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**SPECIES RICHNESS AND COMPOSITION OF BUTTERFLY
ASSEMBLAGES (LEPIDOPTERA: RHOPALOCERA) ALONG THE
ALTITUDINAL GRADIENT OF SERRA DA ESTRELA**

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Mestrado em Biologia da Conservação

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**Riqueza e composição específica das comunidades de borboletas
(Lepidoptera:Rhopalocera) ao longo do gradiente altitudinal da Serra da Estrela**

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Resumo

Os gradientes altitudinais são importantes modelos em ecologia pois permitem, numa escala reduzida, estudar a influência da variação de diversas variáveis ambientais nos padrões de biodiversidade existentes. Estes padrões são influenciados por diversos factores, desde variáveis climáticas às relacionadas com a perturbação humana. Nesta tese é estudada a variação na composição das comunidades de borboletas ao longo do gradiente altitudinal na Serra da Estrela e são testadas duas hipóteses sobre os padrões de riqueza específica ao longo do gradiente altitudinal: a regra de Rapoport e o “Mid Domain Effect” (MDE). Foram assinaladas 70 espécies de borboletas neste estudo e constatou-se que a variação na riqueza de espécies de borboletas apresenta um padrão “mid peak” apoiando o MDE. Verificou-se também que as comunidades de borboletas do mesmo nível altitudinal são mais semelhantes entre si independentemente da vertente em que se encontram e são também mais semelhantes com as comunidades dos níveis altitudinais adjacentes. Finalmente, a análise da distribuição de algumas espécies ameaçadas de borboletas torna evidente a necessidade de realização de mais estudos sobre a biologia da conservação deste grupo de animais em Portugal.

Abstract

Altitudinal gradients are important ecological models because they allow us to study the influence of diverse environmental variables in the existing biodiversity patterns. These patterns are influenced by various factors, from climatic variables to variables related to human disturbance. In this thesis we studied the variations in the composition of the butterfly assemblages along the altitudinal gradient of Serra da Estrela and we tested two hypotheses regarding the species richness patterns along the altitudinal gradient: Rapoport's rule and the Mid Domain Effect (MDE). In this study 70 butterfly species were marked recorded and it was observed that the butterfly species richness presents a mid-peak pattern supporting the MDE. It was also noted that the butterfly assemblages in the same altitudinal level show more similarity within themselves regardless of the slope they are found in, and are also more similar to the assemblages present in the adjacent altitudinal levels. Finally, the distribution of some threatened butterfly species was analyzed and it became evident the need to do more studies regarding the conservation biology of this animal group in Portugal.

Índex

Agradecimientos	3
Resumo	4
Abstract	5
Introduction.....	7
Material and methods	13
Study area.....	13
Study group.....	14
Sampling method	16
Data analysis	18
Results	21
General results.....	21
Altitudinal patterns of butterfly species richness	22
Butterfly assemblages composition.....	26
Butterfly conservation.....	28
Discussion	29
General discussion.....	29
Altitudinal patterns of butterfly species richness	30
Butterfly assemblages composition.....	33
Butterfly conservation.....	35
References	38
Appendix I	46
Appendix II	47
Appendix III	49

Introduction

Mountains are important habitats for many species (Lee *et al.* 2013), and promote adaptation and speciation due to their isolated nature (Hodkinson & Jackson 2005). Mountains are also very distinctive systems that allow the study of ecological and biogeographical theories aiming to explain the drivers of species diversity change (Lee *et al.* 2013) as they offer a wide range of environmental conditions (and habitats) along the altitudinal gradient (Chaverri-Polini 1998).

Altitudinal gradient studies are important because they allow us to study a large number of changing abiotic and biotic factors across a small geographical area and their influence on biodiversity patterns (Ashton *et al.* 2011). Mountains show steep gradients for a large number of environmental variables, like temperature, air pressure, ultraviolet radiation and precipitation, and these factors affect the distribution and the evolutionary dynamics of many species and communities (Hodkinson & Jackson 2005; McCain & Grytnes 2010).

Since the XIX century the altitudinal gradient has been the target of many studies conducted by different naturalists including Darwin and Wallace. Even though altitudinal gradients were, in the past, less studied than other ecological gradients (e.g. the latitudinal gradient) there was a resurgence of altitudinal studies during the last decades, leading them to become a model for gradient studies in ecology (Nogués-Bravo *et al.* 2008). Besides their relevance in ecology, elevational gradients also proved to be very important in the development of biogeography and evolutionary biology (Lomolino 2001).

The particular environmental conditions across the altitudinal gradient of a mountain system, along with the isolation of some habitats that are specific to many mountain areas (e.g. alpine lakes, summit vegetation), contribute to the unique biodiversity that can be found in mountains (Hodkinson & Jackson 2005)

Early studies on the biodiversity along the altitudinal gradient noted that there seems to be a pattern of species richness decreasing with increasing altitude. Since then, four major patterns of species richness change along the altitudinal

gradient have been identified (McCain & Grytnes 2010): 1) monotonical decrease, where species richness tends to decrease monotonically with elevation; 2) low plateau, in which the richness at lower altitudes is high, only starting to decrease at some point in the middle of the mountain; 3) low plateau-mid peak, shows high levels of species richness at low altitudes, a peak around mid elevation of the mountain and then a decrease near the top; 4) mid peak, is the situation where species richness increases until medium elevations and thereafter decreases (McCain 2009)(Fig.1).

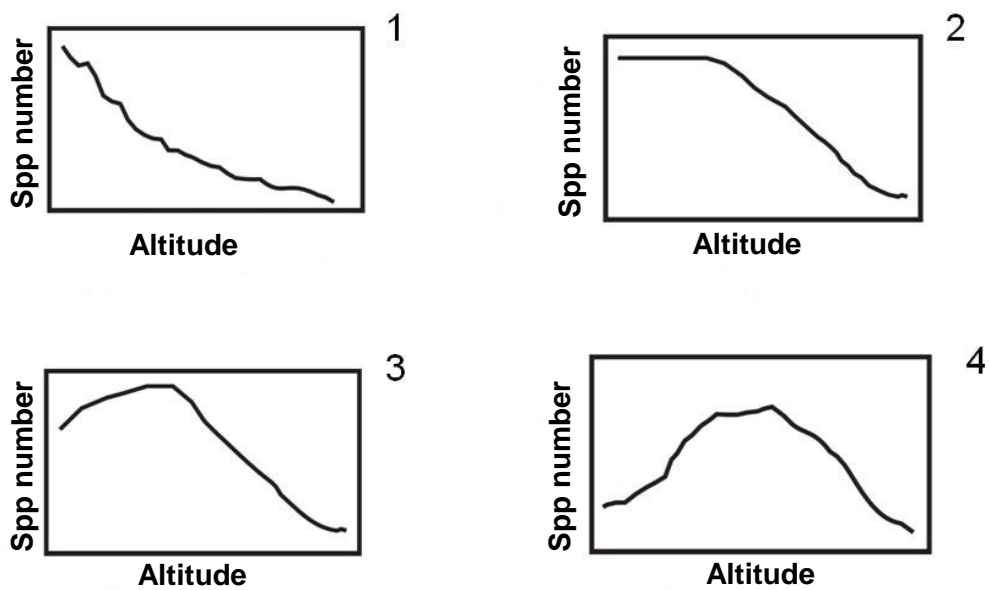


Fig.1. The four major patterns of species richness variation along altitudinal gradients. 1- Monotonical decrease; 2 – Low plateau; 3 – Low plateau-mid peak; 4 - Mid peak (adapted from McCain & Grytnes 2010)

Nevertheless, despite not universally accepted, the mid peak seems to be the most common pattern of species richness change across altitude for different plant and animal groups (McCoy 1990; Bachman *et al.* 2004; Rahbek 2005; Werenkraut & Ruggiero 2011; Lee *et al.* 2013).

There are several explanations for the biodiversity patterns observed on elevational gradients. The relationship between species richness and available area seems to be important because with increasing altitude the area available tends to decrease, which means that the higher altitudinal bands of a mountain have less area and therefore are able to shelter less species (Rahbek 1995).

The climatic gradient along a mountain can also influence species distribution: some authors argue that the differences in temperature and humidity along the elevational gradient can drive species richness patterns while other authors defend that the combination of all the aspects that compose climate can determine a peak of productivity towards the middle of the mountain, and this can then influence the optimal point for different species (Lomolino 2001). Furthermore, since with increasing altitude there is also an increase in the isolation of the communities it was noted that areas at higher altitudes tend to have less species due to the low immigration rates (Lomolino 2001).

Several ecological hypotheses have been proposed to explain the patterns of species richness change along the altitudinal gradients, but two have been more extensively debated: the Rapoport's rule and the mid domain effect (MDE). The Rapoport's altitudinal hypothesis states that species inhabiting higher altitudes must have a broader tolerance to some climatic variables, having a larger altitudinal range than the species at lower altitudes. Thus, as a consequence, overall species richness is magnified at low altitudes and decreases with altitude (Stevens 1992). Rapoport's altitudinal rule was extended from the Rapoport's latitudinal rule (Stevens 1989) that states that individuals found at higher latitude must have a greater latitudinal range as they are capable of tolerating a larger range of environmental conditions. Kwon *et al.* (2014) showed that Rapoport's altitudinal rule supported their findings, as temperature seemed to be a determinant factor in the distribution of ant species in South Korean mountains. They found that the ant species found at higher altitudes supported a wider temperature range and showed a wider distribution range as well.

On the other hand, the mid domain effect hypothesis (MDE) argues that there is a mid elevation peak in species richness due to "the increasing overlap of species ranges towards the centre of a shared geographic domain due to geometric boundary constraints in relation to the distribution of species range sizes and midpoints" (Colwell & Lees 2000), meaning that a mid elevation peak can be due only to geographic constraints and be independent of climatic variables. Several studies have supported the MDE and, for example, according to Lee *et al.* (2013) the MDE was the most powerful variable explaining the

altitudinal patterns of plant species richness on the Baekdudaegan Mountains (South Korea).

Nevertheless, despite the general pattern of biodiversity change along altitudinal gradients, local environmental conditions may significantly influence local biodiversity patterns. For example the inclination and orientation of each slope influences the level of radiation, water runoff and the soil characteristics which ultimately affect the composition and richness of species assemblages (Werenkraut & Ruggiero 2014). Further, the local availability of liquid water may also generate a local peak in species richness (Werenkraut & Ruggiero 2014), and influence the productivity pattern along the altitudinal gradient. Productivity can itself influence species richness in two different ways: 1) as productivity increases so does species richness or 2) as productivity increases, species richness increases, peaks at mid elevation and then tends to decrease at high levels of productivity (Sanders 2002)

Human activities have also played an important role in determining local patterns of species richness variation along the altitudinal gradient. Human activities are linked to mountain areas for centuries: mountains have been used for livestock and dairy production, were subjected to intense agricultural activities, forest harvesting, fire practices and recreational activities such as skiing, making them particularly disturbed habitats, especially at lower and higher altitudes (Hodkinson & Jackson 2005). These disturbances cause the degradation and fragmentation of the habitat and as they occur with higher intensity on the top and bottom of the mountains the mid-altitude areas tend to be less disturbed (Nogués-Bravo *et al.* 2008).

Human activities may have destroyed some natural patterns of species distribution along altitudinal gradients making it more difficult to identify and study the natural distribution of mountain biodiversity. As a consequence it is harder to obtain the knowledge needed to manage and apply conservation programs to protect mountain biodiversity and habitats (Nogués-Bravo *et al.* 2008).

As mountains are great biodiversity centres and have value for humans, many of these areas around the world are, in some way, protected as natural parks or

some similar designation (Price 1998). This is also the case of Portugal, where most mountain areas are classified as “Zonas de protecção especial” and/or “Sítios de importancia comunitaria” (ICNF 2016). Even so, mountain biodiversity is threatened by a variety of factors most of which are related to direct and indirect effects of human activities. The best way to assess the quality of the environment and the impact and rate of human-induced changes is by using bioindicators (Holt & Miller 2011).

Bioindicators are species or communities whose reactions readily reflect the state of the environment (Stewart *et al.* 2007). The biodiversity of any area is incredibly complex making it very complicated to survey its totality, or even assess the changes in the whole natural communities following a disturbance event. Because of this, the use of bioindicators in ecological studies is essential to obtain valuable data in an effective and efficient way (Duelli & Obrist 2003).

Bioindicators have been divided into three main types (McGeoch 1998): environmental indicators, that are used to detect and monitor changes and disturbances in the environment, as they respond to these changes predictably and in a way that is easy to observe; ecological indicators, that demonstrate the effects of disturbance in the habitat such as, habitat fragmentation and climate change; biodiversity indicators are taxonomic or functional groups whose diversity reflects, in some way, the diversity of other higher taxa from the same study habitat (McGeoch 1998; Stewart *et al.* 2007).

Bioindicators must have a series of characteristics to be considered suitable for ecological studies (Rainio & Niemelä 2003):

- they must have a well-known ecology and taxonomy;
- they should have a broad distribution;
- they should present an early reaction to change in the environment and the reaction should reflect that of other species;
- they should be cost-effective to survey and have some economic relevance.

However, when choosing a bioindicator the goal of the study should also be taken into account, as the consistency of the results will depend on the

suitability of the bioindicator to the issue being studied (Hodkinson & Jackson 2005; Gerlach *et al.* 2013).

Many groups of invertebrates have been regarded as very good indicators due to their abundance, broad distribution, sensitivity to change and by having dispersal mechanisms that allow quick ecological responses. Further, they play key functions in the habitat such as decomposition and pollination and can be easily sampled (Rosenberg *et al.* 1986; Hodkinson & Jackson 2005; Gerlach *et al.* 2013). Invertebrates have also been shown to be better biodiversity indicators than vertebrates in several ecological studies as they are more diverse and abundant, are more sensitive to low disturbance effects and respond to changes at small spatial scales (Gerlach *et al.* 2013).

For these reasons, several invertebrate groups, but namely butterflies, are frequently used as bioindicators in ecological studies in a variety of ecosystems, and have also proved to be a suitable target group when studying changes in species richness and composition along ecological gradients (Kessler *et al.* 2011).

The main goals of this work are: 1) the study of the variation in butterfly species richness and composition across the altitudinal gradient of Serra da Estrela; 2) the assessment of the validity of two ecological hypotheses – Rapoport's rule and the Mid Domain Effect – in explaining the observed species richness distribution patterns and 3) the analysis of the abundance and distribution of rare butterfly species to assess their conservation status in Serra da Estrela.

Material and methods

Study area

The study was conducted in Serra da Estrela (N 40° 19' 18,47", W 7° 36' 49,81"), the highest mountain in continental Portugal, reaching 1993m. Along with Serra do Açor and Serra da Lousã, Serra da Estrela forms the western extreme of the Iberian Central System, one of the main mountain systems in the Iberian Peninsula (ICNF 2016). Serra da Estrela is classified as a Portuguese Natural Park since 1976, is also part of Natura 2000 network and its upper limits (the higher plateau and Zêzere headwaters) are included in the Ramsar Convention (ICNF 2016). Serra da Estrela is affected by the Atlantic and Mediterranean climates having a mosaic of habitats that represent different biogeographic regions (ICNF 2016). Along with the intense historical and human influence, this mountain is a unique natural site with characteristics that allow high levels of biodiversity (CISE 2016), being a particularly important spot for several species and habitats associated with high altitude in Portugal (ICNF 2016). In regard to the flora of the region some species can be highlighted due to their importance, rarity or exclusiveness: *Festuca henriquesii* and *Centaurea paniculata* subsp. *rothmalerana* are endemic to Serra da Estrela, *Ranunculus abnormis*, *Echinopartum ibericum* and *Centaurea micrantha* are endemic to the Iberian Peninsula, and the endangered *Taxus baccata* that in Portugal, other than Serra da Estrela, is only accounted for in the Peneda-Gerês National Park. Regarding the fauna, it should be mentioned the presence of *Iberolacerta monticola*, *Chioglossa lusitanica* and *Galemys pyrenaicus*, all endemic to the Iberian Peninsula and classified as Vulnerable by the International Union for Conservation of Nature (ICNF 2016).

Serra da Estrela is a very important site for butterfly studies in Portugal and, due to its altitudinal gradient, there can be found some species that are rare elsewhere in the country (Marabuto *et al.* 2004), like *Cyaniris semiargus* (Rotternburg, 1775), *Argynnis aglaja* (Linnaeus, 1758) and *Satyrus actaea* (Esper, 1781) (Maravalhas 2003). A considerable number of endemic and rare invertebrate species are also known to occur in Serra da Estrela (Grosso-Silva 2000; Ferreira *et al.* 2009; Maravalhas & Soares 2013; Serrano *et al.* 2014),

highlighting the high conservation value of this protected area for nature conservation in the Iberian Peninsula (e.g. Sánchez-Fernández *et al.* 2008).

Study group

There are 98 species of diurnal butterflies (Lepidoptera: Rhopalocera) reported to Serra da Estrela (personal information from Centro de Interpretação da Serra da Estrela; to be available at <http://www.cise.pt/pt/index.php/base-de-dados>) (Fig.2), including a protected species - *Euphydryas aurinia* (Rottemburg, 1775) - listed in the Habitats Directive (Annex 2) and in the Bern Convention (Annex 2) (ICNF 2016). Furthermore, some rare European species, like *Cyaniris semiargus*, *Argynnis aglaja* and *Satyrus actaea*, also occur in Serra da Estrela, being extremely rare in other areas of Portugal (Maravalhas 2003; Marabuto *et al.*, 2004; van Swaay *et al.* 2010). However, the knowledge on the butterflies of Serra da Estrela is still scanty due to the poor information on species abundance, distribution, biology and sensitivity to habitat change, being a serious impediment for the effective conservation of this emblematic insect group in Serra da Estrela (Cardoso *et al.* 2011).



Fig.2. Some species known to be present in Serra da Estrela. 1 –*Thymelicus sylvestris* (photo: José Conde). 2 –*Iphiclides feisthamelli* (photo: Hugo Figueiredo). 3 –*Aricia cramera* (photo: José Conde). 4 –*Euphydryas aurinia* (photo: Hugo Figueiredo). 5 –*Colias croceus* (photo: Hugo Figueiredo)

Butterflies are one of the most suitable organisms in studies regarding biological diversity and conservation biology due to their ecological importance, well known taxonomy and biotope (Kitahara *et al.* 2008). As they are also viewed in a very positive way by the public, in contrast to most invertebrate groups, they are more profitable to study and protect (Kühn *et al.* 2008; van Swaay & Warren 2012) thus being one of the most frequently chosen groups of invertebrates for conservation programs (New 1997).

During their life cycle butterflies occupy several microhabitats in the ecosystem and they also react very quickly to habitat changes making trends identifiable in a short period of time; this makes butterflies very good when it comes to detect environmental changes, like climate and land use changes (van Swaay & Warren 2012). According to Brown (1997) environmental changes due to the effects of agriculture practices, logging, clearing and the introduction of exotic plant species can be very harmful to butterfly assemblages leading to the loss of the most sensitive species. Thus by monitoring butterfly assemblages we can obtain valuable information on how to apply conservation efforts in order to reverse the loss of biodiversity (van Swaay & Warren 2012).

Diurnal butterflies have been used as biodiversity indicators due to their conspicuousness, ease of identification and sensitiveness to environmental changes (Gerlach *et al.* 2013). Butterflies have also proven to be good indicators to assess changes in the environment that affect other invertebrate populations (Thomas 2005; van Swaay & van Strien 2005), and to be good indicators of other taxa richness like beetles, scorpions and centipedes (Gerlach *et al.* 2013). On the other hand, several multi-taxa studies have also concluded that butterflies are good biodiversity indicators and should be selected as a target group in ecological and biological conservation studies (Kessler *et al.* 2010).

Sampling method

The study was carried along six elevational gradients with different orientations (East, West, Southeast, Southwest, Northeast and Northwest) in Serra da Estrela between May and August of 2015. Each elevational gradient was divided into seven altitudinal levels, spaced by 250m and starting at the mountain base (~400m). The method used to sample the butterfly communities was an adaptation of the transect count described by Pollard & Yates (1993). In each site, we selected five transects that represented local habitat heterogeneity (Fig.3), altogether accounting for 210 samples (6 altitudinal gradients x 7 altitudinal levels x 5 replicas) (Fig.4).



Fig.3. Spatial representation of the five transects (replicas) made in each site. This site (SE1900) was chosen randomly.

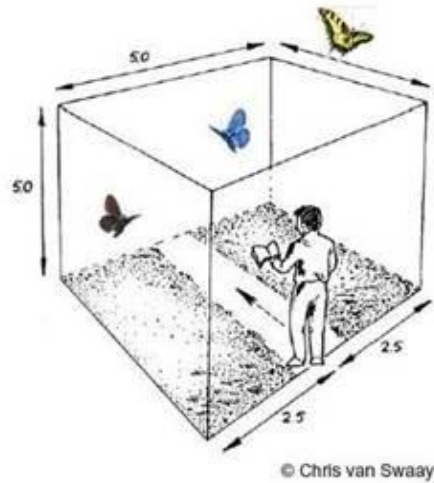


Fig.5. Illustration of the butterfly sampling technique (Image from: The North American butterfly monitoring network <http://www.clfs.umd.edu/lries/NABMN/pages/standardize.html>)

Data analysis

Overall species richness and estimated species richness were calculated for the study area (gamma diversity) jointly with the assessment of local biodiversity metrics, namely species richness, estimated species richness and evenness, for each of the 42 sampling sites.

We used the Incidence-based Coverage Estimator (ICE) (Lee & Chao, 1994) to assess local and regional species richness because this non-parametric richness estimator proved to perform well under different assemblage characteristics, sampling efforts and survey designs (Reese *et al.* 2014). The Incidence-based Coverage Estimator was calculated using the following formula:

$$C_{ICE} = S_{freq} + \frac{S_{infr}}{C_{ICE}} + \frac{q_1}{C_{ICE}} \gamma_{ICE}^2$$

where S_{freq} is the number of frequent species in a sample (found more than 10 times), S_{infr} is the number of infrequent species in a sample (found less than 10 times), q_1 is the number of unique species present, C_{ICE} is the proportion of all incidences of infrequent species that are not unique ($C_{ICE} = 1 - \frac{q_1}{n_{infr}}$), n_{infr} is the total number of incidences of infrequent species and γ_{ICE}^2 is the coefficient of

variation $\gamma_{ICE}^2 = \max \left[\frac{S_{infr}}{C_{ICE}} \frac{m_{infr}}{(m_{infr}-1)} \frac{\sum_{k=1}^{10} k(k-1)q_k}{(n_{infr})^2} - 1, 0 \right]$ where m_{infr} is the number of samples with at least one infrequent species (Magurran *et al.* 2011).

Species richness estimates were obtained for both regional and local scales by setting singletons and doubletons as rare species and by randomizing data on individuals (n=100 runs) with replacement to assess estimator variance. These analyses were carried out using the EstimateS software (Colwell 2013)

Evenness was calculated using the Pielou J index which is a suitable measure of relative evenness and the most widely used in ecological studies (Jost 2010). The Pielou J index compares the observed values of the Shannon-Wiener index (H') against the maximum value this species diversity index can attain ($\ln S$), by using the following expression:

$$J' = \frac{H'}{\ln S}$$

where H' is the Shannon-Wiener diversity index ($H' = -\sum p_i \ln(p_i)$) and S is the number of species in the sample (Heip *et al.* 1998).

Evenness scores were computed for each study site using the statistical package Species Diversity & Richness IV (Seaby & Henderson 2006).

The study of the variation in butterfly species richness and evenness across the altitudinal gradient was carried out using generalized linear models. Butterfly species richness variation across altitude was assessed by plotting estimated species richness for each site and gradient, and the altitudinal variations were modelled by polynomial regression analyses. On the other hand, the overall variation in species evenness across altitude was assessed jointly for all the study gradients and modelled using linear regression analyses.

The differences on species richness and evenness between gradients and altitudinal bands were assessed by hierarchical analysis of variance (*Model I mixed model*) with the factor “altitudinal band” nested in “gradient”. These analyses were performed after ensuring the assumptions of analysis of variance

(data normality, independence and homogeneity of variances). The hierarchical analyses of variance were performed in the R environment using the *stats* v3.1.3 software package (R Core Team 2014).

The analysis of species richness patterns along elevational gradients was carried out by evaluating the validity of two general hypotheses on the variation of species diversity across ecological gradients:

- to test the influence of the Rapoport's rule on species richness patterns along altitudinal gradients, we examined the relationship between the altitudinal ranges of butterfly species and their altitudinal midpoint (i.e. the mean of maximum and minimum altitudes at which each species was recorded). The association between the two variables (altitudinal midpoint and range size) was plotted for each altitudinal band and evaluated by polynomial regression analyses.

- the mid domain effect (MDE) was evaluated by comparing the empirical species richness with computed species richness resulting from a null model that accounts for the geometric constraints on species distributions. We applied the discrete mid domain effect model (Dunn *et al.* 2006) by a simulation process using the MDE algorithm (Colwell & Hurtt 1994) based on 5000 randomizations. Computed species richness for each altitudinal band was obtained jointly with the 95% confidence intervals. This analysis was carried out using the RangeModel software (Colwell 2006).

The study of community similarity across the altitudinal gradients of Serra da Estrela was evaluated by multivariate analysis techniques. Data were square-root transformed to meet the assumptions of the selected multivariate analyses being followed by the performance of clustering and ordination techniques.

Cluster analysis was performed using an agglomerative Ward's method and Bray-Curtis distance measure. Two ordination techniques – Principal Component Analysis (PCA) and Non-metric Multi-Dimensional Scaling (NMDS) - were also applied to explore complementary information on community similarity between sites and species associations to particular sites.

The PCA analysis was performed using a correlation distance matrix and setting the number of vectors shown in the graph (the 85% most informative

species). Then, using the PCA as the initial start position, a NMDS analysis was carried out using a quantitative similarity measure (Bray-Curtis) and allowing the rotation of the output. To ease interpretation, the sites at the same altitudinal band were represented by the same colour symbol, species names are associated to the corresponding vectors and site group perimeters were identified in the graphic. All multivariate analyses were carried out using the Community Analysis Package 4.0 (Henderson & Seaby 2007).

Finally, we assessed the altitudinal range of selected butterfly species of conservation concern in Serra da Estrela. The selected butterfly species were: *Argynnis aglaja*, *Cyaniris semiargus*, *Euphydryas aurinia*, *Hyponephele lycaon* (Kuehn, 1774), *Lycaena bleusei* (Oberthür, 1884) *Lycaena tityrus* (Poda, 1761), *Satyrus actaea* and *Thymelicus acteon* (Rottemburg, 1927). These species were chosen based on their threatened status according to Maravalhas (2003) and van Swaay *et al.* (2010) and considering their protection status by international conventions. All the selected species are included in a threatened category “Moderately threatened” or “In danger of extinction” for Portugal (Maravalhas 2003), with the exception of *Euphydryas aurinia* that has a “Not threatened” status (Maravalhas 2003). Nevertheless, *E. aurinia* is listed in the Bern Convention Annex II and in the Habitats Directive Annex II (IUCN Red List 2016). According to van Swaay *et al.* (2010), who red listed European butterflies, only *Thymelicus acteon* is considered Near Threatened (NT), with all the other species being classified as Least Concern (LC). The differences in species classification between the two studies (Maravalhas 2003; van Swaay *et al.* 2010) are in part due to the differences in the spatial scale of the analysis (regional vs national).

Results

General results

In total 6294 butterflies belonging to 70 species were observed during this study (Appendix I) The use of the ICE estimator allowed us to obtain the value of $74,5 \pm 7,9$ as a reliable estimate of the gamma diversity in our study area in Serra da Estrela. Some particularly important butterfly species for conservation

were observed, as is the case of *Argynnis aglaja* and *Cyaniris semiargus* that are classified as “In danger of extinction” in Portugal (Maravalhas 2003), and *Thymelicus acteon* that as a “Near threatened” status in Europe (van Swaay *et al.* 2010) and a “Moderately threatened” status in Portugal (Maravalhas 2003).

Altitudinal patterns of butterfly species richness

The results of the hierarchical analysis of variance allowed us to conclude that there are differences in species richness and evenness of the butterfly assemblages between altitudinal levels ($F=12.49$; $p<0.001$), but no significant differences were found in species richness and evenness when we compared the butterfly assemblages of different altitudinal gradients ($F=0.76$; $p>0.05$).

In most altitudinal gradients, the butterfly species richness showed a peak towards the middle of the mountain altitudinal range. However the altitudinal level where this peak occurs can vary from 650m to 1400m. Furthermore, two altitudinal gradients (Southeast and Southwest) showed a bimodal distribution of species richness by presenting a second peak at altitudes closer to the top of the mountain (Fig. 6). The northern altitudinal gradients show a very regular mid peak pattern, an increase in species richness at the lower levels, a peak at mid elevation and then a decrease in species richness (Fig.7). Regarding the eastern altitudinal gradient there is the presence a peak in species richness at 650m even though it is not as accentuated as it is in the other gradients; in the western gradient, the estimated species richness seems to have a slight decrease towards the middle before the peak at 1400m, however this is not present in the empirical results (Fig.8). Environmental data concerning nectar availability, flower abundance, site disturbance and habitat type (Appendix II) allowed further discrimination in species richness at local spatial scales.

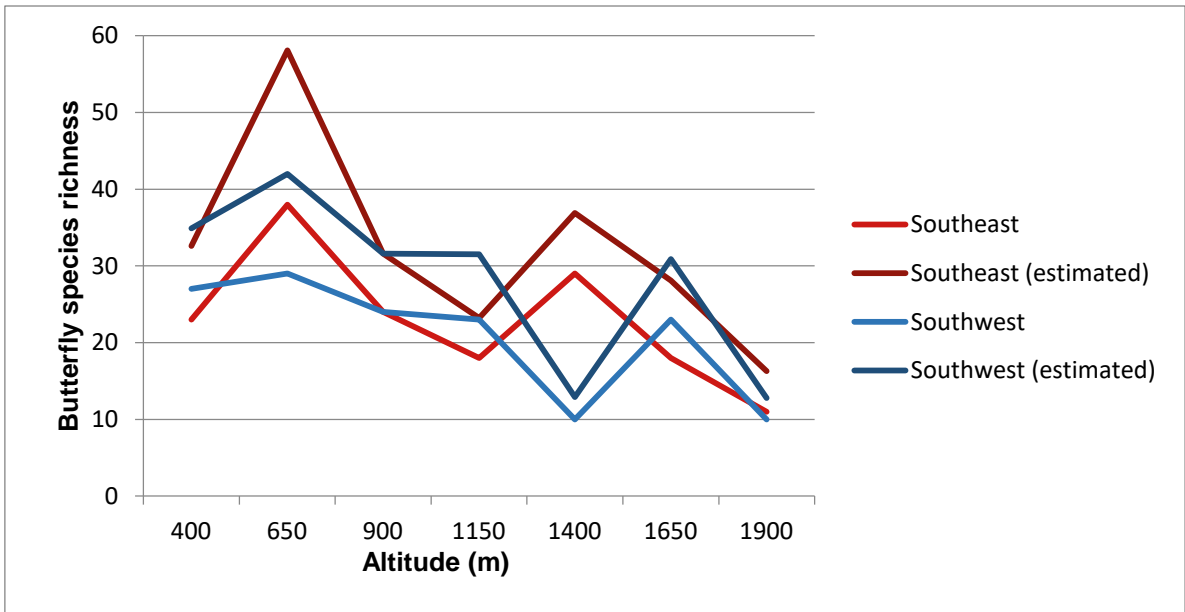


Fig.6. Variations in empirical and estimated butterfly species richness along the southern altitudinal gradients

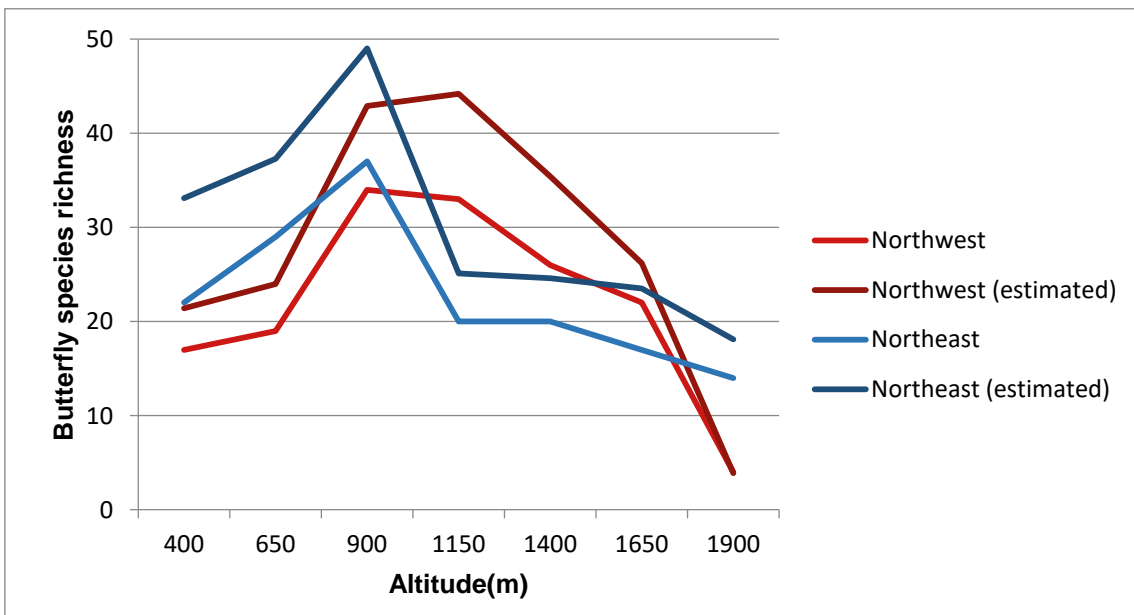


Fig.7. Variations in empirical and estimated butterfly species richness along the northern altitudinal gradients

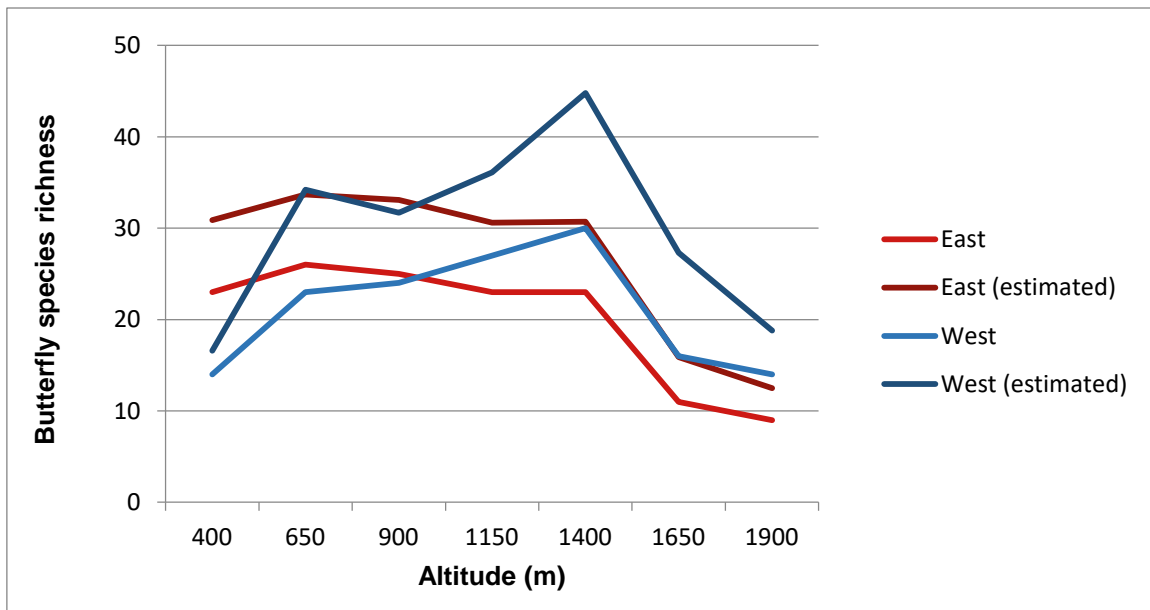


Fig.8. Variations in empirical and estimated butterfly species richness along the eastern and western altitudinal gradients.

In Serra da Estrela species evenness seems to decrease monotonically from lower to higher altitudes (Fig.9) with the less even assemblages being recorded in sites at the highest altitude (1900m) and the most even assemblages were found in sites at the lowest altitude (400m). Nevertheless, in spite of this trend, butterfly species evenness from sites at 1400m was higher than expected.

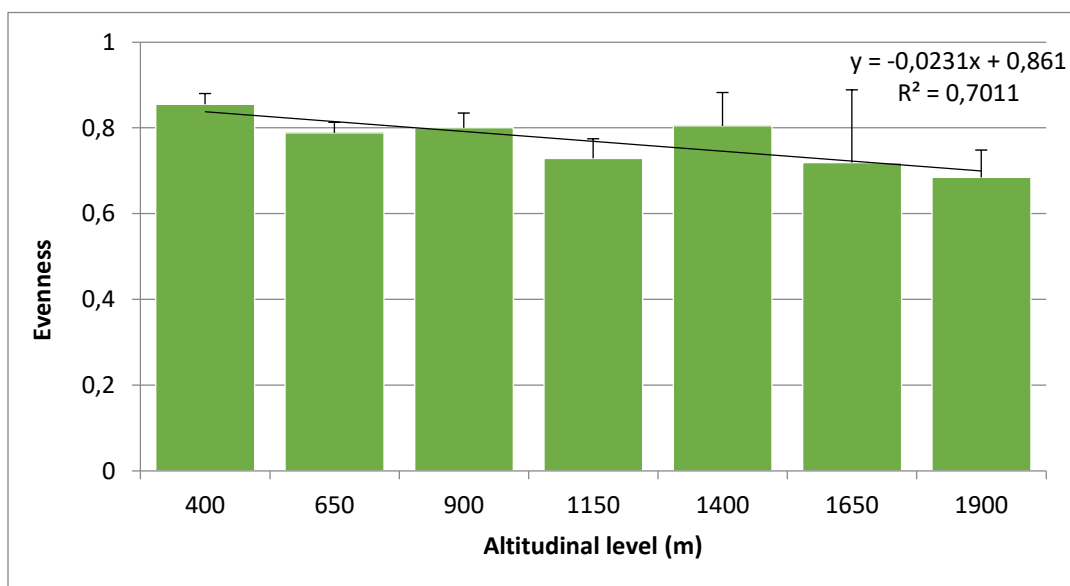


Fig.9. Species evenness variation according to the altitudinal level.

The analysis of the two hypotheses – Rapoport’s rule and the MDE – explaining species richness patterns along elevational gradients in Serra da Estrela allowed us to identify patterns in our data which are important to infer the mechanisms determining variation in species richness across altitude. Contrary to the predictions of the Rapoport’s rule, species from higher altitudes (i.e. found in sites at higher mean altitudes) did not show a wider altitudinal distribution range than species from lower altitudes (Fig.10). In fact, the species having intermediate mean altitudes showed the largest ranges (attaining over 1350m) when compared with species with mean altitudes near the extremes of the altitudinal gradient. This finding enables us to reject Rapoport’s rule as an explanatory hypothesis for the distribution pattern of butterfly species richness across the altitudinal gradient of Serra da Estrela

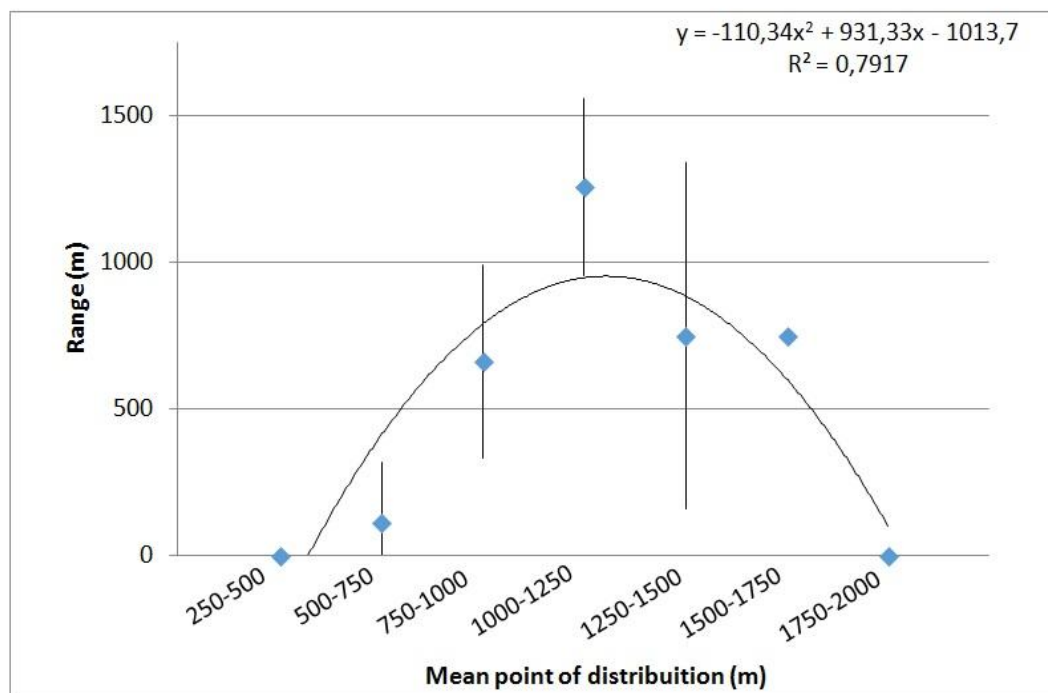


Fig.10. Butterfly species altitudinal range according to their midpoint (i.e. the mean of maximum and minimum altitudes at which each species was recorded) plotted in altitudinal bands with 250m intervals.

The comparative analysis of the predicted species richness using the MDE model with the empirical species richness across the altitudinal gradient of

Serra da Estrela points to some affinities. Both the empirical and the modelled species richness show a similar trend by presenting a peak at medium elevations, even though it is at different altitudinal levels (Fig.11). However, some deviations of the empirical species richness from the computed species richness are clear particularly at lower altitudes where empirical butterfly species richness was much higher than predicted by the MDE model. Nevertheless taking into consideration the matching of both trends (empirical and modelled) of variation in butterfly species richness across altitude, we can state that the MDE hypothesis provides a reasonable explanation for the altitudinal variation in butterfly species richness in Serra da Estrela.

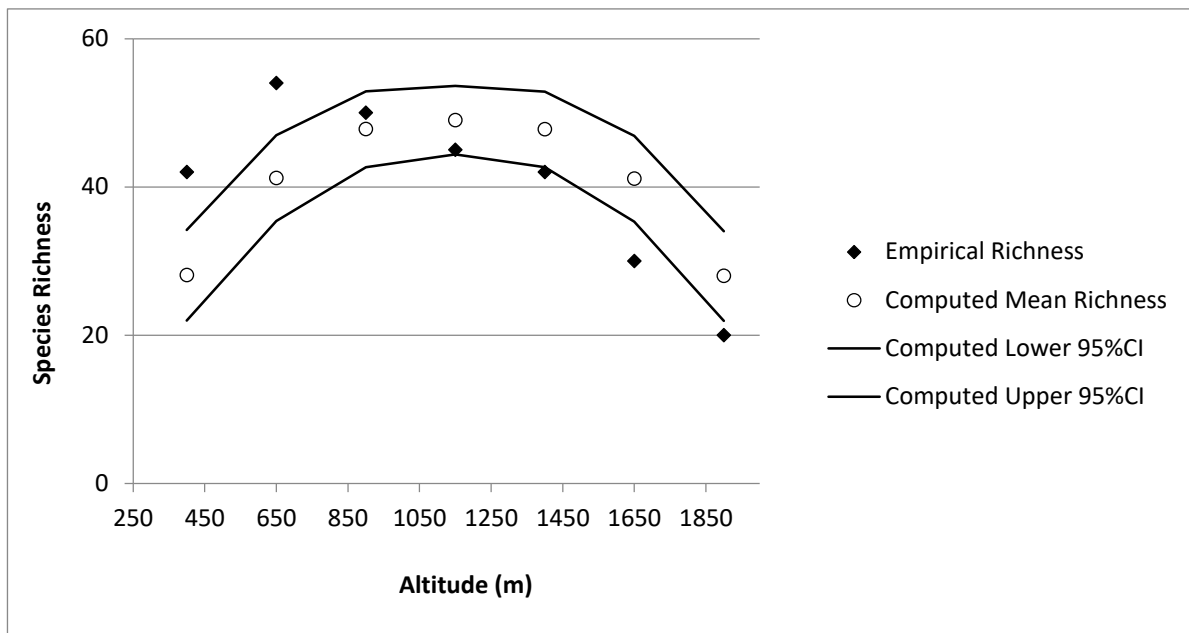


Fig.11. Variation in butterfly species richness across altitude according to the empirical results and the modelled results using the MDE model.

Butterfly assemblages composition

The study of similarity between butterfly assemblages from the different study sites showed that altitude plays a role in explaining the clustering of assemblages. Butterfly assemblages from the same altitudinal levels formed distinct clusters showing higher similarity with species assemblages from contiguous altitudinal levels. Further, the butterfly assemblages from sites at the

base of the mountain (~400m) and at mountaintop (~1900m) showed the highest dissimilarity in species composition. Also important to highlight was the finding that butterfly assemblages at lower elevational levels showed more overlap than those at higher levels. This means that the assemblages at lower levels are more similar between them than the ones present toward the top of the mountain (Fig.12).

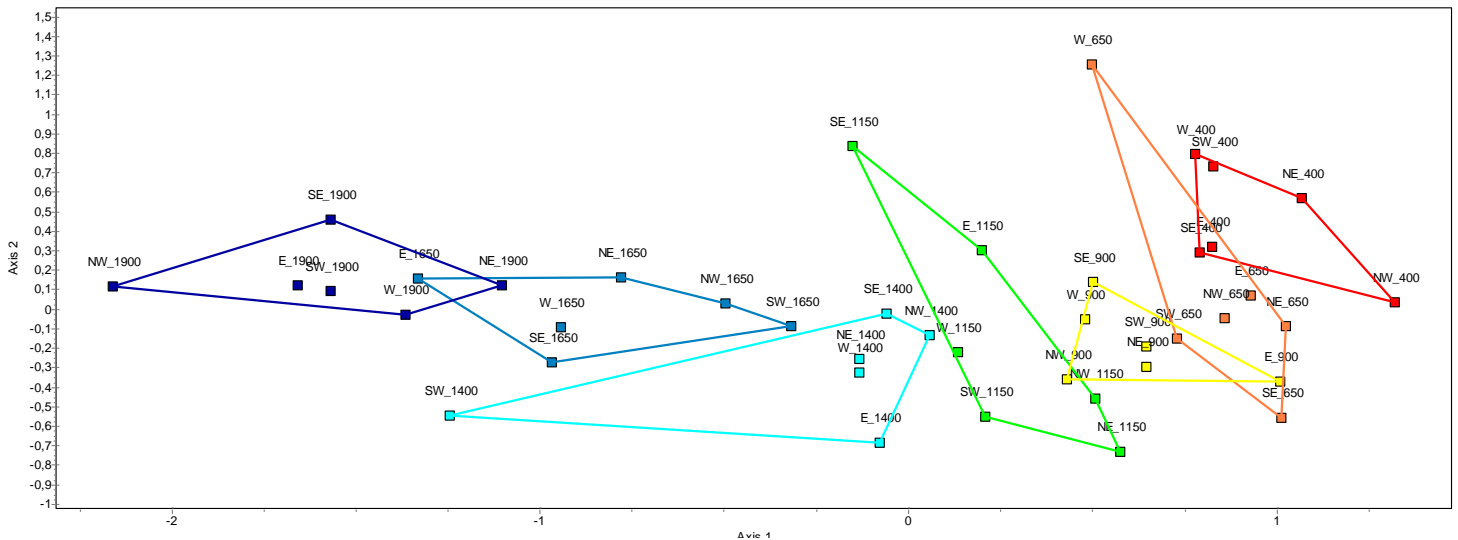


Fig.12. Similarity of butterfly assemblages in relation to the altitudinal levels and gradients. The name of each assemblage includes the name of the gradient (abbreviated) followed by the altitudinal band. Assemblages from the same altitudinal band are linked by the same colour line.

The butterfly assemblages of Serra da Estrela seem to be strictly associated with different altitudinal bands and three main groups presenting identical species composition can be clearly depicted: assemblages from low (400m-900m), medium (1150m-1400m) and high (1650m-1900m) altitudes (Appendix III).

The assemblage groups associated to specific altitudinal bands present some characteristic (indicator) butterfly species. For example, *Satyrus actaea* and *Hesperia* more representative butterfly species. At the mid elevation levels two subgroups of representative species can be recognized, one larger group including *Argynnis aglaja*, *Brintesia circe* (Fabricius, 1775), *Melanargia lachesis* (Hubner, 1790), *Polyommatus icarus* (Rottemburg, 1775), and *Thymelicus*

acteon (associated to the 900m altitudinal band) and the other smaller group composed by *Hyponephele lycaon* and *Issoria lathonia* (Linnaeus, 1758) (associated to the 1150-1400m altitudinal bands). *comma* Linnaeus, 1758) are representative of the top of the mountain showing a high positive association with sites at 1650m and 1900m (Fig.13). At lower altitudinal levels (400m-650m), *Maniola jurtina* (Linnaeus, 1758), *Pieris rapae* (Linnaeus, 1758) and *Pyronia bathseba* (Fabricius, 1793) seem to be the

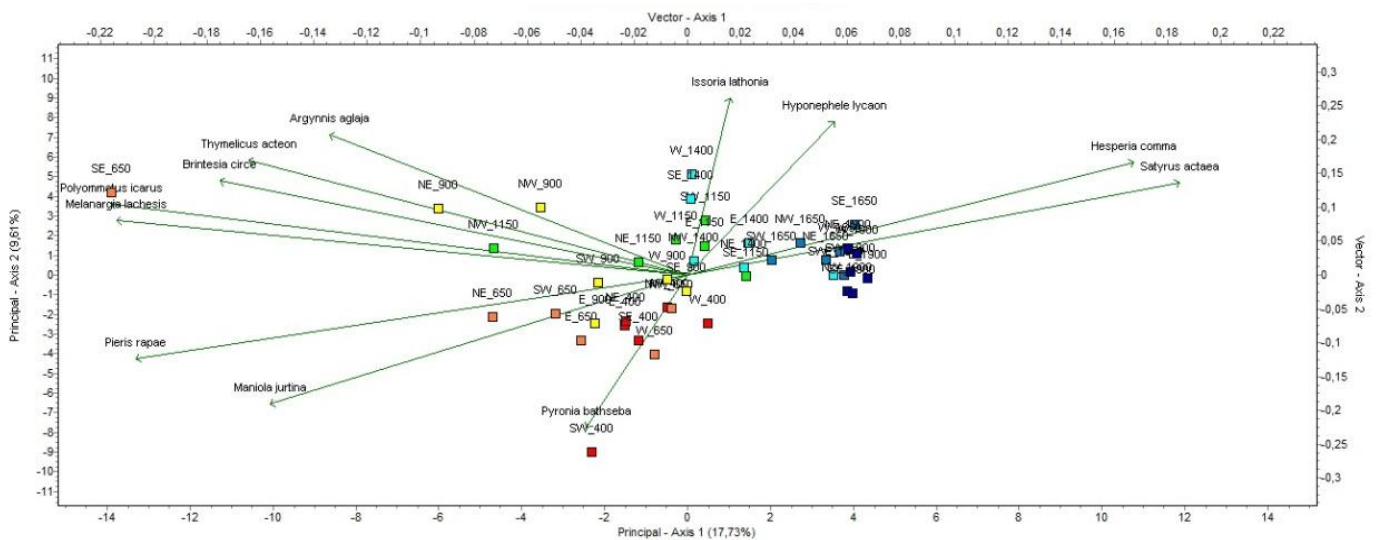


Fig.13. Similarity of the study sites considering butterfly species composition and identification of indicator butterfly species for site groups. The name of each assemblage includes the name of the gradient (abbreviated) followed by the altitudinal band. Assemblages from the same altitudinal band are represented by squares with a same colour.

Butterfly conservation

The altitudinal distribution ranges of selected butterfly species classified as threatened in Portugal (Maravalhas 2003) or being legally protected in Europe (IUCN Red List 2016) shows no apparent pattern. Conservation concern butterfly species were found along the altitudinal gradient, from the base to the mountaintop (Fig.14). Further, while some species seem to be altitudinal range restricted (e.g. *E. aurinia* and *C. semiargus*) others show a much wider altitudinal range of nearly 1000m (e.g. *L. bleusei* and *H. lycaon*). On the other hand, some altitudinal segregation can be depicted when it comes to the

distribution of *Lycaena tityrus* in regards to *Lycaena bleusei*: *L.tityrus* appears more frequently at lower altitudinal levels while *L.bleusei* seems to prefer the intermediate altitudinal levels (Fig.14). In the past, the Iberian endemic *L.bleusei* was often confused with *L. tityrus* and only recently its presence was confirmed in Serra da Estrela (Marabuto *et al.* 2004).

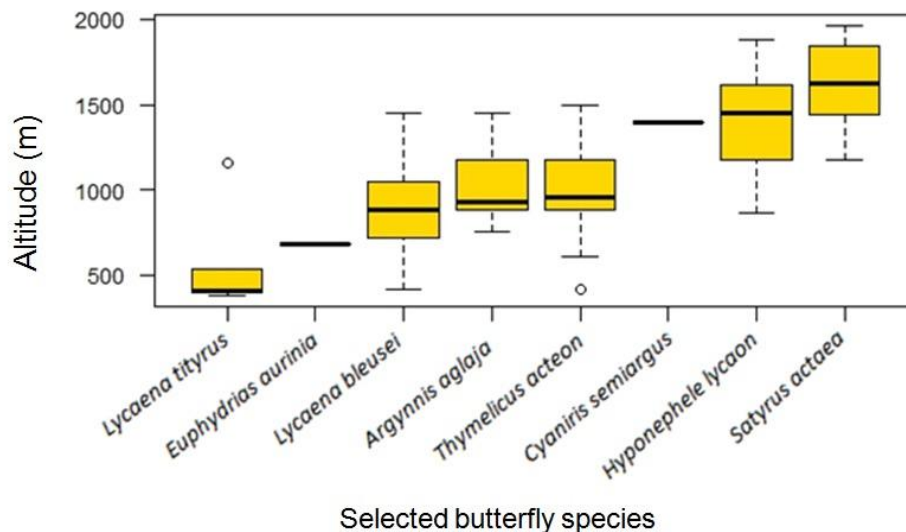


Fig.14. Altitudinal ranges of the selected butterfly species of conservation concern

Discussion

General discussion

The butterfly species richness found in Serra da Estrela was considerable (S=70) and highlights the high value for nature conservation of the natural legacy of this natural park.

According to the ICE estimator the number of butterfly species present in Serra da Estrela is higher than the one we observed, showing that the assemblages were not fully sampled. However the difference between sampled and estimated richness is relatively small, meaning that the chosen sampling method was effective to meet our goals. On the other hand published and unpublished records report the occurrence of 98 species of diurnal butterflies (Lepidoptera: Rhopalocera) in Serra da Estrela Natural Park (personal information from Centro de Interpretação da Serra da Estrela; to be available at <http://www.cise.pt/pt/index.php/base-de-dados>), but this finding results from

multiple studies carried out in the different seasons of the year, covering a wide variety of habitats and encompassing many localities within Serra da Estrela Natural Park since the early XX century (Marabuto *et al.* 2004).

The number of butterfly species reported from Serra da Estrela is relatively high when compared with other Portuguese protected areas and include several species of conservation concern (Araújo & Garcia-Pereira 2003; Maravalhas 2003). The occurrence of such number of species is in part due to the altitudinal gradient of Serra da Estrela which determines the presence of different habitat-types allowing the occurrence of butterfly species with different ecologies. Human activities also contribute to increase the butterfly species pool of Serra da Estrela by favouring the presence of some species (e.g. *Iphiclides feishtameilli* and *Pieris rapae*) that are usually found in disturbed areas, like orchards and farm fields (Garcia-Barros *et al.* 2013)

Altitudinal patterns of butterfly species richness

Our general findings on the variation in species richness between elevational levels and gradients were somehow expected: the significant differences in species richness and evenness between altitudinal levels result from the different biophysical characteristics and disturbance levels found along the elevational gradient; however, when comparing elevational gradients from the different slopes of the mountain, they all present a similar pattern of variation in climatic variables and disturbance levels, so no significant variations in butterfly species richness were detected.

During the last decades several studies have stressed the merits of using the altitudinal gradient as a model to investigate the variation in species richness and composition and the role of the different factors that drive biodiversity patterns. In spite of the different patterns of variation in species richness along the altitudinal gradient (McCain & Grytnes 2010) many studies showed that higher species richness tends to occur at intermediate altitudinal levels (McCoy 1990; Rahbek 1995; Sanders 2002; Lee *et al.* 2013). Our study also shows that butterfly species richness peaked at intermediate altitudinal levels in Serra da Estrela, generally fitting the mid peak pattern. However, for some elevational

gradients (particularly the eastern), the pattern seems to be more similar to the low plateau-mid peak pattern.

There are several ecological hypotheses that explain mid elevation peaks in the species richness of insects associated with plants: 1) "the middle is good hypothesis" (Janzen *et al.* 1976) says that the highest net plant productivity occurs at intermediate levels because temperatures during the day allow for a high photosynthetic rate and the cooler night temperatures permit low respiratory rates; this higher net plant productivity allows for more plant-associated insect species to occur at mid elevations; 2) "the ends are bad" hypothesis (Gagne 1979) states that species tend to accumulate at mid elevations due to environmental constraints; the upper limit is limited by the severity of the climate (very low temperatures) and resource restriction and the lower one by the climate harshness (aridity) and higher predation levels.

More specifically for butterflies several studies showed that species richness is usually positively correlated with nectar availability and plant species richness (Simonson *et al.* 2001; Pywell *et al.* 2004; Holl 1995; Kitahara *et al.* 2008). In our study, despite the variability in the peak in butterfly species richness between slopes, it was more often recorded at altitudes where a higher number and abundance of nectar producing plant species was found (Appendix II).

Also influencing butterfly species richness and abundance at local sites is the level of human disturbance of the site (Schmitt 2003; Kitahara *et al.* 2008), this was also an important factor in our study as in some cases the peak in butterfly species richness occurred in spots where we found a smaller level of disturbance, even though the peak in nectar availability and plant species richness occurred in an adjacent point, (e.g. the north-western slope). Surprisingly in our study the dominant habitat type present at each site does not seem to influence butterfly species richness as the peaks were found in sites where the dominant habitat can vary from disturbed rural areas to undisturbed scrublands and grasslands.

Our results also show a decline in the evenness as we get to the higher altitudinal levels of the mountain; this means that the butterfly species from

assemblages at lower altitudes have more even abundances while at higher altitudes there are some dominant species. Choi and An (2010) have also found a decrease in evenness along an altitudinal gradient. Insect communities present at high altitudes are characterized by few species and a greater abundance of individuals; this can be due to the fact that at higher levels the environment is more severe and less species are able to cope with such conditions, making the high altitude adapted species more abundant than the other co-occurring species (Choi & An 2010).

We tested two general hypotheses - Rapoport's rule and the Mid Domain Effect- on the variation of species richness along altitudinal gradients and concluded that our findings support partially the MDE. In fact, some studies have discredited Rapoport's altitudinal rule during the last two decades, questioning its usefulness to interpret changes in species richness along elevational gradients and have called for the need to the development of ecological theory in this area (Bachman *et al.* 2004; Lee *et al.* 2013). The Rapoport's rule failed to apply to our data because we found that species having higher altitudinal midpoints presented lower altitudinal ranges than those with medium altitudinal midpoints. Rapoport's altitudinal rule may not be supported because it does not take into account some aspects that can influence the range of different species. The knowledge that range sizes are dynamic, correlate with species-specific attributes and are influenced by environmental variables led McCain & Knight (2013) to state that the study of mountain species' range sizes is still an open frontier.

On the other hand, when testing the MDE model, we were able to check that the variation in butterfly species richness across altitude presented a peak at intermediate altitude, which results in part from spatial constraints. The peak in species richness occurred at a lower altitudinal level than the one predicted by the MDE model and butterfly species richness at higher altitudes was also lower than predicted. This clearly indicated that other factors (e.g. climate, disturbance, water availability) operating at local spatial scales influence species distribution patterns along the elevational gradient. Many previous studies have documented that the MDE is an important factor influencing

species richness patterns in altitudinal gradients for a variety of plant and animal groups from different mountain systems of our planet (Sanders 2002; Bachman *et al.* 2004; Watkins *et al.* 2006). For example Watkins *et al.* (2006) and Lee *et al.* (2013) have found that the MDE model largely explained the distribution pattern of species richness in their studies, in comparison to other alternative hypotheses.

Nevertheless, the mismatch between our findings and the MDE model predictions highlights the role played by local environmental factors in driving species richness patterns. Sanders (2002) has shown that even though the MDE model explained some of the variation in species richness pattern observed, in some cases, area availability was the best predictor of the variation in species richness along the altitudinal gradient.

Butterfly assemblages composition

There was a clear discrimination in the composition of butterfly assemblages along the altitudinal gradient, with the assemblages from the same altitudinal level grouping together and being more similar to assemblages of contiguous altitudinal levels. In other studies, butterfly assemblages have also been found to be arranged along the altitudinal gradient (Sawchik *et al.* 2005; Carneiro *et al.* 2014).

The similarity between the assemblages at lower levels can be due to a more heterogeneous habitat. At lower levels the habitat is more disturbed, urbanized and some dominant habitat-types can be found up to 900m, allowing for a wider dispersal of some butterfly species (e.g. *Pyronia bathseba* and *Maniola jurtina*) at the lower levels making the butterfly assemblages less differentiated.

At higher altitudinal levels, many environmental factors present much different values from the ones found at lower levels, leading to quite distinct habitats and plant communities that are specific of mountaintops. Consequently, only a small number of butterfly species adapted to these specific conditions are able to subsist, leading for more homogeneous assemblages towards the top of the mountain which are quite distinct from the ones found at lower levels.

Some butterfly species are representative of specific altitudinal bands, meaning that their abundance is associated with the particular environmental conditions in that altitudinal band. As these conditions change along the altitudinal gradient so does butterfly species presence and abundance, leading to differences in the compositions and richness of butterfly assemblages (Sawchik *et al.* 2005).

Three butterfly species are more representative of the lower altitudinal levels namely *Maniola jurtina*, *Pieris rapae* and *Pyronia bathseba*.

Maniola jurtina is a very common species in Portugal, and can be found in the borders of urbanized sites (Maravalhas 2003). *Pieris rapae* is also very common, and shows very high abundance levels in ruderal environments and in gardens with the presence of brassicas (Maravalhas 2003; Garcia-Barros *et al.* 2013)., while *Pyronia bathseba* is usually associated with grasslands and tall lawns (Garcia-Barros *et al.* 2013). So it makes sense that these species are representative of the lower altitude sites where we can find urbanized areas and the presence of agricultural fields and vegetable gardens that promote the presence of these species.

Two other butterfly species – *Hesperia comma* and *Satyrus actaea* - are representative of the higher altitudinal levels of Serra da Estrela. *Hesperia comma* is frequent in Portugal in the mountains of the North, and can be found mainly in open areas of sclerophyllous scrubland and dry pastures (Marabuto 2003, Garcia-Barros *et al.* 2013). *Satyrus actaea* is usually associated with montane xerophyllous habitats being most common in clear rocky areas, (Maravalhas 2003, Garcia-Barros *et al.* 2013).

The species that appear as representative of the middle altitudinal bands (*Argynnis aglaja*, *Brintesia circe*, *Hyponphele lycaon*, *Issoria lathonia*, *Melanargia lachesis*, *Polyommatus icarus* and *Thymelicus acteon*) are all ecological undemanding and most of them are characteristic of mid mountain altitudes. The group of species that is associated with the 900m altitudinal band is composed by species that show a preference for prairies and forest clearings and edges, for example: *Argynnis aglaja* is characteristic of mountain areas between 600m and 1000m and is usually found on the edges of pathways, prairies and forest clearings (Maravalhas 2003), and the presence of *Brintesia circe* is favoured by the presence of pastures at mid mountain altitudes and is

usually found in dry prairies and on the edges of woods between 300m and 1400m (Maravalhas 2003; Garcia-Barros *et al.* 2013). The group of species associated with 1150-1400m altitudinal band is composed by two species that are favoured by low vegetation: *Hyponephele lycaon* is a typical mid altitude mountain species and can be found in undergrowth areas between 600m and 1550m (Maravalhas 2003; Garcia-Barros *et al.* 2013) and *Issoria lathonia* is a very generalist species that can be found in many habitat types but mainly in prairies and flowering heaths up until 1600m, being characteristic of mid altitudes (Maravalhas 2003; Garcia-Barros *et al.* 2013).

Butterfly species that are representative of particular environments can be useful in providing information of the ecological conditions of that particular site, and constitute a useful tool for conservation purposes (Sawchik *et al.* 2005).

Butterfly conservation

All selected butterfly species show some level of threat status in Portugal with the exception of *Euphydryas aurinia* that, in spite not being considered threatened in some reference works, is listed in the Bern Convention and in the Habitats Directive. Even though in our study the distribution of *E. aurinia* was localized, this species shows a very wide distribution not only in Portugal but also in the rest of Europe and Asia (Maravalhas 2003).

Some other butterfly species are most in need of conservation efforts: *Thymelicus acteon* for instance is a species that has a “Near Threatened” status (van Swaay *et al.* 2010) and in Portugal is “Moderately threatened” (Maravalhas 2003). The species shows a wide distribution but has also shown a decline in populations of Europe and Asia. No studies regarding the population tendencies of this species are known for the Iberian Peninsula (Garcia-Barros *et al.* 2013).

Satyrus actaea has a “Least concern” status for Europe (van Swaay *et al.* 2010) and a “Moderately threatened” status for Portugal (Maravalhas 2013). This species is characteristic of high altitudes and its distribution is very localized in Portugal only appearing in Serra da Estrela and Parque Natural de Montesinho. The species can be threatened by the global warming that is expected to reduce its distributional area (Maravalhas 2003, Garcia-Barros *et al.* 2013).

Lycaena tityrus has a “Moderately threatened” status for Portugal (Maravalhas 2013) and a “Least concern” status for Europe (van Swaay *et al.* 2010). This species is not very common in Portugal and has seen its habitat degraded due to agricultural intensification that can cause a population decline (Maravalhas 2003). The distribution of *Lycaena bleusei* in Portugal is not well known and its threat status has not been evaluated. In Europe it has a “Least concern” status (van Swaay *et al.* 2010). *Lycaena bleusei* is endemic to the Iberian Peninsula, and should be the target of population biology studies to better understand its demographic changes (Garcia-Barros *et al.* 2013).

Hyponephele lycaon has a “Moderately threatened” status for Portugal (Maravalhas 2003) and a “Least concern” status in Europe (van Swaay *et al.* 2010). Its distribution is relatively localized and scattered, and there is the need to improve the knowledge regarding its distribution in Portugal. In Europe the known distribution of the species should be revised because the information regarding its distribution may be flawed due to some confusion between *H.lycaon* and *H.lupinus* (Maravalhas 2003).

Of the selected species, only two of them show a “In danger of extinction” status for Portugal (Maravalhas 2003). *Cyaniris semiargus* is very rare in Portugal and has suffered a decline in many European countries (Maravalhas 2003). It can only be found in less exploited meadows, so the agricultural intensification is harmful for the species (Maravalhas 2003); *Argynnis aglaja* is also very rare and only found in the northern mountains in Portugal and is threatened by deforestation. Its populations in Europe have suffered a decline in the last decades, and even though in Portugal and Spain there are no studies regarding its population tendencies it can be speculated, based on other studies, that the populations are negatively affected by deforestation and global warming (Maravalhas 2003; Garcia-Barros *et al.* 2013;).

We consider that, particularly, *Cyaniris semiargus*, *Argynnis aglaja* and *Lycaena tityrus* should be the target of conservation studies and plans due to their status in Portugal and their restricted distribution in Serra da Estrela. These species are directly affected by agricultural intensification, livestock ranching and deforestation (Sánchez *et al.* 2013) and have seen their habitats reduced during the last decades.

The lack of basic knowledge on some butterfly species biology is a serious impediment for the development of effective conservation plans, so efforts should be added to improve our knowledge on species distribution, abundance and ecology (habitat and host plant species associations) (Cardoso *et al.* 2011). The taxonomic and conservation status of some species that were previously confused (*L.tytirus/L.bleusei* and *H.lycaon/H.lupinus*) should be also clarified to assess the need for the development of additional studies. Further, the acknowledged population decline of many butterfly species merits investigation and should follow a monitoring program fulfilling international research standards. This monitoring program should be applied to conservation concern species (like *A. Aglaja* and *C.semiargus*) and the information collected should be used to address species-specific conservation actions. There are a few good examples of butterfly conservation studies in Portugal (e.g. Arnaldo *et al.* 2013; Gonzales *et al.* 2016) but for most threatened species basic information to support conservation action is still lacking

On the other hand, conservation measures should also be taken in order to protect the habitats of butterflies. The habitats of many rare butterfly species are declining due to human actions and the impact of climate changes (Numa *et al.* 2016). The situation is particularly worrisome in mountain areas, where some restricted habitats are in peril jointly with their associated fauna and flora. In these areas legislation and supervision need to be implemented to protect or mitigate the effects of land use changes and overgrazing on the native habitats (e.g. creation of corridors, in the agricultural fields, with native vegetation). It will be also important that environmental-friendly practices should be adopted in areas that are being used for agriculture since intensive farming and the use of pesticides have been responsible for species decline and local population extinctions (Numa *et al.* 2016).

Nowadays there is biased information when it comes to butterfly communities in Europe because not all countries have the same knowledge about their species (van Swaay *et al.* 2010). In Portugal around 92% of the species are classified as LC (Sánchez *et al.* 2013), but we need to take into account that butterflies are very sensitive to

changes in the environment and their main threats are habitat loss, degradation and fragmentation, agricultural intensification, fires and invasive species (van Swaay *et al.* 2010). To prevent the decline or loss of species due to these factors we need to monitor the communities and properly assess their status in order to apply effective conservation programs.

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Appendix I

List of all the butterfly species found and their correspondent threat status in Europe and Portugal. The distribution of each species is shown for each altitudinal band studied.

Species	Status for Portugal (Maravalhas 2003)	Status for Europe (Van Swaay et al. 2010)	Distribution along altitudinal bands										TOTAL	
			400	650	900	1150	1400	1650	1900					
<i>Aglais urticae</i>	Moderadamente ameaçada	LC											3	3
<i>Argynnis adippe</i>	Moderadamente ameaçada	LC		1	7			8						16
<i>Argynnis aglaja</i>	Em perigo de extinção	LC		1	5		1							7
<i>Argynnis niobe</i>	Moderadamente ameaçada	LC		1										1
<i>Argynnis pandora</i>	Não ameaçada	LC	9	23	8	8	10	4	1	1				63
<i>Argynnis papilia</i>	Moderadamente ameaçada	LC	1		1		1							3
<i>Aricia cramera</i>	Não ameaçada	LC	75	127	144	120	24	9						499
<i>Brintesia circe</i>	Não ameaçada	LC	37	55	51	81	34	16	1					275
<i>Caracharodus alceae</i>	Não ameaçada	LC		1										1
<i>Celastrina argiolus</i>	Não ameaçada	LC	6	10	9	4	7	9	4	49				
<i>Charaxes jasius</i>	Não ameaçada	LC	2											2
<i>Coenonympha pamphilus</i>	Não ameaçada	LC	21	3	3	1	3	1						32
<i>Colias croceus</i>	Não ameaçada	LC	45	49	26	27	37	19	10	213				
<i>Cyaniris semiargus</i>	Em perigo de extinção	LC					4							4
<i>Erynnis tages</i>	Moderadamente ameaçada	LC		4										4
<i>Euphydryas aurinia</i>	Não ameaçada	LC		2										2
<i>Gonopteryx cleopatra</i>	Moderadamente ameaçada	LC			4									4
<i>Gonopteryx rhamni</i>	Não ameaçada	LC	5	32	6	5	2							55
<i>Hesperia comma</i>	Não ameaçada	LC			3	24	13	40	52	132				
<i>Hipparchia fialia</i>	Não ameaçada	LC			5	25	9							39
<i>Hipparchia hermione</i>	Não ameaçada	NT	1	2	28	31	88	25	8	183				
<i>Hipparchia semele</i>	Não ameaçada	LC	1	12	7	4	7							31
<i>Hipparchia statilinus</i>	Não ameaçada	NT	2	4	6	17	18	8	1	56				
<i>Hyponphele lycaon</i>	Moderadamente ameaçada	LC	3	7	4	1	1							58
<i>Iphiclides podalirius feistha</i>	Não ameaçada	LC			5	20	12	22	20	16				95
<i>Issoria lathonia</i>	Não ameaçada	LC			2									2
<i>Laesopis roboris</i>	Não ameaçada	LC	48	15	6	3	20	16	1	109				
<i>Lampides boeticus</i>	Não ameaçada	LC			5	5	4	1	15					
<i>Lasioommata maera</i>	Não ameaçada	LC	4	5	9	5	7	5	5	40				
<i>Lasioommata megera</i>	Não ameaçada	LC	23	24	8	7	4	2		68				
<i>Leptidea sinapis</i>	Não ameaçada	LC	48	95	43	32	47	50	23	338				
<i>Leptotes pirithous</i>	Não ameaçada	LC												
<i>Limnitis reducta</i>	Moderadamente ameaçada	LC		3										3
<i>Lycaena alciphron</i>	Não ameaçada	LC	1	5	7	3								16
<i>Lycaena bleusei</i>	Não avaliada	LC	6	6	23	7	2							44

Species	Status for Portugal (Maravalhas 2003)	Status for Europe (Van Swaay et al. 2010)	Distribution along altitudinal bands										
			400	650	900	1150	1400	1650	1900	TOTAL			
<i>Lycena phlaeas</i>	Não ameaçada	LC	28	19	74	52	5	6	2	186			
<i>Lycena tityrus</i>	Moderadamente ameaçada	LC	4			1				5			
<i>Maniola jurtina</i>	Não ameaçada	LC	40	27	21	2				90			
<i>Melanargia lachesis</i>	Não ameaçada	LC	27	216	172	141	101	11		668			
<i>Melanargia russiae</i>	Moderadamente ameaçada	LC		1	5	22	4			32			
<i>Melitaea deione</i>	Não ameaçada	LC	1	24	6	8	2	1		42			
<i>Melitaea didyma</i>	Moderadamente ameaçada	LC		1	5					6			
<i>Melitaea nevadensis</i>	Não ameaçada	LC		1	1	1				3			
<i>Melitaea parthenoides</i>	Moderadamente ameaçada	LC		1						1			
<i>Melitaea phoebe</i>	Não ameaçada	LC			2	3	1			6			
<i>Melitaea trivia</i>	Moderadamente ameaçada	LC	2	24	14	1				41			
<i>Neozephyrus quercus</i>	Não ameaçada	LC		1						1			
<i>Ochlodes sylvanus</i>	Não ameaçada	LC		2						2			
<i>Papilio machaon</i>	Não ameaçada	LC	1	1						2			
<i>Pararge aegeria</i>	Não ameaçada	LC	17	24	6	4	14	1		66			
<i>Pieris brassicae</i>	Não ameaçada	LC	4	13	1		4	1		23			
<i>Pieris napi</i>	Não ameaçada	LC	4	9	25	3	1	4		46			
<i>Pieris rapae</i>	Não ameaçada	LC	91	138	44	36	18	10		337			
<i>Plebejus argus</i>	Não ameaçada	LC		5	7	16	60	100	50	238			
<i>Polygonia c-album</i>	Moderadamente ameaçada	LC	5	1	1					7			
<i>Polyommatus icarus</i>	Não ameaçada	LC	28	57	23	47	33	6		194			
<i>Pontia daplidice</i>	Não ameaçada	LC	14	67	38	123	31	20	8	301			
<i>Pyrgus malvoides</i>	Não ameaçada	LC					1			1			
<i>Pyronia bathseba</i>	Não ameaçada	LC	3	3	1					7			
<i>Pyronia cecilia</i>	Não ameaçada	LC	56	22	20	21				119			
<i>Pyronia tithonus</i>	Não ameaçada	LC	10	98	152	218	152	17		647			
<i>Satyrium esculi</i>	Não ameaçada	LC		1		1				2			
<i>Satyrium ilicis</i>	Não ameaçada	LC	3							3			
<i>Satyrium spini</i>	Não ameaçada	LC	1	13	5	1				20			
<i>Satyrus actaea</i>	Moderadamente ameaçada	LC				4	79	285	168	536			
<i>Thymelicus acteon</i>	Moderadamente ameaçada	NT	1	10	13	15	4			43			
<i>Thymelicus lineola</i>	Moderadamente ameaçada	LC			4					4			
<i>Thymelicus sylvestris</i>	Não ameaçada	LC	25	35	29	20	7	1		117			
<i>Vanessa atalanta</i>	Não ameaçada	LC		1	1	2		4		8			
<i>Vanessa cardui</i>	Não ameaçada	LC	2	4				5	1	12			

Appendix II

Nectar producing plant species richness, flower abundance and dominant habitat type for each study site. The level of disturbance in each study site is presented in a qualitative scale from 1 (low disturbed site) to 5 (very disturbed site).

Study site		Nectar producing plant species richness	Flower abundance	Disturbance level	Dominant habitat type
Slope	Altitudinal band				
E	400	95	3196	3	Oak forest
	650	68	12547	5	Scrubland
	900	74	3143	4	Mixed forest
	1150	31	1482	4	Scrubland
	1400	24	411	2	Scrubland
	1650	12	5755	3	Scrubland
	1900	17	1452	4	Grassland
W	400	54	992	4	Grassland
	650	88	2148	3	Scrubland
	900	112	3224	5	Pine forest
	1150	62	7170	4	Scrubland
	1400	34	17935	3	Scrubland
	1650	24	50226	3	Grassland
	1900	30	2486	3	Grassland
NE	400	71	1779	4	Rural
	650	89	1207	3	Riparian forest
	900	89	6331	3	Rural
	1150	54	3650	3	Meadow
	1400	10	1270	4	Birch forest
	1650	19	13731	3	Grassland
	1900	22	2775	4	Scrubland
NW	400	84	807	5	Scrubland
	650	82	1320	3	Pine forest
	900	95	4689	4	Grassland
	1150	77	4065	3	Grassland
	1400	37	3211	3	Scrubland
	1650	26	58086	2	Grassland
	1900	16	2775	2	Grassland
SE	400	88	3603	4	Rural
	650	104	1511	2	Rural
	900	51	1145	4	Scrubland
	1150	24	689	4	Scrubland
	1400	67	6989	4	Grassland
	1650	9	5955	3	Grassland
	1900	15	11724	2	Scrubland
SW	400	107	1980	3	Pine forest
	650	120	3303	3	Rural
	900	114	2502	3	Scrubland
	1150	73	3134	3	Scrubland
	1400	6	53	4	Scrubland
	1650	14	7169	2	Grassland
	1900	21	33643	3	Scrubland

Appendix III

Similarity in butterfly species composition between study areas at different altitudes. Three groups of butterfly assemblages (low-, medium- and high-altitude) were identified. Study areas names are abbreviated by indicating the transect and the altitudinal level (p.ex. E_1150 meaning a site on the Eastern transect at 1150m of altitude).

