, the abandonment of traditional practices, such as grazing and mowing activities, became a widespread phenomenon (Tasser et al. 2007; Pellissier et al. 2013; Lasanta et al. 2016) that caused a huge loss of open semi-natural habitats favouring the forest regrowth (Hunziker 1995; Gehrig-Fasel et al. 2007). Simultaneously, some of the remaining open areas, suitable for traditional management, were overexploited (Tasser and Tappeiner 2002; Mottet et al. 2006) threatening the ecological integrity of the grassland.

Grazing activities has an important effect on the structure and composition of grassland vegetation (Olf & Ritchie 1998). Overgrazing determines an increase of nitrophilous species and a dominance of unpalatable species (Edwards & Crawley 1999, Bonato et al. 2005), reducing the floristic diversity and simplifying the vegetation structure. Conversely, low grazing levels act directly on the dominant species, limits the growth of grass encouraging the seed germination and favouring an increase of plant diversity (Olf & Ritchie 1998, Osem et

al. 2002). Therefore, sustainable grazing levels are fundamental to conserve grassland habitats, maintaining high level of biodiversity and preserving the ecological functionality (Vandewalle et al. 2010; Barragan et al. 2011). Several studies have confirmed the importance of traditional pastoral and farming practices in preserving the diversity of invertebrate taxa and grassland habitats (Benton et al. 2003, Tschanrtke et al 2005, Stefanescu et al.2009; Molina et al. 2014). Therefore, invertebrates are important for grassland habitats too responding readily to changes at both landscape and locale scale, acting as good ecological indicators (Viterbi et al. 2013).

The aim of the research consisted in assessing the effects of grazing management at local scale on four macro-invertebrates groups: ground beetles, dung beetles, butterflies and grasshoppers. In particular, I investigated how different grazing levels affected species richness, abundance and functional groups of each taxon.

## **METHODS**

### ***Study area and data collection***

The study was located in a subalpine meadow located at 2000 m a.s.l., in the Ciamosseretto valley, Gran Paradiso National Park (SCI IT1201000; E 7°19'; N 45°32'). The area is characterized by grasslands with different types of herbaceous associations shaped by long time grazing activities. In particular, the investigated pasture is composed of subalpine and alpine grasslands included in Annex I of the Habitats Directive, such as rupicolous calcareous and basophilic grasslands of the *Alyso-Sedion albi* (cod. 6110 HD), siliceous alpine and boreal grasslands (cod. 6150 HD) and species-rich *Nardus* grasslands, on siliceous substrates in mountain areas (cod. 6230 HD). The pasture was historically exploited, but only starting from 1994 the herder conducted regular pastoral activities mainly with cattle and goats. Due to an inappropriate grazing management, the pasture resulted overexploited, and the ecological integrity of the area threatened. The pasture was subjected to different grazing pressures, caused by various factors; i.e., semi-wild cattle management, steep slope location (that enhances the erosion power of cattle trampling), and discharge of the manure overflow along the slope adjacent to the pasture. Consequently, the pasture currently results divided into portions with different pressures and clear differences in their ecological integrity: i) high quality, semi-natural areas, that are well-managed with low intensity, extensive and sustainable grazing (*low grazing*); ii) areas managed by the herder, but with the first traces of



overgrazing (*medium* grazing); iii) highly disturbed areas, with nitrophilous vegetation, bare ground, clear signs of trampling and accumulation of organic waste, in particular near the cattleshed and in the flattest sites, where the cattle usually aggregate and rest (*high* grazing).

In order to evaluate the effects of different grazing levels on the grassland ecosystems, we monitored four taxa considered as good bio-indicators by different authors (McGeoch 1998, Bogliani 2003, Viterbi et al. 2013), such as the Lepidoptera Rhopalocera, Orthoptera, Coleoptera Carabidae and Coleoptera Scarabeidae, in each of the three pressure levels (low, medium, high).

We placed six sampling units (plots, 2 per each pressure level), inside which we tracked a grid-cell (30x30 m) composed of 9 squares (10x10 m) and a linear transect of 200 m in length.

In each plot, we sampled the four target groups with *ad hoc* semi-quantitative census techniques, during summer 2014 and 2015, from June to September.

### ***Sampling techniques***

The butterflies (Lepidoptera Rhopalocera) were monitored using linear transect method within a radius of 2.5 m from the transect (Pollard, 1979). Individuals were captured and released after specific identification, except for specimens of difficult identification, which were retained for subsequent laboratory determination. Five survey rounds were performed from July to September, in the middle of the sunny days.

Grasshoppers (Orthoptera) were sampled according to the "ring counts" method (Gardiner & Chesmore 2005) along a linear transect. The "ring" is a plastic cylinder 50 cm in height, with a 150 cm diameter (about 0.18 m<sup>2</sup>). Walking along the transect, we put on the ground the "ring" 30 times and we collected all the adult individuals, whereas the nymphs were released. We performed five survey rounds carried out between mid-July and the end of September.

We collected ground beetles (Coleoptera Carabidae) using *pitfall traps* (plastic cups, diameter of 7 cm, filled with 10 ml of white vinegar and few drops of detergent as surfactant). The traps were set in the centre of each grid square (10x10 m), making a total of 54 *pitfall traps*. The traps were emptied every two weeks, from June to September, for 7 times per seasons. Specimens were determined at species level by expert taxonomists.

For dung beetles (Coleoptera Scarabeidae) we used *baited pitfall traps* with fresh cattle dung as attractor (Lobo et al. 1998), and we placed them into the centre of each grid square, for a total of 54 traps. *Pitfall traps* were composed of a plastic cup (15 cm of diameter) and they were covered with a metal grid upon which we placed the attractor. We used a mixture of water, salt and few drops of detergent as preservative and surfactant. The traps were active for 48 hours and then inactivated. This operation was carried out every 15 days during, between June and September, for a total of 7 survey rounds.

Microclimatic conditions were measured by data-loggers (Thermochron iButton, DS1922L, Maxim, Sunnyvale, CA, U.S.) that recorded air temperature every hour throughout the field season. They were located in the centre of the study area, at least 1 m above the ground and covered with a white shield.

### ***Data analysis***

To quantify the grazing impact on bio-indicator groups through the whole grazing period (middle of June-middle of September) and to identify potentially different seasonal effects, we did not pool together data coming from the seasonal repetitions and we analysed each taxon independently. I carried out all analysis using R 3.2.2 (R Core Team 2015).

### ***Grazing indicators***

I performed the analyses considering three different time extent: first sampling season (2014), second sampling season (2015) and the two combined seasons (2014-2015). For each taxon an independent analysis were carried out to identify the species indicators of different cattle grazing levels. For this purpose, four species matrices were assembled using abundance data of different species. The Indicator Value (IndVal: Dufrene and Legendre 1997) combines a species mean abundance and its frequency of occurrence in the group of sites. A high indicator value is obtained by a combination of large mean abundance within a group compared to the other groups (specificity) and presence in most sites of that group (fidelity). I combined groups of sites using the function 'multipatt' of the 'indicspecies' package in R (De Cáceres and Legendre 2009, De Cáceres et al., 2010). I performed a logarithmic transformations on all matrices to reduce the influence of extreme values of abundance (Legendre and Legendre 1998) before calculating the index value. I estimated the degree of statistical significance of the association (*p-value*) using a restricted permutation test (n=999).

### *Grazing impacts on species richness and abundance*

To understand which variables affected the species richness and abundance, I analysed them by generalized mixed-effect models (GLMMs) with Poisson distribution, using the day of the year (doy), the day temperatures, the grazing pressure (classified in three categories: low, medium and high) and grazing presence/absence, during the time frame immediately preceding the survey, as covariates. I used "lme4" package (Bates et al. 2015) and including the "plot" and "year" as random effect variables. I selected the best models by using the Akaike Information Criterion corrected for small samples (AICc) and I calculated the  $R^2$ s with "MuMIn" package (Barton 2016), categorized as *conditional* or *marginal*. The *marginal*  $R^2$  represents the variance explained by the fixed factors, whereas *conditional*  $R^2$  could be interpreted as the variance explained by both the fixed and the random factors.

To make all explanatory variables comparable and to verify their independence, I scaled them (Becker et al. 1988) and used only uncorrelated variables (*Pearson correlation*  $r < |0.7|$ ; Dormann et al. 2013) for further analyses. For one case, because "doy" was correlated with temperature (*Pearson correlation*  $r = -0.78$ ,  $p < 0.001$ ), I discarded the second variable from subsequent analyses. Analyses were performed for each taxon separately.

### *Functional traits and grazing pressure*

For the analysis I considered functional groups specific for each taxon. Butterflies were classified according to the feeding habits (monophagous or poliphagous) and vagility (low or high vagility). I summarized ground beetles according to feeding strategies (predators or omnivorous) and by wing length (brachipterous, macropterous or polymorphic form). I combined dung beetles functional traits according to the nesting behaviour and body size (small/large paracoprids or small/large endocoprids). Then, we used Pearson's Chi-squared test in order to assess if functional groups frequencies of each taxon differed significantly among the three classes (low, medium and high) of grazing pressure. I corrected the *p-values* with a conservative Bonferroni's correction.

## **RESULTS**

In the 2014 sampling season we recorded 111 species, among which 42 butterflies, 11 grasshoppers, 41 carabids and 16 dung beetles, whereas in the 2015 we observed 103 species, among which 51 butterflies, 13 grasshoppers and 39 carabids, for a total of. Dung beetles

data are not yet available for the 2015 sampling session, because they are still under analysis by the taxonomist.

### *Lepidoptera Rhopalocera*

During 2014, in the study area I collected 587 individuals of butterflies, belonging to 42 species, whereas in 2015, 656 individuals pertaining to 51 species.

By the IndVal analysis I identified 7 indicators significantly associated to low, medium or high grazing impact (Tab.1). Only *Parnassius apollo* is related to all groups of sites, while three species were associated only to low grazing level and two species linked to medium pasture.

The best generalized mixed-effect model suggested that the cattle grazing is an important factor for butterflies. Indeed, results showed that cow occurrence during sampling session and the grazing levels affected negatively the butterfly richness and abundance (Tab.2). The daily temperature influenced positively both dependent variables (species richness and abundance). The values of  $R^2$  showed that random factors added at least 10% to the variance explained by fixed variables alone.

The Pearson's Chi-squared test showed that butterfly ecological traits differed significantly among different grazing pressures, both in the 2014 and 2015 (Tab. 3). In particular, the results showed highest proportion of poliphagous and less vagile species in low and medium grazing areas, whereas monophagous and more vagile species were mainly linked to high grazing areas.

### *Orthoptera*

In 2014 I collected 332 individuals of grasshoppers, belonging to 12 species, while in 2015, 437 individuals split in 13 species.

Also for grasshoppers, IndVal analysis selected 7 indicator species. Two species were significantly associated to all groups (*Stauroderous scalaris* and *Tettigonia cantans*), four species resulted as indicators for low-medium grazing areas, and one species for high-medium grazing areas (Tab.1).

The grasshopper species richness did not seem to be affected by the selected covariates, having the null model the best AICc value. Conversely, the Orthoptera abundance, compared to overgrazed areas, was significantly higher in medium and low grazing areas, while the day

of the year (doy) had a positive effect.  $R^2$  values confirmed the importance of "plot" and "year" as a random factors (Tab.2).

In both 2014 and 2015, the Pearson's Chi-squared test, did not show any significant differences between Ensifera and Caelifera frequencies, for the different grazing levels (Tab.3).

#### *Coleoptera Carabidae*

In 2014, 1396 ground beetles specimens belonging to 41 species were collected, while in 2015, 1669 for 39 species.

IndVal analysis revealed 7 indicators. Among them only *Laemostenus janthinus coeruleus* was significantly associated to low grazing areas. Three species were associated to medium grazing areas while three other species to the medium-high grazing areas (Tab.1).

The results of the GLMMs were in contrast with the previous results obtained for the other taxa. Indeed, species richness was lower in medium pasture areas compared to the high ones, while the cow occurrence assessed during sampling sessions had a negative effect. Moreover, models highlighted a decrease of species richness through the season and a second order relationship between doys and abundance, that corresponds to a hump-shaped curve, suggesting that the number of individuals peaked during the middle of the grazing period. Another positive and significant factor in affecting abundance was the temperature. The random factors were fundamental to explain variance, mainly for abundance data, increasing of 70% the  $R^2$  value (Tab.2).

The analysis of ecological traits (Pearson's Chi-squared test) showed that feeding strategies were not significantly different between 2014 and 2015. On the contrary, analysing the frequencies of the wing length groups, I observed a significant dominance of brachipterous specimens in high and medium grazed areas (Tab.3).

#### *Coleoptera Scarabeidae*

I performed data analysis for dung beetle group only for specimens collected in 2014. The number of specimens were 1016, belonging to 16 species.

The IndVal analysis identified three indicators. In particular, *Trypocopris vernalis* is indicator of low level of grazing, *Bodilopsis rufa* is associated to high-medium pressure whereas *Tripocopris vernalis* describe all the grazing levels (Tab.1).

The models suggested the importance of the grazing pressure for both species richness and abundance. Like for ground beetles, the best GLMMs showed a negative relationship between doy and species richness and second order doy and abundance. Moreover, species richness was higher in plots characterized by a medium grazing level, respecting to sites more grazed. The presence of cows during sampling session had a negative impact on abundance. Temperature seems to have a negative effect on dung beetle abundance. The random factors were important only for abundance models (Tab.2).

In the analysis of ecological traits, I classified dung beetles according to four functional groups: small paracoprids, small endocoprids, large paracoprids and large endocoprids. Because during the study only one species of large endocoprids was found, I discarded this traits from the analysis. The results of Chi-squared test showed strong differences in frequencies among dung beetles functional groups for the different levels of grazing. Indeed, large paracoprids specimens were significantly more frequent in low grazed areas while small endocoprids in areas with a high grazing level. (Tab.3).

Tab.1 Species indicators of different levels of grazing pressure

Species	IndVal		Groups		
	Index	<i>p</i>	low	medium	high
<b>Lepidoptera</b>					
<i>Aricia eumedon</i>	0.509	0.011		+	
<i>Coenonympha gardetta</i>	0.652	0.002	+		
<i>Erebia alberganus</i>	0.582	0.026	+	+	
<i>Lycaena eurydame</i>	0.551	0.005		+	
<i>Oeneis glacialis</i>	0.340	0.044	+		
<i>Parnassius apollo</i>	0.582	0.035	+	+	+
<i>Plebejus argus</i>	0.593	0.001	+		
<b>Orthoptera</b>					
<i>Chorthippus gr biguttulus</i>	0.714	0.021	+	+	
<i>Gomphoceris sibiricus</i>	0.790	0.022	+	+	
<i>Omocestus rufipes</i>	0.524	0.030		+	+
<i>Omocestus viridulus</i>	0.734	0.027	+	+	
<i>Pseudochorthippus parallelus</i>	0.838	0.023	+	+	
<i>Stauroderus scalaris</i>	0.890	0.007	+	+	+
<i>Tettigonia cantans</i>	0.746	0.014	+	+	+
<b>Coleoptera Carabidae</b>					
<i>Amara lunicollis</i>	0.632	0.001		+	
<i>Calathus fuscipes graecus</i>	0.971	0.001		+	+
<i>Calathus melanocephalus</i>	0.910	0.001		+	+
<i>Laemostenus janthinus coeruleus</i>	0.840	0.001	+		
<i>Metallina lampros</i>	0.650	0.001		+	+
<i>Ophonus laticollis</i>	0.690	0.001		+	
<i>Poecilus lepidus gressorius</i>	0.843	0.001		+	
<b>Coleoptera Scarabeidae</b>					
<i>Bodilopsis rufa</i>	0.706	0.008		+	+
<i>Trypocopris pyrenaicus</i>	0.823	0.033	+	+	+
<i>Trypocopris vernalis</i>	0.662	0.024	+		

Tab.2 Best generalized mixed effect models selected by AIC selection criterion

Response variable	Butterflies		Grasshoppers		Carabids		Dung beetles	
	S	N	S	N	S	N	S	N
intercept	2.056 ( $\pm 0.111$ )***	2.720 ( $\pm 0.130$ )***	2.253 ( $\pm 0.095$ ) ***	1.544 ( $\pm 0.192$ )***	2.042 ( $\pm 0.128$ )***	3.709 ( $\pm 0.415$ )***	1.495 ( $\pm 0.166$ )***	3.330 ( $\pm 0.272$ )***
doy	-0.087 ( $\pm 0.053$ )	-0.026 ( $\pm 0.038$ )		0.110 ( $\pm 0.036$ )**	-0.200 ( $\pm 0.058$ )***	0.001 ( $\pm 0.028$ )	-0.342 ( $\pm 0.084$ )***	-0.364 ( $\pm 0.038$ )***
I(doy <sup>2</sup> )	0.019 ( $\pm 0.061$ )	0.078 ( $\pm 0.044$ )		0.031 ( $\pm 0.039$ )	0.020 ( $\pm 0.056$ )	-0.103 ( $\pm 0.023$ )***	-0.085( $\pm 0.099$ )	-0.156( $\pm 0.046$ )***
temperature	0.203 ( $\pm 0.06$ )***	0.385 ( $\pm 0.039$ )***			0.076 ( $\pm 0.054$ )	0.092 ( $\pm 0.028$ )***	0.017 ( $\pm 0.071$ )	-0.06 ( $\pm 0.030$ )*
grazing (yes/no)	-0.324 ( $\pm 0.111$ )**	-0.452 ( $\pm 0.085$ )***				-0.416 ( $\pm 0.059$ )***		-0.363 ( $\pm 0.118$ )**
intensity (low)				1.17 ( $\pm 0.017$ )***	-0.983 ( $\pm 0.186$ )***		0.0741 ( $\pm 0.193$ )	
intensity (medium)				1.009 ( $\pm 0.168$ )***	-0.082 ( $\pm 0.164$ )		0.393 ( $\pm 0.179$ )*	
df	7	7	3	7	8	7	7	6
R <sup>2</sup> m	0.379	0.472	0	0.584	0.594	0.048	0.437	0.196
R <sup>2</sup> c	0.470	0.622	0.152	0.658	0.631	0.730	0.437	0.565

Tab.3 Person's Chi-squared test performed on ecological traits of indicator groups.

\* low number of frequencies (< 5)

		2014		2015	
<b>BUTTERFLIES</b>		X <sup>2</sup>	p	X <sup>2</sup>	p
stenophagy	mono/poliphagous	16.352	< 0.001	11.319	0.01
vagility	less/more vagile	20.322	< 0.001	34.688	< 0.001
		2014		2015	
<b>GROUND BEETLES</b>		X <sup>2</sup>	p	X <sup>2</sup>	p
feeding strategies	predators/omnivorous	4.748	n.s.	-*	-*
wing shapes	macropterous, brachipterous, polimorphicous	23.391	< 0.001	53.161	< 0.001
		2014			
<b>DUNG BEETLES</b>		X <sup>2</sup>	p		
nesting behaviour and size	large/small paracoprids or endocoprids	271.26	< 0.001		
		2014		2015	
<b>GRASSHOPPERS</b>		X <sup>2</sup>	p	X <sup>2</sup>	p
suborder	ensifera/caelifera	5.536	n.s.	4.423	n.s.



## DISCUSSION

In this study, I tested the effects of cattle grazing on target insect groups inhabiting a subalpine grassland. Since ecological integrity of pasture is threatened by an irregular management of livestock, the aim of this work consisted in evaluating the status of the insect communities and in detecting the best "grazing indicators" in order to improve management activities.

Although biological communities are mainly affected by topographic, climatic and environmental factors, also the intensity of grazing may play an important role in shaping both species richness and abundance (Marini et al. 2009), and the results of my study are consistent with these statements.

All the taxa showed different responses in relation to the grazing regimes, probably due to a different relationship between target groups and vegetation (Bachand et al. 2014). Butterflies and grasshoppers depend generally on floristic structure and composition, whilst ground beetles are largely not directly related to plants. Conversely, dung beetles appeared mainly related to the grazing livestock as their lifecycle can be completed thanks to the manure that accumulates on pastures, although they may be affected by micro climatic conditions too.

Carabids are frequently used to measure the degree of habitat alteration (Rainio and Niemelä 2003). Indeed, also in this case, they showed a higher species richness and abundance in high grazed areas compared to the lower ones. These results were in contrast with many studies, which attested a negative impact of overgrazing regimes on ground beetle communities (Grandchamp et al. 2005, Kaltsas et al. 2013). However, fertilization could indirectly favour ground beetles. Heavy fertilized and stressed plants contain high concentration of aminoacids which attract plant sucking insects (mainly aphids) (Dardeau et al. 2015) which in turn favour the presence of ground beetles that feed on them. Moreover, organic fertilization could favour other soil invertebrates as potential prey of carabids (Bardgett et al. 1998). Indeed, in strongly grazed areas, the most abundant species is the generalist predator *Calathus fuscipes graecus*, which represent about an half of the total specimens collected and resulted as species indicator of high or medium level of grazing.

The overstocking in pasture causes the physical destruction of the sward, the increase of soil nutrients due to the animal fertilization that suppresses the competition among plants, favouring nitrophilous species. As a consequence the resource availability for butterfly are

drastically reduced (Ockinger et al. 2006). Indeed, butterfly species richness was higher in pastures with low and medium level of grazing, where the cow impact did not negatively affect them. Analysing the butterfly ecological traits, poliphagous species colonized mainly pastures with medium and low level of grazing, where the floristic communities were more diversified and structured. Some more vagile species were detected also in more exploited areas but probably their occurrence was due to moving individuals. Among butterflies, *Lyceana eurydame* resulted as IndVal species of medium grazing sites because of the occurrence of some nitrophilous plants, such as *Rumex* sp. and *Poligonum* sp., which are its host plants. In Switzerland, *L. eurydame* adults dispersed towards nearby nectar sources in unmown meadows, before returning to already mown meadows to lay eggs on *Poligonum bistorta* (Erhardt 1992). This behaviour evidenced that heterogeneous environments are needed to support different butterfly life stages (van Swaay et al. 2010). Indeed, some thermophilous species might select grazed areas for thermoregulation or oviposition because attracted by bare ground patches.

Dung beetles are undoubtedly one of the most typical and ecologically relevant group of insects in grazed alpine habitats. Dung beetle habitat selection usually does not depend upon vegetation structure *per se*, as microclimatic conditions are very important for them. Like for other groups, the models showed a negative impact of grazing on dung beetles abundance. Particularly, functional groups with large paracoprids were more damaged by grazing, showing a decrease in their frequencies in highly grazed areas. By exploiting faeces as sources of food, they have an important role in many ecological processes (Hanski 1991, Nichols et al. 2008, Barragan et al. 2011). In particular, they play a crucial role in incorporating the organic material into the soil. The decreasing of large paracoprids beetles compromised the ecological status of pasture, with a consequent loss of some fundamental services, like dung removal, controlling flies and parasites that affect livestock, pet and people and finally seed dispersal (Nichols et al. 2008).

Vegetation composition and structure influence the distribution of Orthoptera species, both at regional and local scale (Fielding et al. 1995) and, according to perturbation, they could shift to macro- and micro-habitat use during their life stages (Huston et al. 1994). In my study, species richness was not affected by any selected variable, whereas the abundance was strongly influenced by different grazing levels. Indeed, I found the highest abundances of individuals in pastures with low or medium level of grazing, which probably offered more suitable microhabitats. IndVal analysis also showed four species associated with pastures with

low and medium level of grazing, because they generally prefer short vegetation (e.g. *Pseudochortippus parallelus*; Guido and Giannelle 2001). Moreover, Orthoptera, laying eggs under the soil, were favoured by short grass. The availability of bare grounds may offer sites for basking (Key 2000), whilst surrounding vegetation provide adequate cover from inclement weather and predation (Gardiner et al. 2002). Both these microhabitat features (bare ground patches and short grass) are typical of pastures with medium or low level of grazing.

## **CONCLUSION**

The results of my work, clearly demonstrate how grazing management influences species presence and richness, also on a local scale. Our compared plots are all located in the same main habitat type (meadows at the boundary between the subalpine-alpine belts) and at the same altitude, but differ in their species composition as a consequence of grazing levels. Indeed, grazing alters micro-habitat, and consequently micro-climate, with a cascading effect on animal biodiversity. It is fundamental, especially in protected areas, where the Park staff can influence the management strategies, to strongly control grazing in order to reduce as much as possible the patches characterised by high grazing levels, and in any case making them surrounded by well-managed portions, with low intensity sustainable grazing. Such areas can act as sources of biodiversity, that can host the most fragile species and furnish starting points for the recolonisation of the most impacted areas.

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