School of Doctoral Studies in Biological Sciences

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Land Abandonment in the Mediterranean
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Effects on Butterfly Communities with Respect to Life History Traits

Ph.D. Thesis

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Annotation

This thesis deals with the effects of changing land use, following landscape abandonment, on butterfly communities in the Mediterranean Basin.

It consists of three case studies. The first focuses on the effects of forest encroachment on butterflies in the Southern Balkans; the second studies butterfly communities in Portuguese 'montados' and the third explores demography and life histories of three co-occurring Papilionidae butterfly species *(Archon apollinus, Zerynthia polyxena* and *Zerynthia cerisy)* in Greek Thrace.

The results describe shifts in butterfly communities, detectable even at the level of individual species life history traits, with increasing forest encroachment. The preference of range-restricted Mediterranean endemics for either grasslands or open woodland formations contributes to falsifying the forested Mediterranean hypothesis, favouring a hypothesis of finely grained landscape mosaic instead. This mosaic is currently threatened by land use change and biodiversity homogenisation. Maintaining habitat and landscape heterogeneity is crucial for conserving the Mediterranean biodiversity hot-spot.

Declaration [in Czech]

Prohlašuji, že svoji disertační práci jsem vypracovala samostatně pouze s použitím pramenů a literatury uvedených v seznamu citované literatury. Prohlašuji, že v souladu s § 47b zákona č. 111/1998 Sb. v platném znění souhlasím se zveřejněním své disertační práce, a to v úpravě vzniklé vypuštěním vyznačených částí archivovaných Přírodovědeckou fakultou elektronickou cestou ve veřejně přístupné části databáze STAG provozované Jihočeskou univerzitou v Českých Budějovicích na jejích internetových stránkách, a to se zachováním mého autorského práva k odevzdanému textu této kvalifikační práce. Souhlasím dále s tím, aby toutéž elektronickou cestou byly v souladu s uvedeným ustanovením zákona č. 111/1998 Sb. zveřejněny posudky školitele a oponentů práce i záznam o průběhu a výsledku obhajoby kvalifikační práce. Rovněž souhlasím s porovnáním textu mé kvalifikační práce s databází kvalifikačních prací Theses.cz provozovanou Národním registrem vysokoškolských kvalifikačních prací a systémem na odhalování plagiátů.

V Českých Budějovicích 2. června 2016

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Jana Šlancarová

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Illustrations by Martina Slouková on book cover are freely inspired by photos by Alain Dervieux, published in Debussche M, Lepart J, Dervieux A. 1999. Mediterranean landscape changes: evidence from old postcards. Global Ecology and Biogeography **8**: 3-15.

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List of papers and author's contribution

The thesis is based on the following papers (listed chronologically):

I. Šlancarová, J., Vrba, P., Plátek, M., Zapletal, M., Spitzer, L., Konvička, M., 2015. Co-occurrence of three *Aristolochia*-feeding Papilionids *(Archon apollinus, Zerynthia polyxena* and *Zerynthia cerisy)* in Greek Thrace. Journal of Natural History 49, 1825-1848 (IF = 0.954).

Jana Šlancarová participated in experiment preparation, led the data collection in the field, was responsible for data assembly and for statistical analysis, as well as for writing the manuscript with contribution of MK.

II. Šlancarová, J., García-Pereira, P., Faltýnek Fric, Z., Romo, H., García-Barros, E., 2015. Butterflies in Portuguese 'montados': relationships between climate, land use and life-history traits. Journal of Insect Conservation 19, 823-836 (IF = 1.911).

Jana Šlancarová participated in preparing of butterfly life-history trait dataset, was responsible for statistical analysis and writing the manuscript with contribution of EGB and ZFF.

III. Šlancarová, J., Bartoňová, A., Zapletal, M., Kotilínek, M., Faltýnek Fric, Z., Micevski, N., Kati, V., Konvička, M., Life History Traits Reflect Changes in Mediterranean Butterfly Communities due to Forest Encroachment. PLoS One 11 (3), e0152026 (IF = 3.702).

Jana Šlancarová participated in experiment preparation, led the data collection in the field, was responsible for data assembly and analysing majority of data, as well as for writing the manuscript with contribution of MK.

Co-author agreement

Martin Konvička, the supervisor of Ph.D. thesis and co-author of paper in Chapter II and III, fully acknowledges the contribution of Jana Šlancarová as the first author and her major contributions as stated above.

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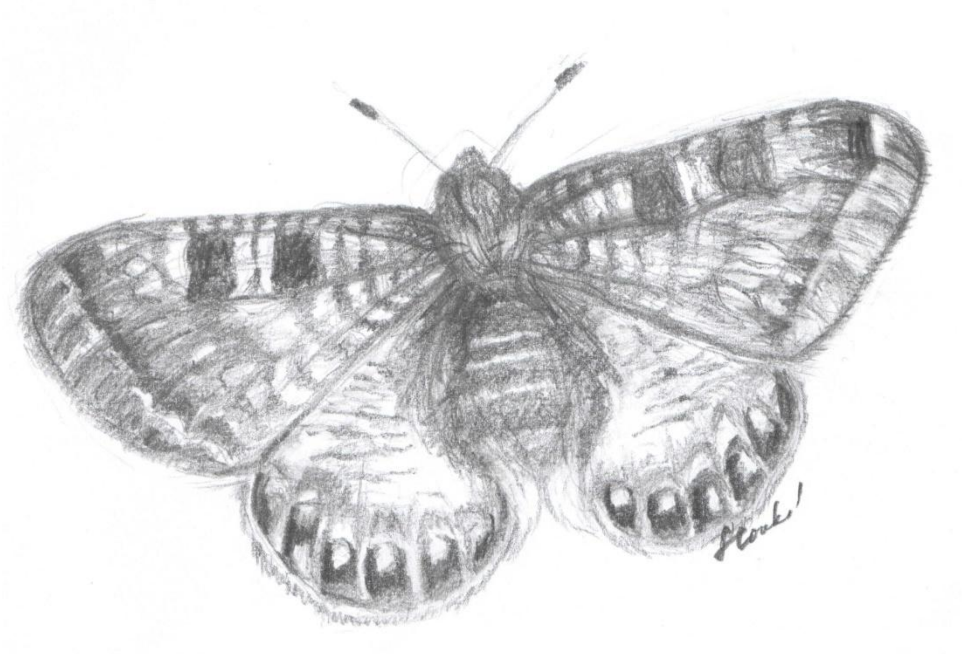
Contents

CHAPTER I

Introduction



Archon apollinus (Herbst, 1789)

The Mediterranean Basin has been cradle to the birth, blooming, and collapse of some of the largest and most powerful civilisations in the world (Blondel 2006). These societies have impacted biota and ecosystems everywhere in the basin for so long that some authors claim that a complex 'co-evolution' has shaped the interactions between these ecosystems and humans (e.g. di Castri 1981; Lepart and Debussche 1992). The high diversity of mountain ranges, gorges, peninsulas and islands creates more complex climatic patterns than anywhere in Europe (Metzger et al. 2005). There is an exceptionally high endemism rate, species richness and threat degree (Myers et al. 2000). These have all played a role in making the Mediterranean Basin one of the world's biodiversity hot-spots (Myers et al. 2000; Thompson 2005).

In the course of history, the region had been maintained by natural disturbances (rainstorms and fires). During the coldest time spans of the Late Pleistocene, mammoth steppe was Earth's most extensive biome (Alvarez-Lao and Garcia 2011a; Guthrie and Guthrie 1990), and cold-adapted large mammal fauna expanded through the southernmost regions of Europe reaching the Iberian Peninsula and northern Greece (Alvarez-Lao and Garcia 2011a; Doukas and Athanassiou 2003; Garcia and Arsuaga 2003; Kahlke 1999). Fauna of that time consisted of a mix of species that would be viewed as peculiar at present consisting of extinct taxa (e.g. woolly rhinoceros *(Coelodonta antiquitatis)*, *Bison* spp., mammoths *(Mammuthus primigenius),* cave bear *(Ursus spelaeus),* Eurasian cave lion *(Panthera leo spelaea),* auroch *(Bos primigenius), Pseudodama* spp., prolago sardo *(Prolagus sardus),* Eurasian wild horse *(Equus ferus ferus),* several species of dwarf hippopotamus and *Elephas* (especially on islands), and, to a lesser extent, species recently distributed in northern latitudes (e.g., reindeer *(Rangifer tarandus),* wolverine *(Gulo gulo)*, arctic fox *(Alopex lagopus)*, musk-ox *(Ovibos moschatus)* and saiga antelope *(Saiga tatarica)*) (Alvarez-Lao and Garcia 2011a, b; Alvarez-Lao et al. 2009; Carbonell et al. 2008; Gomez-Olivencia et al. 2014; Hadjisterkotis and Reese 2008; Martinez-Navarro and Espigares 2003). In the Mediterranean basin, the mammoth steppe reached as far south as to Central Spain, whereas in the Balkan peninsula, the latitudinal mountain ranges separated it from more southern vegetation (Alvarez-Lao and Garcia 2011a; Doukas and Athanassiou 2003), probably formed by a mix of temperate and Mediterranean species and inhabited by interglacial faunal elements (von Koenigswald 2003).

The arrival of *Homo antecessor* together with worldwide Upper Pleistocene megafaunal extinction finally decimated European megafauna (Azanza et al. 2000; Azanza et al. 1999; Carbonell et al. 1995; deCastro et al. 1997; Stuart 1991). Who or what is to be blamed for this extinction, is still under extensive debate, and will not be discussed here in detail. However, according to archeological records, Europe was sparsely populated (Von Koenigswald 1999; Zimmermann 1996), hardly enough to drive healthy populations of mammals to extinction (von Koenigswald 2003). Only if the populations had already been reduced by unfavourable climatic conditions, the last members may have been killed by humans (von Koenigswald 2003).

Following the extirpation of large grazers and the warming of the climate, Mesolithic humans first shifted their activity to coastal areas, sustaining themselves largely on sea resources, supplemented by hunting of forest-tolerant game animals (e.g. red deer, roe deer and wild boar). Later, wild herbivores were supplemented and superseded by domesticated cattle, sheep and goats. Animal domestication in the Fertile Crescent, the origin of most domesticated animals in Mediterranean Basin (Bruford and Townsend 2006; Luikart et al. 2006), continued over a period from ca. 11 000 to 10 000 BP (Zeder 2008). In the Aegean, components of the full Neolithic package (plant and animal domesticates) were carried by maritime colonists at ca. 9 000 to 8 000 BP (Broodbank 1999; Pérles 2001). An additional ca. 1000 to 2000 years were needed for domestic animals to reach France (Fernandez et al. 2006).

Further development of human cultures, epitomised by "Neolithic revolution" affected the landscapes by producing intermediate disturbance regimes through livestock husbandry, grazing and browsing, wood-cutting and coppicing, controlled burning, plant domestication as well as water management and terracing (Blondel 2006). Later, they established sustainable agro-silvo-pastoral ecosystems.

Current views of the relationship between humans and ecosystems in the Mediterranean Basin are split into two contrasting theories. The 'Ruined Landscape' or 'Lost Eden theory,' argues that human-caused deforestation and overgrazing resulted in the cumulative degradation and desertification of Mediterranean landscapes (e.g. Thirgood 1981). The second viewpoint argues that savannah-like landscapes are fairly characteristic of remnant natural vegetation in the Mediterranean Basin. The high representation of endemics depending on non-wooded conditions together with the presence of large herbivores suggests that in a hypothetical pristine state, sizeable parts of the region would have been covered in open steppe habitats (Collins et al. 2012; Grove and Rackham 2003) and agro-silvo-pastoral ecosystems maintained or even enhanced landscape and habitat diversity (Grove and Rackham 2003).

These pastoral systems traditionally took different forms, depending on resource availability, local physical factors, and cultural traditions: (i) sedentary livestock raising, involving a combination of stall feeding and free grazing, (ii) semi-nomadic pastoralism whereby the whole household moved with the herd, or (iii) transhumance where only individual herders moved with the stock (Blondel 2006). Transhumance is remarkably well-adapted to areas with high topographic relief, involving biannual movements of herds and flocks between a lowland area and high summer pastures (Blondel 2006). Over time, two major systems arose in the Mediterranean Basin: *Sylva-saltus-ager* and *Dehesa-montado* systems. The first was widespread over most of the former Roman Empire, including the Balkans, while the later characterised land use practices in Spain and Portugal (Blondel 2006). The two systems provided many of the same basic advantages, but they differed in the spatial organisation of three main activities—cultivation, grazing, and harvesting of forest products (Blondel 2006). In the *Sylva-saltus-ager* system, the three activities were conducted in separate areas, in contrast to the *Dehesa-montado* system where the activities were combined within a single area (Blondel 2006).

Unfortunately, during the last century, the agro-silvo-pastoralism system has gradually transformed (particularly decrease in goat and sheep grazing and wood cutting), leading to new landscape structures and dynamics as well as to an overall decrease in biological diversity (Blondel 2006; Sirami et al. 2010). The scale of the problem varies among countries with different socio-political situations.

In Greece, traditional land use began to decline in the beginning of the 20th century and later in the 1970s (e.g., 43% decline in rough grazing: Hadjigeorgiou et al. 2005), when the agricultural income crisis led to massive migration from hilly and mountainous areas (Petmezas 2009). In Bulgaria, farming declined following the fall of communism in the early 1990s, after the privatisation of the land and the transition process from local to a centralised market economy (Milenov et al. 2014), whereas in the Former Yugoslav Republic of Macedonia (hereafter Macedonia), traditional farming still persists. In France, Sirami et al. (2010) documented that although land cover in their study area changed gradually (70% of the total study area, but 2.2% per year), over 74% changed between 1946 and 2002.

Overall, during the last century, agro-pastoral land use systems have been replaced by systems in which intensive agriculture and urban development occur on the productive plains while the marginal areas at higher elevations have been abandoned (Sirami et al. 2010). Land abandonment triggers vegetation succession, leading progressively to the replacement of grassland by shrubland and eventually woodland (Debussche et al. 1999; Romero-Calcerrada and Perry 2004). Such forest transition involves large changes in landscape structure and functioning. As peasant management of integrated agro-sylvo-pastoral systems disappeared, the landscape became less diverse. (Marull et al. 2015). Moreover, European Union (EU) conservation policies do not always respond appropriately to rapid changes in farming patterns in some regions (Dover et al. 2010). For these reasons, land abandonment along with agricultural intensification (Azcarate and Peco 2012; Donald et al. 2001; European Union 2012; Maes and Van Dyck 2001; Mazzoleni et al. 2004; Sirami et al. 2008; Van Dyck et al. 2009) and subsequent forest encroachment in less fertile remote areas (Beaufoy et al. 1994; MacDonald et al. 2000; Strijker 2005) are recognised as major threats not only for Mediterranean, but even for European biodiversity (Lepart and Debussche 1992; Stoate et al. 2009).

I investigated the impacts of the forest encroachment phenomenon on butterflies, as they represent one of the best model systems in biodiversity research (Settele et al. 2009; Thomas 2005; Van Swaay et al. 2006). They are herbivorous insects, therefore they represent the majority of terrestrial life on earth and play an important role in the functioning of ecosystems. Further, and largely owing to the enthusiastic work of citizen scientists, knowledge of their systematics, phylogeny, biology, and ecology is higher than in any other insect group of comparable species richness, especially in central and northern Europe. On the contrary, in southern Europe, detailed life-history data are still missing for some species, including unique endemics, and therefore also fundamental bionomics studies are still needed.

Existing larger-scale studies about the negative impacts of forest encroachment on butterflies remain restricted to the Iberian (Herrando et al. 2016; Stefanescu et al. 2011; Stefanescu et al. 2004) and Apennine (Bonelli et al. 2011) peninsulas, while only local-scale studies exist for the Balkan Peninsula (Grill and Cleary 2003; Grill et al. 2005; Kati et al. 2012). In general, land abandonment initially increases the species richness, as open woodland species supplement grasslands (Preiss et al. 1997; Sirami et al. 2007), however this results in a rapid open habitats species decline, e.g, due to particular host plant associations, nectar requirements, or temperature requirements (Grill et al. 2005; Kati et al. 2004; Romo et al. 2014; Sirami et al. 2010; Zakkak et al. 2014; Zografou et al. 2015; Zografou et al. 2014). Eventually, as newly established dense forests prevail, only a few closed forest (usually widespread) species persist (Fonderflick et al. 2010). This is well known for birds (Fonderflick et al. 2010; Sirami et al. 2008; Zakkak et al. 2015), but only recently confirmed for butterflies (e.g. Slancarova et al. 2016). Thus, biotic homogenisation is of increasing concern, whereas local beta-diversity is declining (Dormann et al. 2007; Ekroos et al. 2010; Warren et al. 2001). In essence, this means a loss of sensitive species (specialists), which are replaced by generalists (butterflies in Romania: Cremene et al. 2005; Spain: Fileccia et al. 2015; Greece: Slancarova et al. 2016; and Italy: Stefanescu et al. 2011).

Intuitively meaningful, the concept of a generalists-specialist continuum in butterfly ecology obtained strong support from studies of life history (functional) traits (e.g. Bartonova et al. 2014; Borschig et al. 2013; Ekroos et al. 2010; Eskildsen et al. 2015). These are defined as any attributes of individual species that have a potentially significant influence on establishment, survival, and fitness of the species (Reich et al. 2003). At the same time, they evolved as a response to various abiotic conditions and competitive relationships (Reich et al. 2003). Traits should vary more between than within species, preferably be measured on continuous scales (McGill et al. 2006) and can be used at large scales crosswise between different taxa and environment gradients (Statzner et al. 2001; Westoby and Wright 2006). Recently, they gained attention as an alternative path for detecting changes in biological communities from a functional point of view (Carnicer et al. 2013; Dennis et al. 2004), as there is still considerable debate about whether community ecology with classical species turnover approaches will ever produce general principles (McGill et al. 2006).

Functional traits analyses may clarify changes in communities along various environmental gradients (Pekin et al. 2011) or succession progression, often following a generalist-specialist continuum (Carnicer et al. 2013; Dennis et al. 2011; Ockinger et al. 2010). However, the particular direction and intensity of such gradients, the precise traits involved and the responses of the specialist and the generalist species vary across regions on a large scale (e.g. within Europe, cf. Bartonova et al. 2014; Ockinger et al. 2010). For butterflies, this continuum may in fact be correlated with vegetation structure and composition (including forest cover or land management patterns) as well as with altitude, temperature and the density of resources (Bartonova et al. 2014; Menendez et al. 2007; Stefanescu et al. 2011). In the case of butterflies in altered landscapes, traits such as low number of generations, low mobility and narrow trophic range collectively define specialism (e.g. Eskildsen et al. 2015). The butterflies tending to decline are usually associated with small ranges (e.g. Borschig et al. 2013; Curtis et al. 2015). Another major gradient, defined by the association between larval host plant growth form, butterfly body size and yearly generation numbers, defines the tendency to occur in wooded versus non-wooded habitats (Bartonova et al. 2014).

Recently, butterflies and moths life-history traits are often used to predict extinction risk and distribution change. They help to improve the explanatory power of statistical models (Ockinger et al. 2010) and facilitate an understanding of the biological connections between threats and traits (Barbaro and van Halder 2009; Mattila et al. 2008; Slade et al. 2013).

Generally speaking, threatened species are characterised by a combination of the following life traits: restricted range, overwintering as eggs or larvae, low mobility, monophagy and short flight periods (Barbaro and van Halder 2009; Mattila et al. 2008). However, these claims have been obtained by studying relatively impoverished temperate or northern faunas, and evidence from other regions and biomes are much needed.

Objectives and content of the thesis

In this thesis, I studied two phenomena using butterfly life-history traits.

The first – entirely predictable, but newly documented from the eastern Mediterranean – was the impact of forest encroachment on butterflies (Chapter II). Forest encroachment usually follows agro-pastoral land abandonment. I worked on a regional scale, in three countries in the Southern Balkans – Bulgaria, Greece, and Macedonia. Since the landscape changes are long-term processes, time scale was replaced by space replications by comparing matching sites in different successional stages. Specifically, I explored the impact of forest encroachment on (a) butterfly diversity patterns, (b) butterfly community composition, and (c) butterfly life history traits. I hence investigated the ecological and functional response of butterfly communities to land abandonment/forest encroachment in an integrated way, in order to provide specific proposals for their conservation.

The second – less known phenomenon – *Dehesa-montado* system, characteristic of the Iberian Peninsula and increasingly recognised as well-adapted and economically viable multiple-use agro-ecosystems for promoting sustainable modern development in many farming areas of the Mediterranean Basin (Blondel 2006). Chapter III summarises the results of a study on butterfly diversity and life-history gradients carried out in an agricultural system of special interest from several points of view (cultural, historical, conservation, traditional management): the 'montado' oak fields of South Portugal. I followed three threads—one based on species richness, the other on abundance and the last on species community composition.

Finally, I focused on three sympatric Papilionidae butterflies with known life-history details, and explored what more can be revealed by a short-term, but intensive, bionomics survey (Chapter IV). *Archon apollinus* (Herbst, 1789)*, Zerynthia cerisy* (Godart, 1824) and *Zerynthia polyxena* (Denis &

Schiffermüller, 1775*)*, all develop on *Aristolochia* plants and co-occur sympatrically in Greek Thrace. I used mark-recapture to describe adult demography and dispersal, and searched for eggs and larvae to assess host plants and microhabitat preferences.

Because there is, even within Europe, a known disproportion that low-biodiversity northern countries produce high numbers of biodiversity researchers, whereas high biodiversity southern regions remain less explored, I hope that my current work will help to transfer the increasingly good knowledge of insect conservation principles, obtained in northern and central Europe, to the megadiverse southeast.

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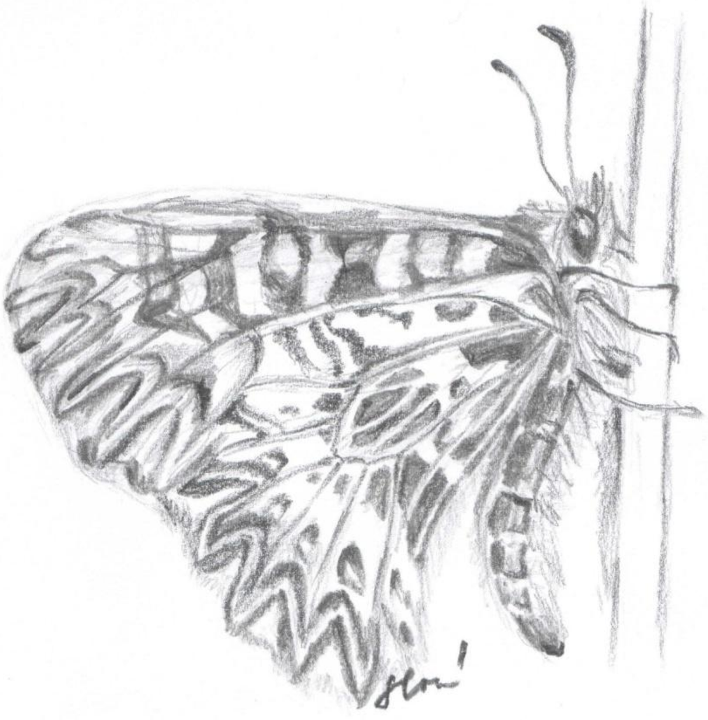
CHAPTER II

Life History Traits Reflect Changes in Mediterranean Butterfly Communities due to Forest Encroachment



Zerynthia polyxena (Denis & Schiffermüller, 1775)

Šlancarová, J., Bartoňová, A., Zapletal, M., Kotilínek, M., Faltýnek Fric, Z., Micevski, N., Kati, V., Konvička, M.

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Abstract

The biodiversity of the Southern Balkans, part of the Mediterranean global biodiversity hot-spot, is threatened by land use intensification and abandonment, the latter causing forest encroachment of formerly open habitats. We investigated the impact of forest encroachment on butterfly species richness, community species composition and the representation of life history traits by repeated seasonal visits of 150 one-hectare sites in five separate regions in three countries – Greece, Bulgaria, and the Republic of Macedonia (FYROM – the Former Yugoslav Republic of Macedonia) – 10 replicates for each habitat type of grasslands, open formations and scrub forest within each region. Grasslands and open formations sites hosted in average more species and more red-listed species than scrub forest, while no pattern was found for numbers of Mediterranean species. As shown by ordination analyses, each of the three habitat types hosted distinct butterfly communities, with Mediterranean species inclining either towards grasslands or open formations. Analysing the representation of life history traits revealed that successional development from grasslands and open formations towards scrub forest shifts the community composition towards species overwintering in earlier stages, having fewer generations per year, and inhabiting large European or Eurosiberian (e.g. northern) ranges; it decreases the representation of Mediterranean endemics. The loss of grasslands and semi-open formations due to forest encroachment thus threatens exactly the species that should be the focus of conservation attention in the Mediterranean region, and innovative conservation actions to prevent ongoing forest encroachment are badly needed.

Introduction

The Mediterranean region of Europe is a global biodiversity hot-spot, due to its exceptional endemism rate, species richness and threat degree [1]. The high diversity of mountain ranges, gorges, peninsulas and islands creates more complex climatic patterns than anywhere in Europe [2]. The great diversity of Mediterranean ecosystems has been further augmented by human activities. As a cradle of human civilisation, the region had been affected, in chronological order, by large herbivores extirpation, forest clearance, pasture, farming, and urbanisation [3]. Although the character of its natural vegetation is still disputed, the high representation of endemics depending on non-wooded conditions suggests that in a pristine state, sizeable parts of the region would be covered by open habitats, maintained by rainstorms, fires, and herbivore actions [4]. In the course of history, traditional agro-pastoral land uses replaced these natural disturbances, maintaining or even enhancing landscape and habitat diversity.

Agricultural intensification in fertile lowlands [5] along with land abandonment and subsequent forest encroachment in less fertile remote areas [6, 7] are recognised as major threats for European biodiversity [8]. Forest encroachment represents a particular risk in the Mediterranean, given the high regional endemism associated with open habitats [9]. The whole situation is complicated by rapid changes in farming patterns in some regions, to which the EU conservation policies do not always respond appropriately [10]. Financial incentives for afforestation too often reflect the "forested Mediterranean" paradigm [cf. 4]. The scale of the problem differs among individual countries. For instance, traditional land use began to decline in Greece with economic growth in the 1970s [e.g., 43% decline in rough grazing: 11], whereas in Bulgaria, farming has declined following the fall of communism in early 1990s, and in the Republic of Macedonia, traditional farming still persists.

The impact of forest encroachment on Mediterranean invertebrate diversity is poorly studied. Forest encroachment is expected to have a negative effect on invertebrate taxa that prefer open habitats, e.g, due to particular host plant associations, nectar requirements, or temperature requirements [12–14]. For butterflies in particular, a large-scale study from the Iberian Peninsula [15] demonstrated the negative effect of marginal land abandonment, and subsequent forest increase – but no such studies exist from the Apennine and Balkan peninsulas, although data from a Greek nature reserve point to the same pattern [16]. Land abandonment initially increases the species richness, as open woodland species supplement grasslands [17]. This is followed by a loss of sensitive species, which are replaced by generalists [butterflies in Romania and Spain: 18, 19]. Eventually, as new dense forests prevail, only a few closed forest species persist.

Species life history traits have recently gained attention as an alternative path to detect changes of biological communities from a functional point of view, at finite and large scales, besides the classical species turnover approaches [20-22]. In butterflies, traits such as a low number of generations, low mobility and narrow trophic range collectively define specialism, associated with small ranges and a tendency to decline in altered landscapes [e.g. 23]. Another major gradient, defined by the association between larval host plant growth form, butterfly body size and yearly generation numbers, defines the tendency to occur in wooded versus non-wooded habitats [24].

This paper examines the impact of the forest encroachment process that usually follows agro-pastoral land abandonment on butterfly communities on a regional scale, considering three countries in the Southern Balkans: namely Bulgaria, Greece, and the (Former Yugoslav) Republic of Macedonia (hereafter Macedonia). We explore the impact of forest encroachment on (a) butterfly diversity patterns, (b) butterfly community composition, and (c) butterfly life history traits. We hence investigate the ecological and functional response of butterfly communities to land abandonment/forest encroachment in an integrated way, in order to provide specific proposals for their conservation.

Materials and methods

Ethics statement

The study was carried out in accordance with the national laws and permits obtained from authorised institutions: Bulgaria (National Museum of Natural History, Sofia), Greece (Ελληνική Δημοκρατία, Υπουργείο Περιβάλλοντος, Ενέργειας & Κλιματικής Αλλαγής, Ειδική Γραμματεία Δασών, Γενική Διεύθυνση Ανάπτυξης & Προστασίας Δασών & Φυσικών Πόρων, Δ/νση Αισθ. Δασών, Δρυμών και Θήρας Τμήμα Γ & Β, No. 170916/1344), and Macedonia (Bird Study and Protection Society of Macedonia). The fieldwork was not carried out in any privately owned nor protected areas. All the butterflies were carefully handled and released after identification; we collected up to five individuals per visit only for taxa not identifiable in the field, for genital preparation and species identification in the lab (not applicable regarding protected species).

Site selection

Our study area was located in the Southern Balkans, encompassing five regions (R1–R5): three in Greece, one in Macedonia, and one in Bulgaria (Fig 1), (S1 Text). We predefined three forest encroachment categories, in terms of woody vegetation cover (> 1.5m), with the help of post-2010 aerial photographs: (a) *Grasslands* – herbaceous vegetation dominance and a woody plant cover less than 5% with tracks of active grazing, (b) *Open formations* – near-even representation of woody and herbaceous cover, (c) *Scrub forest*, with dominance of woody plant vegetation above 70%. We located 30 sampling sites of 1ha standard area in each region (altitude from 10 to 1100 m a. s. l. (mean 440 ± 254 SE)), so as to equally represent the three forest encroachment categories, resulting in an overall number of 150 sites sampled.

Butterfly sampling

We sampled butterflies during four visits in early spring (April/May 2012), late spring (May/June 2012), summer (July 2013), and late summer (August/September 2012), to well cover butterfly phenology [25].

We recorded butterflies in terms of timed surveys [26] lasting 30 person-minutes per site between 9:00–17:00, under suitable weather, using semi-quantitative abundance categories (1, ≤ 5, ≤10, ≤20, ≤50, ≤100, >100 individuals). For taxa not identifiable in the field, we collected up to five individuals per visit for genital preparation and species identification in the lab. Butterfly nomenclature follows Fauna Europaea [27], Red List categorization according to Van Swaay et al. [28] (S3 Table).

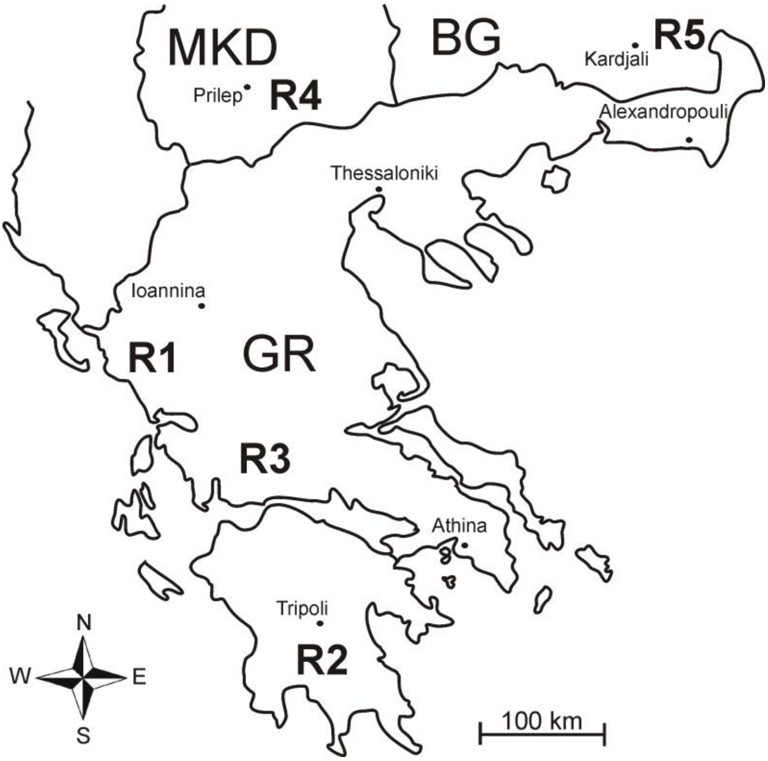


Fig 1. A map of the Southern Balkans showing the five study regions where impacts of Forest encroachment on butterflies were studied.

R1 – foothills of Paramythia Mts, NW Greece (Epirus province); R2 – foothills of Taygetos Mts., S Greece (Lakonia); R3 – southern foothills of Giona Mts, Greece (Sterea Ellada); R4 –Macedonia, Prilep environs; R5 – SE Bulgaria (Kardzhali environs). Average aerial distance among regions was 220 km (range 143 – 320 km)

Life history traits

We considered 15 life history traits for butterflies readily available in literature and reflecting (a) specialism *vs.* generalism (*Feeding index, Flight period, Generation numbers, Migration, Overwintering stage, Wingspan*), (b) larval feeding habits (*Gregariousness, Host plant form, Larval feeding mode, Myrmecophily, Ovum placement*), as well as (c) distribution profile (*Altitudinal range, Mountain distribution, Range size, Range type*). These functional traits are linked with the resilience of the species to environmental or land use change and hence its inherent vulnerability tendency (S4 Table). Information on most of the traits is directly available in literature, or, as in the case of *Feeding index*, easily calculable from published data (see S5 Table). An exception was information on range size, where we used simple numeric coding based on published distribution maps (see S5 Table for references).

Environmental parameters

We collected the following 15 environmental parameters for each site sampled. Two variables were collected to describe the forest encroachment gradient and were inserted in the model as predictors, namely: *Forest encroachment* was categorically variable with tree levels (*Grassland, Open formations, Scrub forest*); *Canopy* cover (percentage cover of woody species >1.5m) was a continuous predictor.

Another set of twelve variables was used as covariables in the model to describe (a) the geographical position (*Latitude* (mean: 22.54 ± 1.70), *Longitude* (39.60±1.78)), (b) the topography (*Altitude* (*m*) (441.89±254.07)), *Slope* (three categories: 1: flat (<15%), 2: sloping (<30%), 3: steep (>30%)) and *Exposure* (ranked: SW, S – 5; SE, W – 4; flat – 3; NW, E – 2; N, NE – 1)), (c) the site humidity (*Water* presence (binary value)), (d) human presence and grazing intensity (*Road* presence (binary value), nearest *Village* distance (m) (1610±1014), nearest *Herdsman's hut* distance (m) (825±631)), as well as (e) *Vegetation composition* (four variables: *Veg1–Veg 4*, which were obtained by recording all vascular plants species with their relative covers (1–3 scale) at each site for a standard time of 60 min during May–

June 2013 [29] , subjecting thus recorded data to principal component analysis (PCA) and extracting values of four PCA axes (details: S2 Figures)). For each site visit, we recorded further parameters describing momentary weather conditions, namely *Air temperature*, *Cloudiness* (1: clear to 3: half-sunny), *Wind* (1: calm to 3: moderate breeze), as well as momentary *Nectar* supply, using a simple ranked scale (1: none or a few isolated flower heads, 2: isolated flowering patches, 3: whole site in bloom).

Data analysis

We transformed the recorded semi-quantitative butterfly abundances to mean numbers of individuals within the respective quantitative intervals, summed this across the four visits, and log-transformed. *Air temperature*, *Cloudiness*, *Wind* and *Nectar* were also summed across the four visits to obtain more detailed scales.

We applied generalised linear models in R [30] (Poisson distribution of the response) to analyze *Forest encroachment* and *Canopy* effects on species richness, numbers of Red-listed species, and numbers of Mediterranean species. For all three response variables, we first tested independent effects of the two primary predictors, considering also polynomial response for *Canopy*. Next, we tested independent effects of all site parameters and visit parameters (i.e., potential nuisance covariables), and used stepwise selection based on all potential covariables, evaluating alternative models' fits according to the Akaike information criterion (*AIC*) to obtain *covariate models*, defined as models best explaining the response variables without referring to the predictor(s) of interest. Finally, we manually forced the predictors *Forest encroachment* and *Canopy* onto the *covariate models,* thus assessing their effects while statistically controlling for variation due to nuisance variables.

To study changes effects on species composition, we used redundancy analysis (RDA), a constrained linear ordination, using CANOCO 5 [31]. We first computed single-term ordinations for both predictors of interest and all covariables. Next, we defined a *covariate model*, based on forward selection from potential covariables. Finally, we computed partial RDAs with predictors *Forest encroachment* and *Canopy*, controlled for effects of *covariate model* terms. We log (x + 1) transformed and centred species abundances in all RDA analyses, and evaluated significances of the ordinations using the Monte Carlo test (999 permutations).

We used the partial RDAs to analyse the life history traits responses. Because life histories co-vary with phylogeny [e.g. 32], we constructed a phylogenetic tree of all recorded species, based on published phylogenies, supplemented by formal classification into genera and subgenera (S6 Text). We turned this tree into a patristic distance matrix, representing the distance of any pair of taxa measured along the branches of the phylogenetic tree. We transformed this distance matrix into a set of descriptors using principal coordinate analysis (PCoA), with PCoA scores centred and standardised. Not all PCoA scores are related to response variable, therefore we used their subset selected by forward selection – only descriptors with $p < 0.04$ were included. Finally, we interpreted the species traits responses to *Forest encroachment* and *Canopy* individually for each trait, after removing the variation explained by phylogenetic descriptors. We evaluated each step using the Monte Carlo test (999 permutations).

Raw dataset for all analyses is available as supplementary material S8 Raw Data.

Results

Species richness patterns

We recorded 128 species in total (R1: 81, R2: 72, R3: 69, R4: 98, R5: 77), including 11 species from the European Red List (R1: 5, R2: 6, R3: 3, R4: 8, R5: 7) (S3 Table). The mean species richness values per site and region were: R1, 23 (SD 7.6, range 12–37); R2, 15 (6.0, 6–30); R3, 17 (4.8, 6–25); R4, 27 (7.6, 11–42); R5, 24 (4.4, 14–32). The regions R4 and R5 hosted significantly higher per site species richness (Kruskal-Wallis $\chi^2 = 50.7$, df = 4, P < 0.0001); whereas the region R2 hosted significantly more endangered red-listed species per site ($\chi^2 = 18.7$, df = 4, P < 0.001).

When tested individually against all predictors and covariables, species richness responded to *Forest encroachment*, being highest in *Grasslands* and lowest in *Scrub forest*, and decreased linearly with *Canopy* cover (Fig 2 (A) and Table 1). Of all potential covariables, *Nectar* had the strongest separate (positive) effect. Regarding site characteristics, richness was highest in intermediate longitudes, and increased with altitude. It also increased with presence of *Water* and *Herdsman's hut*. The strongest site characteristic effect, *Veg1*, pointed to richness increasing with humidity. The combined covariate model (Table 1) explained over 38% of variation in per site species richness. Adding the predictors of interest to this model did not reveal differences among the three stages of *Forest encroachment,* but revealed a significant decline with increasing *Canopy* (Fig 2 (B)).

Numbers of Mediterranean species did not differ between *Forest encroachment* categories nor responded to *Canopy*. They responded to geography covariates and increased at steep *slopes* sites affected by grazing (the combined covariate model explained 39.8 % of variation). Red-listed species, in contrast, responded significantly to both *Forest encroachment* (much lower in *Scrub* forest: Fig 2 (C)) and *Canopy* (Fig 2 (D), polynomial decrease). On the other hand, they did not respond to any covariates except for *Nectar* (3.1% of variation). After controlling for nectar, the effects of *Forest encroachment* and *Canopy* remained significant.

Table 1. Results of regression models assessing the impact of forest encroachment, site environmental variables and visit circumstances on butterfly species richness in the Southern Balkans.

All species					Mediterranean species				Red listed species			
Model	**AIC**	**DF**	**EV %**	**↓↑**	**AIC**	**DF**	**EV %**	**↓↑**	**AIC**	**DF**	**EV %**	**↓↑**
Null model (S ~ 1)	910.93	149	100.0		566.37	149	100		410.01	149	100	
Predictors of forest encroachment												
S ~ *Forest encroachment*	889.11	147	9.30		567.19	147	1.73		405.13	147	6.85	
S ~ *Canopy*	881.26	148	11.40	↓	566.43	148	1.06		402.75	147	8.69	↓↓
Site characteristics												
S ~ *Latitude*	909.94	148	1.07	↓↑	503.32	148	35.41	↓	410.58	148	1.10	
S ~ *Longitude*	836.13	148	27.65	↓↑	560.02	148	4.54	↓	409.88	148	1.64	
S ~ *Altitude*	874.14	148	13.97	↑	561.13	148	3.94	↑	411.8	148	0.16	
S ~ *Water*	901.59	148	4.08	↑	565.03	148	1.82		411.81	148	0.15	
S ~ *Road*	909.48	148	1.24	↑	568.34	148	0.02		411.54	148	0.36	
S ~ *Village*	908.87	148	1.46	↓	567.53	148	0.46		411.89	148	0.09	
S ~ *Herdsman's hut*	897.34	148	5.61	↑	559.37	148	4.90	↓	410.98	148	0.79	
S ~ *Slope*	907.46	148	1.97	↓	558.81	148	5.20	↑	410.35	148	1.28	
S ~ *Exposure*	912.25	148	0.24		568.36	148	0.01		410.38	148	1.26	
S ~ *Veg1*	802.99	148	39.58	↓	565.19	148	1.73	↑	409.67	148	1.81	
S ~ *Veg2*	909.99	148	1.06	↓	567.36	148	0.55		411.19	148	0.63	
S ~ *Veg3*	907.12	148	2.09	↑	567.7	148	0.36		410.73	148	0.99	
S ~ *Veg4*	911.73	148	0.43		564.84	148	1.92	↓	410.83	148	0.91	

Arrows indicate significant (Δ AIC ≥ 2.0) positive (↑), negative (↓), domed polynomial (↑↓) or decreasing polynomial (↓↓) response.

The covariate model, based on stepwise selection from site characteristics and visit circumstances, included the terms *Nectar* + *Latitude*+ *Altitude* + *Veg4*.

EV = Explained Variability, N = Number of butterfly species, S = Species Richness.

Table 1. Continued

All species					Mediterranean species				Red listed species			
Model	**AIC**	**DF**	**EV %**	**↓↑**	**AIC**	**DF**	**EV %**	**↓↑**	**AIC**	**DF**	**EV %**	**↓↑**
Visit circumstances												
S ~ *Air Temperature*	900.33	148	4.54	↓	567.75	148	0.34		411.92	148	0.07	
S ~ *Cloudiness*	910.09	148	1.02	↓	560.38	148	4.35	↓	411.72	148	0.22	
S ~ *Wind*	908.05	148	1.76	↑	557.52	148	5.91	↓	410.21	148	1.39	
S ~ *Nectar*	788.35	148	44.85	↑	568.26	148	0.06		408.04	148	3.06	↑
Covariate model (N ~ *Covariates*)	747.46	145			499.27	146	39.80					
S ~ *Covariates* + *Forest Encroachment*	747.36	143	63.21		501.42	144	40.80		405.63	146	8.01	↑
S ~ *Covariates* + *Canopy*	744.57	144	57.74	↓	499.45	145	40.79		402.84	146	10.16	↓

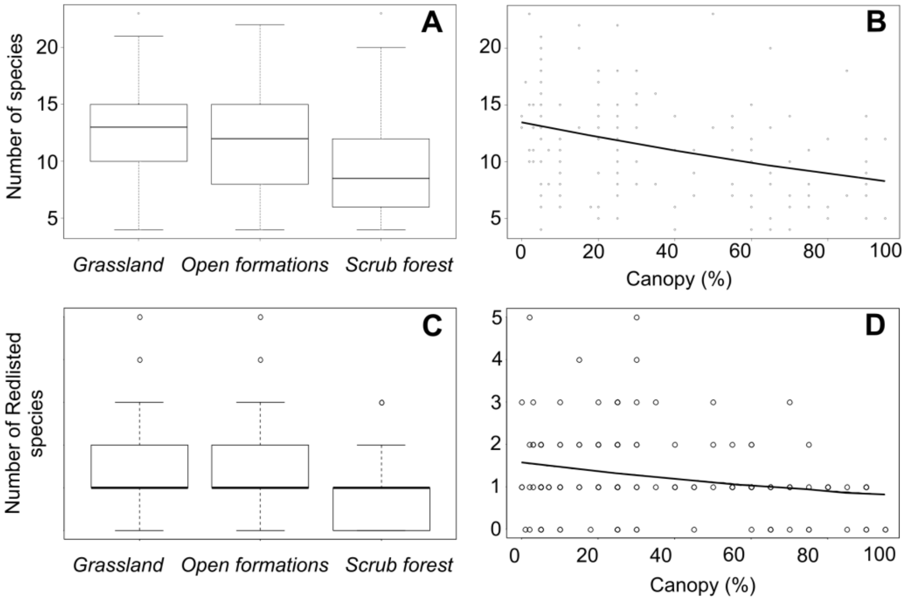
Arrows indicate significant (Δ AIC ≥ 2.0) positive (↑), negative (↓), domed polynomial (↑↓) or decreasing polynomial (↓↓) response.

The covariate model, based on stepwise selection from site characteristics and visit circumstances, included the terms *Nectar* + *Latitude*+ *Altitude* + *Veg4*.

EV = Explained Variability, N = Number of butterfly species, S = Species Richness.

Fig 2. Impact of *Forest encroachment* and *Canopy* on butterfly species richness (A-B) and Red-listed species (C-D) recorded during 2012–2013 from 150 sites in the Southern Balkans.

The box plots show values of species richness (A) and Red-listed species (C) predicted by the generalised linear model (glm) with *Forest Encroachment* treated as 3-level factors, no covariates included. The lines in (B–D) show glm predicted values with *Canopy* covers treated as linear predictor after inclusion of covariates for Species Richness (B) and without covariates for Red-listed species (D). See Table 1 for details.



Species composition

In the single-term ordinations, both *Forest encroachment* and *Canopy* significantly affected species community composition. The explained variations were rather low, however, if compared with covariate predictors such as *Veg1*, *Nectar* or *Longitude* (Table 2). The forward selection procedure selected the following covariate model: *Altitude* + *Cloudiness* + *Latitude* + *Longitude* + *Nectar* + *Slope* + *Veg1–4* + *Water* + *Altitude* × *Latitude* × *Longitude* (36% of variation, F = 6.3, P = 0.001). On residuals of this model, both *Forest encroachment* and *Canopy* retained their significant effects (Table 2).

In the partial RDA with *Forest encroachment* (Fig 3(A)), the gradient described by the RDA axis 1 (1.67% of the variation) separated *Grasslands* from *Scrub forest*, whereas Axis 2 formed a gradient from *Grasslands/Scrub forest* towards *Open formations* (1.37%). The three forest encroachment stages thus hosted distinct sets of species. For *Grasslands*, several Mediterranean species were represented (e.g., *Pieris krueperi*, *Erynnis marloyi*), including red-listed ones (*Carcharodus orientalis*); these were accompanied by species with European or Eurosiberian distribution (e.g., *Polyommatus bellargus*, *Libythea celtis*), including the Red-listed *Iolana iolas*, and by widely distributed generalists (*Polyommatus icarus*, *Papilio machaon*). Open formations hosted distinctly high numbers of Mediterranean species (e.g., *Pyronia cecilia, Polygonia egea*), including Red-listed ones (*Hipparchia syriaca*), and a high number of Red-listed species with more northerly Eurosiberian distribution (*Parnassius mnemosyne*, *Hipparchia statilinus*). Finally, scrub forest hosted prevailingly non-threatened species with northerly ranges (*Argynnis aglaja*, *Aphantopus hyperantus*), although a Mediterranean representative occurred there as well (*Pieris mannii*). In the partial RDA with *Canopy* (1.85%), practically all Mediterranean species, as well as practically all Red-listed ones, inclined towards low *Canopy* (Fig 3(B)).

Table 2. Results of Redundancy analyses analyzing butterfly species composition.

Summary of single-term ordinations of predictors of interest *(Forest encroachment* and *Canopy)* and potential covariates (site characteristics and visit circumstances) as well as partial RDA ordinations assessing the effect of predictors of interest on butterfly species community composition (BSC) after controlling for site characteristics and visit circumstances (see Methods for details).

Table is available on next page.

Footnotes to Table 2.

Covariate model: BSC ~ *Altitude* + *Cloudiness* + *Latitude* + *Longitude* + *Nectar* + *Slope* + *Veg1–4* + *Water* + *Altitude* × *Latitude* × *Longitude.*

AEV = Adjusted explanatory variable (%).

Significance as follows: **** < 0.0001; *** < 0.001; ** < 0.01; * < 0.05; . < 0.1.

Table 2. Continued

Null model (BSC ~ 1)	**AEV**	**F**	**P**
Predictors			
BSC ~ *Forest encroachment*	3.00	3.30	***
BSC ~ *Canopy*	3.30	6.20	***
Site characteristics			
BSC ~ *Latitude*	6.00	10.50	***
BSC ~ *Longitude*	10.90	19.20	***
BSC ~ *Altitude*	4.40	7.80	***
BSC ~ *Water*	0.60	1.90	*
BSC ~ *Road*	0.00	0.90	
BSC ~ *Village*	0.40	1.60	.
BSC ~ *Herdsman's hut*	2.50	4.80	***
BSC ~ *Slope*	2.80	5.30	***
BSC ~ *Exposure*	0.00	0.80	
BSC ~ *Veg1*	12.80	22.80	***
BSC ~ *Veg2*	2.20	4.40	***
BSC ~ *Veg3*	1.40	3.20	**
BSC ~ *Veg4*	2.10	4.20	***
Visit circumstances			
BSC ~ *Air temperature*	2.40	4.70	***
BSC ~ *Cloudiness*	2.20	2.90	**
BSC ~ *Wind*	2.20	4.30	***
BSC ~ *Nectar*	10.70	18.80	***
Covariate model (N ~ *Covariates*)	36.2	6.3	***
BSC ~ *Covariates + Forest encroachment*	1.50	2.00	***
BSC ~ *Covariates + Canopy*	1.00	2.40	***

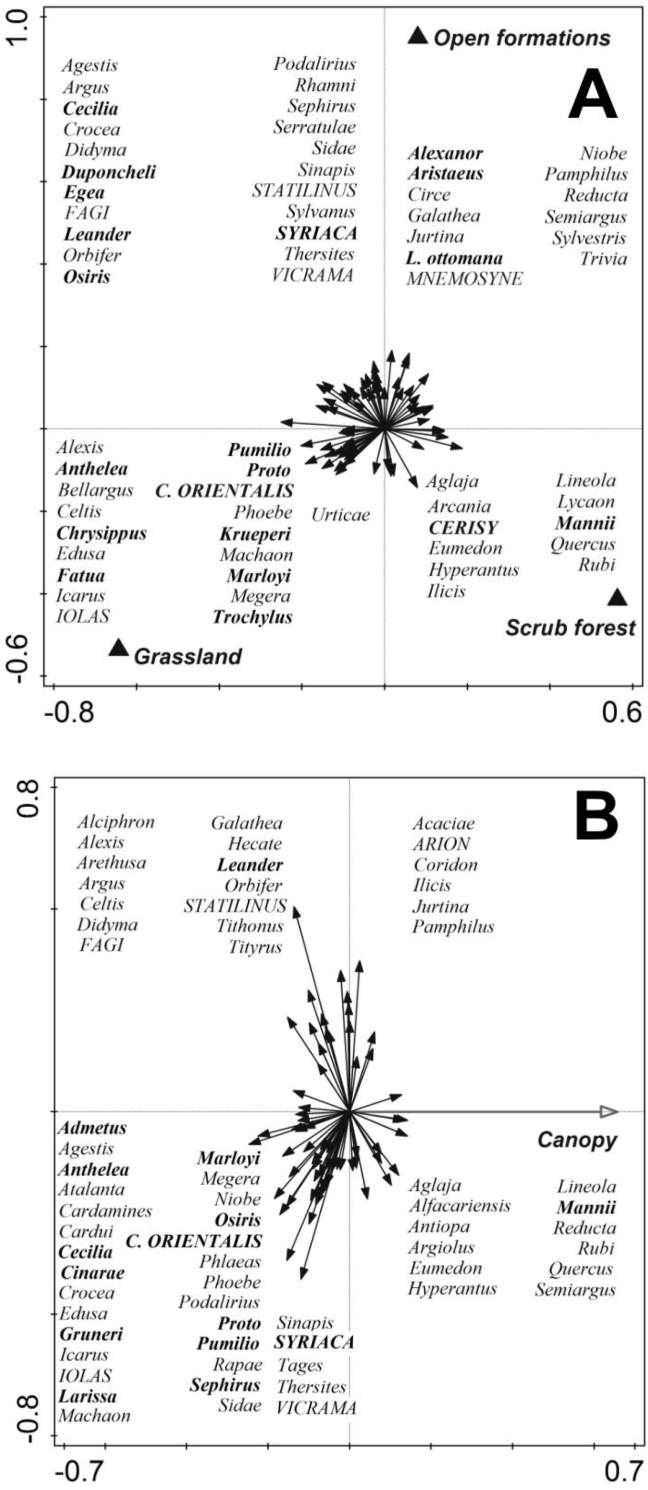


Fig 3. Ordination diagrams (partial redundancy analysis), showing the effect of (A) *Forest encroachment*, and (B) *Canopy* on butterfly species community composition. Both diagrams refer to analyses that statistically controlled for effects of covariates, and removed the effects of phylogeny (covariate model as in Table 2). See Table 2 for results of statistical tests. Species with Mediterranean ranges written in bold, Red-listed species in CAPITALS.

Species traits

Visualization of relationships among the life history traits showed a clear difference between large and mobile species with multiple generations per year, overwintering in later stages, occurring as adults late in season, having wide host plant spectra, and inhabiting large Holarctic or Eurosiberian ranges, and species with opposite traits, typically with restricted Mediterranean or European ranges (S7 Figure). The second gradient distinguished species with multiple generations, developing on forbs and/or consuming generative plant parts, from those forming few generations per year, and feeding on woody plants or grasses.

Only a few traits responded significantly to both *Forest encroachment* and *Canopy* (Table 3). *Generation numbers* responded to both predictors, indicating that closed forests were inhabited by butterflies forming fewer generations per year.

Regarding *Forest encroachment* (Fig 4, (A–D)), species overwintering in earlier stages displayed affinity towards *Scrub forest.* Vegetation closure decreased the representation of Mediterranean and Holarctic species and increased that of European and Eurosiberian species. Spring and autumn species prevailed on *Grasslands*, summer species inclined towards *Open formations* or *Scrub forest.* Regarding *Canopy* (Fig 4, (E–H)), there were marginally significant relationships with *Larval feeding mode* (increase of leaf chewers), *Range type* (decrease of Mediterranean and increase of Holarctic plus European species with increasing *Canopy*), and *Host plant form* (consumers of woody plants or grasses increasing with *Canopy* cover).

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Table 3. Results of life history traits analysis. Traits-based interpretation of partial RDA ordinations of Southern Balkans butterfly community species composition (BSC) that assessed the response to *Forest encroachment* and *Canopy* models including significant covariates and controlled for phylogeny.

BSC ~ Response + [*Trait*] |	***Forest encroachment***			***Canopy***		
Covariates	**AEV**	**F**	**P**	**AEV**	**F**	**P**
Altitudinal range	0.00	0.8		0.00	0.20	
Feeding index	0.00	0.1		0.00	<0.1	
Flight period	3.40	2.0	*	0.70	1.20	
Generation numbers	3.90	5.9	**	3.80	5.60	*
Gregariousness	<0.1	1.1		1.70	3.00	
Host plant form	0.00	0.8		2.10	3.40	.
Larval feeding mode	0.50	1.6		1.90	3.30	.
Migration	1.20	2.5	.	0.00	0.70	
Mountain distribution	0.00	0.1		0.00	<0.1	
Myrmecophily	0.00	0.2		0.00	<0.1	
Overwintering stage	4.40	2.8	**	1.20	2.40	
Ovum placement	0.00	0.8		0.00	0.90	
Range size	0.00	0.9		0.20	1.2	
Range type	3.50	2.4	*	3.40	2.40	.
Wingspan	0.00	0.5		0.20	1.20	

Covariate model structure as in Table 2.
AEV = Adjusted explanatory variable (%)
Significance as follows: **** < 0.0001; *** < 0.001; ** < 0.01; * < 0.05; . < 0.1

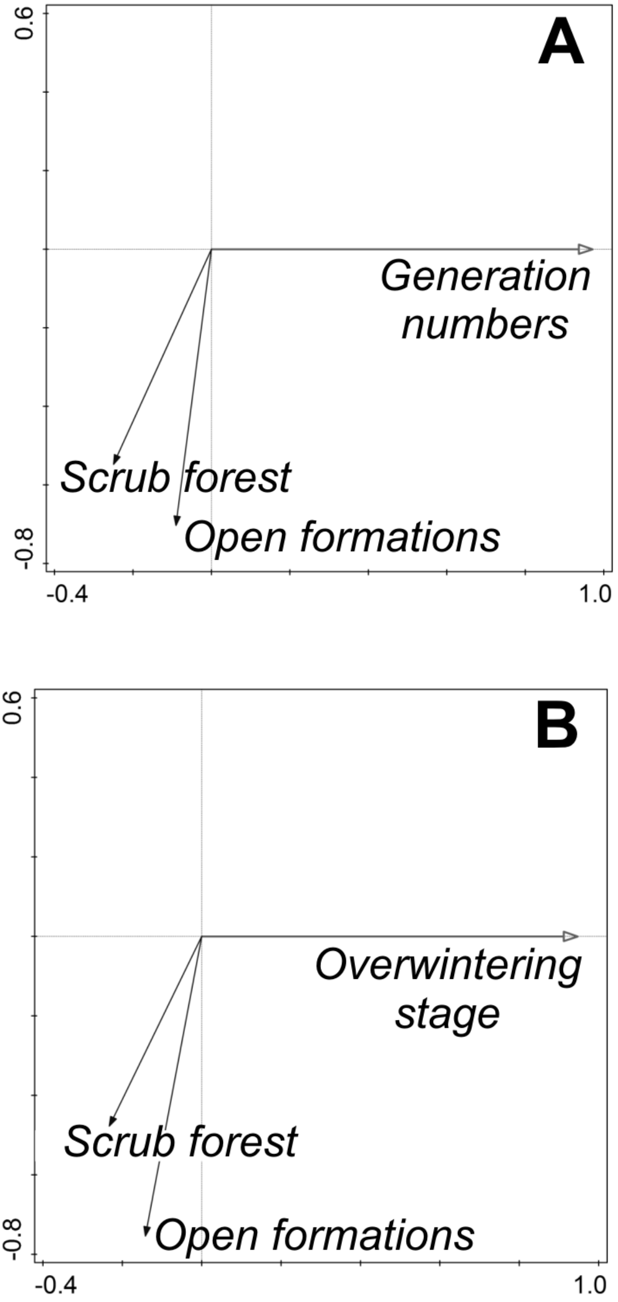
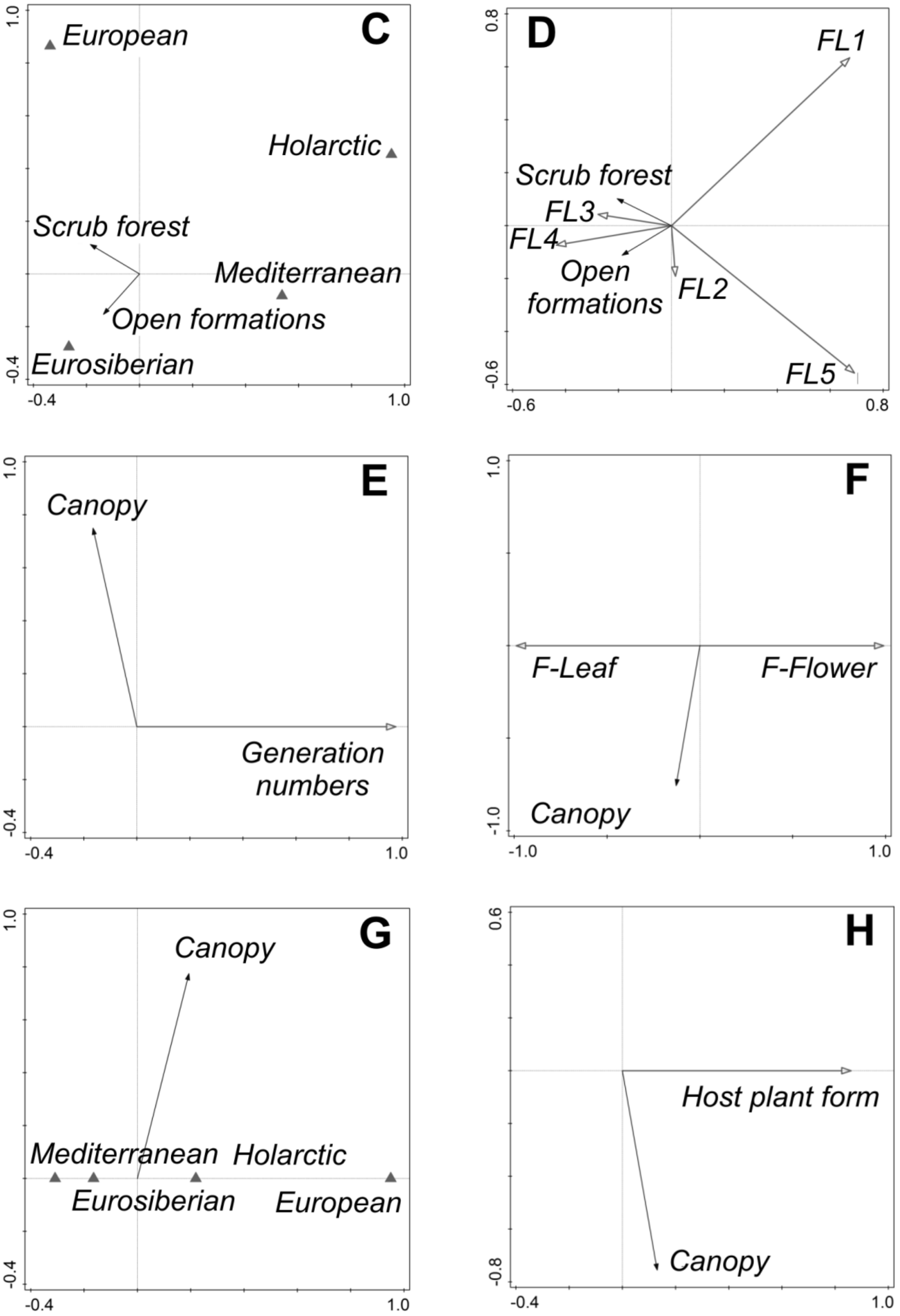


Fig 4. Ordination diagrams showing life-history traits interpretation of analyses of the effects of *Forest encroachment* and *Canopy* on butterfly community composition. Partial redundancy analysis, computed after including covariates (see Table 2 for formulation of covariate model) and removing the effects of phylogeny. The arrows in panels (A–D) stand for horizontal ("*Scrub forest*") and vertical ("*Open formations*") ordination axes in Fig 3(A), whereas panels (E–H) refer to ordination diagrams in Fig 3(B). Statistical tests in Table 3.

Fig 4. Continued



Discussion

The large-scale comparison of South Balkan butterfly communities indicated that compared with grassland and open formations, sites overgrown by scrub forest hosted lower species richness and lower richness of Red-listed species, but the same number of Mediterranean species. In ordination analyses, we found profound changes in the butterfly community composition due to increasing woody vegetation cover. Interpreting these patterns using the butterflies' life history traits showed that encroachment of formerly open landscapes by forest benefits species with fewer generations per year, overwintering in earlier stages, developing on woody plants or grasses [i.e., apparent plants, cf. 32] and inhabiting large Eurosiberian or Holarctic ranges. It harms species forming more generations per year, developing on unapparent plants and inhabiting small Mediterranean ranges.

Species richness and community composition along forest encroachment gradient

Forest encroachment, expressed either as a categorical predictor or as a proportion of woody *Canopy* cover, was associated with local butterfly richness decline regardless of the site characteristics and visit circumstances covariables for the canopy cover case. It is well known that a majority of European butterflies avoid closed-canopy habitats [33], and hence it is hardly surprising that canopy closure represents a direct threat to this insect group. Our results thus corroborate, over a relatively large geographic scale, the dependency of many butterflies occurring in the Mediterranean region on open formations (grasslands, open forests), previously documented for Mediterranean species in local-scale studies [e.g. 13, 16, 34, 35].

Covariables increasing butterfly species richness included *Water* presence and the vegetation gradient *Veg1*, both revealing that lack of humidity restricts local species richness in the Mediterranean [36]; and *Herdsman's hut*, suggesting positive effects of grazing-associated disturbance on species richness. *Village* proximity affected species richness negatively, indicating that species richness was not supported by other human activities than grazing. The negative effect of the vegetation covariable *Veg3* (distinguishing natural and weedy communities) supported the latter conjecture. Notably, species richness increased with altitude, which seems to contradict well known patterns of altitudinal richness decline [37, 38], but this was due to the fact that our sampling was restricted to lower elevations, not covering high mountains, while the biodiversity of the elevations in the Mediterranean seems to be drought restricted [36].

The richness patterns were strikingly different if only Mediterranean species or only Red-listed species were considered. For the former, we failed to detect a dependency on any of the two predictors describing forest encroachment. We also found meaningful responses of this group of species to potential covariates, although sometimes contrasting to those for total species richness (e.g., Mediterranean species increased, rather than decreased, with *Temperature*, and responded oppositely to the major vegetation gradient *Veg1*). For the latter, increasing canopy cover was by far the best predictor restricting their numbers, and the only significant covariate was (rather trivially) *Nectar*. These contrasting results arguably reflect definitions of the two groups. The Red-listed group contains species of all possible distribution ranges, but sharing a high degree of threat within Europe, and loss of open habitats threatens European butterflies in general [28, 39]. In contrast, the Mediterranean group is defined by shared distribution range, independently of habitats, and our samples included species of all possible habitats, from bare grounds (e.g., *Carchardodus orientalis*, *Chilades trochylus*, *Pseudochazara anthelea*) to closed forest (*Kirinia roxelana*, *Zerynthia cerisy*) [cf. 40]. Thus, apart from the low number of Red-listed species in closed canopy sites, analysing mere species numbers does not convey much information regarding individual species requirements.

The ordination analyses focusing on species composition provided deeper insights. Treating forest encroachment levels as a categorical predictor showed that each of the three categories hosted some Mediterranean and some Red-listed species, although both *Grasslands* and *Open formations* hosted apparently more such species than *Scrub forest.* Moreover, each of the two categories attracted distinct species, suggesting that to sustain the whole butterfly diversity associated with traditional Mediterranean landscapes, mosaics of alteration of grasslands open "savannah-like" formations are necessary. Note that even open habitat species may temporarily utilise cooler microclimates provided by close canopy sites [41] , which explains our scrub forest records of such species as *Hipparchia statilinus*, a Mediterranean species requiring near-bare ground for larval development (27 forest presence records out of 85) [cf. 42]. *Open formations* hosted both Mediterranean species, some of them threatened (e.g., *Hipparchia syriaca*), in combination with species that prefer barren surfaces in more northerly parts of their ranges (e.g., *Hipparchia statilinus*, *Pseudopilotes vicrama*) [28, 43].

The patterns found for the numeric predictor *Canopy* were even clearer, revealing avoidance of Mediterranean species, and affinity of northern species, towards increasing *Canopy* cover.

Species traits changes along forest encroachment

The prevailingly European species colonising *Scrub forest* sites form few generations per year, overwinter in early stages, and, counter-intuitively, fly in spring; the prevailingly Eurosiberian species colonising *Open formations* fly mainly in high summer; and the prevailingly Mediterranean species colonising *Grasslands* were mainly spring or autumn flying species. It follows that a link exists between species ranges, habitat successional stage and associated butterflies' development. This was previously suggested by Dennis et al. [21], who related the life histories of British butterflies to the life history strategies of their host plants. Association of slowly developing species forming few generations per annum with late successional habitats has been reported from such disparate regions as Germany [44], Catalonia [45] and Japan [46]. This is sometimes attributed to habitat disturbance dynamics, in that rarely disturbed habitats allow for slower insect reproductive rates in contrast to frequently disturbed habitats. This interpretation, however, fails to explain why woodland species *both* overwinter in early stages and occur as adults early in spring, which forces them to develop rapidly. An alternative explanation, suggested, e.g. by Cizek et al. [32] invokes the nature of antiherbivore defenses in late-successional plants (trees, coarse grasses). In such plants, quantitative defenses (tannin, silica et.) prevail, restricting associated herbivores' development to young plant tissues, available in early season. In parallel, woodland species developing on forbs are constrained to early development by rapid canopy shading, or progressive host plant senescence [47]. The marginally significant effect of host plant form in our analysis circumstantially supports the plant defenses role. Moreover, species with higher generation numbers and species overwintering in later stages inclined towards grasslands, where the combined effects of host plants senescence and canopy shading do not apply.

Similar logic may explain the link between Mediterranean distribution, spring plus autumn adult period, and *Grasslands*. Grasslands receive enough sun early in the year, get hot and dry during high summer, but become inhabitable again with autumn rains [48]. Then, multivoltine species (e.g. *Gegenes pumilio, Pieris krueperi, Chilodes trochylus*) form additional generation(s), whereas univoltine species with long-living adults [cf. 49] locate both nectar and oviposition substrates there. The association of species flying in high summer with *Open formations* is best explained by the structural heterogeneity of such sites, where mosaics of closed and open vegetation offer varying microclimate conditions, supplying some nectar, moisture and shade even during summer.

Conservation implications

Species' ranges result from phylogenetic history, dispersal and habitat requirements [50]. The avoidance of closed canopy sites by the range-restricted Mediterranean species, and their affinity for either *Open formations* or *Grasslands*, agrees with results recently reported for Greek birds [51] and spiders [14]. Grill et al. [16] and Kati et al. [12] reported, for butterflies and orthopterans, respectively, the highest species richness, and highest representations of range-restricted species, from such richly structured habitats as abandoned orchards and wooded pastures in the Greek nature reserve Dadia. Increases of common northern species at the expense of Mediterranean endemics were also detected for southern French birds [52], Sardinian plants [53], and Catalonian (i.e., West Mediterranean) butterflies [15]. Assuming historical conservatism of species life histories, the negative association of Mediterranean species with closed canopy condition falsifies the "forested Mediterranean" hypothesis, highlighting the need to maintain open landscapes across the region.

Notably, the increases of northern species due to forest encroachment contradict the predictions that northern species should decline at their southern range margins due to the current climatic warming [54]. This process is probably counteracted by another development, detected for Greek butterflies by Zografou et al. [55], who found an increase of low-altitude thermophilous species against high-altitude ones. The two processes, increase in the representation of thermophilous species due to warming climate and their decrease due to habitat loss, are likely affecting species individually, depending on their ability to adapt, e.g. by locating sites with suitable microclimates [56]. For global conservation, however, the outcomes are hardly positive, because the majority of the Mediterranean endemics depend on grasslands or open formations, habitats that are rapidly decreasing all over the study region.

Without maintaining rich mosaics of open and semi-open habitats across the southern Balkans, the restructuring of butterfly and other small animal communities due to forest encroachment will gradually replace range-restricted endemic fauna by wide ranging generalists. Maintaining open landscapes is complicated by several factors. First, such widely advocated land management tools as "headage payments" for shepherds [8] or agro-environmental schemes rewarding environmentally benign farming [57], were originally designed in north-western Europe and may be poorly transferable to the conditions of Southern Europe, with much more diverse habitat conditions and declining rural population [58]. Second, financial incentives do not guarantee that human impacts on habitats replicate those existing in the past. For instance, agrotechnology developments such as fodder crops production and vehicle transport relaxed the need to harvest summer coppice, or to move herds across the landscapes (transhumance) [cf. 59]. Cizek et al. [60] documented that current management technologies fail to provide microhabitat heterogeneity needed for reserve management in Central Europe, and the outcomes may be even worse in species-richer Southern Europe. Still worse, relying on subsidies assumes constant economic growth, which is far from guaranteed in the long term. Economic decline might promote returns of urban population to villages, but this would be a long-term process, whereas breakdowns in funding may lead to rapid habitat and species losses.

Without downplaying the subsidised efforts to maintain rural habitats diversity [61], novel approaches which would maintain the open to semi-open conditions across the Mediterranean while being economically sustainable should be sought. At least locally, declining grazing by farm animals might be replaced by free ranging ungulates, including species historically extirpated from the Mediterranean [62]. Such projects would, at least regionally, return to the Mediterranean the key players that had been affecting ecosystem dynamics before the advent of farming, and with which the regionally endemic biodiversity has evolved.

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Supporting information

Additional Supporting Information may be found in the online version of this article: http://journals.plos.org/plosone/article?id=10.1371/journal.pone.0152026 #sec017

S1 Text. Detailed description of study regions, south Balkan, 2013–2014

S2 Figures. Description of the direct ordination used to extract vegetation variables from s pecies composition of the sites

S3 Table. Checklist of butterfly species recorded in individual regions and total numbers of records (+/ – indicate presence/absence)

S4 Table. List of butterfly species life-history traits used to analyse impacts of forest encroachment on South Balkans butterflies, associated hypotheses and relevant references

S5 Table. List of life history traits, used to analyse impacts of forest encroachment on South Balkans butterflies

S6 Text. Reference list to the sources of phylogenetic information

S7 Figure. Unconstrained analysis of butterfly species life history traits

S8 Raw data. Raw data used for analysing butterfly species richness and community composition

CHAPTER III

Co-occurrence of three *Aristolochia*-feeding Papilionids *(Archon apollinus, Zerynthia polyxena* and *Zerynthia cerisy)* in Greek Thrace

Archon apollinus (Herbst 1798)

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Abstract

Comparative studies of co-occurring species using overlapping resources may help in understanding the mechanisms supporting biotic diversity in species-rich regions, such as the Mediterranean region of Europe. Three Papilionidae butterflies, *Archon apollinus, Zerynthia cerisy* and *Zerynthia polyxena*, develop on *Aristolochia* plants and co-occur in Greek Thrace. We used mark–recapture to describe adult demography and dispersal, and searched for eggs and larvae to assess host plants and microhabitat preferences. Adult flight timing followed a sequence from earliest *A. apollinus,* through *Z. polyxena* to late *Z. cerisy*; this was more prominent in 2010 (warm early spring) than in 2011 (cold delayed spring). Population densities were highest for *A. apollinus* and lowest for *Z. cerisy*, whereas dispersal ability followed a reverse pattern. Adults of all three species crossed distances > 3 km and used all habitat types present. Four *Aristolochia* host plants were used at the study locality: small *Aristolochia pallida,* intermediate *A. rotunda* and *Aristolochia hirta,* and bulky, late-sprouting *Aristolochia clematitis.* Both *A. apollinus* and *Z. polyxena* used all four *Aristolochia* species, the former preferring *A. rotunda* and *Aristolochia hirta,* the latter *A. rotunda* and *Aristolochia pallida. Zerynthia cerisy* did not use the early-growing *Aristolochia pallida* while frequently using the late-growing *Aristolochia clematitis.* Further parameters affecting oviposition were biotope and canopy closure: early *A. apollinus* tolerated shady sites but late *Z. cerisy* avoided them. The simultaneous use of several host plants differing in phenology and habitat requirements, combined with rather high dispersal ability, arguably buffers the butterflies' population dynamics against yearly variation in weather, while allowing efficient occupation of the diverse Mediterranean landscapes. The regional habitat diversity, created during millennia of human activity, is currently threatened by land abandonment, which may diminish the resource base for the studied butterflies.

Introduction

The Mediterranean Basin located at the conjunction of Europe, North Africa and western Asia represents one of the global biodiversity hotspots (Myers et al. 2000). Its biotic richness is determined by steep bioclimatic gradients, a long unglaciated history, and millennia of coexistence with humans, whose activity has contributed to the maintenance of diverse habitats (Grove and Rackham 2003; Mazzoleni et al. 2004; Blondel et al. 2010). Currently, Mediterranean landscapes are under interwoven threats of land-use intensification (including urbanization and coastal development) and abandonment (depopulation of remote areas, successional loss of open biotopes) (Debussche et al. 1999; Mazzoleni et al. 2004).

The processes that maintain Mediterranean biodiversity remain little studied, especially if compared with more northerly parts of Europe. This is well illustrated using the model group of butterflies. Although over two-thirds of European butterflies occur in Mediterranean states (cf. Kudrna et al. 2011), only a handful of papers have focused on this region. Apart of studies describing macro-scale patterns (e.g. Hortal et al. 2004; Fattorini 2006), analysing existing threats (e.g. Bonelli et al. 2011), or comparing effects of land uses (e.g. Grill and Cleary 2003; Grill et al. 2005; Kati et al. 2012), only a few studies have targeted the mechanistic details of species occurrence (e.g. Celik 2012; Verovnik et al. 2013). One approach to studying the mechanisms that maintain biodiversity is to compare life histories of sympatric related species overlapping in resource use. Such species may theoretically compete with each other (Thomas and Elmes 2001; Kunte 2008), unless they use different spatiotemporal niches. Alternatively, their resources may be sufficiently abundant to mitigate competitive interactions (e.g. Kalapanida and Petrakis 2012), or varied responses to abiotic factors may generate mutually independent population dynamics (WallisDeVries et al. 2011).

Middle-sized papilionid butterflies developing on birthworts *(Aristolochia spp.)* represent a guild of related, locally coexisting butterflies. Aristolochiaceae, the ancestral host plants of Papilionidae

(Condamine et al. 2013), synthesize aristolochic acids, used by the butterflies both as oviposition clues and chemical protection (Klitzke and Brown 2000; Mebs and Schneider 2002). Seven *Aristolochia*-feeding papilionids occur in the Mediterranean (Tolman and Lewington 2009; Dapporto 2010). They overlap in host plants spectra (cf. Tolman and Lewington 2009), but little is known regarding details of their host preferences. Within Europe, the highest diversity of those butterflies is found in Greek Thrace, where three species co-occur (cf. Kudrna et al. 2011). *Zerynthia polyxena* (Denis and Schiffermüller, 1775) and *Zerynthia cerisy* are native to the region, whereas *Archon apollinus* was transferred there from European Turkey in the 1920s (cf. Tolman and Lewington 2009).

For two seasons, we studied the immature and adult habitat use of the three species in Greek Thrace, to elucidate their coexistence patterns. Here, we report our findings on adult demography, larval and adult phenology, and larval and adult distribution within a heterogeneous semi-cultural landscape, asking the following questions:

(1) Do adults of the three species differ in their use of individual biotope types in the study area?
(2) Do the three species differ in adult demography parameters, including population density, and are these parameters related to adult mobility?
(3) How do immature of the three species use available host plants, are there discernible differences related to plant species, conditions or microhabitat?

Based on these questions, we argue that the sympatric coexistence of the three species is mediated via sequential phenology of their host plants, plus by ability of all three species to use the diverse habitat mosaic at the study locality. As one of the three studied species, *Z. polyxena*, also enjoys legal protection in the EU, we briefly discuss the management implications of our findings.

Material and methods

Study area

Greek Thrace, i.e. the administrative provinces Alexandroupoli, Komotini and Xanthi, is situated on the southern slopes of the eastern Rhodope Mountains facing the Aegean coast in the south and open to penetration by eastern faunal elements, it represents a crossroads of three bioregions, Black Sea, Continental and Mediterranean (EEA 2012).

Our study locality (Figure 1) (40°58' N, 25°50' E; altitude 150–200 m; total area 95 ha) consisted of three stream valleys covered by ancient and partly abandoned cultural landscape near a once important and now almost depopulated village. Abandoned fields, alluvial grasslands, scrubs overgrowing former pastures, gallery groves along creeks and closed woods on steep slopes form a diverse vegetation mosaic (Figure 2j). The bedrock is acidic igneous intrusions overtopped by alkali sediments. The vegetation is submediterranean, with woody plants represented, e.g. by *Juniperus oxycedrus, Pyrus amygdaliformis* and *Quercus coccifera* in scrubby macchia, *Platanus orientalis* along the creeks, and *Quercus pubescens* and *Carpinus orientalis* in woodlands.

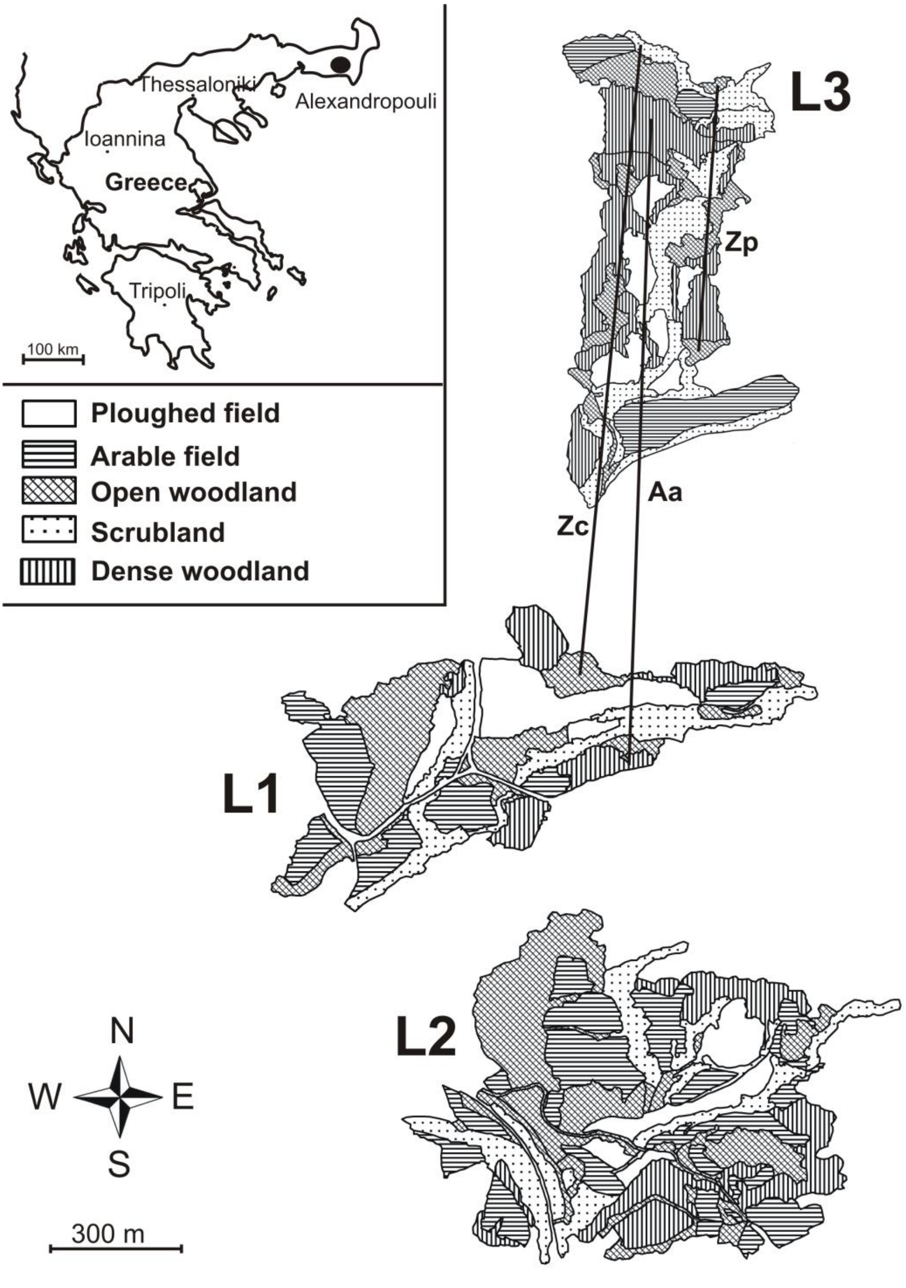


Figure 1. Position of the study area in northeastern Greece (dark dot on the map) and mutual positions of the three study subsites, with the mosaics of individual biotopes. The longest single moves of three study species: Aa, *Archon apollinus;* Zc, *Zerynthia cerisy;* Zp, *Zerynthia polyxena.*

Study species

Archon apollinus (Figure 2a) is distributed from European Turkey and the Aegean islands through southern Turkey to Iraq, Syria, Jordan, Israel and Lebanon (Hesselbarth et al. 1995). It was released to Greek Thrace in 1918 and 1919, from stock originally from the Kuru Dagi Mountains, Turkey, 80 km to the east (Tolman and Lewington 2009). Of multiple *Aristolochia* host species reported in literature (Buresch 1915; Koçak 1982; De Freina 1985; Fuchs 1995), *Aristolochia pallida* (Figure 2d), *Aristolochia hirta* (Figure 2g), *A. rotunda* (Figure 2e) and *Aristolochia clematitis* (Figure 2f) occur in Greek Thrace.

Zerynthia cerisy (Figure 2b) occurs from the southeastern Balkan Peninsula through Turkey and Syria to Jordan, Lebanon and Israel (Hesselbarth et al. 1995). Its habitats range from dry grasslands to open woodlands, depending on the supply of *Aristolochia* host plants. Out of 16 potential hosts reported in literature (Hesselbarth et al. 1995; Tolman and Lewington 2009), in addition to those four mentioned above, *Aristolochia sempervirens* and *Aristolochia parvifolia* occur in Greek Thrace.

Zerynthia polyxena (Figure 2(c)) is distributed from southeastern France through northern Italy, the Balkan Peninsula and northern Turkey to Kazakhstan, reaching southern Central Europe in the north (Kudrna et al. 2011; Zinetti et al. 2013). The habitat range is again wide (cf. Celik 2012). Out of the seven literature-reported *Aristolochia* host plants (Hesselbarth et al. 1995; Tolman and Lewington 2009), *Aristolochia clematitis, A. rotunda, Aristolochia hirta* and *Aristolochia pallida* occur in Greek Thrace.

All the study species are univoltine, they overwinter as pupae and emerge in spring, their females lay eggs in small batches. In Greece, *A. apollinus* is the earliest-flying species (March to April on mainland) followed by *Z. polyxena* and *Z. cerisy* (late March to early July) (Pamperis 1997).

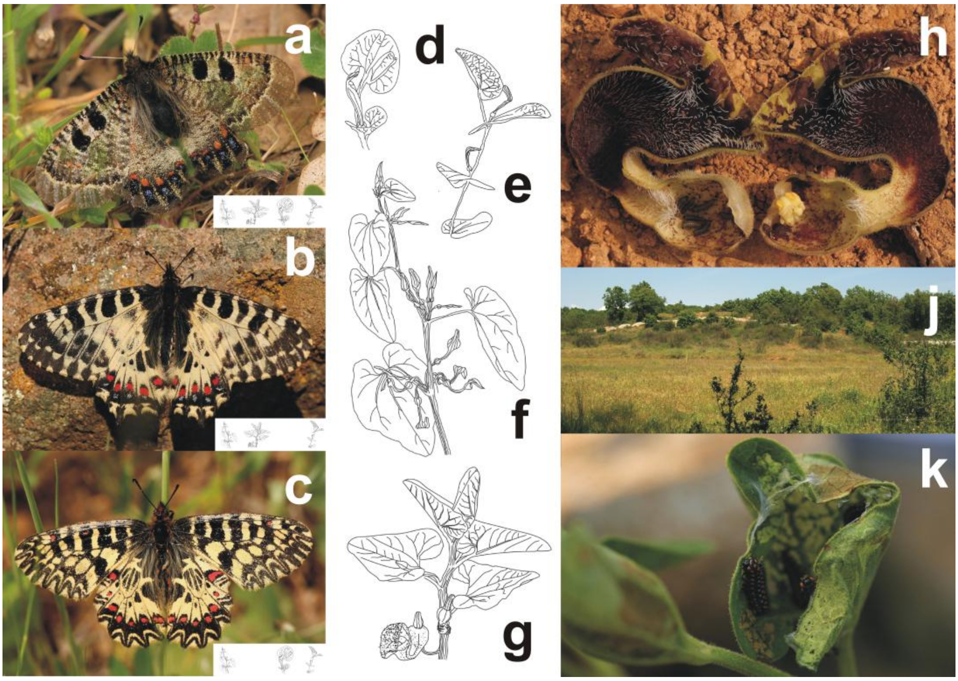


Figure 2. Adults of the studied butterflies: (A) *Archon apollinus;* (B) *Zerynthia cerisy;* (C) *Zerynthia polyxena* (the small inserts stand for host plant species used by the respective species at the study locality); and drawings of their *Aristolochia* host plants (D) *Aristolochia pallida;* (E) *A. rotunda;* (F) *Aristolochia clematitis;* (G) *Aristolochia hirta;* (H) Dissected subterranean *Aristolochia hirta* flower with *A. apollinus* first-instar larvae; (I) Habitat mosaic at the Greek Thrace study site, showing a field in the front, and scrub with open forest in the background; (J) Silk-woven *Aristolochia hirta* leaves with *A. apollinus* larvae.

Mark–recapture

We used mark–recapture to investigate adult demography, marking the butterflies in April–May 2010 (1267 captures over 26 marking days) and 2011 (1596 captures over 29 days). We based the markings on three stream valleys (Figure 1), each encompassing a mosaic of four main land-cover types: scrub (18 ha in total), open woodland (23 ha), close woodland (18 ha) and arable field (13 ha).

On each day with suitable weather (low wind speed, little cloud and air temperature > 14°C) one person marked the butterflies within each valley, paying even attention to the four main land-cover types and capturing as many butterflies of all three species as possible. Captured butterflies were marked with numbers using a waterproof pen and immediately released. For each (re)capture, we recorded the species, the code, sex, date and time (closest hour), habitat type and activity before capture [flying, chasing, patrolling, egg-laying, mating, resting (including basking), nectaring]. In 2011, we also recorded GPS coordinates of each capture event (Garmin GPSmap 60 C with precision of ±3 m).

Comparison of adult activity patterns

Log-linear models, computed in R (The R Core Team 2012) were used to compare the activity before captures with respect to habitat, sex and species. We first defined the null model with activity classes as dependent variable, and then used the information-theory approach Akaike information criterion (AIC) to select the most parsimonious model explaining the activity according to the variables species, sex, biotope and all their interactions.

Adult demography and dispersal

Demography parameters were estimated using the POPAN parameterization of the Jolly–Seber approach for open populations with time-varying deaths and recruitment in MARK (White 1999). The three estimated primary parameters are daily residence rates Φ_i (which combines mortality and emigration), daily catchability pi and daily recruitment (the percentage entering the population) $pent_i$. Derived parameters include daily population size, N_i, and total population N. The primary parameters may be constant in time and sex (.), sex-dependent (g), time-dependent in a factorial (t), linear (T), quadratic (T^2) or cubic (T^3) manners. Time and sex can have additive (g + t, g + T, g + T^2…) or interactive (g × t, g × T, g × T^2…) effects. The best-fitting models are selected from a range of candidate models according to the quasi-Akaike information criterion (qAIC) (Schtickzelle et al. 2002). Average residences were obtained from the best-fitting candidate model containing time-invariant Φ_{avg}. Average longevity was obtained as $-\ln(\Phi_{avg})-1$.

To compare dispersal distances with respect to species and sex, we fitted the 2011 movements data for all individuals recaptured at least once using the inverse power function (IPF), estimating the probabilities (p) of movements to distance (D) (Hill et al. 1996; Baguette 2003), as, $p_{IPF} = a\,D^{-n}$. The parameters a and n were estimated by regressing the natural logarithms of the inverse cumulative proportions of individuals crossing 100-m classes ($\ln p$) on the natural logarithms of distance (in km), i.e. $\ln(p) = \ln(a) - n\ln(D)$. We followed Zar (2009) to compare the slopes of the resulting regression functions.

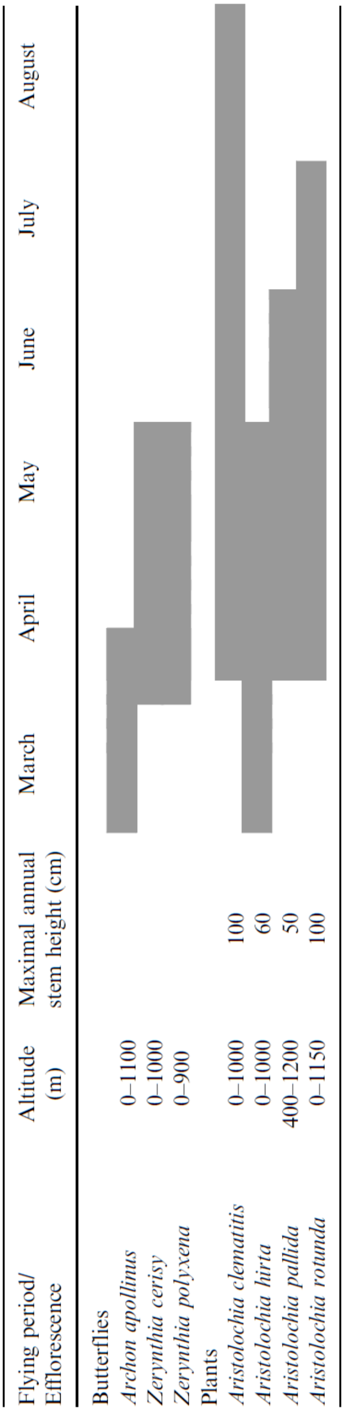
Eggs and larvae search

Direct comparison of egg placements and larval distributions was complicated by the sequential phenology of the three butterflies and their host plants. To overcome this complication, we surveyed the developmental stages twice, early in April (egg-laying period), and early in May (larval period). We did so in the same sites where the mark–recapture was carried out.

From 4 to 10 April 2010, we searched for eggs on as many *Aristolochia* spp. plants as possible (Table 1). For each plant, we recorded habitat, plant species, its phenology (ranked variable: 1, unfolded leaves; 2, folded leaves, but not flowering; 3, flowering; 4, overblown, fruiting); butterfly species, egg number; egg placement (three categories: stalk, ventral leaf side, dorsal leaf side); plant sun exposure [ranked: 1, exposed in short (≤ 0.3 m) vegetation; 2, in scrub mantle or taller herbaceous vegetation; 3, under a scrub]; number of *Aristolochia* stems in surrounding 1 m (a1 m);

proportion of laid/ unlaid *Aristolochia* plants (laid/unlaid plants). We also recorded the covers of forbs and grasses in 1-m and 5-m diameter circles around the focal plant (forb1 m, grass1 m, forb5 m, grass5 m); and covers of scrubs and trees in 10-m diameters (scrub10 m, trees10 m). We labelled each plant that had a positive eggs record with a numbered flag for a subsequent search for larvae. We recorded the same variables for a high number (n = 686) of *Aristolochia* plants not bearing any eggs.

Table 1. Phenology of the studied butterflies and their host plants, according to literature: Strid and Tan (1997), Carbonell (1991), Hesselbarth (1995), Tolman and Lewington (2009).



Flying period/ Efflorescence	Altitude (m)	Maximal annual stem height (cm)	March	April	May	June	July	August
Butterflies								
Archon apollinus	0–1100		▨	▨				
Zerynthia cerisy	0–1000			▨	▨			
Zerynthia polyxena	0–900			▨	▨			
Plants								
Aristolochia clematitis	0–1000	100		▨	▨	▨	▨	▨
Aristolochia hirta	0–1000	60	▨	▨	▨			
Aristolochia pallida	400–1200	50		▨	▨	▨		
Aristolochia rotunda	0–1150	100		▨	▨	▨	▨	

From 3 to 16 May, 2010, we located 85% of the previously labelled egg-bearing plants, searching for butterfly larvae, and searched at further randomly selected plants. If the larvae were found, we recorded their number (larvae number), butterfly species and instar. As for the eggs search, we counted *Aristolochia* species in a 1-m diameter circle (a1 m) and focal plant phenology. Further, we estimated the number of *Aristolochia* leaves in a 1-m diameter circle (leaf1 m). We also recorded a number (n = 39) of *Aristolochia* plants without any butterfly species.

Analysing egg and larval distribution

We applied general linear models in R, following four complementary approaches.

For the eggs (April data), we first asked, which predictors related to the host plant and its surroundings influenced egg placement. Separately for each species, we defined a binomial distribution model with logit link function, contrasting plants bearing eggs of the focal species against plants either bearing eggs of the other two species, or not bearing eggs at all (eggs of a species present versus absent). We first computed single-predictor models for all potential predictors, and then considered interactions between plant species, habitat and phenology. Finally, we constructed a multiple regression model, based on forward selection of predictors, including two-way interactions.

For the eggs, we also compared the preferences of the three species based only on positive eggs records, i.e. working only with plants bearing eggs, but adding the information on egg batch sizes. We first defined a model explaining the number of eggs per batch by butterfly species (Poisson distribution of errors). To this model, we sequentially added one-way interactions with potential predictors, so asking how were the between-species differences in numbers of eggs related to such predictors as plant species or habitat, and we again constructed a multiple regression model based on forward selection of predictors.

Analogous to eggs, we compared separately for each species, plants bearing larvae of the respective species against those bearing larvae of the other two species or not bearing larvae at all (larvae of a species present versus absent), using binomial distribution and logit link. We again started with one-way regressions, and proceeded towards the final model-based forward selection from all nominally significant predictors and two-way interactions.

Finally, we compared the larval counts of the three species, based only on plants with positive records but adding the information on larval group sizes, proceeding analogously to models with egg counts.

Results

Adult activity, demography and dispersal

In 2010, only *A. apollinus* and *Z. cerisy* males were marked with sufficient intensity for reliable demographic estimates, whereas in 2011 the demography of all three species could be directly compared (Table 2).

Table 2. Summary of mark–recapture data obtained during the study of the butterflies *Archon apollinus, Zerynthia cerisy* and *Zerynthia polyxena* in 2010 and 2011.

Butterfly species	Marking days (maximum duration)	Sex	Individuals captured	Individuals recaptured	Proportion recaptured (%)	Capture events
Archon apollinus 2010	14 (16)	M	538	163	30.3	889
		F	124	10	8.0	142
		Total	662	173	26.1	1031
Archon apollinus 2011	25 (33)	M	519	246	47.4	973
		F	182	37	20.3	230
		Total	701	283	40.4	1203
Zerynthia cerisy 2010	12 (16)	M	84	54	64.3	215
		F	21	0	0	21
		Total	105	54	51.4	236
Zerynthia cerisy 2011	19 (33)	M	85	26	30.6	121
		F	27	9	33.3	37
		Total	112	35	31.3	158
Zerynthia polyxena 2011	26 (36)	M	92	37	40.2	158
		F	62	11	17.7	77
		Total	154	48	31.2	235

Archon apollinus adults were active from early mornings, staying at sun-exposed spots while shady vegetation was still under hoar-frost. *Zerynthia cerisy* and *Z. polyxena* appeared later in the day, apparently requiring more sunshine for activity; the highest *Z. cerisy* flight was from noon onwards. Both *A. apollinus* and *Z. polyxena* were often chased from lower vegetation strata, whereas *Z. cerisy* frequently flew higher, overflying taller bushes and trees. When chased from a shady spot, all three species sought a sunny patch for basking.

The log-linear model for adult activity, based on captures from the year 2011, contained all factors of interest (i.e. habitat, sex and activity), plus all two-way interactions (e.g. species*sex, species*habitat, species*activity, habitat*activity, habitat*sex and activity*sex) (Null model: df = 143, deviance = 3959, AIC = 4348; fitted model: df = 52, 91, deviance = 90.79, AIC = 584.5). The interaction terms revealed, first, that although male captures prevailed in all species, relatively more females were captured in *Z. polyxena* (32%) than in *Z. cerisy* (23%) and *A. apollinus* (18%). More *Z. cerisy* captures originated from open woodland (58%), whereas the other two species were captured in similar proportions (30–40%) in open woodland and scrub. *Archon apollinus* patrolled more frequently (30%) than the remaining two species (~20%); *Z. cerisy* displayed direct flight more frequently (59%) than the other two species (~20%); and *Z. polyxena* was encountered while resting more frequently (40% versus *A. apollinus*: 29%, *Z. cerisy* 9%). Patrolling was most frequent in scrub (34%), followed by open woodland (28%) and close forest (17%). Resting was frequent in close forest (41%), followed by fields (31%). Oviposition was most frequent in close forest (9%) and more females were encountered in close forest (40%), than elsewhere (~20% in all habitats).

Modelled demography parameters (Table 3) showed that residence rates were higher for males in all three species, and higher for *A. apollinus* than for the remaining two species. Capture probabilities p always factorially depended on marking day, whereas proportional recruitments pent followed either domed patterns, with additive or multiplicative effects of sex due to the earlier appearance of males *(A. apollinus, Z. polyxena);* or linear decrease of males with progression of season (*Z. cerisy* in both years). Plotting the estimated daily numbers showed a short flight season with prominent peak for *A. apollinus*, 2010, and almost a month delayed and shallower peak for *A. apollinus* in 2011.

Table 3. Summary of best-supported Jolly–Seber models (bold) and models with time-invariant residence, used to estimate demographic parameters and population sizes of *Archon apollinus, Zerynthia cerisy* and *Zerynthia polyxena* in Greek Thrace, 2010 and 2011, plus the respective estimated numbers of individuals, average residence times and maximum residences observed.

Butterfly species	Model	*AIC*	*Δ AIC*	No. of parameters	Estimated males (± SE)	Estimated females (± SE)	Average residence time M/F	Maximum residence observed M/F
A. apollinus, 2010	$\varphi\ (g + t)\ p\ (g + t)\ pent\ (g + T^2)\ N\ (g)$	3022.1	0	34	1326 (±122)	1327 (±122)	6.1/6.1	14/8
	$\varphi\ (g)\ p\ (g + t)\ pent\ (g + T^2)\ N\ (g)$	3027.9	5.8	22	1330 (±64)	1331 (±64)		
A. apollinus, 2011	$\varphi\ (g)\ p\ (g + t)\ pent\ (g + T^2)\ N\ (g)$	3516.6	0	34	992 (±48)	707 (±96)	8.2/4.5	28/14
	$\varphi\ (g)\ p\ (g + t)\ pent\ (g{*}T^2)\ N\ (g)$	3521.7	5.1	36	976 (±49)	672 (±86)		
Z. cerisy, 2010	$\varphi\ (.)\ p\ (t)\ pent\ (T)\ N\ (g)$	382.2	0	16	166 (±31)	–	4.7/–	12/–
	$\varphi\ (.)\ p\ (t)\ pent\ (T^2)\ N\ (g)$	384.4	2.2	17	176 (±27)	–		
Z. cerisy, 2011	$\varphi\ (g)\ p\ (g + t)\ pent\ (g + T)\ N\ (g)$	511.9	0	27	395 (±171)	86 (±27)	14.7/6.3	30/8
	$\varphi\ (g)\ p\ (g + t)\ pent\ (g + T^2)\ N\ (g)$	513.1	1.2	28	373 (±159)	75 (±23)		
Z. polyxena, 2011	$\varphi\ (g)\ p\ (g + t)\ pent\ (g + T^2)\ N\ (g)$	814.8	0	35	337 (±79)	252 (±64)	10.3/3.6	19/9
	$\varphi\ (g)\ p\ (g + t)\ pent\ (g{*}T^2)\ N\ (g)$	818.0	3.3	37	235 (±48)	239 (±58)		

Notes: Model parameters: φ, residence; p, catchability; *pen*, proportion of entering.
Parameter responses: (.), not depending on sex or time; (*g*), sex dependent; *t*, responding to time in a factorial manner; *T*, responding to time in a linear manner; T^2, responding to time in a domed polynomial manner.

Zerynthia cerisy flew later than *A. apollinus* in 2010, when the marking covered only a late tail of its flight. For *Z. polyxena* and *Z. cerisy* in 2011, the adult flight periods overlapped with each other and with *A. apollinus* (Figure 3).

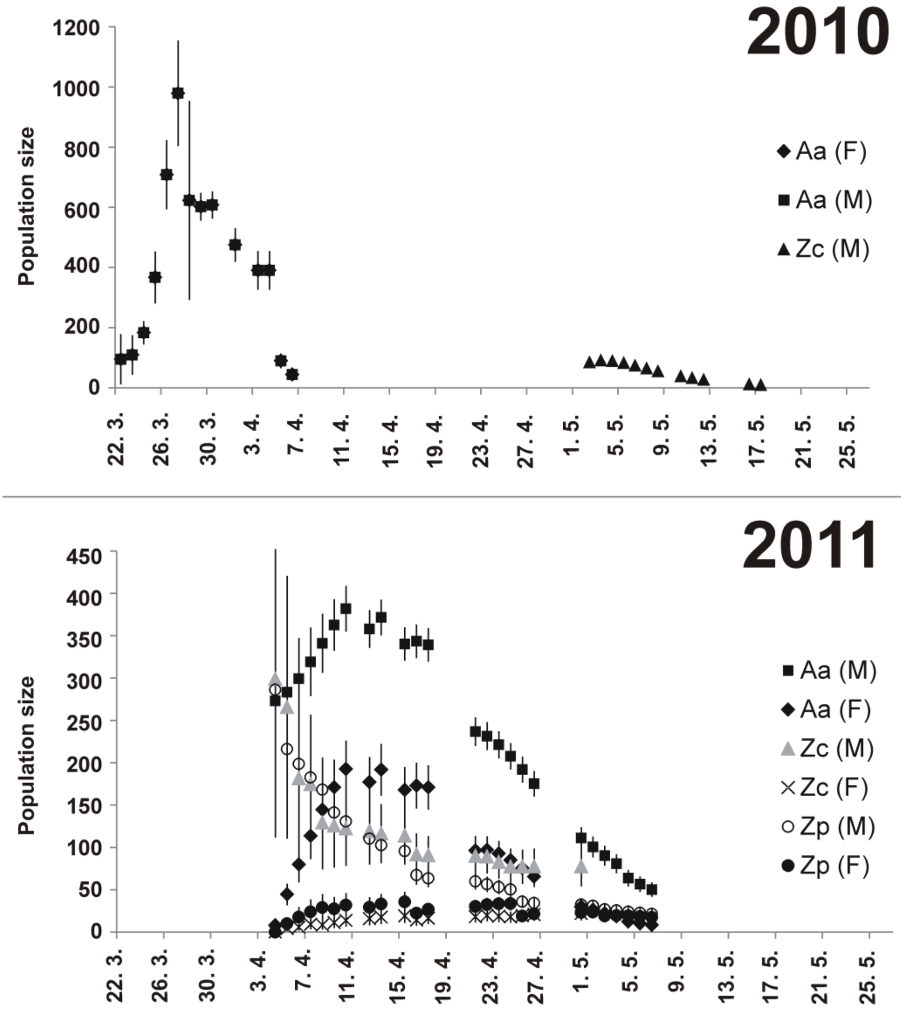


Figure 3. Estimates of the adult daily population sizes based on mark–recapture data: year 2010, when only data for *Archon apollinus* (most of flight period) and *Zerynthia cerisy* (late tail of flight period) allowed the estimation; year 2011, *A. apollinus, Z. cerisy, Zerynthia polyxena*. The error lines present standard errors of estimates, see Table 3 for model parameters.

Both *Z. cerisy* and *Z. polyxena* were also still on wings, although in small numbers, at the end of the first May decade, whereas *A. apollinus* flight terminated at the beginning of May. The estimated population sizes were highest for *A. apollinus* (≈1000 individuals of each sex) in both 2010 and 2011, followed by *Z. polyxena* (≈300 individuals), 2011, and *Z. cerisy* (≈300 males and 100 females, i.e. a male bias), 2011.

The mean and median flight distances were shortest in *A. apollinus*, longer in *Z. polyxena* and longest in *Z. cerisy*. Males of *A. apollinus* appeared to be less mobile than females, whereas the opposite applied to *Z. polyxena* (Table 4). All three species crossed maximum distances of several kilometres, with distances > 5 km crossed by *A. apollinus* and *Z. cerisy* males.

Fitting the inverse power functions provided significant regressions for all species*sex combinations (Table 4). The fitted lines displayed significant differences (slopes: $F = 5.36$, $p < 0.05$, elevations: $F = 6.08$, $p < 0.05$; df = 9, 188). Based on the regression slopes, *A. apollinus* males moved less readily than *A. apollinus* females ($p < 0.01$), *Z. polyxena* males and females (both $p < 0.05$), and *Z. cerisy* males ($p < 0.001$) and females ($p < 0.01$). *Archon apollinus* females moved less than both sexes of *Z. cerisy* ($P < 0.05$). Disregarding sexes, *A. apollinus* dispersed less than *Z. cerisy* ($p < 0.001$) and *Z. polyxena* ($p < 0.001$), and *Z. polyxena* less than *Z. cerisy* ($p < 0.05$). Regardless of these differences, the movement probability × population size products suggested that for each species and sex, at least 10 individuals would cross a 10-km distance.

Egg distributions on host plants

We found 111 egg batches of *A. apollinus* [total 457 eggs, the mean/median batch egg number: 4/2 (± 4.0 SD), maximum 35 eggs], most of them on *A. rotunda* (73 batches), followed by *Aristolochia hirta* (19), *Aristolochia pallida* (18) and *Aristolochia clematitis* (1). For *Z. cerisy*, we found 31 batches [74 eggs, mean/median: 2/2 (± 1.6 SD), maximum 6] situated on *Aristolochia clematitis* (18), *Aristolochia hirta* (10) and *A. rotunda* (3). The 70 egg batches of *Z. polyxena* [total 191 eggs, mean/ median: 3/2 (± 2.6 SD), maximum 11] were found in *A. rotunda* (32), *Aristolochia pallida* (30), *Aristolochia hirta* (5) and *Aristolochia clematitis* (3). We also detected cases of several species batches at the same plant: *A. apollinus* + *Z. polyxena* (60 batches), *A. apollinus* +

+ *Z. cerisy* (6), *Z. polyxena* + *Z. cerisy* (4). These cases were ignored from the analyses below, but recalculation not ignoring them did not substantially change the results. We also ignored batches found on grass near *Aristolochia* plants (*A. apollinus*: 14, *Z. polyxena*: 1). The *A. apollinus* egg batch containing 35 eggs was also ignored, as an outlier.

Placement of *A. apollinus* egg batches (Table 5) depended on plant species (*A. rotunda* and *Aristolochia hirta* preferred, *Aristolochia pallida* intermediate, *Aristolochia clematitis* avoided). Eggs were most frequent at unfolded plants, less so on folded and never at fruiting ones. The significant interaction plant species– phenology revealed that *Aristolochia pallida* and *A. rotunda* were used during later phenological stages. Prominent plants growing in short vegetation were preferred over those growing in scrub mantles. Plants were more likely to be used if surrounded by lower cover of grasses (1-m diameter medians: 10% versus 20%, 5-m diameter: 25% versus 30%), higher cover of forbs (1-m diameter medians: 20% versus 15%), lower cover of scrubs (both medians 30%) and higher canopy closure (medians 65% versus 40%). The plants used were surrounded by more *Aristolochia* plants; this applied to surrounding plants not bearing and bearing butterfly eggs, and the latter predictor yielded the strongest fit of all single-factors models.

The preferred *Z. cerisy* host plant was *Aristolochia clematitis*, followed by *Aristolochia hirta*. Eggs were frequent at fruiting or flowering plants, never at unfolded plants. Plants growing freely or under scrubs were preferred over those in forest mantles. The preferred plants grew under low scrub (medians: 25% versus 30%) and canopy (medians: 10% versus 50%) covers.

The preferred *Z. polyxena* plant was *Aristolochia pallida*, followed by *A. rotunda*; the remaining two plants were rarely used. All four habitats were used, although closed forest and open woodland prevailed. The significant interaction of plant species–habitat revealed that *Aristolochia clematitis* was most often used in scrub. Unfolded and flowering plants were used more often than fruiting ones. Preferred plants were surrounded by higher cover of forbs (5-m diameter medians: 25% versus 20%), lower covers of scrubs in 10 m (both 1-m and 5-m diameter medians: 30%) and lower canopy closure (medians: 30% versus 45%).

Table 4. Basic dispersal statistics and results of fitting the inverse power functions, based on all butterfly individuals recaptured at least once.

Butterfly species	Sex	Mean distance ± SE	Median distance	Max. single move	Max. total distance	Equation $ln(p) = lna(\pm SE) - n(\pm SE)lnD$	*df*	*F, p*	$R^2_{adj.}$	$p_{10\ km}$	$Ind_{10\ km}$
Archon apollinus	M	490 ± 38	190	2790	7610	$\ln(p) = -0.82\ (\pm 0.086)\ \ln D - 2.50\ (\pm 0.121)$	1,41	89.81***	0.68	0.017	17
	F	550 ± 93	270	3080	4510	$\ln(p) = -0.51\ (\pm 0.084)\ \ln D - 2.01\ (\pm 0.132)$	1,17	35.81***	0.66	0.048	19
	Both	490 ± 36	190	–	–	$\ln(p) = -0.83\ (\pm 0.089)\ \ln D - 2.47\ (\pm 0.123)$	1,43	88.34***	0.66	0.020	35
Zerynthia polyxena	M	630 ± 97	460	1920	4330	$\ln(p) = -0.43\ (\pm 0.134)\ \ln D - 1.79\ (\pm 0.212)$	1,13	10.23**	0.40	0.062	21
	F	490 ± 165	300	1830	3690	$\ln(p) = -0.39\ (\pm 0.084)\ \ln D - 1.79\ (\pm 0.177)$	1,7	21.42**	0.72	0.068	17
	Both	600 ± 83	440	–	–	$\ln(p) = -0.51\ (\pm 0.116)\ \ln D - 1.91\ (\pm 0.178)$	1,17	19.41***	0.51	0.046	27
Zerynthia cerisy	M	830 ± 164	400	3110	5570	$\ln(p) = -0.38\ (\pm 0.067)\ \ln D - 1.63\ (\pm 0.110)$	1,15	32.21***	0.66	0.082	32
	F	720 ± 180	640	1690	1880	$\ln(p) = -0.32\ (\pm 0.107)\ \ln D - 1.32\ (\pm 0.207)$	1,8	8.79**	0.46	0.127	11
	Both	810 ± 130	490	–	–	$\ln(p) = -0.39\ (\pm 0.077)\ \ln D - 2.30\ (\pm 0.139)$	1,12	25.24***	0.78	0.041	20

Notes: All distances are in metres. $p_{10\ km}$ and $Ind_{10\ km}$ are inverse power functions-derived probability of crossing 10 km distance, and the number of individuals predicted to cross 10 km distance, based on the estimated population sizes reported in Table 3.

Table 5. Results of general linear models-regressions relating eggs records to predictors related to host plants and habitats.

Model	*Archon apollinus*			*Zerynthia cerisy*			*Zerynthia polyxena*			~ butterfly species*predictor		
	df	AIC	Δ Dev.	df	AIC	Δ Dev.	df	AIC	Δ Dev.	df	AIC	Δ Dev.
Null (egg number~1)	896	669.61		896	271.55		896	493.47		210	926.71	
~ butterfly species [A]	–	–	–	–	–	–	–	–	–	208	922.80	2.27
~ egg placement [B]	–	–	–	–	–	–	–	–	–	204	917.11	4.37
~ a1 m [C]	895	621.91	7.44	895	266.45	2.63	895	471.93	4.79	205	922.73	1.94
~ habitat [D]	893	663.24	1.85	893	272.55	1.85	893	478.57	4.25	201	915.34	6.85
~ laid plants [E]	895	441.06	34.53	895	272.98	0.21	895	495.43	0.01	205	924.43	1.40
~ unlaid plants [F]	895	581.6	13.48	895	273.54	0.00	895	495.33	0.03	205	921.67	2.28
~ phenology [G]	893	594.77	12.11	895	271.28	0.84	895	494.95	0.10	201	921.99	4.73
~ sun exposure [H]	894	638.23	5.30	895	271.73	0.68	895	488.13	1.49	203	928.33	1.43
~ plant species [I]	893	637.37	5.73	895	268.47	1.89	895	416.91	15.98	200	912.08	8.53
~ grass1 m [J]	895	648.94	3.40	895	235.84	10.28	895	478.92	3.37	205	919.52	2.96
~ grass5 m [K]	895	652.26	2.90	893	244.86	12.13	893	490.7	1.78	205	925.52	1.05
~ forbs1 m [L]	895	666.44	0.77	894	260.74	5.49	894	496.92	0.11	205	924.04	1.52
~ forbs5 m [M]	895	670.36	0.19	893	221.48	20.80	893	475.2	4.94	205	924.43	1.42
~ scrub10 m [N]	895	666.24	0.80	895	260.45	4.86	895	488.49	1.42	205	923.30	1.76
~ trees10 m [O]	895	640.62	4.64	895	238.04	13.17	895	490.23	1.07	205	922.07	2.15
Two-way interactions												
~ D: I	881	652.27	1.45	881	231.03	6.77	882	442.48	11.14	186	911.11	9.24
~ I: G	882	586.56	11.57	881	217.55	13.08	882	480.48	3.58	183	922.8	7.43
Multiple-regressions												
~ D + H + G + D + I + O + L	882	292.81	60.63	–	–	–	–	–	–	–	–	–
~ I + G + H + O + C	–	–	–	886	181.49	40.83	–	–	–	–	–	–
~ E + I + O + D + N + C	–	–	–	–	–	–	886	364.64	30.28	–	–	–
~:D + J + B + F	–	–	–	–	–	–	–	–	–	197	895.8	24.14

Notes: Models for *Archon apollinus, Zerynthia cerisy* and *Zerynthia polyxena* compared plants with egg batches of the focal species against all other plants, including those with negative records. Models ~ butterfly species*predictor compared egg batch sizes among the three species, disregarding plants with negative records.

All three forward-selected models contained plant species and canopy closure (trees10); laid plants and biotope entered models for *A. apollinus* and *Z. polyxena*; plant placement and phenology for *A. apollinus* and *Z. cerisy*; and amount of adjoining host plants (a1 m) for *Z. cerisy* and *Z. polyxena*.

Mutual comparison among the three butterflies revealed that *A. apollinus* produced the largest batches and *Z. cerisy* the smallest batches. All three species preferred ventral leaf sides. The significant interaction species–habitat revealed that *Z. cerisy* was the only species ovipositing in arable fields. As for the plant species, *Z. cerisy* preferred *Aristolochia hirta* (Figure 4a). The significant interaction species– phenology showed that *Z. polyxena* used folded plants. *Zerynthia cerisy* favoured plants growing under sparser canopy (median: 10%) than *Z. polyxena* (30%) and *A. apollinus* (60%) (Figure 4b).

Larval counts

We found 112 larval groups of *A. apollinus* [total 278 larvae, the mean/median group size: 2/2 (± 1.9 SD), maximum 10 larvae], 89 groups of *Z. polyxena* [total 196 larvae, mean/median: 2/1 (± 1.9 SD), maximum 10], and 18 groups of *Z. cerisy* [total 28 larvae, mean/median: 2/1 (± 0.7 SD), maximum 3] (Figure 4c). *Archon apollinus* larvae were most commonly found on *A. rotunda* (71), followed by *Aristolochia hirta* (26), *Aristolochia pallida* (10) and *Aristolochia clematitis* (5). *Zerynthia cerisy* larvae were found on *Aristolochia hirta* (9), *Aristolochia clematitis* (6) and *A. rotunda* (3). *Zerynthia polyxena* were recorded on *A. rotunda* (57), *Aristolochia pallida* (23), *Aristolochia clematitis* (8) and *Aristolochia hirta* (1). We also detected cases of more than one species feeding at the same plant (*A. apollinus* + *Z. polyxena*: 2, *A. apollinus* + *Z. cerisy*: 2, *Z. polyxena* + *Z. cerisy*: 1); these cases were ignored in subsequent analyses. Early instars of *A. apollinus* larvae were feeding inside flowers, including the large, subterranean flowers of *Aristolochia hirta* (n = 3) (Figure 2h). Groups of medium-sized larvae built silk-woven tents from host plant leaves (Figure 2k), staying there during the morning and evening hours and leaving the tents to feed and bask at midday. In contrast, *Z. cerisy* and *Z. polyxena* fed in exposed positions.

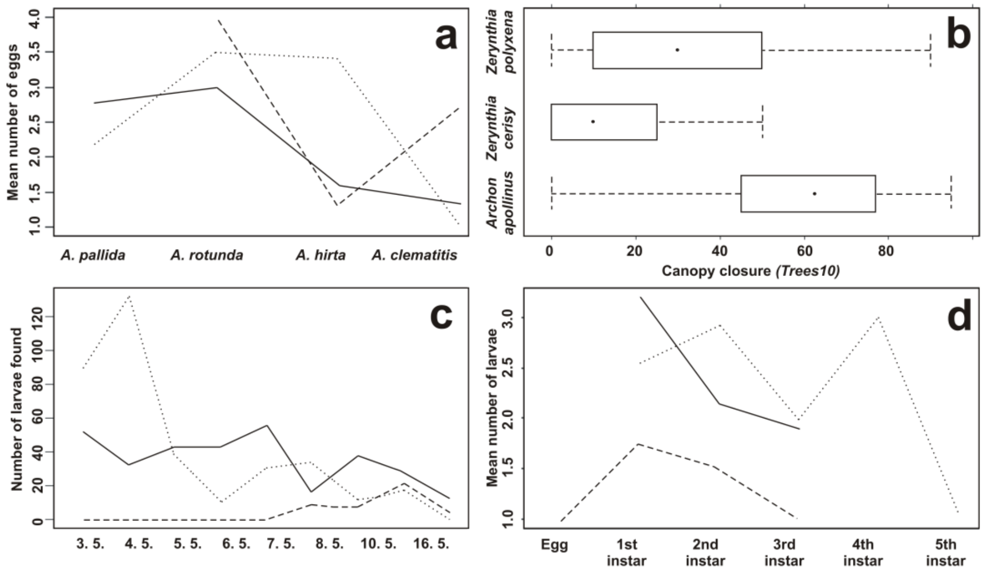


Figure 4. Results of model for eggs and larval records. (A) Interaction plot showing average egg batch sizes for individual butterfly species on individual species of *Aristolochia* plants; (B) box-plots (medians and quartiles) showing the amount of canopy closure (variable Trees10: see Material and methods) above *Aristolochia* plants bearing eggs of the respective butterflies; (C) numbers of larvae of the three studied butterfly species recorded during searches for larvae, note the unbalanced scale on the x-axis; (D) interaction plot showing average number of larvae of the three studied butterfly species in individual instars. For panels (A, C, D) dotted line, *Archon apollinus*; dashed line, *Zerynthia cerisy*; full line, *Zerynthia polyxena*.

As with eggs, *A. apollinus* larvae were most frequent found on *Aristolochia hirta*, followed by *A. rotunda; Aristolochia pallida* and *Aristolochia clematitis* were rarely used (Table 6). Most of the larvae were in third to fifth instars during the search. Most of the plants used were already flowering or fruiting. The larval occupancy was not affected by the density of *Aristolochia* stems in 1-m diameters, but was affected by the number of leaves (occupied ≈70, unoccupied ≈40).

Zerynthia cerisy larvae were found most frequently at *Aristolochia clematitis* and *Aristolochia hirta*, less so at *A. rotunda*, and never at *Aristolochia pallida*. Most of the larvae were in first to second instar, most of the plants used were already flowering or fruiting. The occupied plants were surrounded by low *Aristolochia* stem density (occupied ≈5, unoccupied ≈10), and hence leaf density (occupied ≈30, unoccupied ≈45), but the significant interaction of stem—leaf density revealed that the larvae preferred isolated but richly leafed plants.

Zerynthia polyxena larvae distinctly preferred *Aristolochia pallida* over *A. rotunda* and *Aristolochia clematitis*, whereas *Aristolochia hirta* was least preferred. The larvae were mostly in the first to third instars. Host plant phenology, surrounding stem density and surrounding leaf number had no effects.

Mutual comparison among the three species corroborated that *Z. polyxena* larvae were more abundant on *Aristolochia pallida* and *Aristolochia rotunda*. More *Z. polyxena* larvae were found on plants with high amount of leaves consumed. Considering instars (Figure 4d), the results were roughly the same as in models with negative records. All forward-selected models included plant species and larval instar.

Discussion

The phenology of the system composed of four spring-blooming *Aristolochia* plants and three spring-flying papilionid butterflies reflected yearly weather. In 2010, adults of the earliest-flying *A. apollinus* appeared during the third week of March, rapidly reached peak numbers and ceased flying in early April, whereas the two other species were barely appearing. Three weeks later, the encountered *Z. cerisy* and *Z. polyxena* were often worn individuals. Some adults of the latter two species, however, were still on wings a month later. In 2011, when the spring was unusually cold, no *A. apollinus* appeared before the first week of April, and all three species flew simultaneously.

Comparisons of sympatric species that aimed to disclose details of their resource niches are easier for species occurring simultaneously in time [e.g. space utilization by Eumaeini lycaenids: Prieto and Dahners (2009); or a study of co-occurring dragonflies: Khelifa et al. (2013)]. Analyses of butterfly oviposition preferences with regard to host plant conditions may provide guidance for conservation management (e.g. Jansen et al. 2012; Šlancarová et al. 2012).

Table 6. Results of general linear models-regressions relating larval records to predictors related to host plants and habitats.

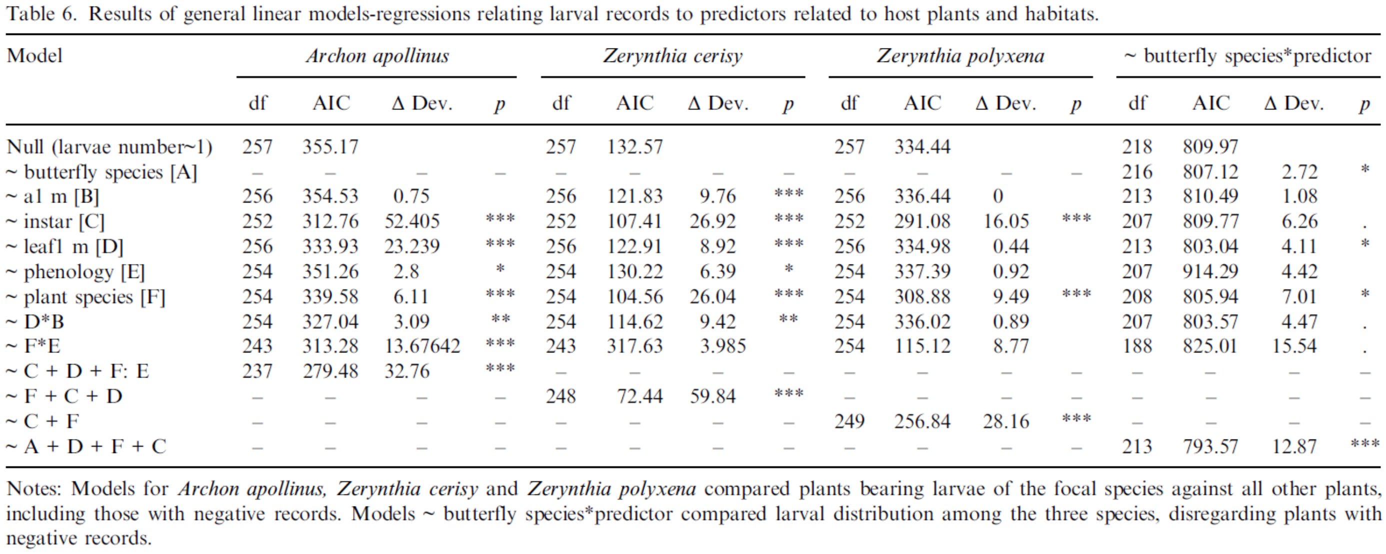


Table 6. Results of general linear models-regressions relating larval records to predictors related to host plants and habitats.

Model	*Archon apollinus*				*Zerynthia cerisy*				*Zerynthia polyxena*				~ butterfly species*predictor			
	df	AIC	Δ Dev.	*p*	df	AIC	Δ Dev.	*p*	df	AIC	Δ Dev.	*p*	df	AIC	Δ Dev.	*p*
Null (larvae number~1)	257	355.17			257	132.57			257	334.44			218	809.97		
~ butterfly species [A]	–	–	–	–	–	–	–	–	–	–	–	–	216	807.12	2.72	*
~ a1 m [B]	256	354.53	0.75		256	121.83	9.76	***	256	336.44	0		213	810.49	1.08	
~ instar [C]	252	312.76	52.405	***	252	107.41	26.92	***	252	291.08	16.05	***	207	809.77	6.26	.
~ leaf1 m [D]	256	333.93	23.239	***	256	122.91	8.92	***	256	334.98	0.44		213	803.04	4.11	*
~ phenology [E]	254	351.26	2.8	*	254	130.22	6.39	*	254	337.39	0.92		207	914.29	4.42	
~ plant species [F]	254	339.58	6.11	***	254	104.56	26.04	***	254	308.88	9.49	***	208	805.94	7.01	*
~ D*B	254	327.04	3.09	**	254	114.62	9.42	**	254	336.02	0.89		207	803.57	4.47	.
~ F*E	243	313.28	13.67642	***	243	317.63	3.985		254	115.12	8.77		188	825.01	15.54	.
~ C + D + F: E	237	279.48	32.76	***	–	–	–	–	–	–	–	–	–	–	–	–
~ F + C + D	–	–	–	–	248	72.44	59.84	***	–	–	–	–	–	–	–	–
~ C + F	–	–	–	–	–	–	–	–	249	256.84	28.16	***	–	–	–	–
~ A + D + F + C	–	–	–	–	–	–	–	–	–	–	–	–	213	793.57	12.87	***

Notes: Models for *Archon apollinus, Zerynthia cerisy* and *Zerynthia polyxena* compared plants bearing larvae of the focal species against all other plants, including those with negative records. Models ~ butterfly species*predictor compared larval distribution among the three species, disregarding plants with negative records.

Due to sequential phenology, however, the system described in this paper does not present an ideal time for studying immature distribution – any recording date would be too early or too late for some butterfly– plant interactions. Our findings on egg and larval preferences therefore describe only snapshots of the whole system dynamics, overcoming this limitation would require following pre-tagged plants, and associated butterfly immature, for the entire spring (cf. Table 1).

Adult biotope use

In the highly heterogeneous submediterranean biotope mosaic, all studied species were encountered in all available habitats, but there were clear differences: the phenologically postponed *Z. cerisy* preferred open woodland over scrub, contrasting with the earlier-appearing *A. apollinus* and *Z. polyxena*; and females of all three species were more frequently than males encountered in close forest, which they probably entered for oviposition. Using adjacent components of vegetation mosaic for different activities is common in butterflies. Slamova et al. (2013) observed that adults of *Erebia aethiops* visited sites with shady microclimates during the hottest time of day, and Bergman (2001) observed that females of *Lopinga achine* spent much time within forest gaps containing host plants, whereas males stayed in dense understorey. Dennis et al. (2003) warned against viewing insect habitats as equivalents to phytosociological units with homogeneous vegetation, because a species may locate different resources in different vegetation types.

Adult demography and dispersal

For 2 years, *A. apollinus* was consistently the most abundant of the three study species, followed by *Z. polyxena* and *Z. cerisy.*

Residency values (and hence, daily and average survival), obtained from mark– recapture data, did not deviate from those reported for other univoltine spring-flying butterflies (e.g. Vlasanek et al. 2009; Celik 2012). In cases when sexes differed, males displayed higher residency than females, contrasting with the distantly related spring-flying papilionid

Parnassius mnemosyne (Vlasanek et al. 2009) or with a population of *Z. polyxena* in Slovenia (Celik 2012). Lower female residency indicates either increased mortality or increased emigration. As we detected higher female mobility in all three species, emigration provides a plausible explanation.

Archon apollinus was the most sedentary species, followed by *Z. polyxena* and *Z. cerisy*. Abundances (and hence local densities) followed the reverse pattern, pointing to a density–mobility trade off. Inverse mobility–density relationships were detected, e.g. for British (Cowley et al. 2001) and Central European (Bartonova et al. 2014) butterfly faunas, and observed empirically by Konvicka et al. (2012) for butterflies co-occurring in humid grasslands. Butterflies using widespread but sparsely distributed resources employ higher mobility to compensate for low resource density.

The low *A. apollinus* mobility agrees with Hesselbarth et al. (1995), who nevertheless expected females to be more sedentary than males, contrary to our finding. The low mobility might be linked to the short time available for dispersal in early spring (cf. Vlasanek et al. 2009). For *Z. polyxena*, the mobility reported here is higher than that observed in Slovenia (Celik 2012), or in the closely related Italian species *Zerynthia cassandra* Geyer, 1828 (Vovlas et al. 2014). Detecting long-distance movements using mark–recapture depends on the study area size (cf. Zimmermann et al. 2011), which might explain these discrepancies. Still, *Z. polyxena* is generally viewed as a sedentary species in more northerly parts of Europe (Weidemann 1995; Dapporto 2010), possibly because of regional differences in resources supply and distribution. Whereas four host plants with varying phenology are used in Greek Thrace, a single host *(Aristolochia lutea)* was used by the Slovenian population (Celik 2012), and another single host *(Aristolochia clematitis)* is used in Central Europe (Benes et al. 2002). With several potential larval hosts growing in various habitats, ovipositing females may benefit from wider landscape exploration, whereas in single-host situations, females should be faithful to host plant patches. Finally, the high *Z. cerisy* mobility may reflect its flight in later spring, when the weather becomes more favourable, but locating sun-exposed host plants becomes increasingly difficult.

Requirements of pre-adult stages

Each of the three butterflies oviposited and fed on several phenotypically different *Aristolochia* species, suggesting that chemical clues, rather than larval morphology, are used to locate oviposition substrates (e.g. Mebs and Schneider 2002). The four *Aristolochia* plants consumed varied in phenology (*Aristolochia pallida* was the earliest sprouting, *Aristolochia clematitis* the latest sprouting) and biotope association (*Aristolochia hirta* prevailed in closed forest, *Aristolochia pallida* in open forest, *Aristolochia rotunda* in scrub, *Aristolochia clematitis* on disturbed grounds), and their use by the butterflies reflected these patterns.

Archon apollinus and *Z. polyxena* used all four *Aristolochia* species, corroborating reports on their wide host ranges (cf. Tolman and Lewington 2009) and refuting the claims that *Aristolochia clematitis* is toxic for *A. apollinus* (Carbonell 1991; Köstler 1993). Immatures of the late-flying *Z. cerisy* did not use *Aristolochia pallida*, which was mostly senescent during its flight. Immatures of *A. apollinus* were relatively frequent in closed forest, arguably because its adults oviposit before the leaf flush, similarly to other early-spring woodland butterflies [e.g. *Anthocharis cardamines* (Linnaeus 1758): Dempster (1997); or *Hamearis lucina* (Linnaeus 1758): Sparks et al. 1994]. On the other hand, some immatures of the late-flying *Z. cerisy* were located on *Aristolochia* plants growing in recently abandoned fields, or on stream gravel beds. These sites do not get shaded later in the season and using disturbed habitats is often linked to increased mobility (Dennis et al. 2004), which was the case here.

All three butterflies preferred sites with high *Aristolochia* shoot densities, similarly to the Cretan endemic *Zerynthia cretica* (Dennis 1996). Plants growing in higher densities, or just larger plants, are better detected by females, provide more food for the larvae, and offer better cover from parasitoids or predators (Renwick and Chew 1994). *Archon apollinus* and *Z. polyxena* eggs were frequent at plants with other egg-bearing plants in their vicinity. Such accumulations of eggs may result from visits to an identical spot by several females (Hesselbarth et al. 1995), or from recurrent visits of a female to the same plant (Tolman and Lewington 2009). Restricted time available for flight in early spring might be responsible for recurrent visits.

Another trait shared by *A. apollinus* and *Z. polyxena* was their preference for host plants surrounded by forbs, rather than grasses. As both species oviposit on young plants, the surrounding vegetation probably serves as clue for future host plant size. For *Z. cerisy*, both Tolman and Lewington (2009) and Hesselbarth et al. (1995) describe oviposition at shady spots, in contrast to our records. It is possible that their descriptions reflected conditions in warmer parts of the *Z. cerisy* range, where the females might prefer cooler spots than at our locality, situated near the northern border of the species' range.

The use of several host plant species that appear sequentially in different habitats clearly enhances the regional resource base for the butterflies, arguably buffering their population dynamics against risks of phenological mismatches with their plants (Cushman et al. 1994; Van Asch and Visser 2007). This highlights the importance of a finely structured habitat mosaic, where alternative host plants grow in relatively close proximity, within reach of dispersing butterflies (cf. Dennis et al. 2003).

Different situations, however, exist elsewhere in Europe. Jordano and Gomariz (1994) reported from Spain that because potential host plants of *Zerynthia rumina* (Linnaeus 1758) are rarely sympatric, host plant use by the butterfly varies among its populations. *Zerynthia cretica* is reportedly monophagous on the Cretan endemic *Aristolochia cretica*, although other congeneric plants occur on the island (Dennis 1996). A single host plant is also used by the *Z. cassandra* population studied by Vovlas et al. (2014) in Italy, the Slovenian *Z. polyxena* studied by Celik (2012), and all *Z. polyxena* populations in Central Europe (Batary et al. 2008).

Conclusion

As the three *Aristolochia*-feeding papilionids follow a phenological sequence in adult and larval emergence and use overlapping ranges of host plants, which themselves differ in phenological patterns and prevail in different successional stages of Mediterranean vegetation, the co-occurrence of the butterflies appears to be facilitated by their ability to locate resources in different, but closely adjacent, environments (cf. Oliver et al. 2010).

Considering the local population sizes and dispersal ability of the three studied butterflies relative to the patchiness of landscape mosaic in the area studied, none of them seems to be currently threatened by host plant or habitat loss. This certainly applies for the EU-protected *Zerynthia polyxena*, a species that is still widespread in Greece (Pamperis 1997). Still, the heterogeneous patchworks of open, semi-open and closed vegetation, typical for ancient cultural landscapes of the Mediterranean, may be lost to spontaneous forest encroachment if left unmanaged, or even actively planted by trees. The crucial role of landscape heterogeneity for invertebrates is increasingly recognized from human-altered areas of northern Europe (e.g., Samways 2007; Shreeve and Dennis 2011; Slancarova et al. 2014) and might be even more prominent in the Mediterranean, a region hosting many more species and currently experiencing rapid land use changes (Atauri and De Lucio 2001; Kati et al. 2012; Fernández-Chacón et al. 2014).

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CHAPTER IV

Butterflies in Portuguese 'montados': relationships between climate, land use and life-history traits

Zerynthia polyxena (Denis & Schiffermüller, 1775)

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Abstract

Butterfly life-history features are expected to co-vary along environmental gradients related to changes in the vegetation structure or composition; however the direction and intensity should vary across regions at the large scale. This study focuses on the butterfly communities of Portuguese 'montados'. Sixteen sites (mostly cork oak fields) were selected, reflecting a succession gradient in the vegetation of the understorey after human intervention. While controlling for vegetation and broader geographical and climate effects, we looked for trends in butterfly species richness and abundance (using generalised linear models) and for trends in species composition (using redundancy analyses). Moreover, we tried to uncover the co-variation between the butterfly life-history characteristics and succession. The results revealed that butterfly species richness was not significantly influenced by any of the considered variables. In contrast, abundance depended on geographic and oceanity–continentality gradients as it increased towards the East and with more marked temperature annual ranges and less dry summer conditions. Species composition was influenced by temperature ranges and by shrub coverage. There was no strong evidence in favour of fast–slow or generalist–specialist syndromes co-varying along human imposed environmental gradients. However, after controlling for the broad scale variables (geography and climate) shrub cover emerged as a relevant factor. This reinforces the idea that late successional stages are not optimal for butterfly communities. It implies the importance of the extensive methods of traditional management and the negative effects of long-term abandonment.

Introduction

Species population densities are generally expected to respond to changes in the habitat according to the interactions between the species requirements (i.e. their 'ecological niches' in a broad sense) and their resources (e.g. Price 2002). The degree of specialization can be measured to a reasonable extent by quantifying the life-history traits, hence the species composition would be generally expected to involve a response (via the life-history traits) to some key variables affecting their habitats. Butterflies (diurnal Lepidoptera belonging to the superfamily Papilionoidea) depend strongly on specific plants as larval food, on ambient temperature and structure due to their ectothermic condition and comparatively small sizes, and on broad habitat features and connectivity, as the adults are flying insects with relatively complex behaviour (e.g. Boggs et al. 2003; Dapporto and Dennis 2013; Dennis et al. 2004). Since the broad aspects of the life-histories of the European butterflies are well known, they represent an interesting material for investigating the co-variation of life-history features along environmental gradients related to changes in the vegetation structure or composition (e.g. Krauss et al. 2003).

For several decades ecologists have been interested in identifying basic sets of properties of the organisms (life-history traits which essentially represent demographic tactics) that would be objectives for selection and an expression of their capabilities while facing a changing environment. More or less precise combinations of such traits are generally expected to result in a graded response across different species. They could be ordered along a gradient or continuum, be it termed r–K (from species maximising intrinsic rate of population growth towards those conforming to carrying capacity of their environments), fast–slow, or generalist–specialist (see among others: Borschig et al. 2013; Gaillard et al. 1989; Pianka 1970). Any such 'continuum' may in turn be correlated with spatial or climatic variables. In ectotherms, an increase in temperature has generally resulted in smaller adult sizes, higher growth rates, shorter life spans and shorter development times (Atkinson 1994; Karlsson and Wiklund 2005; Sibly and Atkinson 1994); however, these traits may be modified by habitat fragmentation (Karlsson and van Dyck 2005).

Evidence has shown that butterfly life-histories co-vary along environmental gradients, often following a generalist–specialist continuum (Carnicer et al. 2013; Dennis et al. 2011; Ockinger et al. 2010). However, the particular direction and intensity of such gradients, the precise traits involved and the responses of the specialist and the generalist species vary across regions on a large scale (e.g. within Europe, cf. Bartonova et al. 2014; Ockinger et al. 2010). For butterflies, this continuum may in fact be correlated with vegetation structure and composition (including forest cover or land management patterns) as well as with altitude, temperature and the density of resources (Bartonova et al. 2014; Dapporto and Dennis 2013; Menendez et al. 2007; Stefanescu et al. 2011).

Here we focus on the butterfly communities of a semi-natural type of landscape, the oak woods (namely, cork oak) in the western Mediterranean across the south of Portugal. Within this relatively homogeneous habitat, we analyse the changes in species diversity along the gradient of plant diversity which reflects plant succession after human intervention. Our study covers a relatively large geographic area (roughly 300 × 150/200 km) which, in spite of being located in one of the biodiversity hotspots of the Mediterranean Region (Myers et al. 2000), features a comparatively poor butterfly fauna in the context of the western Mediterranean (97 species from a total of 139 in Portugal: Garcia-Barros et al. 2004; Maravalhas 2003). Moreover, the biogeographic variation in the South Por-tuguese butterfly fauna is lower than in other Mediterranean areas, so that the faunal regionalization of the area is shallow for these insects (Garcia-Pereira et al. 2003; Romo and Garcia-Barros 2010).

Extensive areas in the south of Portugal have been traditionally devoted to a mixed, non-intensive, land use consisting of retaining the trees while managing the remaining vegetation for cultivation or livestock grazing, which has resulted in a characteristic savannah-like landscape, the 'montados' (equivalent to the Spanish 'dehesa'). The trees themselves (namely two species of evergreen oaks: *Quercus suber L.*, the cork oak and *Q. ilex L.,* the holm oak) are subject to exploitation: the cork is periodically harvested without killing the trees, the acorns are a fodder for pigs, and the ground remaining is used for crop cultivation or sheep grazing. Thus, this agricultural system (accounting for ca. 35 % of the forest coverage in that country: DGF 1993; Oliveira 1995) is economically important. Moreover the 'montados' are the result of a complex, long-term interaction between humans and their environment, dating back several centuries (Blondel and Aronson 1999; Costa et al. 2009; Pinto-Correia et al. 2011; Ribeiro 1993) and have been classified as a habitat of conservation interest under the European Union policies with both socio-economic and biodiversity values (Bugalho et al. 2011). The abandonment of traditional management practices due to depopulation during the second half of the 20th century resulted in shrub and forest encroachment; which in turn causes the decline of species associated with early succession or ecotone habitats (Bugalho et al. 2011; Moreira and Russo 2007; Verdasca et al. 2012). Such effects are in part compensated by forest fuel management to prevent fire hazards (Pausas et al. 2008; Ramirez and Diaz 2008; Bugalho et al. 2011). Although the elimination of the understorey probably negatively affects the Mediterranean oak forest regeneration (Porto et al. 2011; Santana et al. 2011), it might have—paradoxically—positive effects for the diversity of taxa such as the butterflies (Verdasca et al. 2012).

Based on the ideas above we focused on a much wider area than the one scrutinised by Verdasca et al. (2012) (i.e. the 'montados' across the South of Portugal) and attempted to (1): identify how the gradient of plant diversity that features the natural regeneration of the vegetation in cork oak fields affects the species diversity, richness, abundance and community composition of butterflies; (2) analyse co-variation between butterfly life-history traits and the succession sequence and (3) alternatively, to identify any broad-scale environmental patterns (e.g. in climate, geography, geology and human disturbance at much wider scales than the study sites) that could provide alternative explanations for the patterns found.

Materials and methods

Study area and site selection

The study area was the south of Portugal, including the regions of Alto Alentejo, Alentejo, Baixo Alentejo and Algarve. The fieldwork was carried out at 18 sites (Fig. 1), but two of them were later excluded (see below). While all the study sites corresponded to 'montado' areas (i.e., cork oaks or holm oaks as tree layer), the sampling sites were selected to cover three basic degrees of human intervention in the oak woods which would also reflect a successional gradient in the vegetation of the understorey. Herbaceous and shrub cover would vary while the tree layer remained constant, although in different densities. The three types of understorey vegetation consisted of: *Grassland*, where the non-tree layer was dominated by annual or perennial herbs and the typical use is cattle grazing; *Shrubland* where grazing did not occur (generally for more than 5 years) which allowed for the development of at least small (<0.50 m) woody plants where the use of the oak trees persisted; and *Scrub forest* where there were no traces of recent human exploitation, leading to a dense and complex vegetation cover which in some instances approached the climax state.

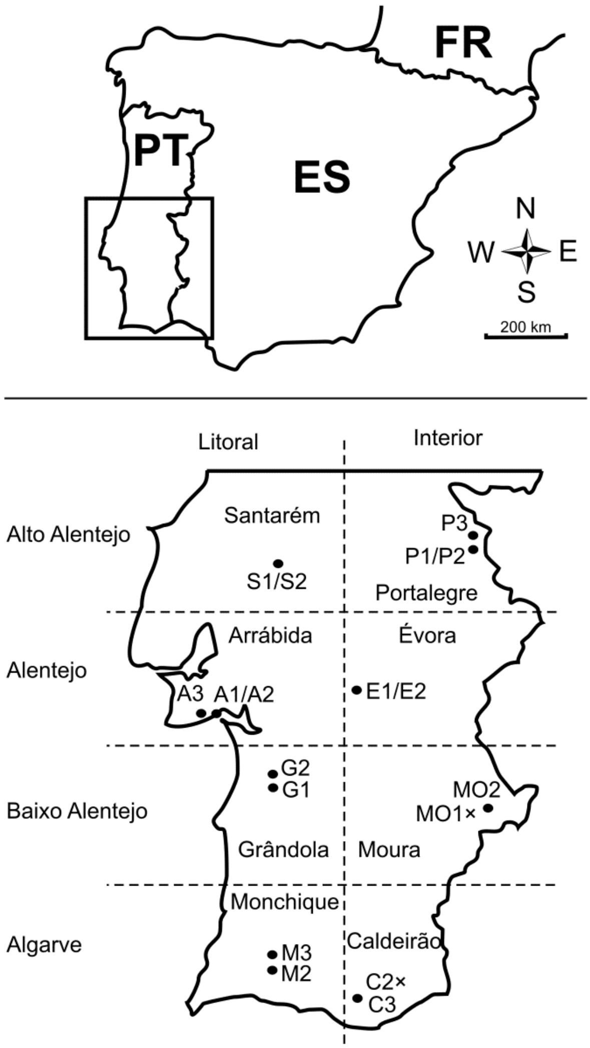


Fig. 1 Location of study sites in southern Portugal (n = 16; C2 and MO1 were excluded because of unexpected changes in land use).

An effort was made to select the three types of vegetation within a relatively short distance (1–30 km) within a grid of eight broad areas covering both the longitudinal and the latitudinal gradients all across SW Portugal (Fig. 1). However a fully hierarchical design was not possible for two reasons. The first was the occurrence of unexpected changes in land use in two of the sites (ploughing and clearing of the shrubs) in Caldeirão (C2) and Moura (MO1). The second was the absence of truly mature woodland in most plains so that this vegetation stage was represented only in the mountainous or hilly regions such as Arrábida, Portalegre, Monchique and Caldeirão.

Environmental variables and measures of vegetation

A degree of between-site variation in insect communities would be expected to result from responses to environmental and biogeographical factors acting far beyond the local scale and superimposed on local patterns of management. To account for such patterns we collected information on several characteristics that are known to influence faunas such as the geographic position, climate, geological substrate and broad-scale human footprint (e.g. Kadlec et al. 2009). These characteristics, used as explanatory variables, were (a) *Altitude;* (b) *Longitude;* (c) *Latitude,* (d) *Landcover* (obtained from the European Environment Agency, Corine Programme 1985–1990: Anonymous 2000); (e) *Geological substrate* [coded as: (1) clay, (2) limestone, (3) siliceous (from DGA 1998)]; and 19 climatic variables (from the BIOCLIM subset of WorldClim: Hijmans et al. 2005): (f) *Annual Mean Temperature;* (g) *Mean Diurnal Range* [Mean of monthly temperature ranges (maximal temperature - minimal temperature)]; (h) *Temperature Seasonality* (standard deviation 9 100); (i) *Isothermality* (the ratio between the mean diurnal range and temperature annual range); (j) *Maximum Temperature of Warmest Month;* (k) *Minimum Temperature of Coldest Month;* (l) *Temperature Annual Range* (difference between the means of the coldest and warmest month); (m) *Mean Temperature of Wettest Quarter;* (n) *Mean Temperature of Driest Quarter;* (o) *Mean Temperature of Coldest Quarter;* (p) *Mean Temperature of Warmest Quarter;* (r) *Annual Precipitation;* (s) *Precipitation of Wettest Month;* (t) *Precipitation of Driest Month;* (u) *Precipitation Seasonality* (coefficient of variation);

(v) *Precipitation of Wettest Quarter;* (w) *Precipitation of Driest Quarter;* (x) *Precipitation of Warmest Quarter;* (y) *Precipitation of Coldest Quarter.*

To measure the broad aspects of the vegetation diversity in each sampling station, we plotted a random square (5 9 5 m) in each site every month between May and July (1999). Then, the numbers of identifiable species of vascular plants were counted and the mean cover (%) of the herbaceous, shrub and tree layers was estimated. A detailed report on the plants recorded may be found in Malveiro (2001). The following vegetation variables were recorded: (a) *Number of plant species*—total number of vascular plant species (pooled for the whole period); (b) *Trees*— relative cover of the tree layer (% of the total area). In all instances the tree species were the *Quercus* species mentioned above; (c) *Shrub*—relative cover of the bush/shrub layer (% of the total area). This accounted for any non-tree woody plants as well as for the perennial semi-woody species whose presence would indicate the absence of human intervention for a period of at least 5 years; (d) *Herbs*—relative cover of the herbaceous layer (% of the total area) which accounted for the non-woody plants of any size and growth form.

Field counts and life-history data

Butterfly counts were recorded at each site once a month between April and September following the transect method of Pollard and Yates (1993), during two consecutive years (1998 and 1999). The counts were recorded along transect routes (1000 m long and 5 m wide, carried out at constant speed until a butterfly was intercepted, with ambient temperature equal or above 15 °C, cloud cover less than 75 % and no wind or just a slight breeze, between 10:00 and 16:00 h mean solar time).

Each butterfly species was classified for several life-history and geographical traits (see Online Resource 1). Depending on their nature these variables were categorised by means of fuzzy coding, e.g. when a species displays multiple membership of different categories in which a trait was divided. For quantitative numerical features such as the altitudinal ranges, the proportional membership of a species for each category of the trait was estimated.

The following variables were used: (a) *Altitudinal range* [fuzzy coded: (1) 0–500 m; (2) 501–2000 m; (3) over 2001 m]; (b) *Range size* [ranked scale: 1—less than 1000 km^2 (including mountain ranges and peninsulas smaller than the Iberian); 2—widespread across Europe or the Mediter-ranean area; 3—Western Palaearctic (Europe as far as the Urals, Western Kazakhstan, Pontomediterranean region); 4—Eurosiberian (further than the Ural Mountains, including the circumpolar area); 5—large (larger than the Palearctic)]; (c) *Feeding index* (FI)—measured as the number of plant families (F) and genera (G) used as larval hosts using the formula: FI = (G 9 Fa)1/2, where a = (F)/ 2 9 G. The index assumes higher values for any number of hosts when the hosts represent a greater number of genera and families (i.e. increased FI); (d) *Flight period* [fuzzy coded: (1) FL1—early spring: February–March; (2) FL2— late spring: April; (3) FL3—summer: June; (4) FL4—late summer: July–September; (5) FL5—autumn: October or later]; (e) *Gregariousness* (scored binarily); (f) *Host plant apparency* (ranked scale: 1—ephemeral plants and small herbs; 2—higher herbs and grasses; 3—shrubs and small trees; 4—trees); (g) *Migration* (scored binarily); (h) *Myrmecophily* (scored binarily); (i) *Number of generations* per year; (j) *Voltinism* (scored binarily) (i.e. uni-voltine/bivoltine or polyvoltine); (k) *Overwintering stage* [scale 1–4: (1) 1—egg; (2) 4—imago (including species without overwintering)]; (l) *Egg placement* on the host plant [categorical: (1) on the flower; (2) on the leaf; (3) anywhere on the host plant (meaning non-specific part of host plant); (4) off the host plant]; (m) *Range type* [categorical: (1) European; (2) Eurosiberian; (3) Mediterranean; (4) Holarctic]; (n) *Wingspan* (adult forewing length in mm). The information was compiled from existing literature (for details see Online Resource 2).

For the analyses of phylogenetic correlation, we built a supertree from the available phylogenetic evidence (presented in Online Resource 3).

We studied the effects of environmental and climatic variables on each of the following attributes of the butterfly communities (herein response variables): (a) *Species richness* (i.e. the number of species), (b) *H index* (ecological diversity estimated by the Shannon diversity index, e.g. Magurran 2004), (c) *Abundance* (i.e. number of individuals), and (d) *Species composition* with interpretation of life-history traits after considering the phylogeny.

Statistical analyses

Preliminary exploration

Firstly, in order to avoid spurious effects of co-linearity in the subsequent statistical analyses we explored the linear correlation between 19 BIOCLIM climatic variables (Table 1). Only variables with a Pearson correlation coefficient less than 0.75 were considered (Rissler and Apodaca 2007). Among the correlated variables, we selected those potentially most relevant given the climatic conditions in the study area, i.e. *Temperature Annual Range* and *Precipitation of Driest Month*. *Temperature Annual Range* reflects differences between mild coastal or plain areas and more heterogeneous hilly or (sub) montane landscapes. *Precipitation of Driest Month* is meaningful in the Mediterranean area as an estimate of the intensity of the summer drought, a period of stress for an organism's growth.

In the second step, the linear correlations between the climate variables, response and vegetation variables were inspected (Table 2). In some instances, when pairs of related variables were highly and significantly correlated (such as in the case of *H index* and *Species richness* or *Longitude* and *TAR*), this evidence was used to select just one variable with potential biological relevance in the study area (i.e. *Species richness* and *Longitude*).

Species richness and abundance

Species richness was analysed using generalized linear models (GLM) in R (The R Core Team 2012) with an assumed Poisson distribution of the dependent variable (i.e. number of butterfly species). The same was done for analysing Abundance except that here a Gaussian distribution was adopted. Analyses were not controlled for phylogeny. We tested the independent effects of all variables. Moreover, we performed forward selection from all significant variables using the add1 function in R. Model fits were assessed using the Akaike information criterion (AIC).

Scatterplots showing the relationships between selected pairs of variables (such as those in Fig. 2) were created using values predicted by the respective generalized linear regression model in R.

Tab. 1 Bivariate correlations (Pearson r) between BIOCLIM climate variables.

	Mean diurnal range	Isothermality	T seasonality	Max. T of warmest M	Min. T of coldest M	T annual range	Mean T of wettest Q	Mean T of driest Q	Mean T of warmest Q
Annual mean T	0.2	0.2	−0.06	0.27	0.44	0.05	0.52*	0.36	0.4
Mean diurnal range		−0.21	0.76**	0.89***	−0.7**	0.89***	−0.48	0.8***	0.81***
Isothermality			−0.78***	−0.57*	0.6*	−0.62*	0.79***	−0.63**	−0.6*
T seasonality				0.92***	−0.86***	0.97***	−0.85***	0.9***	0.88***
Max. T of warmest M					−0.68**	0.97***	−0.62*	0.98***	0.98***
Min. T of coldest M						−0.85***	0.87***	−0.61*	−0.57*
T annual range							−0.75**	0.92***	0.92***
Mean T of wettest Q								−0.59*	−0.53*
Mean T of driest Q									0.99***
Mean T of warmest Q									
Mean T of coldest Q									
Annual P									
P of ettest M									
P of driest M									
P seasonality									
P of wettest Q									
P of driest Q									
P of warmest Q									

	Mean T of coldest Q	Annual P	P of wettest M	P of driest M	P seasonality	P of wettest Q	P of driest Q	P of warmest Q	P of coldest Q
Annual mean T	0.56*	−0.36	−0.46	−0.23	0.04	−0.4	−0.31	−0.31	−0.39
Mean diurnal range	−0.5*	−0.34	−0.56*	0	−0.34	−0.56*	0.02	−0.07	−0.46
Isothermality	0.78***	−0.8***	−0.59*	−0.95***	0.9***	−0.56*	−0.95***	−0.94***	−0.64**
T seasonality	−0.86***	0.31	0.04	0.61*	−0.78***	0.02	0.62*	0.57*	0.12
Max. T of warmest M	−0.6*	0.05	−0.24	0.39	−0.65**	−0.23	0.39	0.31	−0.12
Min. T of coldest M	0.94***	−0.18	−0.023	−0.42	0.56*	0.04	−0.45	−0.41	−0.03
T annual range	−0.77**	0.1	−0.17	0.43	−0.67**	−0.18	0.44	0.37	−0.08
Mean T of wettest Q	0.96***	−0.48	−0.28	−0.67**	0.76**	−0.24	−0.73**	−0.6**	−0.32
Mean T of driest Q	−0.56*	0.12	−0.19	0.46	−0.73**	−0.18	0.45	0.39	−0.07
Mean T of warmest Q	−0.52*	0.11	−0.18	0.44	−0.67**	−0.16	0.41	0.35	−0.06
Mean T of coldest Q		−0.45	−0.29	−0.65**	0.71**	−0.23	−0.69**	−0.66**	−0.2
Annual P			0.94***	0.91***	−0.56*	0.94***	0.91***	0.93***	0.97***
P of ettest M				0.74**	−0.28	0.99***	0.73**	0.77***	0.98***
P of driest M					−0.83***	0.72**	0.98***	0.99***	0.79***
P seasonality						−0.24	−0.84***	−0.82***	−0.34
P of wettest Q							0.71**	0.74**	0.99***
P of driest Q								0.99***	0.79***
P of warmest Q									0.81***

T temperature, *P* precipitation, *M* month, Q quarter

* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; **** $p < 0.0001$

Table 2 Bivariate correlations (Pearson r) between the climate variables, response and vegetation variables (n = 16 in all instances).

	H index	Herbs	Longitude	NPS	Precipitation	Shrub	Species richness	TAR	Trees
Abundance	−0.13	0.20	0.60*	0.15	0.64**	−0.35	0.289	0.54*	0.14
H index		−0.06	−0.28	0.36	−0.34	0.31	0.78****	−0.39	−0.16
Herbs			−0.12	−0.24	0.17	−0.30	−0.29	−0.07	−0.69**
Longitude				0.12	0.56*	−0.15	0.06	0.92****	0.24
NPS					−0.03	0.31	0.33	0.03	0.18
Precipitation						−0.15	0.01	0.43	0.07
Shrub							0.27	−0.18	−0.39
Species richness								−0.08	0.02
TAR									0.15

NPS number of plant species, *TAR* temperature annual range

· < 0.1; * < 0.05; ** < 0.01; *** < 0.001; **** <0.0001

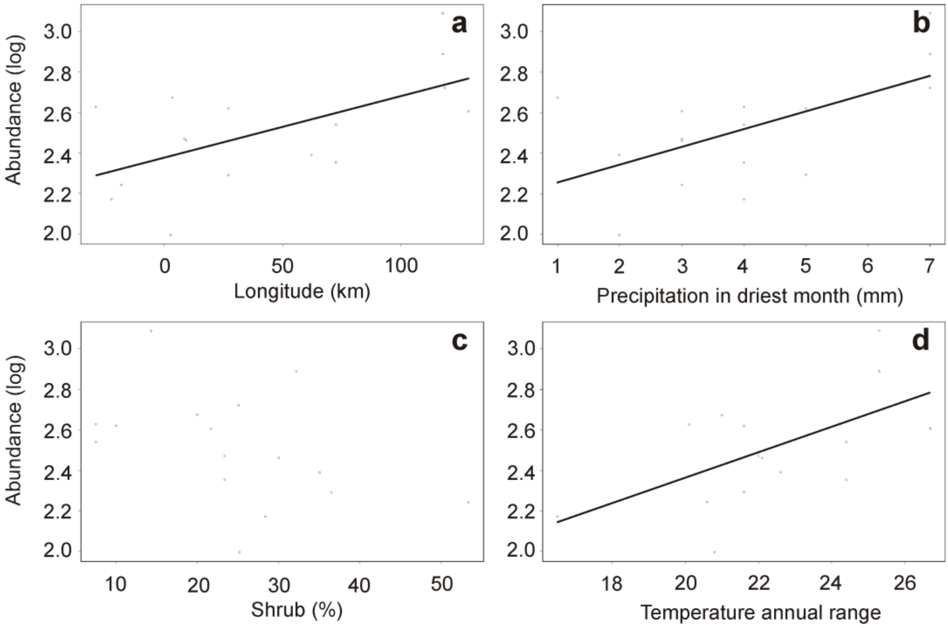


Fig. 2 The effects of *Longitude* (a), *Precipitation of Driest Month* (b), *Shrub cover* (marginally significant) (c) and *Temperature annual range* (d), on butterfly species abundance in southern Portugal. Scatterplots showing the relationships between the selected pairs of variables were created using R software (The R Core Team 2012). Lines represent values predicted by the respective generalized linear regression models. See Table 3 for results.

Species composition

Changes in butterfly *Species composition* were evaluated by redundancy analysis (RDA) using CANOCO 5 (Ter Braak and Smilauer 2012). We carried out single-term ordinations for individual environmental variables. Species numbers at each site were log(x + 1) transformed and centred during the analyses. The significance of the environmental variables was evaluated by the Monte Carlo permutation test (999 permutations). Secondly, forward selection from all significant variables was performed to select the most relevant predictors.

The RDA models of species response to *Temperature annual range* formed the basis for life-history traits analysis. Because life-histories co-vary with phylogeny, the phylogenetic tree was turned into a patristic distance matrix, representing the distance of any pair of taxa measured along the branches of the phylogenetic tree. The distance matrix was turned into a set of descriptors using principal coordinate analysis (PCO), with PCO scores centred and standardized. Not all PCO scores are related to the response variable, therefore we used their subset selected by forward selection—only descriptors with $p < 0.04$ were included. Finally, the species responses to explanatory variables were explained by the traits, after removing the variation explained by phylogenetic descriptors. Each step was evaluated by the Monte Carlo test (999 permutations). Analyses were carried out individually for each trait.

To find any patterns hidden by the climatic gradient, another set of partial RDAs was conducted with *Temperature annual range* as a covariate. Analyses were performed separately for all each remaining previously significant explanatory variables.

Results

We recorded 52 butterfly species (6451 individuals). The most widespread species were *Leptotes pirithous* (Linnaeus, 1767), *Maniola jurtina* (Linnaeus, 1758) (also the most abundant species) and *Pieris brassicae* (Linnaeus 1758). The mean number of species per site was 21 (±3.95 SD). Sites C3 and G2 were the most diverse (27 species). C3 lies within a biotically rich region characterised by basic rocks in the southern Algarve, which hosts some Mediterranean-type species not found elsewhere in the country south of the Tagus valley such as *Euphydryas desfontainii* (Godart, 1819) or just there and in the northern parts, e.g. *Pseudophilotes abencerragus* (Pierret, 1837), *Plebejus argus* (Linnaeus, 1758), *Melitaea didyma* (Esper, 1778) and *Aporia crataegi* (Linnaeus, 1758) (Maravalhas 2003). The poorest site was E1 (Évora) with only 12 species. The highest number of individuals (1229 individuals) was recorded at site P1 (Portalegre), located in the innerand northernmost part of the region studied.

Species richness

Single-term models revealed that butterfly species richness was not significantly influenced by any environmental (i.e. climatic, geographical, geological or vegetation) variable (Table 3).

Abundance

Butterfly abundance significantly increased with increasing *Temperature Annual Range* (Table 3; Fig. 2d) and *Precipitation of Driest Month* (Fig. 2b). Moreover, it tended to increase towards the East (Fig. 2a). On the other hand, it seemed to decline along with higher *Shrub cover*, although this relationship was only marginally significant (see data points in Fig. 2c). Forward selection showed that the strongest predictor was *Longitude*.

Species composition and life-history traits

Univariate ordinations revealed that species composition was significantly influenced by the following variables (with signs indicating the direction of the relationship relative to the first canonical axis): *Longitude* (+), *Precipitation of driest month* (+), *Shrub* (+), and *Temperature Annual Range* (-) (Table 3).

Forward selection from all significant variables selected *Temperature Annual Range* as the only relevant predictor; revealing that increasing difference between winter and summer temperatures, or increasing continentality, was the most important predictor of the species composition of butterfly community structure. Species such as *Satyrium esculi* (Hübner, 1804), *Gonepteryx cleopatra* (Linnaeus, 1767) or *Muschampsia proto* (Ochsenheimer, 1808) inclined towards lower climate continentality, whereas species such as *Glaucopsyche melanops* (Boisduval, 1828), *Hipparchia statilinus* (Hufnagel, 1766) or *Argynnis pandora* (Denis and Schiffermüller, 1775) inclined towards areas with larger winter and summer differences (Fig. 3).

Visualization of relationships between *Temperature Annual Range* and the life-history traits are shown in Fig. 4a–d, where Response 1 represents the negative gradient of *Temperature Annual Range*.

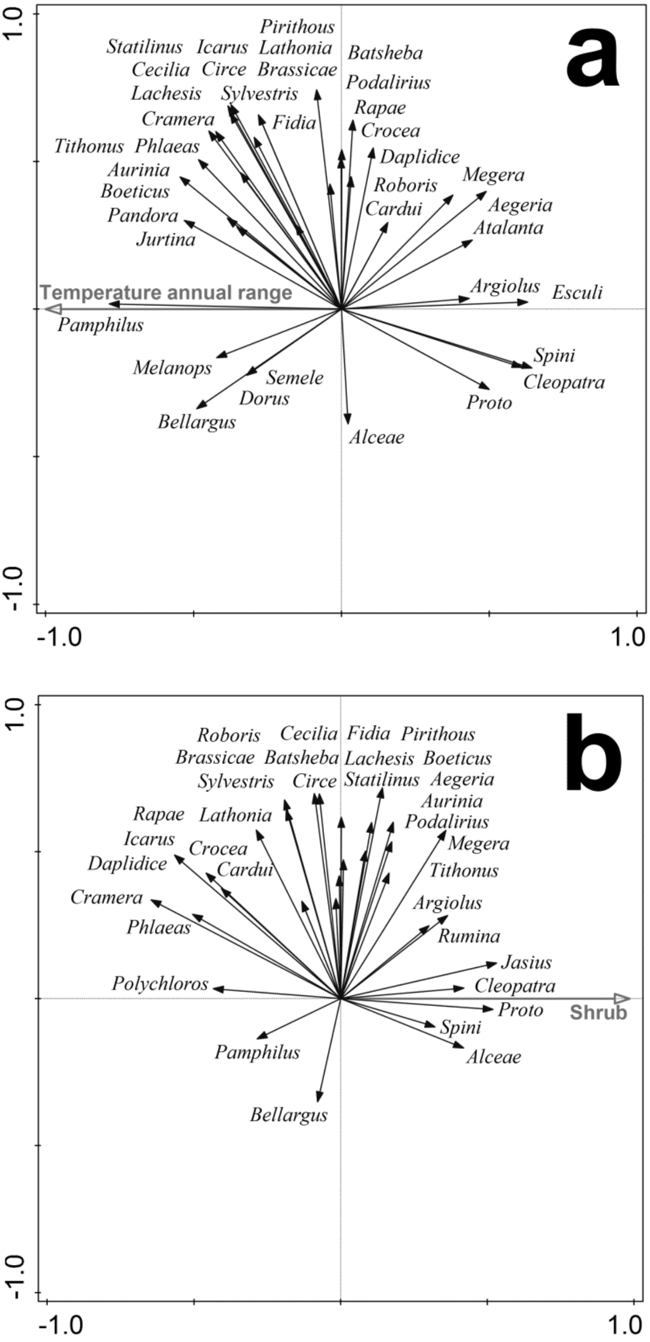
Table 3. Results of generalized linear model (GLM) regressions and redundancy analysis (RDA) ordinations assessing the impact of various predictors on butterfly *Species richness* (Poisson distribution of dependent variable), *Abundance* (Gaussian distribution) and *Species composition* during the research in 1998–1999 in southern Portugal

Model	Species richness				Abundance				Species composition		
	AIC	df	Explained variation %	*p*	AIC	*df*	Explained variation %	*p*	AEV %	F	*p*
Null	91.52	15			6.95	15					
~Altitude	93.45	13	0.66		6.37	14	14.88		2.20	1.30	
~Geological substrate	95.26	13	2.27		9.81	13	6.86		0.00	0.90	
~Herbs	92.54	14	8.46		8.35	14	3.66		0.30	0.00	
~Landcover	92.28	14	10.69		7.50	14	8.61		2.50	1.40	
~Latitude	93.42	14	2.92		6.82	14	12.46		0.80	1.10	
~Longitude	93.49	14	0.38		1.42	14	37.52	*	10.70	2.80	**
~Number of plant species	92.28	14	10.75		8.91	14	0.23		0.70	1.10	
~Precipitation in driest month	93.52	14	0.01		2.07	14	34.93	*	7.90	2.30	*
~Shrub	92.70	14	7.14		5.58	14	18.99	.	6.20	2.00	*
~Temperature annual range	93.46	14	0.54		1.65	14	36.61	*	11.10	2.90	**
~Trees	93.52	14	0.05		8.55	14	2.43		0.00	0.40	
~Shrub | Temperature annual range	–	–	–	–		–	–	–	5.00	1.70	*

AEV adjusted explanatory variation, ***AIC*** Akaike information criterion

. < 0.1; * < 0.05; ** < 0.01; *** < 0.001; **** <0.0001

Fig. 3 Ordination (partial redundancy analysis), showing the effect of *Temperature Annual Range* (a) and *Shrub* (after including *Temperature Annual Range* as covariate), both after removing the effect of phylogeny on butterfly species composition. Only species with weight higher than 10 % are displayed (See Table 3 for results)



Interpretation of this gradient (Table 4) revealed that the lower *Temperature Annual Range*, the more species tend to overwinter in later stages (Fig. 4a), to oviposit rather on flowers or anywhere on host plants (Fig. 4b), and—marginally—having a smaller wingspan.

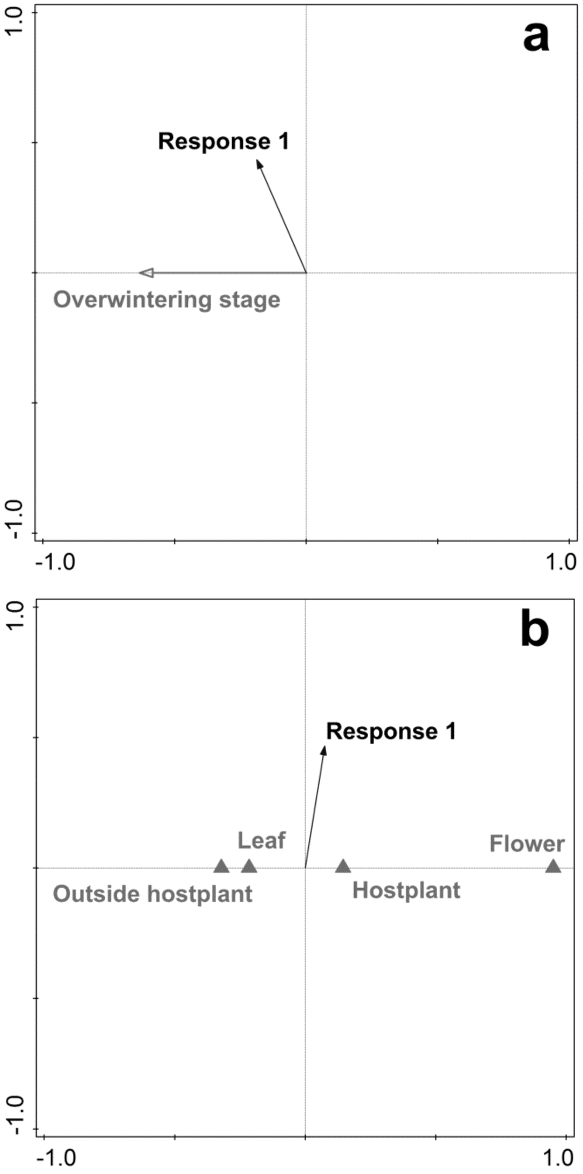


Fig. 4 Ordination (partial redundancy analysis), showing life-history traits interpretation of the analysis of the effect of *Temperature Annual Range* on butterfly species composition after removing the effect of phylogeny: *Overwintering stage* (a); *Egg placement* (b). Response 1 represents the response of respective Axis 1 of the above-mentioned analyses (see Fig. 3a; Table 4 for results)

Table 4 Summary of partial redundancy analysis (RDA) ordinations assessing the impact of *Temperature Annual Range* and *Shrub* (after including *Temperature Annual Range* as covariate), both after phylogenetic correction, interpreted by butterfly life-history traits. Data obtained during the research in 1998–1999 in southern Portugal

Model	~Temperature annual range			~Shrub | temperature annual range		
	AEV %	F	*p*	AEV %	F	*p*
Altitudinal range	0.00	0.40		2.70	1.70	
Range size	0.00	0.40		8.80	5.70	*
Egg placement	11.10	2.90	**	0.00	0.80	
Feeding index	0.00	<0.1		0.00	0.80	
Flight period	9.30	2.00		0.00	0.90	
Gregariousness	0.00	0.60		0.00	0.20	
Host plant apparency	0.00	0.70		5.10	3.60	.
Migration	0.00	0.40		16.60	10.70	**
Myrmecophily	0.00	0.10		0.00	<0.1	
Number of generations	0.50	1.20		12.60	8.10	**
Overwintering stage	13.50	7.30	*	5.80	4.00	*
Range type	5.80	1.80		14.10	3.70	*
Voltinism	0.00	0.20		2.00	2.00	
Wingspan	5.30	3.20	.	0.00	0.20	

AEV adjusted explanatory variation

. < 0.1; * < 0.05; ** < 0.01; *** < 0.001; **** <0.0001

After considering the *Temperature Annual Range* as a covariate, the only significant predictor was *Shrub* (Fig. 3b). Interpretation of this vegetation gradient showed that the higher the Shrub cover is, the more species of either Mediterranean or European distribution range occur (Fig. 5e). Moreover, species occurring in more Atlantic conditions tend to have a smaller range size (Fig. 5a), be more sedentary (Fig. 5c), having fewer generations per year (Fig. 5d) and oviposit anywhere on host plants or on non-host substrates (Fig. 5b).

Discussion

Our results were unexpected as we found that in the 'montados' habitats of W Mediterranean Portugal, the relationship between plant succession and butterfly fauna diversity and composition holds only after considering the effect of the most important regional gradient, that of Temperature Annual Range. These patterns are more apparent on the composition of butterfly communities than on the number of species, and are neatly reflected in butterfly community life-history traits.

Species richness

The lack of patterns in the relationship between species richness and the management of the cork oak fields might be explained in part by the relatively low species richness and heterogeneity of the faunas in the southern half of Portugal (as documented by Araujo and Garcia-Pereir 2003 and Hortal et al. 2004). However, an association between overall diversity (of plant species, to start with) would be generally expected to occur along the gradient between a recently managed and a late successional 'montado'. Comparable patterns have already been described, for instance of steady levels in richness and abundance (density) combined with changing community composition across a succession gradient (e.g. Sanford 2002). On the other hand, Verdasca et al. (2012) found increases in both overall butterfly species richness and abundance shortly after fuel management, i.e. in the very early stages.

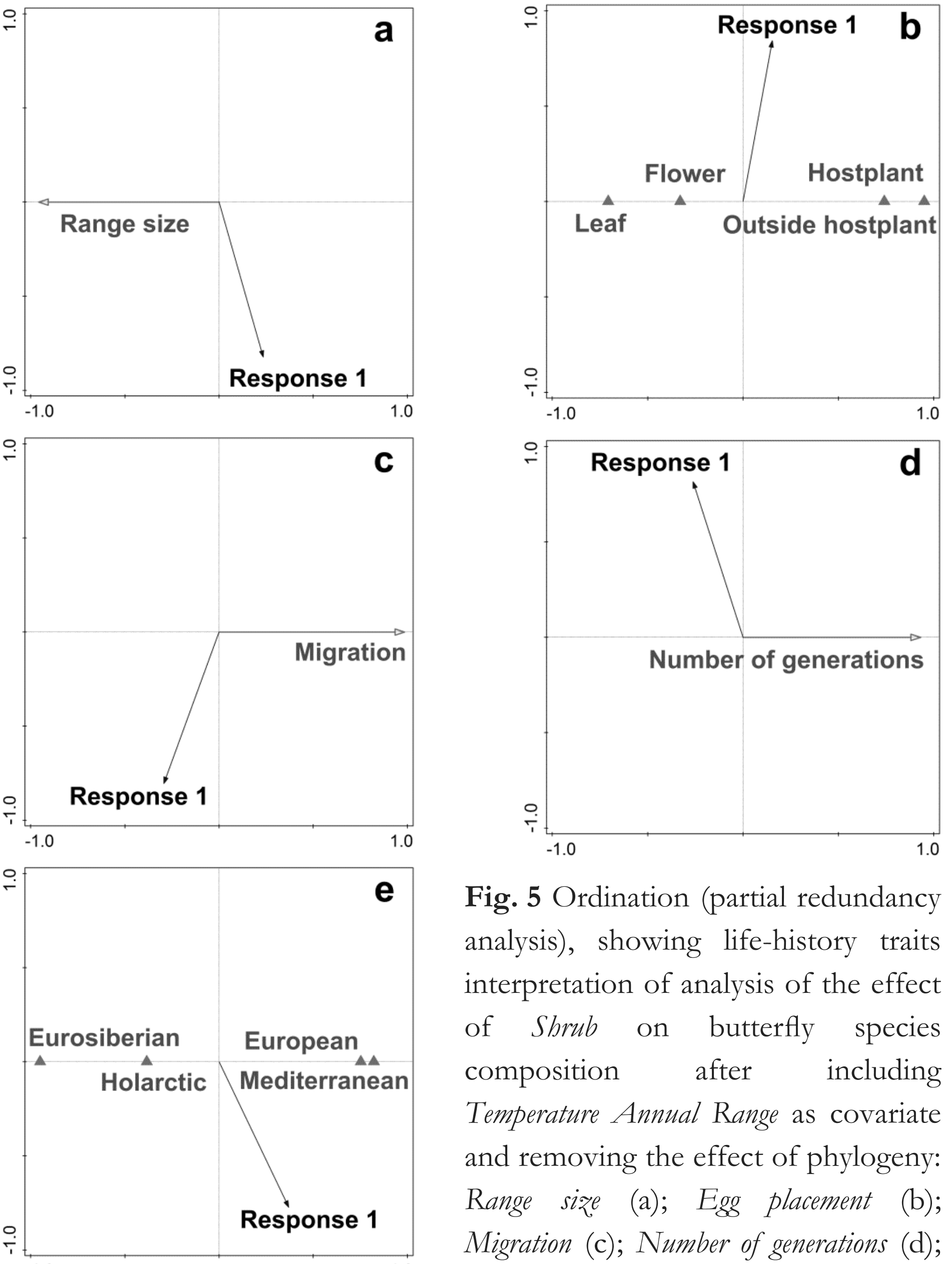


Fig. 5 Ordination (partial redundancy analysis), showing life-history traits interpretation of analysis of the effect of *Shrub* on butterfly species composition after including *Temperature Annual Range* as covariate and removing the effect of phylogeny: *Range size* (a); *Egg placement* (b); *Migration* (c); *Number of generations* (d); *Range type* (e). Response 1 represents the response of respective Axis 1 of the above-mentioned analyses (see Fig. 3a; Table 4 for results)

Abundance

At the wide scale the trends in abundance indicate an increasing number of individuals (total population density) towards the inland (or with increasing distances from the Atlantic coast; oceanity–continentality gradient), which means a comparatively higher total precipitation and cooler winter temperatures (e.g. Honrado 2003). Together with the negative (although marginally significant) relationship with the *Shrub cover*, this may be interpreted as a result of increased productivity in terms of the biomass of low size, ephemeral or early succession plants (e.g. annuals including grasses). Thus, although in a tentative way, our results seem to agree with those from Verdasca et al. (2012) who found that the number of individuals increased to a marked peak just about 2–3 years after abandonment, then declined to stabilise at a lower plateau after about 20 years. In short, increasing *Shrub cover* is related to decreasing density of adult butterflies in the long term. According to the further results (discussed below) this may be due to highest population densities of the comparatively widespread, large and predominantly univoltine species like several Satyrinae butterflies (cf. Dover et al. 2011, who—however—found a positive effect for the presence of scattered shrub inside hay meadows in northern Spain).

Species composition

The trend in species composition varied roughly as described for abundance above, from low coastal areas with relatively low differences between summer and winter temperatures ('Atlantic climate') towards 'continental' inland areas (see e.g. Honrado 2003). As there were no accompanying trends in butterfly species richness, it is clear that there are changes in representation of individual species, or beta and gamma diversity, as one travels from coastal areas inland (Araujo and Garcia-Pereira 2003; Garcia-Pereira et al. 2003; Hortal et al. 2004). The fact that an average of only 20 species per site was recorded from the pool of 52 recorded across the whole study area prospected indicates a relatively high spatial replacement of species. The localities with a more Atlantic climate hosted species such as *Satyrium esculi*, *Gonepteryx cleopatra* or *Muschampsia proto*, all known to follow coastal areas in the W Mediterranean (cf. Tolman and Lewington 2009).

In contrast, localities with a more continental climate hosted some distinctly dry adapted species, such as *Glaucopsyche melanops* and *Hipparchia statilinus*, or species with more continental distribution in the SW Palaearctic, such as *Argynnis pandora* (cf. Tolman and Lewington 2009). Our results thus highlight the important role of oceanity–continentality in structuring local butterfly communities even on a relatively small scale and within the homogeneous habitat of Portuguese 'montados'.

Life histories, climate and geography

The oceanic–continental pattern identified above corresponded with the representation of life-history traits of the constituent butterflies. In particular, the lower representation of species overwintering in earlier stages in areas with a more oceanic climate was earlier noted by WallisDeVries et al. (2011) from the Netherlands. The authors claimed that more oceanic conditions with mild winters represent a higher risk for butterflies overwintering as eggs and larvae. In contrast, species overwintering in early stages of development (often associated with univoltinism and late adult phenology: Dennis et al. 2005; Shapiro 1975) predominate inland. This is compatible with the finding that adult wingspan marginally decreases eastwards. Although it would be tempting to invoke time constraints in development for an explanation (review and references in Nylin (2009)) it is unlikely that the differences in the time window available for development are so contrasting across the region studied, which is in the south–west of the Mediterranean with a minimal altitudinal gradient. The complementary trend would be that the comparatively large-bodied, polyvoltine species, which overwinter at late stages, would predominate along the southwestern coastal fringe (corresponding to the warmest locations).

Another unexpected result was the absence of any relationships between subsets of traits and summer water stress (via e.g. the *Precipitation of Driest Month*, or this together with the *Temperature Annual Range*), since aridity represents a factor of stress for both animals and plants throughout the Mediterranean (Blondel and Aronson 1999). It appears that all the species occurring within the region are able to withstand summer water stress, or to evade it via summer diapause (as in the case of large satyrines) or emigration (as in the case of such migrants as *Lampides boeticus* (Linnaeus 1767) or *Vanessa cardui* (Linnaeus 1758)) (Stefanescu et al. 2013).

Life-histories and vegetation structure

The effect of vegetation structure on the composition of Portugese 'montados' butterflies was apparent only after statistical controlling of the prevailing oceanity–continen-tality gradient. Moreover, the vegetation gradient did not follow a clear successional pattern; instead, it followed a 'non-shrub' to 'shrub' cline, revealing that shrub abundance was important for structuring butterflies communities. The representation of butterfly life-histories responds, if anything, to increasing coverage of shrub land, which would a priori be thought of as the intermediate succession level. The trend is built from a combination of what would a priori be thought of as specialised and generalist traits. At one of the ends would be species with Mediterranean and relatively wide distribution types (e.g. widespread in Europe), with sedentary adults, which lay single eggs rather non-specifically on the host or on non-host objects, and are predominantly univoltine. Non-migratory habits may be linked to intermediate or late succession habitats while these retain comparatively high resources and are subject to low disturbance (Brown and Kodric-Brown 1977; Dennis et al. 2005; Steffan-Dewenter and Tscharntke 1997). This supports conclusions from other parts of the Mediterranean (Stefanescu et al. 2005, 2009) and elsewhere (Steffan-Dewenter and Tscharntke 1997) that decreasing voltinism tends to occur in later successional stages, perhaps implying a reduction in the pressure to compensate for unpredictably high mortality rates (Brown 1985; Brown and Southwood 1987). Alternatively, decreasing voltinism may be associated with feeding on 'apparent' plants, defined as plants that are relatively long living, protected by quantitative substances, and competitively strong (Bartonova et al. 2014). A relatively low degree of specificity for the egg-laying substrate may have to do with a high density and continuous spread of the larval resources as well as with the habit of overwintering as eggs (e.g. Dennis et al. 2005 and discussion above; Wiklund 1984).

Conclusions

We failed to discover a relationship between butterfly species diversity and plant succession in the habitat of South Portugese 'montados'. On the other hand, we found a clear succesional effect on the representation of individual butterfly species, and the representation of butterfly life-history traits. The latter effects, however, were apparent only after statistical control of an oceanity–continentality gradient, which was by far the strongest predictor of butterfly community structure, revealing the importance of climatic variables even at the relatively small scale of this study. Although different from a study of fuel management impacts on butterfly communities conducted in the same region (Verdasca et al. (2012), our results are compatible with the idea that the most relevant changes in butterfly communities in the area may concentrate in the earliest stages of vegetation recovery after intense management.

Given the overall change in species composition across sites, we cannot conclude that our results reflect species poor butterfly fauna across the south Portuguese 'montados'. However there seems to be little change in structure besides the effect of the shrub cover, which is superimposed on that of temperature (in turn the effect of temperature and other climate variables may be difficult to analyse, as these are highly spatially structured in Portugal: Hortal et al. 2004). The lack of correlations between species diversities and management state across the study sites may indicate a relatively random species replacement operating at distances well above the between-site distances in our study, or a dependence of the butterfly communities on habitat types different than those represented by the 'montados' (e.g. marginal non-managed habitats, edges). Thus, the whole system might be requiring the combination of the three vegetation stages (herbs, shrub, trees) for the whole 'pool' of species to persist, even with changing population densities along the succession. From this point of view, any extensive changes (i.e. spreading across very wide areas including e.g. extensive wildfires) should impose a negative effect in the pool of the butterfly species (as well as on their vegetal hosts: Farris et al. 2010), while periodical changes at local scales, especially in interconnected areas, would be expected to result into neutral or even positive effects (as pointed out by Sanford 2002). However more attention is required to understand the dynamics of butterfly communities in the relatively poorly studied but diverse Mediterranean areas; for instance Verdasca et al. (2012) found significant increases of diversity after human disturbance while evidence from the Eastern Mediterranean indicates declining butterfly diversities after pasture abandonment (Slancarova et al. unpublished data).

We found no evidence for a generalist–specialist continuum to co-vary along a human imposed environmental gradient. We cannot discard the possibility that our present interpretation and coding for butterfly life-histories is imperfect (hence failing to recover the actual responses of these insects to habitat change), a problem hampered by imperfect knowledge of the geographic variation in their biology (e.g. Dennis et al. 2008). Within these limits, the shrub cover emerged as the most relevant element: species with comparatively small range sizes tend to predominate at the intermediate stages of the plant community succession. This, together with increasing general evidence from European butterflies on the negative effects of both intensive management and long-term abandonment in traditionally managed European landscapes (Settele et al. 2009) supports the idea that a limited (spatially) but regular level of management is required to sustain the diversity of these insects across the area and to avoid the replacement of the regionally characteristic species by widespread ones.

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Supporting information

Additional Supporting Information may be found in the online version of this article: http://link.springer.com/article/10.1007%2Fs10841-015-9801-6

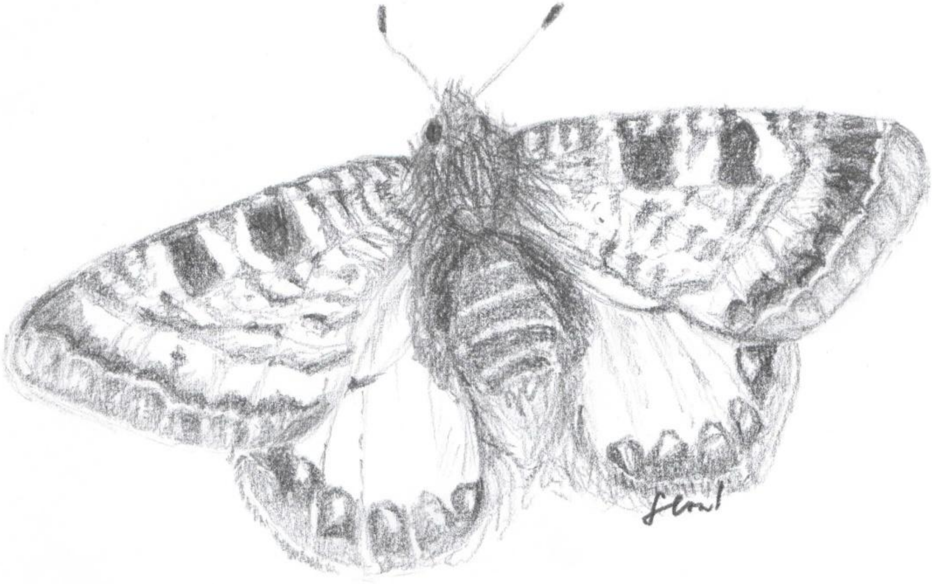
Online Resource 1 List of species and life-history traits used in the analyses of data from southern Portugal. Abbreviations as follows: FL1 (Flying period): February–March, FL2: April, FL3: June, FL4: July–September, FL5: October and onward; AL1 (Altitudinal range): 0–500 m, AL2: 501–2000 m, AL3: over 2001 m; Range type: EUS – Eurosiberian, EUR – European, GLOB – global, HOL – Holarctic, MED – Mediterranean; 10841_2015_9801_MOESM1_ESM.docx

Online Resource 2 Reference list to the sources of information on the life-history traits used in the analyses of data from the butterflies in southern Portugal; 10841_2015_9801_MOESM2_ESM.docx

Online Resource 3 Reference list to the sources of phylogenetic information; 10841_2015_9801_MOESM3_ESM.docx

CHAPTER V

Summary of results and future perspectives



Archon apollinus (Herbst 1798)

This thesis emphasises the negative effects of changing human land use systems, following landscape abandonment and the risk of biodiversity homogenisation in the Mediterranean Basin. The regional habitat diversity, created during millennia of human activity, is currently threatened by land abandonment (especially in less productive areas), which may diminish the resource base for the studied butterflies. Rapid decline in Mediterranean butterfly populations poses a severe threat for the conservation of European biodiversity.

In chapter II, I detected a clear effect of forest encroachment in the Southern Balkans on the representation of individual butterfly species, and the representation of butterfly life history traits. With proceeding succession, species of non-forest habitats are predictably replaced by forest-tolerating species. Less predictably, Mediterranean endemics occurred both on grasslands and semi-open formations. Most importantly, the change in community composition was reflected in the shift of life history traits. Later successional sites contained more species overwintering in earlier stages and having fewer generations per year, but inhabiting large European or Eurosiberian (e.g. northern) ranges; this decreases the representation of Mediterranean endemics. Therefore, loss of open, non-forested habitats increases the representation of more northerly species, which somehow contradicts the frequent predictions that northern species should retreat northwards from their southern distribution margins due to ongoing climate change.

Chapter III shows that successional effects on butterfly communities became apparent only after statistical control of the oceanity–continentality gradient, which was by far the strongest predictor of butterfly community structure in Portuguese 'montados'. This reveals the importance of climatic variables even at the relatively small scale of this study. It also supports the idea that a spatially limited but regular level of management is required to sustain butterfly diversity across the area and to avoid the replacement of the regionally characteristic species by widespread ones.

Chapter IV documents that three related and sympatric butterflies using overlapping larval resources (several *Aristolochia* spp. plants differing in growth forms and preferring different habitats) co-exist via partially shifted phenology of both the butterflies and the plants. Each butterfly species mainly uses the host plant which is best available for oviposition during its adult flight, but each can use several hosts, and good dispersal ability allows location of the host plants patches within the diverse Mediterranean landscape. Mobility of individual species was inversely related to their local densities, ensuring that the least abundant species (*Zerynthia cerisy*) was still able to form an interconnected population within the study landscape. To sustain the whole butterfly – host plants system, maintenance of finely scaled habitat mosaic is necessary.

As the negative trend of forest encroachment could vary for different taxa or groups of species – specialists and generalists, sedentary and migratory, endangered and non-endangered species (Bender et al. 1998, Thomas 2000, Stefanescu et al. 2011a, Sirami et al. 2008), the importance of considering life-history traits has become recently indisputable (e.g., Borschig et al. 2013, Ekroos et al. 2010, Eskildsen et al. 2015, Ockinger et al. 2010, Stefanescu et a 2011a). Responses of communities to landscape changes are more likely to be revealed by an analysis of ecological traits, than species richness (Scalercio et al. 2012, Slancarova et al. 2016, Bartonova et al. 2014). Even for understanding the meta-community assembly processes, Pavoine et al. (2014) prefer explicit trait-based approaches before the more indirect approach based on phylogenetic conservatism. However, caution is still needed, when working with trait databases. Missing data could considerably influence the results (Taugourdeau et al. 2014) and proper interpretation is needed as well (Summerville 2015).

The continuous manipulation and redesigning of landscapes and habitats by humans has had profound consequences for the distribution, dynamics, and turnover of species and communities (Blondel 2006). Conservation of landscapes with a long history of human use needs to take into account the role of humans in shaping ecological features and biodiversity (Marul et al. 2015). Heterogeneous patchworks of open, semi-open and closed vegetation, typical for ancient cultural landscapes of the Mediterranean, may be lost to spontaneous forest encroachment if left unmanaged, or even actively planted by trees. Moreover, there is a high risk of the restructuring of butterfly and other small animal communities –

range-restricted endemic fauna might be gradually replaced by wide ranging generalists. Our results stressed the problem of biotic homogenisation. In abandoned and forest covered Mediterranean region, the Mediterranean endemics as well as species with a southern distribution are replaced by species with a northern distribution.

Assuming historical conservatism of species life histories, the negative association of Mediterranean species with closed canopy conditions falsifies the "forested Mediterranean" hypothesis, highlighting the need to maintain open landscapes across the region. Although we can prove neither the 'Ruined Landscape' nor the 'forested Mediterranean' theory, the reality almost certainly lies between the two extremes, we cannot deny the exceptional diversity and persistently dynamic structure of Mediterranean habitats (Blondel 2006).

Sylva-saltus-ager and *Dehesa-montado* systems, which have been in use for many centuries without resulting in a depletion in the production of resources, are an illustration that sustainability may be achieved, provided that management techniques do not result in dramatic changes in certain major functions of the ecosystems (Blondel 2006).

Conservation measures for butterflies should focus on promoting traditional agro-pastoral practices, including small-scale cultivation and light livestock grazing in order to preserve open and semi-open rural mosaics. Rotational grazing allows spatial and temporal heterogeneity in vegetation structure to be maintained at farm scale, which could be far more beneficial (Fonderflick et al. 2014). Especially in the case of *Dehesas-montado* systems, livestock grazing is an essential driver, it should be promoted after shrub cutting in cleared sites to restore the characteristic assemblages, species interactions and ecosystem function (Garcia-Tejero 2013). Such measures should help not only butterflies, but also birds and other insect taxa (Zakkak et al. 2014, Garcia-Tejero 2013, Fonderlick et al. 2014, Pereira et al. 2014).

Nevertheless, maintaining open landscapes is complicated by several factors. First, such widely advocated land management tools such as "headage payments" for shepherds (Stoate et al. 2009) or agro-environmental schemes rewarding environmentally benign farming (Knop et al. 2006), were originally designed in north-western Europe and may be poorly transferable to the conditions of Southern Europe, with much more diverse habitat conditions and declining rural population (Bunce et al. 1998, Sirami et al., 2008). Fortunately, efforts have already been made to bridge the knowledge gap in the understudied Balkan area (Dyulgerova et al. 2015, Popgeorgiev et al. 2014, Sokos et al. 2013, Zakkak et al. 2015). Overall, the effectiveness of agro-environmental schemes to halt biodiversity decline has been disputed (Kleijn et al. 2011, Konvicka et al. 2008, Popgeorgiev et al, 2014, Wrbka et al. 2008). The EU has recently agreed on a new headline target for biodiversity in the EU as well as on Common Agriculture Policy reform 2014-2020 (EU Regulation 7536/10, European Commission, 2014). Member states should be able to refine rural development programs according to different local landscape contexts, which is highly required (Dyulgerova et al. 2015, Popgeorgiev et al. 2014, Sokos et al. 2013, Zakkak et al. 2015), however is doubtful that biodiversity will benefit much in practice, as small scale farmers are often excluded from receiving direct payments because of strict regulations (Pe'er et al. 2014).

Second, financial incentives do not guarantee that human impacts on habitats replicate those existing in the past. For instance, agro-technology developments such as fodder crops production and vehicle transport relaxed the need to harvest summer coppice, or to move herds across the landscapes (transhumance) (cf. Van der Leeuw 2004). Cizek et al. (2012) documented that current management technologies fail to provide the microhabitat heterogeneity needed for reserve management in Central Europe, and the outcomes may be even worse in species-richer Southern Europe. Still worse, relying on subsidies assumes constant economic growth, which is far from guaranteed in the long term. Economic decline might promote the return of urban populations to villages, but this would be a long-term process, whereas breakdowns in funding may lead to rapid habitat and species losses.

Without downplaying the subsidised efforts to maintain rural habitats diversity, novel approaches (e.g. logging, fire disturbance, rewilding) which would maintain the open to semi-open conditions across the Mediterranean while being economically sustainable, should be sought (Dover et al. 2011, Pons et al. 2003). Current policies to maintain extensive farming landscapes underestimated the human labour needed to sustain these landscapes and the recent and future dynamics of the socio-economic drivers behind abandonment (Navarro and Pereira 2012). At least locally, declining grazing by farm animals might be replaced by free ranging ungulates, including species historically extirpated from the Mediterranean (Dobson 1998, Navarro and Pereira 2012). Such projects would, at least regionally, return to the Mediterranean the key players that had been affecting ecosystem dynamics before the advent of farming, and with which the regionally endemic biodiversity has evolved. The positive effects of rewilding on biodiversity, has already been proved not only in Northern Europe (Kuiters and Slim 2003), but very recently also in the Mediterranean (Regos et al. 2016).

Future perspectives

Although the fates of endemic butterflies safely falsify the "forested Mediterranean" hypothesis, there is legitimate concern, to what extend are the butterfly results applicable to the entire insect diversity of the Mediterranean hot-spot. Habitat indicators based on characteristic species might be associated with habitat types (Stefanescu et al. 2011b), and butterflies acting as good surrogates for grassland biodiversity, might not be applicable to woodlands. To obtain a more complete picture of the Mediterranean situation, the approach presented here should be expanded to night-active Lepidoptera, and perhaps other insect groups. Moths, in particular, have been sampled from the same sites as the butterflies sampled for Chapter II of this dissertation. Analysing moths results may or may not provide further support for my claim that forest encroachment disfavours endemic Mediterranean fauna.

My nearest future perspectives remain associated with life-history traits. I'm preparing a life-history database of European butterflies, primarily intended for the manuscript: 'Population trends and habitat associations of Mediterranean fauna', which is in rough preparation. Model groups are butterflies, reptiles and birds, individual traits reflect similar biological phenomena at individual model groups. The hypotheses are still in their infancy, however, one option is to ask: 'Which traits define rarity?', the second one is to define rarity by habitat type, but using the traits as covariates.

Very recently, I got the opportunity to join the Separating Environmental Changes and their effects on commUnity tRaits in European butterflies project (sECURE). The overarching goal of sECURE is to identify analytical pathways allowing the best use of butterfly species traits, ecological characteristics and phylogenetic information to predict the impacts of drivers of global change on species co-occurrence, community assembly and biodiversity. Right now, the project has been submitted, and decision should be announced during the summer.

In addition, I would like to continue in cooperation with the Czech Union for Nature Conservation in managing the locality of *Polyommatus damon* (Denis & Schiffermüller, 1775) at Kamenný Vrch near Kurdějov, and integrate more Czech applied nature conservation projects.

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APPENDIX

Author's Curriculum Vitae

Zerynthia polyxena (Denis & Schiffermüller, 1775)

Author's Curriculum Vitae

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2012	MSc. in Teaching Biology to High School, Faculty of Science, University of South Bohemia in České Budějovice
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2011	MSc. in Animal Ecology, Faculty of Science, University of South Bohemia in České Budějovice
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Publications

Šlancarová, J., Bartoňová, A., Zapletal, M., Kotilínek, M., Faltýnek Fric, Z., Micevski, N., Kati, V., Konvička, M. 2016. Life History Traits Reflect Changes in Mediterranean Butterfly Communities Due to Forest Encroachment. PLoS ONE 11(3): e0152026. doi:10.1371/journal.pone.0152026

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Grants acquired

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Grant Agency of University of South Bohemia, 2013
Grant Agency of University of South Bohemia, 2012
The Joan Mosenthal DeWind Award, The Xerxes Society, 2012

Projects

Participation on several projects of Grant Agency of Academy of Science of the Czech Republic, Grant Agency of the Czech Republic, Ministry of Education, Youth and Sports:

2015	Revising the state of populations of *Euphydryas aurinia* in Western Bohemia, Czech Republic. Research on Relict Asian steppe communities in the Balkans – a butterfly as an indicator, Croatia.
2013–2015	Monitoring of *Erebia* species in Krkonoše and Jeseníky mountains

2012–2013	Research on Land Use Abandonment in Eastern Mediterranean – effects on butterfly and moth communities, Macedonia, Bulgaria and Greece.
2010–2011	Research on three swallowtails butterflies (*A. apollinus, Z. cerisy and Z. polyxena)*, Greece.
2009–2011	Monitoring of Sites of Community Importance with focus on Damon blue (*Polyommatus damon)* for ANCLP CR, Czech Republic.
2008–2009	European Conservation Action Network Conservation Visit to Zdanice, Czech Republic

International conferences and oral presentations

Šlancarová, J., Novotný, D., Beneš, J., Zapletal, M. Konvička, M. 2015. Macromoths light trap catches on intensive farmland: Relative effects of crops, land covers and landscape heterogeneity. In: 19th Euroepan Congress of lepidopterology. Book of abstracts. Radebeul, 27th September–2nd October 2015, Germany.

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