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Diversity and distribution patterns of Lepidoptera and Orthoptera in Greece and their

responses to local and global climate change

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Summary

Our planet is currently changing. Climate warming has already had profound effects on ecological communities such as changes in species abundances, distributional shifts towards higher latitudes or altitudes, and on trophic interactions. However, species responses to climate change can vary greatly. There is no universal trend embracing all habitats, time periods and taxonomic groups. In addition, due to limited human and economic resources, we lack knowledge, and thus evidence, on how climatic change impacts certain regions and habitats, including some of those expected to experience the most profound impacts (e.g. mountains in Mediterranean). Further research is needed about even the basic ecology of less well-studied groups (e.g. Orthoptera) or most vulnerable species (montane). First we need to define current distributions, secondly to study species responses to climate change and find their potential linkages to finer scale species-habitat associations or other taxonomic groups that could be used as surrogates and third to establish baselines to assess future changes.

In this thesis, I focus on species responses to climate change and on the biodiversity patterns along environmental gradients such as altitude. I chose to study butterflies¹ and Orthoptera because they are ectothermic organisms and are expected to react faster to temperature variation than warm-blooded organisms such as mammals. In addition, there is evidence of their strong congruent species richness patterns, which might be a simplified option to decrease complexity of the study system and overcome the lack of permanent monitoring schemes. High biodiversity, sensitivity to climate change and availability of historical data were the main criteria for the selection of my three study systems. All three areas are located within Greece, part of the broader Mediterranean Basin. The urgent need for better understanding, interpretation and conservation in this area is underlined by the following three facts: (i) our study area is a hotspot of biodiversity, yet poorly studied (ii) it has a warmer climate than most of the rest of the Northern Hemisphere and (iii) it has been losing biological patterns hitherto considered 'Mediterranean'.

In this thesis, I explore the structure, diversity and possible impact of climatic change in a little-studied arthropod community in Greece. I start with a comparable study using historical and current butterfly data and searching for signals of climate change (Chapter

¹ The term butterflies will be used throughout this thesis instead of the more general term Lepidoptera which was used in the title of the thesis; the term butterflies referred to diurnal butterflies only (or Rhopalocera).

2), zoom in on phenological responses and their associations to different habitats and habitat-specific variables using historical and current data of both butterflies and Orthoptera (Chapter 3), continue with butterfly and Orthoptera species-turnover across different spatial scales using data from two mountainous regions (Chapter 4) and finally concentrate on current and future phenological patterns of butterfly community and individual species tested along an elevational gradient (Chapter 5).

It is suggested that the European protected-area network will cease to be efficient to conserve biodiversity, as species may leave existing nature reserves in search of more climatically-suitable habitats. The second chapter of this thesis investigates whether there is a significant temperature increase the last 20-year period and examines long-term (1998-2011/2012) and short-term (2011-2012) changes in the butterfly fauna within a protected area of north-eastern Greece, Dadia National Park. Despite the protected status of Dadia National Park (Dadia NP hereafter) and the subsequent stability of land use regimes, we found marked changes in butterfly community composition over a 13 year period accompanying a significant temperature increase of 0.95°C. Nevertheless, our analysis gave no evidence of significant year-to-year (2011-2012) variability on butterfly community composition, suggesting that community-composition change might be attributed to longer-term environmental factors, such as climate warming. We further documented species mainly occurring at low elevations increasing their abundance whereas species mainly occurring at higher elevations declined. Possible conservation options for this Mediterranean protected area studied, and new approaches for increasing species' resilience, were discussed in the light of these changes.

The *third chapter*, analyzes thoroughly the phenological patterns of both butterflies and Orthoptera and investigates how these patterns have change over a 13 and 12-year period respectively in Dadia NP. Given the great taxonomic variability of insects' phenological patterns, we tried to interpret how and why this variability occurs using a novel technique (Standardized Major-Axis analysis), testing also the possibility for congruent phenological patterns *vis-a-vis* the environmental gradient of canopy cover. Although no congruent phenological patterns were found to occur, we found an advancement of both groups' mean date of appearances from the first to the second survey, while the duration of flight periods decreased for butterflies and did not change for Orthoptera. Further evidence that species phenological responses can be dictated by different habitats or habitat-specific variables,

reinforced the idea of incorporating both these elements into such analyses; in a sense, these elements may lead to clearer pictures of species phenological responses to regional warming, as this was shown in this chapter for butterflies.

Chapter four deals with the diversity patterns of butterfly and Orthoptera across spatial scales using an additive partitioning framework. In this study data were collected from two mountainous areas (Rodopi and Grammos) located in the north and west of Greece respectively, and sites were selected hierarchically. High mountains are likely to be particularly vulnerable to climate change but may also become *refugia* in periods of climate change. Whether different taxonomic groups are distributed randomly across montane ecosystems remains unclear and further empirical evidence is needed, as well as tests regarding the congruency of diversity patterns. This chapter gives the first detailed description for the diversity of the two insect groups, partitioned into four hierarchical levels: ecoregions (mountains), elevational zones, habitat types, and transects for butterflies or plots for Orthoptera. We found higher levels of beta diversity among elevational zones and to a lesser extent between ecoregions and habitat types. These results lead to the recommendation that when monitoring montane nature reserves, all elevational zones must be represented and adequate replicates of different habitat types out of the two ecoregions studied must be considered. Our work revealed incongruent diversity patterns between the two insect groups, thus it is clear that butterflies are of limited use as a surrogate group for Orthoptera diversity and vice versa. Further suggestions on monitoring schemes for commonness and rarity in these groups are made.

The *fifth chapter* deals with the basic assumption that increasing altitude is expected to lead species' activity to be shorter, later in the season and more synchronized; this phenomenon could provoke trophic mismatches leading to regional extinctions if two or more groups that are closely connected, as for example if butterflies and their host-plants respond differently to climate change. Using butterfly data collected along the elevational gradient (in Rodopi and Grammos), we analyzed if and how the phenology changed along this gradient. We calculated the lapse rate of the temperature, meaning how temperature decreased as we move to the upper altitudes, and we predicted how future climate warming would affect butterfly community. Our results, as we would expect, supported a delay in the timing of butterfly appearances and a shortening of the duration of their flight period along the altitudinal gradient. But also they supported a different rate at which

phenological change occurs in the two study regions. Based on a temperature lapse rate of approximately 3°C per 1km elevation increase, our findings suggest that a 1°C decrease in mean seasonal temperature could be associated with a 5-day and a 6- to 8-day phenological delay at community and species level respectively. We conclude that elevational gradients can be important predictors of phenological responses to climate change, especially if time-dependent records are lacking. The use of space-for-time substitution has been highlighted, but other issues such as the different rates between geographical locations or the synchronization of timing of appearances of univoltine species with increasing altitude have now been raised. If we are to detect more evidence for climate-induced phenological shifts, and to make predictions about how butterfly and Orthoptera communities will respond under future global climate scenarios further research is desirable.

Περίληψη

Ο πλανήτης μας μεταβάλλεται συνεχώς. Ανάμεσα στις περιβαλλοντικές αλλαγές που υπόκειται, η κλιματική αλλαγή επηρεάζει έντονα τη βιοποικιλότητα ωθώντας τα είδη σε αλλαγές της αφθονίας τους και της κατανομής τους, με τη μετακίνησή τους προς μεγαλύτερα υψόμετρα και γεωγραφικά πλάτη, ενώ μπορεί ακόμα να προκαλέσει την διατάραξη των τροφικών αλληλεξαρτήσεων του ενός είδους (ή μιας ομάδας ειδών) με ένα άλλο. Ακόμα και σήμερα που έχουμε καλύτερη επίγνωση του τί συμβαίνει, οι αποκρίσεις των ειδών στην κλιματική αλλαγή φαίνεται να διαφέρουν σημαντικά μεταξύ των διαφορετικών ενδιαιτημάτων, των χρονικών περιόδων, των ειδών ή και των ομάδων. Επιπλέον, εξαιτίας των περιορισμένων χρηματοδοτικών πόρων αλλά και του περιορισμένου αριθμού σε εξειδικευμένο ανθρώπινο δυναμικό, υπάρχει μια σημαντική έλλειψη γνώσης και πληροφοριών σε ένα μεγάλο τμήμα της Ευρώπης και ιδιαίτερα στη Μεσόγειο, όπου τα αποτελέσματα της κλιματικής αλλαγής αναμένονται να είναι δριμύτερα. Συνεπώς, περαιτέρω έρευνα σε θέματα βασικής οικολογίας λιγότερο διερευνημένων ομάδων (π.χ. Ορθόπτερα) ή πιο ευπαθών ομάδων (π.χ. αυτών που κατοικούν σε ορεινές περιοχές με μεγάλο υψόμετρο) καθίσταται απαραίτητη. Με αυτόν τον τρόπο θα μπορέσει να καθοριστεί πρώτον η σημερινή κατανομή των ειδών, δεύτερον να μελετηθούν οι αποκρίσεις των ειδών στην κλιματική αλλαγή και να ανιχνευθούν οι πιθανοί δεσμοί με χαρακτηριστικά του ενδιαιτήματος που διαβιούν αλλά και με άλλες ομάδες που θα μπορούσαν να υποκαταστήσουν η μία την άλλη και τρίτον να μπουν οι βάσεις για τη διερεύνηση μελλοντικών αλλαγών που δύναται να προκαλέσουν περαιτέρω μεταβολές στο κλίμα στη Μεσόγειο.

Σε αυτή τη διατριβή, επικεντρώνομαι στις αποκρίσεις των ειδών στην κλιματική αλλαγή και στα πρότυπα ποικιλότητάς τους ως προς περιβαλλοντικές παραμέτρους, όπως για παράδειγμα την υψομετρική διαβάθμιση. Επέλεξα τη μελέτη των ημερόβιων πεταλούδων και των Ορθοπτέρων διότι είναι εξώθερμοι οργανισμοί και αναμένεται να αντιδρούν γρηγορότερα στη θερμοκρασιακή μεταβολή συγκριτικά με άλλες ομάδες ενδόθερμων οργανισμών, όπως τα θηλαστικά. Επιπροσθέτως, υπάρχουν ενδείξεις για το ότι η μία ομάδα μπορεί να υποκαταστήσει σε ένα μεγάλο βαθμό την άλλη, κάτι που αν επαληθευθεί, θα μπορούσε να μειώσει κατά πολύ την πολυπλοκότητα του συστήματος που εξετάζουμε και ίσως να υπερπηδήσει τα προβλήματα που προκύπτουν από την έλλειψη μόνιμων επιφανειών παρακολούθησης για τα είδη που απαντώνται στην ελληνική επικράτεια.

Τα βασικά κριτήρια για την επιλογή των δειγματοληπτικών περιοχών ήταν: (α) η υψηλή βιοποικιλότητα, (β) ο βαθμός ευαισθησίας στην κλιματική αλλαγή των οικοσυστημάτων και (γ) η ύπαρξη ιστορικών δεδομένων. Όλες οι περιοχές αποτελούν τμήμα του ελληνικού χώρου και άρα της ευρύτερης περιοχής της Μεσογείου. Το γεγονός ότι η Μεσόγειος θεωρείται ζώνη υψηλής βιοποικιλότητας, ότι η θερμοκρασιακή άνοδός της είναι μεγαλύτερη από το υπόλοιπο βόρειο ημισφαίριο και ότι ένα μέρος των μεσογειακών ειδών ενδέχεται να μετοικήσει σε βορειότερες περιοχές, επιφέρει την επιτακτική ανάγκη να κατανοήσουμε, να ερμηνεύσουμε και να διατηρήσουμε την πλούσια βιοποικιλότητα αυτού του τόπου.

Η παρούσα διατριβή ξεκινά με μια συγκριτική μελέτη κάνοντας χρήση ιστορικών και σύγχρονων δεδομένων που αφορούν στις ημερόβιες πεταλούδες και στο πώς αυτές αντιδρούν στην κλιματική αλλαγή (Κεφάλαιο 2), εστιάζει στις φαινολογικές αποκρίσεις των πεταλούδων αλλά και των Ορθοπτέρων ως προς τα διαφορετικά ενδιαιτήματα και τις διαφορετικές περιβαλλοντικές μεταβλητές (Κεφάλαιο 3), συνεχίζει με το πώς τα πρότυπα ποικιλότητας των δύο υπό μελέτη ομάδων διαφοροποιούνται σε διαφορετικές χωρικές κλίμακες σε δύο ορεινές περιοχές (Κεφάλαιο 4) και στο τέλος μελετά τις φαινολογικές αποκρίσεις των πεταλούδων κατά μήκος του υψομέτρου και προβλέπει το πώς αυτές δύναται να αλλάξουν σύμφωνα με την προβλεπόμενη αύξηση της θερμοκρασίας (Κεφάλαιο 5). Το Κεφάλαιο 6 αποτελεί μία σύνοψη των βασικών αποτελεσμάτων στους επιμέρους ερευνητικούς στόχους, έτσι όπως αυτοί τέθηκαν ανά κεφάλαιο.

Έρευνες έχουν δείξει ότι το ευρωπαϊκό δίκτυο προστατευόμενων περιοχών μπορεί να μην είναι σε θέση να διατηρήσει τη βιοποικιλότητα που βρίσκεται εντός των ορίων του καθώς τα είδη προβλέπεται πως θα μετακινηθούν προς το βορρά, στην προσπάθειά τους να βρουν κατάλληλα για την επιβίωσή τους ενδιαιτήματα. Το δεύτερο κεφάλαιο της διατριβής, αρχικά μελετά κατά πόσο η θερμοκρασία στην περιοχή του εθνικού πάρκου της Δαδιάς έχει αυξηθεί τα τελευταία 20 χρόνια και στη συνέχεια διερευνά τις αλλαγές στη σύνθεση της κοινότητας πεταλούδων σε δύο περιόδους διαφορετικής διάρκειας (μακροπρόθεσμα: 1998-2011/2012 και βραχυπρόθεσμα: 2011-2012). Παρά το καθεστώς προστασίας του δάσους της Δαδιάς και άρα της σταθερότητας στις χρήσεις γης, καταγράψαμε σημαντική αλλαγή στη σύνθεση της κοινότητας των πεταλούδων ακολουθούμενη από αύξηση της θερμοκρασίας κατά 0.95°C. Αντιθέτως, δε βρέθηκε καμία ένδειξη σημαντική ετήσιας αλλαγής (2011-2012), υποδηλώνοντας ότι η αλλαγή στη σύνθεση της βιοκοινότητας που καταγράψαμε πιθανότατα να οφείλεται σε άλλους πιο μακροπρόθεσμους παράγοντες, όπως η κλιματική αλλαγή. Επιπλέον, τα είδη που σε εθνικό επίπεδο απαντώνται κυρίως σε χαμηλό υψόμετρο βρέθηκαν να αυξάνουν την αφθονία τους σε σχέση με αυτά που απαντώνται σε μεγάλο υψόμετρο. Με βάση τις αλλαγές που βρέθηκαν, πιθανά μέτρα διατήρησης των πληθυσμών των πεταλούδων για την υπό μελέτη περιοχή προτείνονται στο τέλος του κεφαλαίου.

Στο τρίτο κεφάλαιο αναλύονται τα φαινολογικά πρότυπα των πεταλούδων και των Ορθοπτέρων και συγκεκριμένα το πώς αυτά έχουν αλλάξει στο πέρασμα 13 και 12 χρόνων αντίστοιχα στην περιοχή της Δαδιάς. Δεδομένης της μεγάλης διακύμανσης στα φαινολογικά πρότυπα των εντόμων, προσπαθήσαμε να ερμηνεύσουμε πώς και γιατί υπάρχει αυτή η τεράστια διακύμανση. Εξετάσαμε επίσης, το κατά πόσο τα φαινολογικά πρότυπα συγκλίνουν μεταξύ των δύο ομάδων ως προς το παράγοντα δενδροκάλυψη, ώστε να μπορέσει η μία να υποκαταστήσει σε ένα βαθμό την άλλη και αντίστροφα. Ακόμα κι αν το τελευταίο δεν επιβεβαιώθηκε, εντούτοις βρήκαμε μια πρόωρη εμφάνιση των ειδών από έτος σε έτος καθώς και μία μικρότερη διάρκεια πετάγματος των πεταλούδων κατά την ίδια χρονική περίοδο. Περαιτέρω ενδείξεις σχετικά με την αλλαγή των φαινολογικών προτύπων των πεταλούδων ως προς τα διαφορετικά ενδιαιτήματα ή όταν περιβαλλοντικές μεταβλητές (π.χ. θερμοκρασία, υγρασία) που συνοδεύουν την κάθε δειγματοληπτική επιφάνεια συμμετέχουν στην ανάλυση, ενισχύουν την ιδέα ότι αυτά τα στοιχεία είναι ικανά να επηρεάσουν με διαφορετικό τρόπο τη φαινολογία των πεταλούδων. Κατά μία έννοια, τα διαφορετικά ενδιαιτήματα και οι περιβαλλοντικές μεταβλητές που χαρακτηρίζουν καθένα από αυτά, είναι σε θέση να μας δώσουν μία πιο λεπτομερή εικόνα σχετικά με τις αποκρίσεις των ειδών στην κλιματική αλλαγή, όπως αυτό αποδείχθηκε στην περίπτωση των πεταλούδων.

Το τέταρτο κεφάλαιο πραγματεύεται τα πρότυπα ποικιλότητας των πεταλούδων και Ορθοπτέρων σε διαφορετική χωρική κλίμακα. Για αυτήν τη μελέτη τα δεδομένα συλλέχθηκαν από δύο ορεινές περιοχές (Ροδόπη και Γράμμος) που τοποθετούνται στη βόρεια και δυτική πλευρά της Ελλάδας, ενώ η επιλογή των δειγματοληπτικών επιφανειών ακολούθησε τη δομή ενός ιεραρχικού μοντέλου. Υποστηρίζεται ότι τα βουνά είναι ιδιαίτερα ευαίσθητα στις αλλαγές του κλίματος, ενώ παράλληλα έχουν αναγνωριστεί ως καταφύγια για είδη που αλλάζουν την κατανομή τους προς μεγαλύτερα υψόμετρα, ως αποτέλεσμα της αύξησης της θερμοκρασίας. Χρειάζεται όμως περαιτέρω έρευνα και γνώση σχετικά με το αν οι διαφορετικές ταξινομικές ομάδες κατανέμονται ή όχι τυχαία στους ορεινούς αυτούς όγκους και στο κατά πόσο τα πρότυπα ποικιλότητας δύναται να συγκλίνουν υπό αυτές τις συνθήκες. Αυτό το κεφάλαιο δίνει για πρώτη φορά μια λεπτομερή περιγραφή των προτύπων ποικιλότητας δύο διαφορετικών ομάδων εντόμων διαχωρισμένα σε τέσσερα ιεραρχικά επίπεδα: οικοσυστήματα (βουνά), υψομετρικές ζώνες, τύποι ενδιαιτημάτων, διαδρομές για τις πεταλούδες και πλαίσια δειγματοληψίας για τα Ορθόπτερα. Βρήκαμε μεγαλύτερα επίπεδα β-ποικιλότητας μεταξύ των υψομετρικών ζωνών και σε μικρότερο βαθμό μεταξύ των οικοσυστημάτων και των διαφορετικών ενδιαιτημάτων. Εκτιμώντας αυτά τα αποτελέσματα, προτείνεται ένας μελλοντικός σχεδιασμός για την παρακολούθηση των ειδών σε ορεινές περιοχές όπου όλες οι υψομετρικές ζώνες θα πρέπει να αντιπροσωπεύονται πλήρως, ενώ ένας επαρκής αριθμός δειγμάτων θα πρέπει επίσης να λαμβάνεται από κάθε βουνό και κάθε τύπο ενδιαιτήματος. Δε βρέθηκαν όμοια πρότυπα ποικιλότητας μεταξύ των δύο ομάδων περιορίζοντας έτσι τη δυνατότητα του να χρησιμοποιηθεί η μία ως υποκατάστατο της άλλης. Περαιτέρω προτάσεις σχετικά με την παρακολούθηση των κοινών και των σπάνιων ειδών πεταλούδων και Ορθοπτέρων δίνονται στο τέλος του κεφαλαίου.

Το πέμπτο κεφάλαιο διερευνά την υπόθεση ότι με την αύξηση του υψομέτρου τα είδη πεταλούδων θα έχουν κάποια χρονική καθυστέρηση στην εμφάνισή τους, μικρότερη και πιο συγχρονισμένη διάρκεια πτήσης. Αυτό το φαινόμενο, θα μπορούσε να οδηγήσει σε διακοπή των τροφικών αλληλεξαρτήσεων μεταξύ ειδών προκαλώντας ακόμα και εξαφανίσεις σε τοπικό επίπεδο, αν δύο ή περισσότερες ομάδες που είναι στενά συνδεδεμένες όπως για παράδειγμα οι πεταλούδες με τα φυτά-ξενιστές τους, αποκρίνονται σε διαφορετικό χρόνο ή τόπο στην κλιματική αλλαγή. Αρχικά υπολογίστηκε η διαφορά θερμοκρασίας από τα χαμηλότερα στα υψηλότερα υψόμετρα και στη συνέχεια έγινε μια πρώτη πρόβλεψη για το πώς η θερμοκρασιακή αλλαγή θα επηρεάσει τη βιοκοινότητα των πεταλούδων. Τα αποτελέσματα της έρευνας, όπως αναμενόταν, υποστήριξαν μία καθυστέρηση στην εμφάνιση των ειδών και μία μικρότερη διάρκεια πετάγματος με την άνοδο του υψομέτρου. Όμως, ο ρυθμός αλλαγής στη φαινολογία βρέθηκε να διαφοροποιείται σημαντικά μεταξύ των δύο περιοχών. Βάσει της μείωσης της θερμοκρασίας κατά 3 βαθμούς κελσίου ανά 1000μ υψομέτρου, προκύπτει ότι για κάθε βαθμό μείωσης της θερμοκρασίας θα σημειώνεται καθυστέρηση στη μέση ημερομηνία εμφάνισης κατά πέντε μέρες και κατά έξι με οχτώ μέρες σε επίπεδο βιοκοινότητας και

μεμονωμένων ειδών αντίστοιχα. Συνεπώς, η υψομετρική διαβάθμιση είναι ένας πολύ σημαντικός παράγοντας για τη μελέτη των φαινολογικών αποκρίσεων των πεταλούδων, ιδιαίτερα όταν δεν υπάρχουν διαθέσιμα δεδομένα μεγάλης χρονικής κλίμακας. Σε αυτό το κεφάλαιο επισημαίνεται η χρήση της υποκατάστασης του χρόνου με το χώρο, όμως προκύπτουν άλλα ζητήματα που χρίζουν προσοχής όπως για παράδειγμα ο διαφορετικός ρυθμός αλλαγής της φαινολογίας της βιοκοινότητας των πεταλούδων μεταξύ των δύο ορεινών περιοχών ή η συγχρονισμένη πτήση των ειδών με μία μόνο γενεά που παρατηρήθηκε ως προς το υψόμετρο. Περαιτέρω έρευνα στα ζητήματα αυτά θα μπορούσε να διευκολύνει την ερμηνεία των προτύπων που παρατηρήθηκαν και σίγουρα να ενισχύσει μελλοντικές προβλέψεις που θα αφορούν στις αποκρίσεις των πεταλούδων και των Ορθοπτέρων στην ελληνική επικράτεια.

-Chapter 1-

Introduction

Biodiversity crisis and insect communities

Humanity is now facing possibly the greatest crisis in its history, a crisis that inevitably is shared by all the other organisms with which we share the earth. Over the last 150 years, the increase of human population size, unsustainable resource utilization and technological development has been causing increasing levels of threat to the ecosystem services that are critical to the maintenance of the current conditions on this planet. These services are "the conditions and processes through which natural ecosystems, and the species that make them up, sustain and fulfill human life" (Daily, 1997). For example, insect pollination of crops is essential for the production of many fruits and vegetables and also some biofuels (Franzén & Nilsson, 2008). Or the maintenance of the quality of the atmosphere by plants is vital for human health and crops (Monks et al. 2009). In addition, the presence of vegetation enables the recycling of nutrients and helps to mitigate erosion and floods (Sekercioglu 2010). Although there are plenty of examples of past civilizations that are also known to have overstressed their environment, now, the concept of "overshoot and collapse", namely that the world economy might expand so rapidly as to overshoot the physical limitations of planet Earth (Randers 2008) is a very real possibility and consistent with current patterns (Johansen & Sornette, 2001).

Insects are responsible for important biotic interaction such as pollination and herbivory and contribute more than 60% of all plant and animal species in Europe, an important numerical fraction of global biodiversity (Bale et al., 2002). Despite their ecological value on ecosystem services and their economic value, a little less than one tenth of one percent of all described insect species (1255) evaluated for inclusion in the 2007 International Union for Conservation of Nature (IUCN) Red List of Threatened Species. There is a rich bibliography demonstrating insects' downward trajectory, yet, only the last decade the world has demonstrated its commitment to conserving its insect fauna.

As conditions change rapidly around the world and the genetic library of animals, plants and microorganisms with which we share Earth is declining, the European Union agreed to a global target of significantly reducing the rate of biodiversity loss by 2010 (SCBD, 2003). The Parties to the Convention on Biological Diversity failed to meet their goals for halting the biodiversity loss and new commitments were created with new goals to be met by 2020 in the Strategic Plan for biodiversity (2011-2020). However, a recent study

suggested that despite accelerating policy, the impacts of these efforts may not lead to improved trends in the state of biodiversity by the end of the current decade (Tittensor, 2014). The challenges faced by scientists such as to preserve biodiversity and natural resources are continuously changing as new threats emerged. In recent years, the threats of global climate change have necessitated the development of new, creative solutions to protect our valuable resources.

Global warming and insect communities

According to Intergovernmental Panel on Climate Change (IPCC) Global surface temperature change for the end of the 21st century is likely to exceed 1.5°C (IPCC, 2013). However, this rise will not occur at a constant rate across space or time (Root and Schneider, 2002). It is therefore important to understand how species respond to climate change in the context of their environments (Primack et al., 2009). The inevitable rise in global temperatures is expected to be a serious threat to all organisms and especially to insects (Settele et al., 2008). It is suggested that insects will suffer more severely from climate change than other organisms due to their exothermic nature (Maes et al., 2010). As poikilothermic animals, insects have limited ability to regulate their body temperature and the changes in the surrounding temperature regimes cause alterations in the development rates, number of broods per year and survival (Karuppaiah and G.K. Sujayanad). Besides development and reproduction, climate change is likely to affect species phenology by altering the onset or the duration of the flight period and species distribution range (Root 2003). Regarding distribution range shifts, it is expected that species will expand their range at the cold edge of their distribution; in parallel a loss of their populations at the southern edge will occur (Parmesan et al., 1999; Settele et al., 2008) though there may be a delay before this happens due to extinction debt (Jackson & Sax, 2010). In this vein, many organisms originally constrained to the Mediterranean region are now making their way northward in Europe, under the new conditions associated with global warming (Blondel et al., 2010).

For well-known groups such as butterflies inhabiting the well-studied regions of North or Central Europe and of North America (e.g. Forister & Shapiro, 2003; Roy & Sparks, 2000; Van Strien et al., 2008; Westwood & Blair, 2010), the environmental requirements are fairly well known, and so it is possible to model the new climatic niches (Settele et al., 2008). But for a number of other less well-studied taxa (e.g. Orthoptera) in barely studied regions (such as Meditterenean) information is scarce and the ability to model species responses and make further predictions is limited. Especially threatened considered to be organisms occurring in ecosystems with "nowhere to go", such as mountains (Colwell et al., 2008; Nogués-Bravo et al., 2007). While the rate of warming in mountains has been twice the global average (Brunetti et al., 2009) and species shift their distribution upwards in order to find climatically-suitable habitats, species distribution range is reducing because land area is usually decreasing with altitude (Körner, 2007; Lenoir et al., 2008). Therefore, studying and forecasting "vulnerable" montane assemblages becomes a priority.

Butterflies as target group

Butterflies constitute an important biodiversity component, recognized to be the best known and most frequently studied early warning indicator of environmental change (Thomas, 2005). Butterfly ecology is very well known: they occur in nearly all terrestrial ecosystems, in disturbed as well as undisturbed areas, utilize most of the successional stages of vegetative growth, and their trophic preferences -both at the larval and adult stages- cover a wide variety of plant species (Caldas & Robbins, 2003). The life cycle of a butterfly comprises four distinct stages in its life-cycle: the egg (ovum), caterpillar (larva), chrysalis (pupa) and adult butterfly (imago). The process of this transformation is known as metamorphosis and it is usually lasts one year (but there are exceptions). Ova are usually laid on plants, also known as their host-plants, upon which the larvae subsequently feed. Ovum shapes and external appearances vary according to the family of origin; thus, may be bottle-shaped, disc-shaped, spherical or dome-shaped (Tolman & Lewington, 1997). The ovum stage lasts a few weeks or several months if hibernation (over-wintering) occurs in this stage. The development stage of the larval progressively continues with instar-stages. Most European species hibernate as larvae, although it is possible to hibernate at the ovum stage (e.g. Parnassius mnemosyne, Argynnis adippe), as chrysalis (e.g. Aporia crataegi, Papilio machaon) or as adults (e.g. Nymphalis polychloros, Gonepteryx rhamni). The life-cycle of a butterfly often lasts one year but there are species with more than one brood during the year (i.e. polyvoltine species). The duration of the flight period of an adult butterfly has a considerable variation between the species; for example the duration of the flight period of Anthocharis cardamines, Erynnis tages and

Pararge aegeria is 20 days, of *Erebia aethiops* 21 days, while for *Argynnis paphia* is almost 35 days (Pamperis & Stavridis, 2009).

Due to their complex and short life cycles and ecological requirements, butterflies can easily track environmental change (Mac Nally et al., 2003; Thomas, 2005). As they are easy to identify and survey compared to other invertebrate taxa (e.g. beetles) they can be monitored cost-effectively at a national or a European scale (Roy et al., 2007). As a result, butterflies are among the best target group for monitoring programs in complex ecosystems with a variety of different habitat types and land-uses, fulfilling adequately the criteria for a good "flagship taxon when researchers aim to raise public awareness about the effects of environmental change on native fauna (New, 1997). Their surrogate value towards other target taxa has often been tested and proved to be either sufficient (e.g. Pearman & Weber, 2007; Zografou et al., 2009) or not (e.g. Gutiérrez & Menéndez, 1998; Lovell et al., 2007). For example, in a study examining changes in distribution of butterflies, plants and birds through time, butterflies found to experience the most rapid rates of extinction and authors argued that this rate may be indicative of declines experienced by other, less well-studied groups (Thomas, 2005). Furthermore, butterflies, as early warning indicators of environmental change, are an excellent group to study the effect of climate change (Settele et al., 2008). They are highly sensitive to environmental factors, especially those associated with climate change (Parmesan et al., 1999). Recent studies prove that they react faster than other groups such as birds (Devictor et al., 2012) as a result of their short generation times and their exothermic nature, meaning that their population dynamics may respond to temperature changes more directly and more rapidly (Van Swaay et al., 2012).

Along with many other attributes of global biodiversity, butterflies are also predicted to decline, to go extinct or to change their geographical distribution (Maes et al., 2010; Parmesan, 2007; Root et al., 2003). About 9% of European butterflies are threatened in Europe and a further 10% of butterflies are considered Near Threatened (Van Swaay et al., 2010). Despite the lack of good trend data in some European countries, the study of Van Swaay (Van Swaay et al., 2010) shows that about a third (31%) of the European butterflies has declining populations, while 4% are increasing and more than half of the species are stable. Although butterflies benefit from a detailed dataset including relatively fine-resolution information on species' distributions and abundance on the north of Europe (Van Swaay et al., 2012), they are still far less studied in the Mediterranean (Dell et al.,

2005). Given also that changes in abundance and species richness in response to climate change can greatly vary from declines, to no change, to increases, depending on the group studied (e.g. lepidopterans, plants, beetles) (Morecroft et al., 2002) it is suggested that more research is needed to quantify how species are responding to climatic variation; to understand better why their responses vary and to define at what extent their communities can be resilient to climate change.

Orthoptera as target group

Orthoptera (grasshoppers and bush crickets) are a major component of rural biodiversity (Ryszkowski et al., 1993); they play a central role in food webs, as primary herbivores and abundant food resource for other taxa. The life cycle of Orthoptera is quite complex, going through a number of stages or instars before becoming adult. The immature stages are referred to as nymphs. As in all exopterygote insects the nymphs closely resemble adults, both in appearance and mode of life, although the wings and genitalia are only functional in the adult. All instars are active and there is never a pupal stage as in butterflies or moths (endopterygote insects). Most grasshoppers and bush crickets have a single generation a year: the eggs hatch in late spring or early summer when the temperature is suitable for nymphal development and the young vegetation provides a high quality food source. Nymphal development normally takes a couple of months and the adults emerge from July onwards depending on the altitude and latitude: the precise timing of these events is very variable (Brown, 1990). Two main types of annual cycles can be distinguished in Greece. The first one is the most common where the eggs hatch in the spring when climatic conditions are ideal and resources available for nymphal development. Adults of this first category occur in the field from late spring to late summer and disappear again before late summer or autumn leaving their eggs to overwinter before hatching the next year during spring. The second category however, includes species for which eggs hatch right after oviposition and there is nymphs or even adults that hibernate (Willemse, 1985).

The complex life cycle of Orthoptera and their life history traits can support their use as appropriate bio-indicators for several regions and biomes (Kati et al., 2004b; Sauberer et al., 2004) or as indicators of land use change and disturbance (e.g. Andersen et al., 2001; Báldi & Kisbenedek, 1997; Kati et al., 2006; Kruess & Tscharntke, 2002). However, their value as surrogates of other target taxa is usually poor (e.g. Duelli & Obrist, 1998). While

some species are tolerant of a range of habitat types, others have very precise habitat preferences (Brown, 1990). This is particularly evident in South Africa, where they have been found to respond significantly to management practices such as burning (Chambers & Samways, 1998), grazing (Gebeyehu & Samways, 2003; Rivers-Moore & Samways, 1996), and mowing (Chambers & Samways, 1998). However, the species-level taxonomy, ecological requirements, distribution patterns and vulnerability status of this group are still poorly known, and great expertise is needed for their identification (Green, 1998). For these reasons, conservationists consider them only rarely for conservation management and monitoring schemes (Kati et al., 2004a).

Furthermore, Orthoptera are thought to be an excellent model group to study the effect of climate change (Nufio et al., 2010). The thermal environment is extremely important to Orthoptera because their life-history characteristics and biological processes such as clutch size, development rate, adult size, reproduction, digestive ability, ability to avoid predators, all are linked and hence, can be affected by temperature (Dearn, 1977; Pitt, 1999). For example, it has been found that both the length and the predictability of the growing season affect the clutch size of the three grasshoppers investigated along an altitudinal gradient (Dearn, 1977) or that the number of growing degree days (GDDs) is associated with the time to adulthood (Nufio et al., 2010). In fact, the authors of this latter study (Nufio et al., 2010) proposed that the phenological advancement for the grasshopper community tested, depended on when a set number of GDDs was reached during a season.

The need of a two-taxa approach

Both butterflies and Orthoptera' life history traits, including their direct association with climatic conditions, render them adequate indicators of environmental change. They have different ecological requirements in some aspects and they hence reflect environmental change differently. For example, grasshoppers are leaf chewers throughout their life cycles, whereas butterflies are leaf chewers as larvae but use nectar resources as adults as the resources become available during the season (Kruess & Tscharntke, 2002; Shreeve, 1992; Stefanescu & Traveset, 2009). In addition, Orthoptera do not specialize on specific host-plants although they might change their location in order to find abundant high-quality food sources or climatically suitable microhabitats (Harrison & Fewell, 1995). Both groups have different spatial requirements, as butterflies are much more mobile as adults but their

caterpillars often specialize on one or few food plant species (Wettstein & Schmid, 1999). Considering that the negative effects of environmental changes have been demonstrated on insects at different rates, showing no consistent trend between areas, time periods, species or groups (Primack et al., 2009), it is important to understand how and why this variability occurs (O'Connor et al., 2012). In addition, different taxa vary on their responses to environmental factors as a function of their life-history traits (Kotliar & Wiens, 1990) and limit their distribution according to their climatic tolerances (Woodward, 1990). As taxonomic variation increases the complexity of the study system, an option to simplify the species model would be to use taxa that can also reflect the distribution patterns of other taxa (Gregory et al., 2005; Noss, 1990). To our knowledge, a few quantitative studies have tried to combine taxa or taxonomic groups with different ecological characteristics, in order to study the effects of climate change (Gibson et al., 1992). In this context, we chose to study butterflies and Orthoptera because of their congruent species richness patterns on one hand (Bazelet & Samways, 2012; Zografou et al., 2009) and their differentiation in terms of spatial and ecological requirements, so as to capture different aspects of climate change effects on insect communities.

Study area

The Mediterranean Basin is one of the richest and most complex regions on Earth: geologically, biologically, and culturally. The Romans named the sea *mediterraneus*, which means "in the middle of the earth" (Blondel et al., 2010). As the effects of global change are likely to be especially severe in Mediterranean (Blondel et al., 2010), an area that it has already warmed more than most of the rest of Northern hemisphere, it will become increasingly important to determine the severity of this change, defining first the proportion of temperature rise on a regional scale (see Chapter 2). Due to our limited knowledge in a particularly diverse and complex group of organisms like Mediterranean insects, we need to start filling the gaps in order to deal effectively with these climatic-dependent species changes, better understand species responses and the characteristics of the environmental systems in which they exist.

Data were collected in Greece, a biodiversity hotspot for butterflies and Orthoptera, including more than 48% (234 species) of all European butterfly species (482) (Van Swaay et al., 2010) and almost 30% (300 species) of all European Orthoptera species (> 1000)

(Willemse & Willemse, 2008). However, monitoring schemes are only recently under development, and therefore species distribution patterns are only available in terms of atlas studies (Pamperis & Stavridis, 2009; Willemse, 1985), whereas, species abundance data as well as basic knowledge on communities composition and structure are scarce (e.g. Grill & Cleary, 2003; Kati et al., 2004a; Zografou et al., 2009).

Data collection was accomplished in the period 2011-2013, considering three study areas, which all fall within the Natura 2000 network of protected area. Dadia-Leukimi-Soufli national park (Dadia NP hereafter, NE Greece, sampled in 2011-12), Rodopi mountainous ranges national park (N Greece, sampled in 2012), Grammos mountain (NW Greece, sampled in 2013) (**Fig. 1**).

Dadia NP (SPA, GR1110002) is situated in northeastern Greece (40°59'-41°15'N, 26°19'-26°36'E) (Fig. 1). It is a hilly area extending over 43000 ha with altitudes ranging from 20 to 650 m, including two strictly protected core areas (7290 ha), where only low-intensity activities such as periodic grazing and selective wood-cutting are allowed, under the control of the local Forest Service of Dadia NP. The core areas are surrounded by a buffer zone where certain human activities are also allowed such as domestic livestock grazing, small agriculture fields and controlled logging. The climate is sub-Mediterranean with an arid summer season (approximately July-September) and a mean annual rainfall ranging from 556 to 916 mm (Maris & Vasileiou, 2010). Mean annual temperature is 14.3°C with lowest values in January and the highest in July-August (Maris & Vasileiou, 2010). We selected Dadia NP as our study area, because its long conservation history has limited the scale of land use changes (Maris & Vasileiou, 2010), and so differences in species composition can reasonably be attributed to factors other than land use change. Previous studies in this area have already assessed its high biodiversity on raptors (Schindler et al., 2008), passerines (Kati & Sekercioglu, 2006), amphibians and reptiles (Kati et al., 2007), Orthoptera (Kati et al., 2004a), orchids (Kati et al., 2000), vascular plants (Korakis et al., 2006), beetles (Argyropoulou et al., 2005) and butterflies (Grill & Cleary, 2003) and this is the second reason for which we chose to study Dadia NP. Not only its high biodiversity but also the existence of historical data on butterflies (Grill & Cleary, 2003) and Orthoptera (Kati et al., 2004a) allowed us to proceed on a comparison with current data.

On the other hand, Rodopi (SCI: GR1140004, SPA: GR1140009) is a mountain-chain situated in NE Greece (1731km²: between 41°12' and 41°36'N and 24° and 25°06'E) and

Grammos (SCI and SPA: GR1320002) is a mountain situated in NW Greece (350 km²: long. 20°50', lat. 40°21') (**Fig. 1**). Both mountain ranges include areas protected by the European network NATURA 2000. Climate in Rodopi is transitional from the sub-Mediterranean type to central European with a strong continental character (mean annual temperature is 11.4°C, mean annual precipitation 1200 mm), while climate in Grammos is of the mountainous type (mean annual temperature 8-12°C and mean annual rainfall 800-2200 mm). We selected these mountain ranges because of their considerable conservation value (Mertzanis et al., 2005; Xirouchakis, 2005; Zografou et al., 2009) and because of their similarities in vegetation structure and in low human impact; they characterized by scattered human settlements, where logging, periodical livestock grazing and small-scale cultivations constitute the main activities. However, their climate and geographical position differs: Rodopi is located to the Greek-Bulgarian border (NE) and Grammos to the Greek-Albanian border (NW). In addition, Rodopi Mountain range has been designated as a National Park from 2009 and a management body consisting of special scientists and representatives of local entities has been activated since then.

Research outline

Given that the human-induced crisis is growing and continues to destroy the supporting life-system of this planet, either through climatic or land-use changes, it has become increasingly apparent that a fundamental knowledge of how the world works is vital to preserve a habitable planet. As a result, many scientists attempt to collect information from different geographical areas on how species or communities respond to human-induced climate change in order to better understand and forecast species' future distribution and life-history processes under the climate change scenarios (e.g. Ekroos et al., 2010; González-Estébanez et al., 2011; Isaac et al., 2011; Stefanescu et al., 2003; Tscharntke et al., 2012).

Our research attempted to investigate the effect of climate change on insect communities in time (Part A: Chapter 2 and 3) and space (Part B: Chapter 4 and 5) for the poorly studied area of Southern-Eastern Europe. We considered butteflies and Orthoptera as our model groups, because of their particular life history traits that render them adequate indicators of environmental change with regards to climatic conditions. We conducted our research in the Mediterranean basin, a biodiversity hotspot that is expected

to be particularly threatened by global warming. We particularly focused on Greece, a country of great biological wealth but with particularly poor biodiversity knowledge for insect communities, in an attempt to contribute to basic ecological knowledge gain for the target groups, besides our hypotheses testing.

Part A. Species and communities' responses to climate change: time dimension

Regarding the time dimension, we compared previous with current databases in Dadia NP, collected under the same methodology, in order to investigate the species responses and communities' composition turnover, as well as the phenological shifts of the model groups associated to regional climate change (Chapters 2 and 3). In the second chapter, we handled the community composition issue focusing solely on butterflies and we addressed the following research questions: (1) Has mean annual temperature trend significantly increased since the 1990s? and (2) Has butterfly community composition and species richness have changed across a thirteen-year period in response to climate warming in this area, given that is largely free of major changes to land use? In the third chapter we handled the phenological issue for both butterflies and Orthoptera and we addressed the following questions: (3) Did phenology change significantly for a period of 13 and 12 years for butterflies and orthopterans respectively (1998-2011: butterflies, 1999-2011: orthopterans)? (4) Is the rate at which the phenology changed consistent for butterflies and orthopterans? (5) Is there a significant effect of habitat type (grasslands, forests and agriculture) on phenological patterns? Then, considering data of only one sampling period (2011) we also investigated (6) whether there is a significant effect of habitat-specific variables (microclimatic: temperature, humidity) on species phenology per habitat type, and (7) whether the phenological patterns of both target groups are congruent vis-a-vis the environmental gradient of canopy cover.

The part of our research presented in the *second chapter* is of particular importance, since empirical evidence for the effect of global warming on insect communities is still scarce for the Mediterranean biome (Peñuelas et al., 2002) compared to temperate latitudes, inspite of the scientific endeavour to elucidate this issue worldwide. This is hence the first comparative study of community composition turnover in the light of climate change in Greece and the Balkan region (Zografou et al., 2014). Furthermore, our research has a

great practical conservation value, attempting to provide tangible conservation management suggestions, in particular under the light of the debatable efficiency of the current network of protected areas as species are expected to be driven out of protected areas by climate warming (Araújo et al., 2011).

To our knowledge, the part of our research presented in the *third chapter* is the first attempt to elucidate the phenological patterns of insect communities vis-a-vis climate change in the eastern Mediterranean basin. It is also the first time that butterflies and Orthoptera are examined on dual basis, in terms of congruent or not phenological patterns and ecological explanations are discussed. Given that an emerging line of research investigates currently the extent to which local habitat conditions can buffer ecological communities against coarse-scale trends and patterns in climate change (Gillingham et al., 2012; Suggitt et al., 2011), our research attempted to investigate the extent to which habitat-specific variables (microclimatic such as temperature or humidity and environmental such as canopy cover and flower heads) could influence species' phenological responses. Within this framework and using a novel statistical approach (Standardized Major Axis technique) we tried to vizualize and interpret species' responses to regional warming.

Part B. Species and communities' responses to climate change: spatial dimension

Regarding the space dimension, we collected data from the two mountain ranges (Rodopi and Grammos) in order to investigate the diversity patterns of butterflies and Orthoptera across different spatial scales. Once we defined the most important spatial scale for diversity patterns (elevation gradient) we investigated how phenology changes along this gradient focusing on the butterfly group (Chapters 4 and 5). In the *fourth chapter*, we studied the diversity patterns of both taxa and we addressed the following research questions: (1) How diversity patterns of butterfly and Orthoptera are distributed across different spatial scales (ecoregions, elevational zones, habitats, transects/plots)? (2) Is there a consistent pattern between the two insect groups? (3) Whether species assemblages within each group (common, rare species) are responsible for the observed patterns of diversity? In the *fifth chapter* we substituted space-for-time to handle the phenological shifts of butterflies and we addressed the following questions: (4) Do butterfly species

have a gradual delay in the timing and a progressive shortening on the duration of the flight period as we move to upper altitudes at community and species level? (5) Is there a significant differentiation on elevational patterns of butterfly communities between the two mountainous areas and across the different habitat types.

The part of our research presented in the *fourth chapter* is of particular importance in the light of climate change where habitats are changing or disappearing and mountains have gained special attention as potential "refugia" for species that shift their distribution to higher altitudes (Hardy et al., 2010; Parmesan, 2007; Root et al., 2003). To protect these important systems effectively we first need to determine the patterns of beta diversity of the local fauna, that is to define the species turnover along environmental gradients within the region of interest (Gering et al., 2003) and to interpret the observed patterns (Gering et al., 2003; Marini et al., 2012; Ribeiro et al., 2008; Wu et al., 2010). Assessing the mechanisms that specify species distribution at different spatial scales and identifying the contribution of each component to the overall diversity is crucial to understand better the driving forces influencing species distribution and thus to propose the appropriate conservation measures for these fragile yet of great importance ecosystems.

The part of our research presented in the *fifth chapter* is one of the few attempts where a proxy of space-for-time is used to study phenological responses of butterflies in the eastern Mediterranean basin. Although projections based on population time series is a commonly used method for the north and central Europe (Altermatt, 2012; Roy & Sparks, 2000; Van Strien et al., 2008), other areas like Mediterranean lack this "privilege" of high quality monitoring data and time series projections are limited (but see Stefanescu et al., 2003). An alternative to studying temporal variability in phenology, when long-term data are not available, is the space-for-time-substitution approach (Banet & Trexler, 2013). Considering the complex species' phenological patterns along altitudinal gradients (Brakefield, 1987; Verity, 1920) and the future increase of temperature, we documented butterfly phenological patterns along altitudinal gradients and we compared them among different habitat types and between the study systems. We attempted to predict, for the first time, how these patterns will change under the future climatic scenarios in the Mediterranean basin, thus setting the cornerstone for future phenological studies in the relatively unknown Greek insect fauna.



Figure 1. The location of Greece within Mediterranean Basin and the three study systems within Greece. Starting in 2011, we first conducted samplings in Dadia-Leukimi-Soufli national park; in 2012 we continued our field samplings in Rodopi mountain ranges national park and for a short-period only we revisit Dadia national park; finally in 2013 we finished our field work by sampling Grammos mountainous area. All areas were sampled in terms of butterfly and Orthoptera species during the spring and summer period.

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- Chapter 2 -

Signals of climate change in butterfly communities in a Mediterranean protected area

Abstract

The European protected-area network will cease to be efficient for biodiversity conservation, particularly in the Mediterranean region, if species are driven out of protected areas by climate warming. Yet, no empirical evidence of how climate change influences ecological communities in Mediterranean nature reserves really exists. Here, we examine long-term (1998-2011/2012) and short-term (2011-2012) changes in the butterfly fauna of Dadia National Park (Greece) by revisiting 21 and 18 transects in 2011 and 2012 respectively, that were initially surveyed in 1998. We evaluate the temperature trend for the study area for a 22-year-period (1990-2012) in which all three butterfly surveys are included. We also assess changes in community composition and species richness in butterfly communities using information on (a) species' elevational distributions in Greece and (b) Community Temperature Index (calculated from the average temperature of species' geographical ranges in Europe, weighted by species' abundance per transect and year). Despite the protected status of Dadia NP and the subsequent stability of land use regimes, we found a marked change in butterfly community composition over a 13 year period, concomitant with an increase of annual average temperature of 0.95°C. Our analysis gave no evidence of significant year-to-year (2011-2012) variability in butterfly community composition, suggesting that the community composition change we recorded is likely the consequence of long-term environmental change, such as climate warming. We observe an increased abundance of low-elevation species whereas species mainly occurring at higher elevations in the region declined. The Community Temperature Index was found to increase in all habitats except agricultural areas. If equivalent changes occur in other protected areas and taxonomic groups across Mediterranean Europe, new conservation options and approaches for increasing species' resilience may have to be devised.

Key-words: butterflies, climate change, nature reserves, community composition, elevational distribution, species richness

Introduction

Major changes in climate worldwide have been identified as the cause of recent shifts observed in species' geographical distributions [1,2,3,4,5]. Many such shifts follow a

poleward range expansion pattern [6,7,8]. Climate warming results in locations becoming generally more favourable for species near the "cool", high-latitude limits of their distributions, but it may be less favourable for species near their "warm", low-latitude limits [9], with consequent changes in relative species' abundance and community composition [10]. There is a documented pattern where widespread species (that are better able to expand their distributions through human-modified landscapes) or species associated with warm conditions are becoming more abundant due to warming, at the expense of habitat specialists or species restricted to higher latitudes or elevations [4,11,12]. Yet, different taxonomic groups and different regions have shown different levels of evidence of tracking changes to the climate [1,13].

Butterflies are known to be highly sensitive to climate change [6] and recent studies prove that they react faster than other groups such as birds [13]. A reason for this is because butterflies have relatively short generation times and are ectothermic organisms, meaning that their population dynamics may respond to temperature changes more directly and more rapidly [14]. Butterflies are among the most well-studied taxa in Europe, benefiting from a detailed dataset including relatively fine-resolution information on species' distributions and abundance [14], but they are still far less studied than vertebrates, although the latter comprise only a small fraction of global biodiversity. While further increases in the earth's temperature are anticipated [15] and are expected to lead to serious changes in diversity patterns worldwide, empirical evidence for such changes is still scarce for the Mediterranean biome [16] compared to temperate latitudes. Some evidence that the species composition of Mediterranean butterfly communities has not responded to climate warming as rapidly as expected based on the biogeographic associations of species [17] suggests that these communities may be comparatively resilient to climate change, but more research is needed to test this hypothesis. In addition, an urgent applied question related to climate-driven changes to ecological communities is whether European protected area networks may cease to be effective for conservation, if species are driven out of protected areas by climate warming [18]. So far, there is no empirical evidence on how climate change during the last decade has influenced species communities in Mediterranean nature reserves: precisely this kind of information is likely to be increasingly important for conservation planning in a global climate change scenario.

In this study, we assess if and how butterfly species richness and community composition have changed in response to climate change in the Greek nature reserve, Dadia-Leukimi-Soufli National Park. Greece is considered to be a biodiversity hotspot for butterflies, including more than 40% (234 species) of all European butterfly species (535) [19]. We selected Dadia-Leukimi-Soufli National Park (Dadia NP hereafter) as our study area, because its long conservation history has limited the scale of land use changes [20], and so differences in species composition can reasonably be attributed to factors other than land use change. In the case of Dadia NP, it has been acknowledged that in the absence of traditional activities (such as logging, livestock grazing), especially in the strictly protected core areas, forest encroachment at the expense of clearings and grasslands would have a negative impact on biodiversity, and particularly on species associated with open habitats [21,22]. Thus, the Specific Forest Management Plan of Dadia NP [23] considers the importance of landscape heterogeneity and open habitats, allowing controlled wood-cutting and grazing within the core areas. As a result, two of the most influential factors in the composition of butterfly communities, the intensity of livestock grazing and logging [24,25,26] have remained quite stable over the last decade (D. Vassilakis, Soufli Forest Department, pers comm). Moreover, preliminary data of an ongoing study on land cover changes in Dadia NP shows that forest cover remained quite consistent (72-74%) from 2001 to 2011 (K. Poirazidis, WWF Greece and P. Xofis, Inforest, unpublished data), implying that forest encroachment has been minimal during the period of study.

Sampling of butterfly communities was conducted in 2011 and 2012 and results were compared to an earlier study we carried out in 1998 [24]. The present paper is the first comparative study of community composition turnover in the light of climate change in Greece and the Balkan region. We investigate (a) if mean annual temperatures in the study area have increased since the 1990s and (b) if butterfly community composition and species richness have changed across a thirteen year period as a response to climate warming in a protected area, which is largely free of major changes to land use. Finally, we discuss how to implement our findings in a tangible conservation context for nature reserve management.

Materials and Methods

Ethics Statement

Specific permission for the field study described in Dadia-Leukimi-Soufli National Park was given by the Ministry of Environment Energy and Climate Change (Greece). Dadia forest has been owned and managed by the local government from 1980 when it was officially declared a Nature Reserve. The field observations included protected butterfly species but all individuals were released immediately after identification.

Study area

The study area of Dadia NP is situated in northeastern Greece (40°59'-41°15'N, 26°19'– 26°36'E) (Fig. 1). It is a hilly area extending over 43000 ha with altitudes ranging from 20 to 650 m, including two strictly protected core areas (7290 ha), where only low-intensity activities such as periodic grazing and selective wood-cutting are allowed, under the control of the local Forest Service of Dadia NP. The core areas are surrounded by a buffer zone where certain human activities are also allowed such as domestic livestock grazing, small agriculture fields and controlled logging. The climate is sub-Mediterranean with an arid summer season (approximately July-September) and a mean annual rainfall ranging from 556 to 916 mm [27]. Mean annual temperature is 14.3°C with lowest values in January and the highest in July-August [27]. The forest is characterized by extensive pine and oak stands [28] and a heterogeneous landscape [29] supporting a high diversity of raptors [30], passerines [31], amphibians and reptiles [32], grasshoppers [33] orchids [34], vascular plants [28], beetles [35] and butterflies [24]. Dadia was established as a nature reserve in 1980 mainly due to its great variety of birds of prey and since then, it has become acknowledged as a region of interest for other groups of organisms as well.



Figure 1. Map of the study area, Dadia National Park in NE-Greece. The map illustrates the geographic location of Dadia National Park where butterflies were sampled in seven habitat types (3 transects per habitat type) in 1998, 2011 and 2012.

Temperature data

Meteorological data (mean annual temperatures) were obtained from two stations, one located within the study area (Dadia NP station, functioning from 1994-2004) and a second one located 56 km away from the study area (the meteorological station in Alexandroupoli has been operating from 1964 until now [36]).

Butterfly sampling

To test for changes in community composition, the butterfly dataset recorded in 2011 followed exactly the same methodology as that used in 1998 [24], i.e. transects of 200m standard length at 3 locations per habitat type (7 habitats on the whole) were carried out, with transects in the same habitat type a minimum of 300m distance and maximum of 1 km from one another (Fig. 1). Each transect was repeated 15 times, approximately every 10 days between May 14 and September 14. Habitat selection was representative of the predominant land use types in Dadia NP [37], containing 7 habitats which were: pine forest, oak forest, mixed forest (of mainly *Quercus spp.* and *Pinus brutia* stands), wet meadow, dry grassland, grazed pasture and agricultural fields. We conducted additional samplings in the broader Dadia NP area in 2011, to complete the NP species inventory, without considering them in the data analysis. Comparisons for the long-term period were conducted between the 21 transect sites for the years 1998-2011.

In addition, a third sampling was conducted in 2012, in order to clarify whether any longterm (1998-2011/12) community composition change can be attributed to long-term environmental changes such as climate change, or to short-term variation in community composition between successive years. To do so, a subset of six habitats out of seven (18 transects) was visited once (June 2012) at the same time and date as in 2011. Comparisons for the long and short-term period were conducted among these 18 transects for the years 1998-2011/2012 and 2011-2012 respectively.

Data analysis

Analysis of temperature

To estimate the temperature trend in Dadia NP during the last decades, a 22 year period (1990-2012) was considered. Because meteorological data for Dadia NP are only available for 1994-2004, a linear model (period 1994-2004) was run using Dadia NP station data as

the response variable and Alexandroupoli station data as the independent variable. The obtained model was then used to estimate the temperature in the study area for all three butterfly surveys. Finally, a linear trend model with randomization (1000 times) was used to test for significant temperature change in the 22 year period. All these analyses were performed with Minitab® Statistical Software (ver.16.1.1).

Community composition change

To check the completeness of the sampling with respect to species detectable by each observer during 1998 and 2011, we assessed sampling efficiency in terms of proportion of species diversity sampled versus the species diversity estimated by non-parametric estimators (Chao 1) [38,39]. Based on this procedure, sampling efficiency was greater than 95% for both years (1998, 2011).

First, an Analysis of Similarities (ANOSIM test) was carried out to explore whether there was a significant change in community composition on a long-term (1998-2011) and short-term (2011-2012) period [40]. The ANOSIM test is based on the ranks of Bray-Curtis dissimilarity index and ranges from -1 to +1, where values greater than zero mean that community composition differs significantly between the years. We created two datasets, one for the long and one for short-term periods, and we treated each one separately. We assessed the significance of the null hypothesis, namely equal similarity among replicates between groups (sampling periods) and within groups (21 transects) after conducting 999 permutations.

Secondly, the non-parametric method for multivariate analysis of variance based on permutation tests [41] was used, in order to determine the main influences on community composition changes. The permutation analysis of variance (PERMANOVA) for the 13 year period (1998-2011) was run for the 21 transects using species' abundances (counts during the 15 visits in each year) as the response variable, the year factor as a fixed effect and the repeated transects as the random effect in the model.

In order to create equivalent comparisons between the long and short-term periods, additional PERMANOVA were conducted for the 18 transects using the single June visit for (a) 1998 - 2011/2012 and (b) 2011 - 2012 respectively.

To pinpoint those species that contributed most to community composition changes, a separate univariate Poisson regression model was fitted for each species and the likelihood

ratio statistic was used as a measure of change strength [42]. These analyses were carried out in R (R Development Core Team, 2009) using the *vegan* library [43] and *mvabund* package [44].

Measures of species' thermal associations

The first measure used for the regional thermal associations of butterflies was defined by three categories, in terms of their elevational distribution on Greek national territory, following the example of Wilson et al. [4] in Spain. We used the Greek Butterfly Atlas [45] and the 1260 actual localities $(6' \times 6')$ recorded by the author or referred to in the bibliography, covering 61.19% of Greece. We classified species that occurred in more than 50% of these 1260 localities as "widespread". Species that occurred in fewer than 50% of the localities were classified according to their elevational associations. Those for which > 50% of the records came from localities with an elevation of more than 1000m, were classified as "high-altitude". Those for which > 50% of the records came from localities with elevations below 1000m were considered as "low-altitude" (Table S1). The elevation threshold of 1000m was used for consistency with the four-grade scale provided in the Greek butterfly Atlas (0-500, 500-1000, 1000-1500 and >1500) [45]. Low and highaltitude species have been adequately sampled in the Greek butterfly Atlas in terms of sampling effort (number of localities) for the Greek territory below and above 1000m. For each elevational zone, we took the ratio between the number of localities and the area covered by the Greek territory (km²). The ratio ranged from 0.02 to 0.1, and a strong correlation emerged between the number of localities and the area at each elevational zone (Spearman rho= 1, n=4, P < 0.001) (Table S4).

The second measure for thermal associations of butterflies was the Species Temperature Index (STI), based on species' biogeographical associations in Europe. The STI is a species-specific value calculated as the average annual temperature across the 50×50 km grid squares where the species has been recorded in Europe [13,14,46,47,48]. At transect level, the average Species Temperature Index of all species was weighted by species' total abundance, in order to estimate a Community Temperature Index for each year. Then the respective transect community temperature indices for the years 1998 and 2011 were compared using a Wilcoxon rank sum test, to conclude whether there has been a significant change in butterfly community thermal structure.

European STI and our elevation-based measure of Greek butterfly thermal associations appeared to give a consistent measure of relative thermal associations of the species observed (Mann-Whitney U test for STI for high versus low-altitude species, n = 88 species, W = 1796, P = 0.02).

Species diversity change

Considering the two butterfly surveys of 1998 and 2011 separately, alpha-diversity (Shannon–Wiener Index H') was calculated for each of the 21 transects for all butterfly species and for high and low-altitude species separately. Beta-diversity was also used to quantify species turnover within each habitat type (3 transects each), using Whittaker's formula, $b = (S/\bar{a})-1$, where S is the total species number within each transect in each habitat type and \bar{a} is the average species number in that habitat type [39]. To test whether the values of alpha and beta-diversity differed between the sampling years we ran general linear models.

To pinpoint whether any significant differences between the two years for the high-altitude and low-altitude species were due to changes in species richness or abundance, Monte-Carlo permutation tests were used. Assuming for the null hypothesis that both years were equivalent and that high-altitude and low-altitude species had the same probability of occurrence in a given sample, the following test statistics for species richness (T_{sp}) and abundance (T_{ab}) were used:

$$T_{sp} = \frac{L_2}{L_2 + H_2} - \frac{L_1}{L_1 + H_1}, \quad T_{ab} = \frac{l_2}{l_2 + h_2} - \frac{l_1}{l_1 + h_1}$$

where *L* is the number of low-altitude species and *H* the number of high-altitude species for the years (1) 1998 and (2) 2011, and *l* is the abundance of the low-altitude species and *h* the abundance of the high-altitude species for the years (1) 1998 and (2) 2011. Thus, if the relative proportion of low-altitude species increases, we expect T_{sp} or T_{ab} to be positive. These steps were repeated 1000 times with no replacement. If the observed value (T_{sp} or T_{ab}) falls within the range of the randomly generated values (two-tailed test for *P*< 0.025) we cannot reject the null hypothesis, namely that both high and low-altitude species have the same probability to occur in the sampling years (in terms of species richness or abundance). We carried out these analyses in Minitab and R using libraries *vegan* and *nlme* [49].

Results

Butterfly diversity of Dadia NP

A total of 78 species (3248 individuals) were recorded in 2011, 35 species (427 individuals) in 2012 and 75 (2855) in 1998. The number of species and the number of species of European conservation concern (SPEC) [50] per habitat type for each sampling period (1998-2011-2012) are given in the supporting information (**Fig. S1**).

Community composition change

A significant difference in community composition over the long-term period (1998-2011) and a non-significant difference over the short-term period (2011-2012) was found, according to ANOSIM results (R = 0.32, n = 42, P = 0.006 and R = 0.02, n = 42, P = 0.4respectively). The PERMANOVA analysis for the 13 year period indicated a significant effect of the year x transect interaction on community composition ($F_{1,168} = 1.2$, P = 0.01, Table S2). A *posteriori* test among levels of the factor 'year', within levels of the factor 'transect', showed significant differences in time only for five transect sites (Table S3). Contrasting results of the single repetition in June between the long and short-term period were found with an additional PERMANOVA. A significant year x transect interaction emerged for the long-term period (1998-2011: $F_{1,24} = 4.63$, P = 0.001; 1998-2012: $F_{1,24} =$ 3.42, P = 0.001), indicating that differences among transects affected the response of community composition to different years over the longer period, while a non-significant year x transect interaction emerged for the short-term period (2011-2012: $F_{1,24} = 0.56$, P =0.9). This result suggests that the lack of difference between 2011-12, in contrast to the difference between 1998 versus both 2011 and 2012, is not simply due to a lack of power in using the single June transect counts for comparisons involving 2012. A posteriori test among levels of the factor year, within levels of the factor transect, showed no significant differences.

Nineteen species which contributed most importantly to the difference between the years 1998 and 2011 (**Table 1**) were pinpointed, out of which 10 species had decreased in abundance. The species with the strongest changes in abundance were the widespread

species *Aporia crataegi* (decrease), and *Argynnis paphia* (decrease). *Arethusana arethusa* has become totally extinct in all study sites since 1998, *Melitaea trivia* considered to be a low-altitude species showed a strong decline (over 90% of its abundance compared to 1998), while species like *Hipparchia fagi, Kirinia roxelana* and *Aricia agestis* almost doubled their abundance.

| Species names | LR | SC | PC |
|-----------------------|---------|----|------|
| Arethusana arethusa | 215.01 | HA | -100 |
| Melitaea trivia | 405.52 | LA | -95 |
| Argynnis paphia | 1125.59 | HA | -85 |
| Aporia crataegi | 2662.47 | HA | -85 |
| Pieris mannii | 293.70 | HA | -84 |
| Vanessa cardui | 258.51 | W | -83 |
| Brenthis daphne | 461.67 | HA | -74 |
| Brintesia circe | 91.47 | HA | -56 |
| Issoria lathonia | 243.78 | HA | -29 |
| Coenonympha pamphilus | 126.65 | HA | -28 |
| Maniola jurtina | 1395.40 | LA | +5 |
| Colias crocea | 361.90 | W | +8 |
| Melitaea didyma | 238.92 | HA | +11 |
| Polyommatus icarus | 615.09 | W | +25 |
| Satyrium ilicis | 455.98 | LA | +34 |
| Thymelicus sylvestris | 200.98 | HA | +79 |
| Hipparchia fagi | 303.96 | HA | +109 |
| Kirinia roxelana | 151.81 | LA | +187 |
| Aricia agestis | 126.65 | LA | +511 |

Table 1. Results from univariate Poisson regression models fitted to each taxon.

LR: Likelihood ratio test statistic used as a measure of species strength of between-years effect, *SC*: species categories (HA: high-altitude, LA: low-altitude, W: widespread) created using species elevational distributions in Greece, *PC*: proportional change (%) of species abundance among 1998 and 2011 (formula used N_{2011}/N_{1998}). Only statistically significant species (*P*<0.05) are shown, while species are ranked from those with the greatest declines to those with the greatest increases in abundance between 1998 and 2011 (%).

Temperature trend

A significant increase of mean annual temperature in Dadia NP was found between 1990 and 2012, of 0.95° C (**Fig. 2**). The null hypothesis (no significant change in temperature) was rejected after conducting 1000 randomizations (*P* = 0.003).



Figure 2. Temperature trend analysis plot for temperature in Dadia National park. The solid line illustrates the mean annual temperatures from 1990 to 2012 in Dadia National Park, and the dotted line the fitted trend line after 1000 repetitions (randomization). The mean annual temperatures show a general upward trend.

Changes in species diversity and thermal associations

Using the first measure of species' regional thermal associations, 40 high-altitude species were observed in both 1998 and 2011 (1557 individuals in 1998, versus 1161 in 2011), whereas 25 low-altitude species (913 ind.) were observed in 1998, versus 31 (1657 ind.) observed in 2011. Only 7 (1998) and 5 (2011) species were classified as "widespread" (**Table S1**). A significant increase in alpha-diversity for the low-altitude species and respectively a significant decrease for high-altitude species was found. The alpha-diversity increase was not significant, when considering all species regardless of whether they were high or low-altitude (**Table 2**). None of the beta-diversity changes between 1998 and 2011 were significant (**Table 2**), with slight increases for the overall butterfly community and

the low-altitude species, versus a slight decrease for the high-altitude species. According to the Monte-Carlo permutations, the changes in species diversity were due to species abundance differences ($T_{ab} = 0.2$, P < 0.025) and not to species richness ($T_{sp} = 0.05$, P = 0.086).

Table 2. Alpha-diversity (mean Shannon index at transect level) and beta-diversity (Whittaker index at habitat level) for (a) all butterfly species, (b) high-altitude species and (c) low-altitude species and respective general linear models testing their significant change between the years 1998 and 2011.

| | | Year | (a) All species | (b) HA species | (c) LA species |
|-----|---------------|-----------------|-----------------|----------------|----------------|
| Ś | a. dimansitar | 1998 | 2.5 | 1.94 | 1.73 |
| ect | a-diversity | 2011 | 2.7 | 1.68 | 1.95 |
| suu | | F | 1.26 | 5.61 | 4.67 |
| Tra | GLM | <i>p</i> -value | 0.26 | 0.02 | 0.03 |
| | 0 1' ' | 1998 | 0.45 | 0.62 | 0.45 |
| ats | p-alversity | 2011 | 0.51 | 0.57 | 0.48 |
| bit | | F | 0.37 | 0.14 | 0.07 |
| Ha | GLM | | | | |
| , , | | <i>p</i> -value | 0.55 | 0.71 | 0.78 |

Using the second measure of the species' European thermal associations, the community temperature index was found to change significantly between the years 1998 and 2011 (Wilcoxon rank sum test W = 344, n = 42, P = 0.0036). In fact, a significant increase of community temperature indices was found in all habitats except for the agricultural areas where the community temperature index had decreased (**Fig. 3**). To ensure that the CTI change did not result from phenological change, we repeated the process of index calculation for all visits during the summer except for the first in 1998 and the last in 2011. CTI again showed a significant increase between time periods, implying that changes in butterfly community composition were independent of any advancement in mean flight dates by the constituent species.



Figure 3. Community Temperature Index (CTI) among the sampled habitats in 1998 and 2011.

A Community Temperature Index (CTI, y-axis) was calculated for each one of the seven habitats (x-axis) as the average Species Temperature Index (calculated after the average temperature of each species' geographical range in Europe, see [13,14]) weighted by species' total abundance, sampled in 1998 (filled circle) and 2011 (empty circle) in each of the habitats. Figure shows significant increase of CTI in all habitats except for the agricultural areas.

Discussion

Signals of climate change

Butterfly community composition changed significantly over the 13-year period in conjunction with a recent temperature increase. We found significant changes in the abundance of regionally high versus low-altitude species, as well as a significant increase

of the Community Temperature Index based on the thermal associations of species' distributions in Europe. In the later recording period, species associated with warm conditions (i.e. low-altitude species) came to dominate over species associated with cool conditions (i.e. high-altitude species). This suggests that butterfly communities in the study area may have responded to climate warming, even in as short a period as 13 years. Of course, it is well known that there are changes over all timescales in temperature timeseries due to local or regional changes that need not be attributed to a prevailing global-warming trend [51]. It is also well established that the expansion of forest owing to land abandonment in the Mediterranean region during the last century may threaten open habitat species [21,22,52]. However, the protected status of Dadia NP and the subsequent stability of land use regimes over the last decade (see Introduction) suggest that our results are nonetheless consistent with the global warming interpretation.

We found marked changes in butterfly community composition over a 13 year time period, but on the other hand our analysis gave no evidence of significant short-term year-to-year variability in butterfly community composition. Butterfly community composition was most influenced by the factors year and transect, when comparing datasets over the longterm period (1998-2011 and 1998-2012). Different habitat types naturally host different butterfly communities [53,54], explaining the transect factor effect. On the other hand, the long conservation history of Dadia NP, where habitat quality and land use have been kept quite consistent, support our hypothesis that changes in community composition between the sampling periods might be attributable to climate change rather than land use change and therefore explaining the factor of year. A *posteriori* test showed that when a specific habitat type is considered, butterfly communities seem to remain the same between years, suggesting minor changes within the same habitat type (Table S3). Small changes within the same habitat type could be due to more than just a direct impact of climate on the butterflies. Climate can influence the relative abundance of species through direct effects on physiology, growth or survival (e.g. [50,55]), or through indirect effects on the insects by influencing the availability of larval foodplants (e.g. [56,57]). Further investigation into how climate may influence butterfly population dynamics and community structure in Mediterranean terrestrial habitats is needed.

Low-altitude species showed a significant increasing trend in terms of alpha-diversity (see Table 2). This suggests a community response to climate warming, where a shift towards a

dominance of lower-elevation species is expected [4,8,10]. Only one species, *M. trivia*, a Near Threatened species at the European Union (EU27) level [58], was a distinct exception. It is a low-altitude species but suffered a dramatic population decline of over 95% (based on its abundance in 1998). Similarly, other *Melitaea* species such as *M.cinxia* have experienced a significant population decline in the Mediterranean (NE Spain) from 1994 to 2008 [17].

High-altitude species showed a significant decreasing trend in terms of alpha-diversity. This represents further evidence of a change in the distribution and abundance of such species towards cooler locations at higher latitudes or elevations [1,6,7]. Two high-altitude species that contributed much to the between-year-difference declined over 80% over the 13-year period, A. crataegi and Pieris mannii. Recent changes in the distribution of A. crataegi in Europe appear to reflect effects of both climate and land use change [59]: in central Spain the species has declined at low elevations, leading to an upward altitudinal shift [55]; in Scandinavia it has expanded its range, whereas in central Europe it has suffered serious declines [59]. P. mannii is known to have expanded its northern range limit in Switzerland and Germany in association with climate warming [60]. Regional warming cannot, however, explain the significant decline of Vanessa cardui, a migrant and 'widespread' species, whose population size is largely regulated by climatic conditions in its overwintering habitat in Africa [61]. Finally, two more high-altitude species in our study area, Melanargia galathea and Coenonympha leander, were only recorded 6.7 km away to the north-west (800m altitude) from their site of observation in 1998 (mixed forest, 350m altitude), suggesting maybe the first signals of some species' movement to higher altitudes.

Our results showed a significant increase in the butterfly Community Temperature Index of sample sites (see Fig. 3). In contrast with the non significant trends observed in NE Spain [17], our findings suggest that butterfly communities may indeed have responded to regional warming in the Eastern Mediterranean basin, even during a relatively short period of 13 years. Our findings are consistent with similar patterns of increasing Community Temperature Index observed in northern Europe [13,14,62]. Agricultural habitats were the exception to the above general pattern. Here, the butterfly community changed from hotter (1998) to cooler (2011) thermal associations. We attribute this pattern to both the presence of natural hedges and tree lines providing shade at field edges, as well as to irrigation

systems, which have recently been found to buffer butterfly communities against the effects of drought in the Mediterranean [63]. Water availability is a key factor determining the distribution of butterflies and many other taxa in dry, low latitude, ecosystems [64,65] prolonging the "green season" of the field margins and therefore the food resources until late summer. Despite their anthropogenic origin, our evidence suggests that cool or moist microhabitats provided by mosaic agricultural landscapes may play a role in supporting butterfly populations under the increased thermal stress of the summer over a period of climate warming. These anthropogenic features may have enabled populations of butterfly species associated with relatively cool or moist conditions to "bounce back" from the effects of preceding hot years during the relatively cool conditions of the field survey in summer 2011 (see Fig. 2).

Conservation implications

New approaches for species conservation in existing protected areas may be needed as the climate warms [18]. Our study showed that artificially cooled or moist habitats such as in traditional agriculture can support species associated with cooler conditions (low temperature index), through possible effects of irrigation during the dry and hot summers of the South-east Mediterranean (see Fig. 3). Perhaps, preserving traditional small agricultural plots with hedges and tree lines and maintaining the current irrigation system could be a useful approach for increasing resilience to climate change [66]. In addition, in order to accommodate the possible distributional movement of species towards higher altitudes (we observed this for two species, *M. galathea, C. leander*, that formerly occurred in the study transects), we propose the future expansion of the existing reserve's borders to the west, towards the South-Eastern hills of the Rhodopi mountains.

Our results demonstrate that a 13 year period of assessment may be adequate to detect responses of butterfly communities in terms of species abundance and thermal structure. Although it is possible that a longer time period may be needed to detect changes in species richness or communities in cold ecosystems of higher latitudes [67], the documented signals even in this relatively short period underline the necessity for systematic research into hotter, low latitude, Mediterranean ecosystems.

The buffer zone of Dadia NP is of greater conservation importance for butterflies than the core areas constituted mainly by pinewoods and designed for the needs of raptors and the

black vulture in particular. More than 55% of the regional butterfly species of European conservation concern were recorded in the park's buffer zone. Likewise, the most speciesrich sites with the highest conservation importance for Orthoptera [33], orchids [34], passerines, amphibians and reptiles [32] as well as butterflies in 1998 [24] are situated in the buffer zone. Importantly, this research provides further evidence that 'buffer zones' are not only transition zones to unprotected areas, but essential parts of a reserve, contributing to its value for nature conservation. Considering that only a small proportion of total land area can ever be realistically protected in the form of nature reserves, conservation efforts must also comprise the surrounding area of nature reserves considering all components of biodiversity [29]. This becomes particularly important in a changing climate scenario, when species – as we have shown here for butterflies – may leave existing nature reserves or alter their habitat associations in search of more climatically-suitable habitats [18,68].

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Supplementary Information

Table S1. Table S1. Presence absence data of all butterfly species for the 7 habitat types (21 transects), SPEC_EU: SPecies of European conservation Concern (SPEC) in the whole of Europe; SPEC_27: Species of European conservation Concern (SPEC) in the 27 countries of the European Union; HA: High-altitude species; LA: Low-altitude species; Wd: Widespread species, A: Agriculture; D: Dry grassland; G: Grazed pasture; M: Mixed forest; O: Oak forest; P: Pinus forest; W: Wet meadow; Open circle: species present in 1998; Filled circle: species present in 2011; Open square: species present in 2012; Species with asterisk (*) found outside the predefined transects during 2011; the symbol (⁺) in Species number in 2012 means that the total number corresponds to the single June visit (in contrast to 1998 and 2011 where the total Species numbers corresponds to 15 visits).

| Taxonomy | SPEC_EU | SPEC_27 | HA | LA | Wd | Α | D | G | Μ | 0 | Р | W |
|------------------------|---------|---------|----|----|----|-----------------|--------------------------|-----------------|--------------------------|--------------------------|-------------------------|-----|
| HESPERIIDAE | | | | | | | | | | | | |
| Carcharodus alceae | | | | 1 | | • | | | 0 | 0 | | |
| Carcharodus lavatherae | 2 | 2 | 1 | | | | • | | 0 | | | |
| Carcharodus orientalis | 1 | | 1 | | | | | | 0 | | | |
| Erynnis tages * | | 4 | | | | | | | | | | |
| Ochlodes sylvanus | | | | | | • | • | | | | | |
| Pyrgus armoricanus | | 3 | 1 | | | | 0 | | $\circ \bullet$ | 0 | $\circ \bullet$ | 0 |
| Pyrgus malvae | | 4 | 1 | | | • | | | • | | | |
| Pyrgus serratulae | | 2 | 1 | | | | | • | | | | |
| Pyrgus sidae | | | 1 | | | | | | 0 | 0 | | • |
| Spialia orbifer | | | 1 | | | • | | | $\circ \bullet$ | 0 | | |
| Tarucus balkanicus | | | | 1 | | | | | | | | • |
| Thymelicus acteon | 2 | 2 | 1 | | | • | 0●□ | | • | | $\circ \bullet \square$ | |
| Thymelicus lineola | | | 1 | | | • | | | • | | | |
| Thymelicus sylvestris | | | 1 | | | 0● | 0●□ | | 0. | 0●□ | 0 | 0•□ |
| LYCAENIDAE | | | | | | | | | | | | |
| Aricia agestis | | | | 1 | | $\circ \bullet$ | $\bigcirc ullet \square$ | | $\bigcirc ullet \square$ | $\circ \bullet$ | | 0•□ |
| Aricia anteros | 2 | | 1 | | | | | | 0 | | | |
| Callophrys rubi | | | | 1 | | | • | | $\bigcirc ullet$ | • | • | |
| Celastrina argiolus | | | | 1 | | $\circ ullet$ | 0 | $\circ \bullet$ | $\circ ullet$ | $\bigcirc ullet$ | | |
| Cyaniris semiargus | | 4 | 1 | | | • | | | | • | | |
| Favonius quercus | | | | 1 | | • | $\bigcirc igodot$ | • | $\bigcirc ullet \square$ | • | 0 | • |
| Glaucopsyche alexis | 3 | | | 1 | | • | | • | • | | | |
| Iolana iolas | 2 | 2 | | 1 | | | | | • | | | |
| Leptotes pirithous | | | | 1 | | • | | • | | | | |
| Lycaena alciphron | | 2 | 1 | | | | | | 0 | • | | ٠ |
| Lycaena ottomana | | | | 1 | | • | | | 0 | $\bigcirc ullet$ | | • |
| Lycaena phlaeas | | | | 1 | | $\circ ullet$ | • | $\circ \bullet$ | $\bigcirc ullet \square$ | $\bigcirc ullet \square$ | 0 | ●□ |
| Lycaena thersamon | 3 | | | 1 | | $\circ ullet$ | | • | | | 0 | • |
| Lycaena tityrus | | | 1 | | | • | • | • | $\circ ullet$ | $\bigcirc ullet$ | 0 | ●□ |
| Maculinea arion * | 1 | 1 | | | | | | | | | | |
| Plebejus argus | | | 1 | | | 0• | | | • | | | |

| Plebejus pylaon | 2 | | 1 | | | 0 | | | | | | |
|------------------------|---|---|---|---|---|-----------------|------------------|-----------------|-----------------|------------------|-----------------|-----------------|
| Polyommatus icarus | | | | | 1 | $\circ \bullet$ | $\circ \bullet$ | 0•□ | $\circ \bullet$ | $\circ \bullet$ | $\circ \bullet$ | $\circ \bullet$ |
| Polyommatus thersites | | | 1 | | | • | • | | 0•□ | $\circ \bullet$ | | • |
| Pseudophilotes vicrama | 2 | 2 | 1 | | | | $\bigcirc ullet$ | $\circ \bullet$ | | • | 0 | |
| Satyrium acaciae | | | | 1 | | $\circ \bullet$ | 0 | 0 | | 0 | 0 | 0 |
| Satyrium ilicis | 3 | 3 | | 1 | | $\circ \bullet$ | 0•□ | 0•□ | 0•□ | 0•□ | 0 | • |
| NYMPHALIDAE | | | | | | | | | | | | |
| Aglais urticae | | | 1 | | | | | 0 | | | | |
| Arethusana arethusa | | | 1 | | | | | | | | 0 | 0 |
| Argynnis niobe * | 3 | 2 | | | | | | | | | | |
| Argynnis pandora | | | 1 | | | $\circ \bullet$ | | 0•□ | $\circ \bullet$ | 0 | • | 0 |
| Argynnis paphia | | | 1 | | | $\circ \bullet$ | 0 | 0•□ | 0•□ | 0•□ | 0● | |
| Brenthis daphne | | | 1 | | | $\circ \bullet$ | | 0 | $\circ \bullet$ | 0•□ | | 0● |
| Brenthis hecate | | | 1 | | | | | | | | | 0 |
| Brintesia circe | | | 1 | | | • | | • | | 0 | 0● | 0•□ |
| Coenonympha arcania | | | 1 | | | | | | $\circ \bullet$ | | | |
| Coenonympha leander * | | | 1 | | | | | | 0 | | | |
| Coenonympha pamphilus | | | | 1 | | | | | | 0 | | 00□ |
| Euphydryas aurinia | 4 | 4 | 1 | | | • | | • | | | | |
| Hipparchia fagi | 2 | 2 | 1 | | | 0 | $\bigcirc ullet$ | | $\circ \bullet$ | $\bigcirc ullet$ | $\circ \bullet$ | 0● |
| Hipparchia fatua | | | | 1 | | | $\bigcirc ullet$ | • | $\circ \bullet$ | • | 0● | 0● |
| Hipparchia senthes | | | | 1 | | • | ●□ | | | • | • | • |
| Hipparchia statilinus | 2 | 2 | 1 | | | | • | $\circ \bullet$ | $\circ \bullet$ | $\circ \bullet$ | • | 0● |
| Hipparchia svriaca | | | 1 | | | | $\bigcirc ullet$ | | 0 | | 000 | 0 |
| Inachis io | | | 1 | | | 0● | | | 00 | | | |
| Issoria lathonia | | | 1 | | | 0● | $\bigcirc ullet$ | 0•□ | 0•□ | $\circ \bullet$ | 000 | ●□ |
| Kirinia roxelana | | | | 1 | | 0● | $\bigcirc ullet$ | $\circ \bullet$ | 0•□ | 0•□ | 000 | 0● |
| Lasiommata maera | | | 1 | | | | 0 | • | 0 | | 0● | 0 |
| Lasiommata megera | | | | 1 | | | • | $\circ \bullet$ | | | • | |
| Libythea celtis | | | | 1 | | | | | • | | | |
| Limenitis reducta | | | | 1 | | 0● | • | • | 0•□ | 0•□ | 0● | 0● |
| Maniola iurtina | | | | 1 | | 0● | 0•□ | 0•□ | 0•□ | 0•□ | 000 | 000 |
| Melanargia galathea * | | | 1 | | | | | | 0 | | | |
| Melanargia larissa * | | | | | | | | | | | | |
| Melitaea cinxia | | 3 | 1 | | | 0● | | • | $\circ \bullet$ | $\circ \bullet$ | | 0● |
| Melitaea didyma | | - | 1 | | | • | • | 0•□ | 0•□ | 0•□ | | 000 |
| Melitaea phoebe | | | 1 | | | 0● | | • | $\circ \bullet$ | | | • |
| Melitaea trivia | | 2 | | 1 | | 0● | 0 | 0 | 0 | 0 | 0 | 0● |
| Nymphalis antiopa | | | 1 | | | • | | | | | • | |
| Nymphalis polychloros | | | 1 | | | | | | • | • | | • |
| Pararge aegeria | | | | 1 | | | | • | | • | • | |
| Polvgonia c-album | | | 1 | | | 0● | | | 0 | 0 | • | |
| Vanessa atalanta | | | | 1 | | 0 | 0 | $\circ \bullet$ | | $\circ \bullet$ | • | • |
| Vanessa cardui | | | | 1 | 1 | 0● | 0 | 0•□ | $\circ \bullet$ | $\circ \bullet$ | 0 | 000 |
| PAPILIONIDAE | | | | | | | | | | | | |
| Iphiclides podalirius | | | | | 1 | • | • | • | • | • | П | • |
| Papilio machaon | | | | 1 | | 0● | | • | 0● | • | • | |
| Parnassius mnemosvne | 2 | 3 | 1 | | | | | | • | | | |
| Zervnthia cerisv | 2 | 2 | | 1 | | 0. | | | | | | |
| Zervnthia polyxena | | | | 1 | | | | | • | | | |
| PIERIDAE | | | | | | | | | | | | |
| Aporia crataegi | | | 1 | | | 0● | 0● | 0. | 0. | 0. | 0 | 0. |
| 1 | | | | | | | | | | | | |

| Colias crocea | | | 1 | $\circ \bullet$ | $\circ \bullet$ | 0•□ | 0•□ | 0•□ | | ٠ |
|-------------------------------------|---|---|---|-----------------|-----------------|-----------------|-----------------|--------------------------|-----------------|----|
| Euchloe ausonia | | 1 | | • | • | $\circ \bullet$ | | | | • |
| Gonepteryx cleopatra | | 1 | | | | | 0 | | | |
| Gonepteryx rhamni | 1 | | | • | | • | 0 | • | | |
| Leptidea duponcheli | 1 | | | | | | 0 | | | |
| Leptidea sinapis | 1 | | | $\circ \bullet$ | • | • | $\circ \bullet$ | $\bigcirc ullet \square$ | $\circ \bullet$ | • |
| Pieris brassicae | 1 | | | $\circ \bullet$ | • | $\circ \bullet$ | $\circ \bullet$ | $\bigcirc ullet$ | 0 | • |
| Pieris mannii | 1 | | | $\circ \bullet$ | 0 | $\circ \bullet$ | $\circ \bullet$ | $\bigcirc ullet$ | 0 | 0 |
| Pieris napi | | | 1 | $\circ \bullet$ | | 0 | 0 | $\bigcirc ullet$ | 0 | |
| Pieris rapae | | | 1 | $\circ \bullet$ | | $\circ \bullet$ | $\circ \bullet$ | $\bigcirc ullet$ | | • |
| Pontia chloridice | | 1 | | | | 0 | | | | |
| Pontia edusa | | 1 | | $\circ \bullet$ | 0 | $\circ \bullet$ | 00 | $\bigcirc ullet \square$ | | • |
| Species number in 1998 | | | | 36 | 25 | 28 | 54 | 39 | 29 | 26 |
| Species number in 2011 | | | | 54 | 31 | 42 | 50 | 44 | 27 | 39 |
| Species number in 2012 ⁺ | | | | - | 6 | 13 | 22 | 16 | 10 | 11 |

Table S2. Results of permutational multivariate analysis of variance (PERMANOVA).

| | df | SS | MS | F | P(perm) |
|-------------|-----|--------|-------|-----|---------|
| Year | 1 | 32992 | 32992 | 7.8 | 0.0001 |
| Transect | 20 | 139220 | 6961 | 1.9 | 0.0001 |
| Interaction | 20 | 84160 | 4208 | 1.2 | 0.0197 |
| Residual | 168 | 609218 | 3626 | | |
| Total | 209 | 865590 | | | |

df, degrees of freedom; SS, sum of squares; MS, mean square; F statisitic; P (perm), P-value after permutation procedure. The main effects fitted in PERMANOVA are the years (fixed factor) and transects (random effect), which explained the changes on community composition found on the long-term period (1998-2011, 21 transect locations).

| Transects | t | P_perm |
|------------------|------|--------|
| Agriculture.1 | 1.18 | 0.10 |
| Agriculture.2 | 1.54 | 0.01* |
| Agriculture.3 | 0.94 | 0.54 |
| Dry meadow.1 | 1.29 | 0.09 |
| Dry meadow.2 | 1.04 | 0.37 |
| Dry meadow.3 | 1.39 | 0.06 |
| Grazed pasture.1 | 1.14 | 0.22 |
| Grazed pasture.2 | 0.95 | 0.61 |
| Grazed pasture.3 | 1.36 | 0.01* |
| Mixed forest.1 | 1.26 | 0.10 |
| Mixed forest.2 | 1.34 | 0.03* |
| Mixed forest.3 | 1.18 | 0.15 |
| Oak forest.1 | 1.54 | 0.02* |
| Oak forest.2 | 1.32 | 0.06 |
| Oak forest.3 | 1.52 | 0.01* |
| Pine forest.1 | 1.24 | 0.14 |
| Pine forest.2 | 1.19 | 0.10 |
| Pine forest.3 | 1.15 | 0.16 |
| Wet meadow.1 | 1.12 | 0.27 |
| Wet meadow.2 | 1.26 | 0.09 |
| Wet meadow.3 | 1.05 | 0.35 |

Table S3. Results of pair-wise a posteriori test of permutational multivariate analysis of variance (PERMANOVA).

t, value of *t*-statistic (based on distances); *P* (*perm*), *P*-value after using 9999 permutations in each case, (*) asterisk indicates significant pairs among levels of the factor year (1998-2011) within levels of the factor transect (21 levels).

Table S4. Distribution of the 1260 actual localities (corresponding to 5193 points observed by the author or referred to the bibliography on Greek butterfly Atlas) among 4 elevation zones of Greek territory.

| Elevation | Area (sqkm) | Ν | Ratio |
|-----------|-------------|------|-------|
| 0-500 | 79795 | 1965 | 0.02 |
| 501-1000 | 34942 | 1430 | 0.04 |
| 1001-1500 | 13588 | 1135 | 0.08 |
| > 1500 | 3843 | 663 | 0.17 |

Area: corresponds to km² of the cover of Greek territory among the 4 elevation zones, extracted by a Digital Terrain Model (DTM, 30x30m pixel size).

N: corresponds to 5193 observation points of the Greek butterfly Atlas.

Ratio: N (observation points) per Area (km²).

Spearman correlation between Area and N was found to be significant (Spearman rho= 1, n=4, P <

0.001), implying an adequate sampling effort per elevation zone.



Number of SPEC: number of species of European conservation value.

Note that in the third year (2012) we sampled all habitats except agriculture fields and only for one repetition (June). No SPEC species were found in this last sampling period.

Figure S1. Number of species and number of SPEC (Species of European conservation concern) per habitat type (7), per sampling year (1998-2011-2012).

- Chapter 3 -

Who flies first? - Habitat-specific phenological shifts of butterflies and orthopterans in the light of climate change: a case study from south-east Mediterranean

Abstract

1. Insects undergo phenological change at different rates, showing no consistent trend between habitats, time periods, species or groups. Understanding how and why this variability occurs is crucial.

2. We analyzed phenological patterns of butterflies and Orthoptera using a novel approach of Standardized major axis (SMA) analysis. We investigated whether: 1) phenology (mean date, duration of flight) of butterflies and Orthoptera changed from one survey (1998 and 1999 respectively) to another (2011), 2) the rate at which phenology changed differed between taxa and 3) phenological change was significantly different across habitat types (agriculture fields, grasslands, forests). Using the 2011 dataset, we investigated relationships between habitat-specific variables and species phenology.

3. Both groups advanced mean dates of appearance from the first to the second survey, while the duration of flight periods decreased for butterflies and did not change for Orthoptera. Although the rate at which phenology changed was consistent between the two groups, at the habitat level, a longer duration of flight period emerged for butterflies in agriculture fields whilst Orthoptera showed no differentiation in flight duration between habitats. We found an earlier emergence of butterflies in grasslands compared to forests, attributed to habitat-specific temperature, while spatial variation in humidity had a significantly lower effect on butterflies' phenology in grasslands compared to forests. We also found a gradual delay of butterfly appearances as canopy cover increased.

4. We demonstrated the utility of SMA analysis in phenological studies and detected evidence that both habitat type and habitat-specific variables refine species' phenological responses.

Keywords: arthropods, butterflies, Orthoptera, phenology, habitat type, global change, Mediterranean

Introduction

As global temperatures are predicted to increase by 1.5–4.5°C by the end of the century (IPCC, 2013) and changes in species' responses and landscape structure are expected to intensify, it is important to understand how species respond to climate change in the context of their environments (Primack et al., 2009). There is ample evidence that climate

warming is linked to a changing onset of phenological events for a variety of taxonomic groups (Menzel et al., 2006; Parmesan, 2007; Primack et al., 2009), including acceleration of spring leaf unfolding and first flowering of wild plants (Badeck et al., 2004; Menzel et al., 2006), changed arrival dates of migrant bird species (Huin & Sparks, 2000; Robson & Barriocanal, 2011) and advanced appearance of butterflies, orthopterans and other insects (Forister & Shapiro, 2003; Nufio et al., 2010; Roy & Sparks, 2000; Stefanescu et al., 2003; Walther et al., 2002). The phenology of insects can be particularly sensitive even to minor changes of temperature, considering that their life-history traits (e.g. development rate and adult emergence) are directly linked to temperature (Ratte, 1985). Habitat warming has a direct effect on the growth rate of ectothermic organisms (Precht et al., 1973). In butterflies for example, warming-related life-history changes include prolongation of the flight period, alterations of the number of generations per year and acceleration of appearance dates (Altermatt, 2010; Roy & Sparks, 2000; Walther et al., 2002). Although there is a rich bibliography studying changes in butterfly phenology, only a few articles represent Mediterranean environments (de Arce Crespo & Gutiérrez, 2011; Gutiérrez & Menéndez, 1998; Stefanescu et al., 2011a; Wilson et al., 2007), and even fewer have combined analyses of changes of more than one taxonomic group (but see Gordo & Sanz, 2006). This is, however, crucial because differences in rates or directions of phenological response by different taxa could lead to asynchrony in species interactions (Memmott et al., 2007; Visser et al., 2012). Considering that insects undergo phenological change at different rates, showing no consistent trend between areas, time periods, species or groups (Primack et al., 2009), it is important to understand how and why this variability occurs (O'Connor et al., 2012).

An emerging line of research investigates to what extent local habitat conditions can buffer ecological communities against coarse-scale trends and patterns in climate change (Gillingham et al., 2012; Suggitt et al., 2011). Local attributes of topography and vegetation structure influence microclimatic conditions (Suggitt et al., 2011), whereas local temperature can be influenced even by very local variables such as canopy cover and moisture (Ashcroft & Gollan, 2012). Consequently, phenology does not only vary markedly over regional elevation gradients (e.g. Gordo et al., 2008; Illán et al., 2012) but also within an altitudinal belt or across different habitat types. Therefore, the use of habitat-specific variables (microclimatic such as temperature and humidity, and

environmental such as canopy cover and the availability of flower heads) could help us to better understand species' phenological response in the context of their surrounding environment. As an example, current research dealing with phenological changes uses sitespecific information [e.g. POSITIVE project (Menzel et al., 2006)] to predict general trends and patterns of species responses to climate change. Altermatt (2012) in northern Switzerland showed that seasonal appearance of butterflies has been influenced by both temperature and habitat type. Nevertheless, there is a limited body of research studying the effect of different habitat types on species' phenology, and even fewer studies have incorporated habitat-specific variables.

Here, we used a novel approach of Standardized major axis (SMA) technique to interpret species' responses to regional warming (see Bishop et al. (2013) for a methodological approach). Although SMA is a highly recommended technique for allometric studies (Warton et al., 2006), in recent years it has been broadly used in functional ecology for estimating bivariate trait relationships between plant species and/or communities (e.g. Adamidis et al., 2014; Wright & Sutton-Grier, 2012). SMA is a slope-fitting technique that shows how one variable scales against another: slopes are fitted by minimising the residual variance in X and Y dimensions simultaneously rather than Y alone (Domínguez et al., 2012; Falster & Westoby, 2005) resulting in a less biased outcome compared to traditional approaches such as ANCOVA (Warton et al., 2006), given that all variables are subject to both measurement and species-sampling error (Wright & Westoby, 2002). We used this technique to quantify bivariate relationships and describe how phenological processes scale from one survey to the other, between two taxonomic groups (butterflies and Orthoptera), across different habitat types and under the effect of habitat-specific variables. Specifically, testing for significant differences in slopes and intercepts we addressed the following questions: (a) Did phenology change significantly over a period of 13 and 12 years for butterflies and orthopterans respectively (1998-2011: butterflies, 1999-2011: orthopterans)? (b) Is the rate at which the phenology changed consistent for butterflies and orthopterans? (c) Is there a significant effect of habitat type (grasslands, forests and agriculture) on phenological patterns? Considering data of only one sampling period (2011) we also investigated the effect of habitat-specific variables on species phenology per habitat type and the congruency of both taxonomic groups' phenological patterns vis-avis the environmental gradient of canopy cover.

Materials and methods

Study area

The study area of Dadia-Leukimi-Soufli National Park (Dadia NP hereafter) is situated in northeastern Greece (41°07'-41°15'N, 26°19'–26°36'E). It is a hilly area extending over 43000 ha with altitudes ranging from 20 to 650 m, including two strictly protected core areas (7290 ha), where only low-intensity activities such as extensive grazing and selective wood-cutting are allowed on a periodical basis. The climate is sub-Mediterranean, with a mean annual rainfall of 652.9 mm, a mean annual temperature of 14.3°C, presenting a minimum in January and a maximum in July-August, while the arid summer season extents from July to September (25°C, 210 mm) (Maris & Vasileiou, 2010). Along with the dry and hot summers that characterize Dadia NP, a significant increase of temperature by 0.95°C has been documented for a 22-year-period (1990–2012) (Zografou et al., 2014). The Dadia area was established as a nature reserve in 1980 mainly due to its great variety of birds of prey, and since then it has been recognized for its high biodiversity value for other taxa (Kati et al., 2007; Korakis et al., 2005; Grill & Cleary, 2003; Kati et al., 2004; Kati et al., 2007; Korakis et al., 2013).

Species datasets

We considered two butterfly datasets from 1998 (Grill & Cleary, 2003) and 2011 (Zografou et al., 2014), which followed exactly the same sampling strategy (standard line transects; Pollard & Yates, 1993). Species diversity and abundance were recorded along 21 transects of 200 m length and 5 m width, covering three major habitat types (forests, grasslands and agriculture fields). Transects were laid out at a minimum of 300m distance and a maximum of 1 km from the nearest neighboring transect, and were repeated 15 times (approximately every 10 days) between May and September of each survey.

Similarly, we compared two orthopteran datasets from 1999 (Kati et al., 2004) and 2011, when species diversity and abundance were recorded in 36 sites (100 m minimum distance apart) in which two transects of 30 m length and 2 m width were sampled in late spring (June), summer (July–August) and autumn (September–October) per year. Adult specimens of Orthoptera were caught in a sweep net, counted and identified *ex situ* using
the Greek Orthoptera guide (Willemse, 1985). The later start of sampling for Orthoptera is due to the fact that they turn into adults much later in the year than butterflies.

According to our methodology, the sampling window was the same per insect group. We included in the analysis only those species present in both surveys (**Appendix**), while tests for the effect of habitat type considered only species present in all three habitats. Sampling efficiency was greater than 95% for butterflies in both 1998 and 2011 and more than 99% for Orthoptera in both 1999 and 2011 (Chao 1; Colwell et al., 2004; Magurran, 2004), allowing database comparison from different observers.

Habitat-specific variables

In 2011, we collected air temperature and soil humidity data for each transect replicate (315 transects for butterflies, 216 transects for orthopterans). We used a Hobo (U12) data logger, recording data every minute for the period that each transect lasted (60 min for butterflies, 15 min for orthopterans), so as to extract the average temperature and humidity values per transect. We estimated the average proportion of canopy cover per transect, considering the cover values in three plots (5 x 2 m) evenly located along butterfly transects (every 100 m: 63 plots) and in four plots along orthopterans' transects (every 10 m: 288 plots). To do so we used a spherical densiometer to estimate canopy cover (measures at four cardinal directions) in July 2011. Finally in the same plots, we estimated the number of flower heads (May 2011) using a ten grade scale ($1 \le 10, 2:11-20, 3:21-50, 4:51-100, 5:101-200, 6:201-300, 7:301-400, 8:401-500, 9:501-600, 10:>600$).

Phenological descriptors

Two different phenological descriptors were calculated for every species in each survey and each habitat type; the first describes the timing of the flight period (species appearances) and the second the duration of the flight period (Brakefield, 1987). The timing of the flight period was calculated as the weighted mean date of adult appearances (mean date hereafter) as follows:

$$T = \sum_{k=1}^{S} p_k t_k \quad , \quad p_k = \frac{n_k}{N}$$

Where p_k is the relative abundance of species k per survey and habitat type (n_k is the number of individuals of species k per visit, N is the number of all individuals per survey or habitat type), t_k is the date of adult appearance in Julian dates (1=January 1).

This is a widely used descriptor in butterfly studies, and considered to be more reliable than other descriptors such as the first day of adult appearance (Illán et al., 2012; Van Strien et al., 2008).

The second descriptor measures the degree of synchronization or duration of the flight period (duration hereafter) and was calculated as the standard deviation about the mean date of species flight period (Brakefield, 1987; de Arce Crespo & Gutiérrez, 2011; Stefanescu et al., 2003). The greater the variance about the mean date is, the more asymmetrical the flight periods from one survey to the other or across the different habitats will be. The final set of phenological descriptors and their values for each species and each year is shown in the **Appendix**.

Data analysis

SMA analysis of Phenological change

To investigate our research questions we quantified and compared bivariate relationships using Standardised Major Axis analysis (hereafter SMA). SMA is a robust slope-fitting technique that compares bivariate relationships in terms of slope differentiation (via a permutation test) reflecting the different rate at which the phenology changed (Warton et al., 2006). When SMA presented no significant slope differentiation, we further tested for significant differences in intercepts, which in our case reflected different initial values of the two phenological descriptors (mean date and duration). A SMA slope is generated by a cloud of points (species) when two normally distributed variables (e.g. mean date 1998 vs mean date 2011) are plotted against each other. First, for each taxonomic group separately, and considering only common species between the two years, we employed SMA to test for significant differentiation of the phenological descriptors (mean date and duration) between the two surveys (1998/1999 and 2011) and line 1:1 (slope=1 and intercept=0). A slope < 1 indicated that species advanced their mean date or reduced the duration of the flight period from the first to second survey. On the contrary, a slope > 1 signified a later mean date of species appearances and a longer flight period. Second, to test whether the rate of phenological change of the two groups was consistent between the two surveys, we

plotted SMA results for both groups together. No significant differentiation of their slopes indicated a consistent rate at which their phenology changed from the first to the second survey. Third, again considering each group separately for species that were common to both years and all three habitat types, we investigated the effect of different habitats on species phenology, comparing SMA slopes per habitat. Shallow slopes indicated slow rates of phenological change from the first to the second survey compared to steeper slopes that reflected fast rates of phenological change. Habitats with low intercept values indicated an advancement of mean date or a reduction of the duration of the flight period from the first to the second survey compared to habitats with higher intercept values.

Habitat-specific variables vs phenological descriptors

To investigate the effect of habitat-specific variables (microclimatic: temperature and humidity) on species phenology (mean date and duration), we used SMA analysis to test the relationships between these variables and phenological descriptors per habitat type (data from 2011).

We also employed general linear models, in order to investigate whether habitat-specific variables (microclimatic: humidity, temperature and environmental: flower-heads, canopy cover) had the same impact on the phenology of butterfly and orthopteran communities. To do so, we first employed Principal Component Analysis (PCA), in order to pinpoint the main habitat-specific variables that regulated site ordination. Since canopy cover (first PCA axis) explained most of the variance (92.4%: for butterfly PCA and 94.8%: for orthopterans' PCA), corresponding to a gradual transition from sites of low to high canopy cover, it served as the continuous explanatory variable in the models. Then, we considered the community-weighted mean values for the mean date (CWM_{mean date}) and for the duration of the flight periods (CWM_{duration}) as the response variables. We calculated CWM_{mean date} and CWM_{duration} for each sampling site of butterflies (21) and Orthoptera (36) separately, using the following equation:

$$CWM = \sum_{i=1}^{n} p_i \times t_k$$

Where p_i is the relative contribution of species *i* to the abundance of the community, *n* is the number of species in the community and t_k is the mean date or the duration of the flight periods respectively.

We used the free software PcOrd for principal component analysis (McCune & Mefford, 1999). The rest of the analyses were conducted in R (R & CoreTeam, 2014), specifically using the SMATR 3 package (Warton et al., 2012) for SMA analysis, *lm* function (Chambers, 1992; Wilkinson & Rogers, 1973) for general linear models, library *nortest* for residual normality tests and *ggplot2* library for graphical representation of the SMA results.

Results

Phenological changes

For both groups, the mean date of adult appearance significantly decreased from the first to the second survey, since the slopes of the relationships between years were positive and significantly lower than slope=1 ($R^2 = 0.39$, P < 0.001; **Fig.1a** for butterflies **and** $R^2 = 0.25$, P < 0.001; Fig.1b for Orthoptera). The relationship between the duration of butterfly flight periods in 1998 and in 2011 had a positive slope and was lower than slope=1, indicating a decrease of the flight period (**Fig. 1c**), while this did not happen for orthopterans, where no significant relationship appeared (P > 0.05, n=33) (**Table 1**).

Table 1. Results of standardized major axis (SMA) analysis for relationships between phenological descriptors (mean date and duration) of different surveys for butterflies (1998-2011) and Orthoptera (1999-2011).

| | | | | | | sig. of difference to slope=1 and intercept=0 | | |
|-------------|--------------------------|-------|-----------|---------|----------------|---|--------------|--|
| | Phenological descriptors | Slope | Intercept | Р | R ² | in slope | in intercept | |
| Butterflies | mean date | 0.80 | 26.74 | < 0.001 | 0.39 | 0.04 | 0.10 | |
| | duration | 0.78 | 2.02 | < 0.001 | 0.78 | 0.04 | 0.45 | |
| Orthoptera | mean date | 0.68 | 61.43 | < 0.001 | 0.25 | 0.02 | 0.01 | |
| | duration | -0.65 | 31.37 | 0.87 | 0.00 | - | - | |

 R^2 : proportion of the explained variance, Bold numbers represent significant differentiations (P < 0.05) from line 1:1 (slope=1 and intercept=0, indicating no difference or change between surveys).

We found no significant differentiation in slopes between the two insect groups (P > 0.05, n=91), indicating that the rate at which phenology changed for the two groups was consistent (**Table S1**).

Considering both surveys across habitat types, we found no significant differentiation of the mean date of emergence of butterflies from the first to the second survey (the relationships for agriculture and grasslands explained no more than 20% of the variance; **Table 2**); on the contrary, we found a significant differentiation of the duration of flight periods, which were longer in the agricultural fields (significantly higher intercept) compared to forests (**Fig. S1**). For Orthoptera, there was no significant differentiation in mean date or duration between habitats and the relationships were always weak (explaining no more than 20% of the variance; **Table 2**).

Table 2. Results of standardized major axis (SMA) analysis for relationships between phenological descriptors (mean date and duration) of different surveys across habitat types for butterflies (1998-2011) and orthopterans (1999-2011). Only species present in both years and the three habitats were considered (n=63, butterflies; n=39, Orthoptera).

| - | | Agriculture | | | Forest | | | Grassland | | | | Sig. of difference | | | |
|-------------|---------------|-------------|------|---------|----------------|------|------------|-----------|----------------|------|-------|--------------------|----------------|-------------|---------------------|
| | Relationships | Sl | Int | Р | R ² | Sl | Int | Р | R ² | Sl | Int | Р | R ² | in slope | in intercep t |
| Butterflies | mean date | 0.6 | 58.8 | 0.1 | 0.1 | 0.8 | 22.5 | < 0.05 | 0.4 | 0.8 | 21.4 | 0.1 | 0.2 | 0.6 | 0.9 |
| | duration | 0.8 | 8.3 | < 0.001 | 0.3 | 1.1 | - 10.62 | < 0.001 | 0.3 | 0.6 | 2.9 | 0.5 | 0.0 | 0.2 | < 0.001 |
| Orthoptera | mean date | 0.6 | 68.2 | 0.1 | 0.2 | 0.5 | 102.3 | 0.3 | 0.1 | 0.4 | 109.6 | 0.2 | 0.1 | 0.7 | 0.4 |
| | duration | -0.7 | 38.9 | 1.0 | 0.0 | -0.9 | 31.7 | 0.3 | 0.1 | -0.9 | 46.7 | 0.5 | 0.0 | 0.9 | 0.1 |

SI: Slope, Int: Intercept, R^2 : proportion of the explained variance. The last two columns show differentiations in slopes and intercepts respectively (via a permutation test). When no significant differentiations in slopes emerged, tests for differences in intercept were conducted. Bold numbers represent significant differentiations (P < 0.05) in slope or intercept.



Figure 1. Variation in relationships between phenological descriptors (mean date (a,b) and duration (c) of flight) for different periods for butterflies (1998 versus 2011) and Orthoptera (1999 versus 2011). Black line corresponds to 1:1 line (slope=1 and intercept=0, indicating no difference between surveys) and white line to the observed change in mean date or duration of flight. Panels a and c correspond to butterflies, and panel b to orthopterans. Only species present in both considered surveys were (n=58, butterflies; n=33, Orthoptera).

Phenological changes vs habitat-specific variables

Across the different habitats, slopes between habitat-specific humidity and butterfly species' phenology differ significantly (**Table 3**); forests had a steeper slope compared to grasslands suggesting a higher rate at which species' phenology changed in forests for a given change in humidity (**Fig. 2**). In addition we found a delayed appearance of butterflies in forests compared to grasslands, as forests had significantly higher intercepts, when testing the relationship between temperature and mean date. For orthopterans, the only significant differentiation in intercept was between temperature and mean date but the relationship was significant only for grasslands and therefore we did not further analyse this relationship (**Table 3**).

We found butterflies to gradually delay their mean date of appearance as canopy cover increased (**Figure S2**) but no significant effect emerged for orthopterans, suggesting the lack of congruency between the two insect groups along the canopy gradient.



Figure 2. Variation in the relationships between phenological descriptors (flight mean date and duration) and habitat-specific variables (temperature and humidity) across different habitat types. Black dots (and lines) are for agriculture fields, white dots for forests and grey dots for grasslands. Lines were drawn only for the significant models. Only butterfly species (2011) present in the three habitats were considered (n=63).

Table 3. Results of standardized major axis (SMA) analysis for relationships between phenological descriptors (mean date and duration) and habitat-specific variables (temperature and humidity) across different habitat types. Only butterfly and orthopterans' species (2011) present in the three habitats were considered (n=63 for butterflies, n=39 for orthoptera).

| roup | ological criptors | | A | Agricultı | ıre | | | Foi | rest | | | Grass | sland | | sig. of c | lifference |
|---------|----------------------|-----------------------------------|------|-----------|-----|----------------|------|-------|---------|----------------|------|-------|---------|----------------|-----------|-----------------|
| 9 | Phen desc | Habitat- specific variables | Sl | Int | Р | R ² | Sl | Int | Р | R ² | Sl | Int | Р | R ² | in slope | in intercept |
| | an ite | temperature | 11 | -121 | 0 | 0 | 13 | -156 | < 0.05 | 1 | 17 | -252 | < 0.05 | 1 | 0.2 | < 0.001 |
| llies | me da | humidity | -6.4 | 584.5 | 1 | 0 | -4.2 | 432.1 | < 0.001 | 0 | -2.9 | 353.1 | < 0.05 | 1 | < 0.001 | |
| Butterf | ration | temperature | 6.9 | -160 | 1 | 0 | 7.1 | -164 | < 0.001 | 0 | 8.8 | -201 | 0.1 | 0 | 0.7 | 0.4 |
| | np | humidity | 3.9 | -217 | 0 | 0 | -2.3 | 160.3 | < 0.001 | 0 | -1.5 | 106.8 | < 0.001 | 0 | < 0.001 | |
| a | an te | temperature | -6.1 | 379.5 | 0 | 0 | -5.1 | 355.2 | 0.9 | 0 | -7 | 384.9 | < 0.001 | 1 | 0.7 | < 0.001 |
| ter | me da | humidity | -2.3 | 322.3 | 0 | 0 | -1.6 | 294.4 | 0.2 | 0 | -3 | 380.6 | 0.6 | 0 | 0.3 | 0.1 |
| hop | ion | - | | | | | | | | | | | | | | |
|)rt | rati | temperature | -4.5 | 140.9 | 0 | 0 | -4.5 | 136.5 | 0.2 | 0 | 5.1 | -111 | 0.5 | 0 | 0.9 | 0.4 |
| | np | humidity | 1.7 | -69.4 | 0 | 0 | 1.4 | -57 | < 0.001 | 0 | 2.2 | -108 | 1 | 0 | 0.5 | 0.2 |

Sl: Slope, Int: Intercept, R^2 : proportion of the explained variance. Column 16 shows differentiations in slopes (via a permutation test) and when no significant differentiations emerge, tests for differences in intercept were conducted (column 17). Bold numbers represent significant differentiations (P < 0.05) in slope or intercept.

Discussion

Here, we used a novel approach of Standardised major axis analysis (SMA) in order to interpret species' phenological responses to regional warming. Using this approach we were able to show an advanced appearance of adult butterfly and orthopteran species over 12 and 13 year periods respectively. Our results showed a general reduction of the duration of butterfly adult appearances from the first to the second survey. However, significant alterations of butterfly phenological events emerged when habitat type and habitat-specific variables were included in the analysis, while for orthopterans no significant patterns emerged. We showed that butterflies delay their appearance with canopy cover increase, indicating that the phenological responses of some taxa (but not necessarily all) could possibly be modified by habitat management.

Phenological change

The results of this study are consistent with those of many authors (e.g. Alternatt, 2012; de Arce Crespo & Gutiérrez, 2011; Nufio et al., 2010; Parmesan & Yohe, 2003; Roy & Sparks, 2000; Stefanescu et al., 2003; Weiss et al., 1993) who found earlier adult insect emergence under warmer or drier conditions. Although some individual species emerged later in our 2011 survey (species located above the 1:1 line in Fig 1 a,b), we considered phenological change on average and analysis at species level was not conducted. In contrast, the overall reduction of butterfly flight periods does not correspond with the lengthened flight periods found recently in Northern Europe for most butterfly species (Roy & Sparks, 2000). This qualitative difference could be due to the heterogeneous effects of climate warming at the northern and the southern edges of butterflies' ranges in Europe (Settele et al., 2008). Dadia NP is located in the east Mediterranean where extreme summer temperatures are anticipated in the future (Lelieveld et al., 2013). The rise of local temperature previously found (Zografou et al., 2014) might intensify the extreme dry summer of the Mediterranean, causing ecological consequences to butterfly populations in Dadia NP by limiting adult nectaring and/or food availability (González-Estébanez et al., 2011). As temperature has long been known to influence lifespan (Loeb & Northrop, 1917), the shorter flight period we found might be a response to summer Mediterranean weather extremes. Trotta et al. (2006) in their experiments showed that a short-lived invertebrate (Drosophila melanogaster) lived up to 29 days when raised at 31.2 °C,

whereas flies from the same population lived up to 247 days when reared at 12 °C. The metabolic theory of ecology is also known to influence lifespan (Van Voorhies, 2001). Considering that the metabolic rate of invertebrates increases exponentially with temperature (Gillooly et al., 2001) small changes in temperature could result in relatively large changes in lifespan. Munch & Salinas (2009) using the metabolic theory indicated that over the next 100 years ectotherm lifespans are expected to shorten by 3-19% for a climate warming scenario with a lower (1.1°C) temperature rise and by 8-42% for a higher scenario (a 2.9°C rise in average temperature) within the bounds of those predicted by the IPCC (2013).

Phenology changed between the two periods at similar rates for butterflies and orthopterans, despite the substantial ecological and life history differences between the two groups. For example, many butterfly species are more specialised in comparison to orthopterans in terms of larval resources and have a greater mobility (Wettstein & Schmid, 1999), although our research suggests that both insect groups are influenced at Mediterranean latitudes by common ecological factors such as flowerheads (Zografou et al., 2009).

Phenological changes across habitat types

The duration of butterfly flight periods appeared to be longer in agriculture fields compared to forests from the first to the second survey (1998 - 2011). The duration of the flight period can be influenced by the degree of habitat openness (García-Barros, 2000). Agricultural fields are more open areas than forests and receive more direct radiation, maximizing their ground temperature and hence providing warmer conditions to species. Apart from the warmer microclimate, the longer duration of flight period in agriculture fields could be attributed to the extended availability of nectar resources; agricultural areas in Dadia NP have a traditional character with natural hedges and tree lines providing shade and flowering plants at field margins. At the same time, these areas provide cooler patches due to the irrigation system, prolonging the "green season" and thus nectar availability until late summer (Zografou et al., 2014). Nectar constitutes the basic diet for temperate-zone butterflies (Wheeler, 1996), supplies them with the required energy for flight (Stefanescu & Traveset, 2009) and increases longevity through carbohydrate intake

(Murphy et al., 1983). Extended longevity, in turn, provides an explanation for the longer flight period we found within agriculture fields.

Phenological changes vs habitat-specific variables

We detected a significant effect of habitat-specific variables (temperature and humidity) on butterflies' phenology. Interpreting the relationship between temperature and mean date, we showed an advanced mean date of adult appearances in grasslands compared to forests. Grasslands and forests are heterogeneous habitats and vary considerably in their microclimatic conditions, especially as the summer season unfolds. Grasslands provide greater nectar resources and warmer conditions in early spring, accelerating the development rates and thus the emergence date of adult butterflies (Precht et al., 1973; Sparks et al., 2005; Weiss et al., 1993). However, grasslands' openness can be more prone to Mediterranean summer drought, which has been shown to influence species behaviour; a comparable study in the Mediterranean revealed earlier emergence of the endemic butterfly species compared to their non-endemic counterparts, as a protective mechanism for the vulnerability of caterpillars to summer drought (López-Villalta, 2010). Another example of how much Mediterranean drought can influence species behaviour is the summer dormancy of adult butterflies, which prolongs females lifespan compared to their conspecifics from cooler climates (Grill et al., 2013). Forests on the other hand are less exposed to solar radiation, and microclimatic conditions can be less favourable in early spring time but more favourable in the second half of the hot summer, when species seek shadier and cooler microhabitats (Ashton et al., 2009; Suggitt et al., 2012). Given that adult butterflies use nectar resources as they become available during the season (Shreeve, 1992; Stefanescu & Traveset, 2009), earlier adult appearance in grasslands and later in forests may be an adaptation to the different flowering periods of plants in each of the two habitats. Our models provide further evidence for butterflies' earlier appearance in open habitats. Although we did not find a congruent phenological pattern between the two taxonomic groups along the canopy gradient, we did find butterflies to gradually delay their mean date of appearances as canopy cover increases.

Habitat-specific humidity was found to influence butterflies' phenological responses between habitats. A shift in humidity seems to have a minor impact on the mean date and the duration of flight period of butterflies in grasslands compared to their counterparts in

forests. This could be the result of a possible adaptation of species' thermal tolerance, resulting from the different range of microclimatic conditions recorded per habitat. There is indeed evidence that humidity records in forests have a narrower range compared to those in grasslands (see x-axis in Fig. 2). Due to the narrow range, species may have adjusted their thermal tolerance to specific climatic conditions and hence not be so responsive to variations in humidity compared to their counterparts in grasslands. On the other hand species found in grasslands may be able to withstand the wide range of humidity recorded herein and thus to have developed a broader band of thermal tolerance. It has been shown that each habitat has a distinct thermal character which probably could shape species' thermal tolerance (Suggitt et al., 2011). Additionally, Rapoport's rule suggests that species at high elevations are able to withstand a broad range of climatic conditions which, in turn, has lead them to have a wide elevational range, while species at low altitudes are adapted to narrow climatic conditions and so their climatic tolerance and their distribution range are also narrow (Stevens, 1992). However, it remains unclear if the pattern observed in our data is simply an artefact resulting from differences in the microclimates associated with different habitats or an actual species adaptation. Further studies are needed in order to elucidate to what extent habitats' thermal range can influence species thermal tolerance and hence, the rate of species phenological responses.

Our results show a concurrent advancement of the mean date of butterfly and orthopterans' emergence over a 13 and a 12 year period respectively. In addition, our results emphasize that the SMA technique can be successfully used when analysing phenological data in insects. The incorporation of habitat type and habitat-specific variables into such analyses may lead to clearer pictures of the phenological responses of species to warming in Mediterranean areas, as we showed here for butterflies¹.

¹ The present chapter is under review in the journal of Ecological Entomology.

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Appendix

Butterfly species (58) present in both surveys (1998-2011). Each species shows the values of its phenological descriptors (mean date and duration of flight period, see Material and Methods section for details).

| | First survey | | Second | survey |
|--|--------------|----------|-----------|----------|
| | mean date | duration | mean date | duration |
| HESPERIIDAE | | | | |
| Carcharodus alceae (Esper, 1780) | 187 | 34 | 177 | 1 |
| Carcharodus lavatherae (Esper, 1783) | 153 | 1 | 178 | 1 |
| Pyrgus sidae (Esper, 1784) | 154 | 9 | 156 | 1 |
| Spialia orbifer (Hübner, 1823) | 214 | 44 | 197 | 37 |
| Thymelicus acteon (Rottemburg, 1775) | 169 | 11 | 174 | 9 |
| Thymelicus sylvestris (Poda, 1761) | 169 | 9 | 171 | 11 |
| LYCAENIDAE | | | | |
| Aricia agestis (Dennis & Schiffermüller, 1775) | 221 | 36 | 195 | 29 |
| Celastrina argiolus (Linnaeus, 1758) | 194 | 34 | 178 | 18 |
| Callophrys rubi (Linnaeus, 1758) | 143 | 10 | 136 | 9 |
| Lycaena alciphron (Rottemburg, 1775) | 175 | 9 | 177 | 15 |
| Lycaena ottomana (Lefèbvre, 1830) | 198 | 38 | 168 | 33 |
| Lycaena phlaeas (Linnaeus, 1761) | 229 | 32 | 204 | 31 |
| Lycaena thersamon (Esper, 1784) | 138 | 8 | 203 | 37 |
| Lycaena tityrus (Poda, 1761) | 210 | 33 | 172 | 28 |
| Polyommatus icarus (Rottemburg, 1775) | 208 | 34 | 179 | 28 |
| Polyommatus thersites (Cantener, 1835) | 244 | 9 | 199 | 33 |
| Pseudophilotes vicrama (Moore, 1865) | 178 | 47 | 161 | 35 |
| Favonius quercus (Linnaeus, 1758) | 219 | 41 | 222 | 22 |
| Satyrium acaciae (Fabricius, 1787) | 162 | 6 | 171 | 4 |
| Satyrium ilicis (Esper, 1779) | 158 | 6 | 163 | 12 |
| NYMPHALIDAE | | | | |
| Argynnis pandora (Dennis & Schiffermüller, 1775) | 208 | 33 | 236 | 34 |
| Argynnis paphia (Linnaeus, 1758) | 216 | 28 | 193 | 22 |
| Brintesia circe (Fabricius, 1775) | 180 | 18 | 181 | 21 |
| Brenthis daphne (Bergsträsser, 1780) | 163 | 16 | 179 | 12 |
| Coenonympha arcania (Linnaeus, 1761) | 179 | 1 | 172 | 7 |
| Coenonympha pamphilus (Linnaeus, 1758) | 209 | 39 | 175 | 35 |
| Hipparchia fagi (Scopoli, 1763) | 249 | 8 | 213 | 27 |
| Hipparchia fatua (Freyer, 1844) | 238 | 16 | 226 | 18 |
| Hipparchia statilinus (Hufnagel, 1766) | 232 | 23 | 228 | 23 |
| Hipparchia syriaca (Staudinger, 1871) | 223 | 22 | 190 | 8 |
| Inachis io (Linnaeus, 1758) | 171 | 12 | 177 | 1 |
| Issoria lathonia (Linnaeus, 1758) | 160 | 32 | 159 | 16 |
| Kirinia roxelana (Cramer, 1777) | 196 | 42 | 174 | 19 |
| Lasiommata maera (Linnaeus, 1758) | 176 | 40 | 148 | 11 |
| Lasiommata megera (Linnaeus, 1767) | 250 | 1 | 163 | 29 |
| Limenitis reducta (Staudinger, 1901) | 180 | 37 | 184 | 29 |
| Melitaea cinxia (Linnaeus, 1758) | 150 | 17 | 142 | 8 |
| Melitaea didyma (Esper, 1778) | 215 | 30 | 185 | 24 |

| Maniola jurtina (Linnaeus, 1758) | 171 | 37 | 190 | 33 |
|--|-----|----|-----|----|
| <i>Melitaea phoebe</i> (Dennis & Schiffermüller, 1775) | 214 | 37 | 187 | 27 |
| Melitaea trivia (Dennis & Schiffermüller, 1775) | 169 | 26 | 176 | 23 |
| Polygonia c-album (Linnaeus, 1758) | 164 | 13 | 193 | 36 |
| Vanessa atalanta (Linnaeus, 1758) | 178 | 27 | 165 | 40 |
| Vanessa cardui (Linnaeus, 1758) | 179 | 36 | 176 | 33 |
| PAPILIONIDAE | | | | |
| Papilio machaon (Linnaeus, 1758) | 216 | 52 | 208 | 29 |
| Zerynthia cerisy (Godart, 1824) | 165 | 7 | 170 | 1 |
| PIERIDAE | | | | |
| Anthocharis cardamines (Linnaeus, 1758) | 138 | 3 | 133 | 7 |
| Aporia crataegi (Linnaeus, 1758) | 152 | 9 | 160 | 9 |
| Colias crocea (Geoffroy, 1785) | 195 | 36 | 171 | 30 |
| Euchloe ausonia (Hübner, 1804) | 154 | 5 | 135 | 10 |
| Gonepteryx rhamni (Linnaeus, 1758) | 180 | 1 | 170 | 29 |
| Leptidea duponcheli (Staudinger, 1871) | 199 | 41 | 166 | 30 |
| Leptidea sinapis (Linnaeus, 1758) | 183 | 27 | 161 | 25 |
| Pieris brassicae (Linnaeus, 1758) | 189 | 30 | 194 | 25 |
| Pontia edusa (Fabricius, 1777) | 215 | 39 | 208 | 30 |
| Pieris mannii (Mayer, 1851) | 186 | 36 | 173 | 28 |
| Pieris napi (Linnaeus, 1758) | 185 | 35 | 129 | 9 |
| Pieris rapae (Linnaeus, 1758) | 184 | 37 | 168 | 23 |

Orthoptera species (28) present at both years of surveys (1999-2011). Each species shows the values of its phenological descriptors (mean date and duration of flight period, see Material and Methods section for details).

| | First | survey | Second | d survey |
|---|--------------|----------|--------------|----------|
| | mean date | duration | mean date | duration |
| ACRIDIDAE | | | | |
| Acrida ungarica (Herbst, 1786) | 215 | 38 | 214 | 11 |
| Calliptamus barbarus barbarus (Costa, 1836) | 211 | 40 | 232 | 4 |
| Chorthippus parallelus parallelus (Zetterstedt, 1821) | 198 | 35 | 198 | 24 |
| Dociostaurus maroccanus (Thunberg, 1815) | 178 | 12 | 161 | 1 |
| Oedipoda caerulescens (Linnaeus, 1758) | 226 | 40 | 218 | 9 |
| Oedaleus decorus decorus (Germar, 1826) | 187 | 17 | 207 | 1 |
| Oedipoda germanica (Latreille, 1804) | 211 | 34 | 227 | 11 |
| Omocestus minutus (Brulle, 1832) | 206 | 45 | 200 | 16 |
| Omocestus rufipes (Zetterstedt, 1821) | 258 | 42 | 198 | 32 |
| Paracaloptenus caloptenoides caloptenoides (Brunner v.W., 1861) | 197 | 17 | 215 | 10 |
| Pezotettix giornai (Rossi, 1794) | 259 | 32 | 214 | 20 |
| Sphingonotus caerulans (Linnaeus, 1767) | 235 | 38 | 235 | 1 |
| PAMPHAGIDAE | | | | |
| Paranocarodes chopardi (Peshev, 1965) | 202 | 38 | 199 | 26 |
| GRYLLIDAE | | | | |
| Melanogryllus desertus (Pallas, 1771) | 194 | 20 | 159 | 1 |
| Oecanthus pellucens pellucens (Scopoli, 1763) | 213 | 7 | 216 | 5 |
| TETTIGONIIDAE | | | | |
| Bucephaloptera bucephala (Brunner v.W., 1882) | 183 | 16 | 197 | 25 |
| Conocephalus hastatus hastatus (Charpentier, 18250 | 200 | 15 | 218 | 7 |
| Decticus verrucivorus (Linnaeus, 1758) | 180 | 16 | 189 | 33 |
| Metrioptera oblongicollis (Brunner v.W., 1882) | 202 | 28 | 210 | 17 |
| Poecilimon brunneri (Frivaldsky, 1867) | 179 | 10 | 173 | 17 |
| Pholidoptera fallax (Fischer, 1853) | 189 | 20 | 175 | 8 |
| Platycleis incerta (Brunner v.W., 1882) | 196 | 36 | 218 | 7 |
| Platycleis intermedia intermedia (Serville, 1839) | 190 | 33 | 213 | 1 |
| Platycleis sepium (Yersin, 1854) | 209 | 15 | 217 | 8 |
| Poecilimon zwicky (Ramme, 1939) | 206 | 73 | 184 | 1 |
| Rhacocleis germanica (Herrich-Schaeffer, 1840) | 247 | 32 | 218 | 8 |
| Tylopsis lilifolia (Fabricius, 1793) | 192 | 19 | 200 | 23 |
| Tettigonia viridissima (Linnaeus, 1758) | 177 | 7 | 201 | 17 |

Supporting Information

Table S1. Results of standardized major axis (SMA) analysis for relationships between phenological descriptors (mean date and duration) of different surveys for butterflies (1998-2011) and Orthoptera (1999-2011).

| | | | | | | sig. of d slop inte | ifference to e=1 and rcept=0 |
|-------------|--------------------------|-------|-----------|---------|-------|---------------------------|------------------------------------|
| | Phenological descriptors | Slope | Intercept | Р | R^2 | in slope | in intercept |
| Butterflies | mean date | 0.80 | 26.74 | < 0.001 | 0.39 | 0.04 | 0.10 |
| | duration | 0.78 | 2.02 | < 0.001 | 0.78 | 0.04 | 0.45 |
| Orthoptera | mean date | 0.68 | 61.43 | < 0.001 | 0.25 | 0.02 | 0.01 |
| | duration | -0.65 | 31.37 | 0.87 | 0.00 | - | - |

 R^2 : proportion of the explained variance, Bold numbers represent significant differentiations (P < 0.05) from line 1:1 (slope=1 and intercept=0, indicating no difference or change between surveys).

Table S2. Results of standardized major axis (SMA) analysis for relationships between phenological descriptors (mean date and duration) of different surveys across insect groups (n=91). No significant differences imply no different rate at which their phenology changed.

| | | | | | | sig. of |
|-------------|--------------|---------|-----------|---------|----------------|------------|
| | | | | | | difference |
| Group | Phenological | | | | | |
| | descriptors | Slope | Intercept | Р | \mathbf{R}^2 | in slope |
| Butterflies | mean date | 0.8038 | 26.74 | < 0.001 | 0.385 | 0.278 |
| Orthoptera | mean date | 0.6806 | 61.43 | 0.003 | 0.254 | 0.578 |
| Butterflies | duration | 0.784 | 2.023 | < 0.001 | 0.255 | 0.409 |
| Orthoptera | duration | -0.6487 | 31.373 | 0.871 | 0.001 | 0.408 |

R²: proportion of variance explained



Figure S1. Variation in relationships between phenological descriptors (mean date and duration) of different surveys for butterflies (1998-2011) across different habitat types. Black dots (and lines) are for agriculture fields, white dots for forests and grey dots for grasslands. Lines were drawn only for the significant models. Only butterfly species present in the three habitats were considered (n=63).



Figure S2. Significant trend of community-weighted mean date (CWM_{mean date}) along the canopy gradient for the butterfly group. Linear regression statistics are given in the top right corner of the panel: ***, P < 0.0001;**, P < 0.01; *, P < 0.05; ^{NS}, not significant.

- Chapter 4 -

Arthropods turnover across different spatial scales: a hierarchical additive partitioning model of two mountainous systems in Mediterranean basin

Abstract

Aim Mountains are complex - highly heterogeneous- ecosystems supporting a variety of taxa that respond to the environment in different ways across different spatial scales. Previous studies have recognized mountains as "species refugia" in the light of climate change and species' geographical movement to higher altitudes. The primary objective of the present chapter is to identify the patterns of butterfly and Orthoptera diversity across spatial scales using an additive partitioning framework.

Location The Rodopi mountain ranges (north-central) and Mt. Grammos (north-west), Greece.

Methods We sampled butterflies at 68 transects and Orthoptera at 268 plots distributed over the two mountains during 2012 and 2013. Diversity (species richness and Shannon index) was partitioned into four hierarchical levels: ecoregions (mountains), elevational zones, habitat types and transects or plots within habitats and we compared permuted values expected by chance to those we estimated. We further give each species an ecological identity as "common" or "rare" and explored the diversity patterns arising in the different species assemblages. Our null hypothesis was always the random distribution of species across the study system. General linear mixed models (GLMM) were used to evaluate the effect of ecoregion, insect group and species categories into alpha and beta components of the overall diversity (gamma).

Results The total diversity was significantly attributed to beta diversity component at high spatial levels: ecoregions accounted for 20.94% and 26.25% of butterfly and Orthoptera diversity (% gamma) and elevational zones accounted for 28.94% and 35.87% respectively. At smaller spatial scales, beta diversity for both groups was significantly higher than expected by chance, in terms of the Shannon index, and common species were recognized for shaping overall species diversity. For rare species beta diversity was higher only at the higher spatial level (ecoregion). We found a strong effect of ecoregion in alpha diversity patterns between butterflies and Orthoptera but not between common and rare species, while the "insect group" and "species categories" effect was always significant.

Main conclusions Our results show significantly higher levels of beta diversity among elevational zones and to a lesser extent between ecoregions and habitat types. In order to preserve the regional diversity, when designing montane reserves, all elevational zones must be represented with adequate replicates of different habitat types from both

ecoregions. In addition, monitoring schemes should target common species, rather than focusing only in rare species. Our work revealed incongruent diversity patterns between the two insect groups, limiting thus the ability of butterflies to serve as surrogate group of Orthoptera's diversity and vice versa. Diversity between taxonomic groups seems to be scale-dependent in our study system.

Keywords: partitioning, diversity patterns, species turnover, butterflies, Orthoptera, spatial scale

Introduction

Understanding the factors influencing the spatial patterns of species richness and composition is an essential component of conservation biology (Carroll et al., 1999). Describing these patterns using biodiversity hotspots such as mountainous ecosystems (Wu et al., 2010) is especially complicated given that distribution of biological diversity can vary considerably at different spatial levels (e.g. elevational zones, habitats) and between different taxa (Pearman & Weber, 2007). In the light of climate change and habitat loss, mountains have gained special attention as potential refuges for species that shift their distribution to higher altitudes (Hardy et al., 2010; Parmesan, 2007; Root et al., 2003). To effectively protect these important systems we need to determine the patterns of beta diversity of the local fauna, that is to define the species turnover along environmental gradients within the region of interest (Gering et al., 2003) and to interpret the driving forces of the observed patterns (Gering et al., 2003; Marini et al., 2012; Ribeiro et al., 2008; Wu et al., 2010). High beta diversity within an ecosystem consisting of diverse habitats or environmental gradients reflects a high degree of habitat and/or food specialization among the inhabitants (Jankowski et al., 2009). To be able to capture the whole spectrum of the local diversity we need to design reserves areas or to modify them according to species' needs improving thus viability of regional populations (Jankowski et al., 2009; Kattan et al., 2006; Wu et al., 2010). In cases where beta diversity is low, meaning that species assemblages are more similar per spatial unit, a more compact reserve network might be sufficient (Kattan et al., 2006).

Therefore to define and interpret how species diversity varies across multiple spatial scales (Gering et al., 2003) and between taxonomic groups (Fleishman et al., 2003) we used the

additive diversity partitioning framework (Lande, 1996). This approach, first conceived in the 1960s, was demonstrated by Lande (1996) and more recently, was reviewed by Veech (Veech et al., 2002). It consists of partitioning the total species diversity (gamma) into two different components of diversity corresponding at two different spatial scales; α -diversity (alpha) that defines the within-community diversity and β -diversity (beta) that defines the between-community diversity (Lande, 1996). The additive relationship of the different spatial components arises from the following model: alpha + beta = gamma, where all components have the same units and therefore can be compared. Although the richness of an area (alpha) is likely to remain a key variable for single land-use planning, yet gives no information about the composition of species across space (Dainese & Poldini, 2012). It is the species turnover (beta) among the sampling units that identifies the spatial patterns of biodiversity arrangement and it is with this component that we can evaluate how optimal a community species richness model is (Kattan et al., 2006).

A well established factor known to influence species turnover is environmental heterogeneity (Kerr & Packer, 1997). In particular, the more heterogeneous the environment of the study system is, the more niches and resources can provide to the local organisms resulting thus to a rich local diversity and vice versa (Ruggiero & Hawkins, 2008; Tews et al., 2004). Montane ecosystems consist of complex environments along their typical elevational gradient, which has often been related to climatic and habitat heterogeneity (Dainese & Poldini, 2012). Because of their environmental heterogeneity and their more recent climatic-role as "species refugia", mountains are thought to play a key role in shaping species diversity patterns and they should be included more often in the analysis of species diversity patterns (Marini et al., 2011).

Different taxa vary on their responses to environmental factors as a function of their lifehistory traits (Kotliar & Wiens, 1990) and limit their distribution according to their climatic tolerances (Woodward, 1990). As taxonomic variation increases complexity of the study system because the location of diversity hotspots may vary among taxa (Prendergast et al., 1999), an option to simplify the species model would be to use taxa that can also reflect the distribution patterns of other taxa (Gregory et al., 2005; Noss, 1990). In this context, we chose to study butterflies and Orthoptera because of their strongly congruent species richness patterns (Bazelet & Samways, 2012; Zografou et al., 2009). However, there are studies supporting no surrogate value of one group to the other (Lovell et al., 2007; Niemela & Baur, 1998). In addition, each species was given an ecological identity as "common" or "rare" based on the positive abundance-occupancy relationship; according to this relationship abundant species have a tendency to be more widespread than species with low abundance and restricted occurrence (Gaston, 1996). As rarity/commonness is usually linked to low/high densities respectively, we expect that this trait will determine to some extent species turnover across space (Orme et al., 2005). Therefore, we further explored diversity patterns arising on the different species assemblages within each group. In this vein, Gering et al. (2003) found that rare species of beetles account for a larger proportion of the total species richness than the common and considered to be responsible for the patterns of the entire community. Ekroos et al., (2010) and Marini et al., (2012) found that beta diversity patterns were strongly influenced by habitat generalists butterflies and high mobile Orthoptera species respectively. Variability in species turnover, signifies that further work might be needed to establish more general and applicable trends especially to biologically rich -yet poorly studied- regions in the world such as the Mediterranean (Blondel & Aronson, 1999).

Environmental heterogeneity and variation on taxonomic responses suggest a mechanism partially explained by the distance decay of similarity relationship (Nekola & White, 1999; Soininen et al., 2007). According to this relationship the longer the distance is between two observations the smaller the proportion of the common species shared between them (Nekola & White, 1999). Thus, as biological similarity decreases with geographical distance (Soininen et al., 2007) we expect to find higher beta-diversity across large spatial scales (e.g. landscapes, elevational zones) than across small spatial scales (e.g. transects, plots) and greater beta diversity for species assemblages corresponding to rare species than common. To increase the robustness of our findings, we pooled together species recorded in two distinct geographically mountainous ranges and from two insect groups.

Our first objective was to identify patterns of butterfly and Orthoptera diversity across different spatial scales (ecoregions, elevational zones, habitats, transects/plots) and compare the patterns found between the two insect groups. To do so, we used the additive partitioning analytical framework in two mountainous ranges (Grammos and Rodopi) in Greece and we tested the null hypothesis that diversity is generated randomly and thus the individuals follow random mechanisms to distribute across the different spatial scales.

Alternatively, there are hidden mechanisms that specify species distribution at different spatial scales. In particular, we expect microclimatic conditions characterizing the smallest spatial scale (transect or plot) and vegetation structure as well as food supply imposed by different habitat types to have a strong impact on species diversity (Scherrer & Körner, 2011). Otherwise, community composition and species richness is determined by high spatial scales such as elevation gradients (Bhattarai & Vetaas, 2006; Stevens, 1992) or mountainous ranges because of differences in management regimes and in biogeographic gradients (e.g. latitude, climate). Our second objective was to assess whether species assemblages within each group (common, rare species) are responsible for the observed patterns of diversity across our study system. Knowing the contribution of each component to the overall diversity we were able to better understand the mechanisms affecting species distribution and thus to effectively propose the appropriate conservation measures for the study area.

Materials and Methods

Study area

Our study area consisted of two mountainous regions: Mt. Grammos situated in NW Greece (350 km²: long. 20°50', lat. 40°21') and the Rodopi mountain-chain situated in NE Greece (1731km²: between 41° 12' and 41° 36' N and 24° and 25° 06' E). Both mountain ranges include areas protected by the European network NATURA 2000 (GR1320002, GR1140008, GR1140003, GR40002, GR1140001, GR1140004). Vegetation follows the same structure: low elevation (0-500m) is dominated by riparian forests (Salix spp., Populus spp., Platanus orientalis), agricultural fields and human settlements, midelevation (500-1000m) is dominated by deciduous broadleaf forests and scrublands (Quercus coccifera, Q. frainetto, Q. pubescens), grasslands and abandoned agricultures, high-elevation (>1000m) is dominated by beech, pine forests (Fagus sylvatica, Pinus nigra), conifer forests (Pinus sylvestris, Abies spp.) and grasslands, and above the treeline (>1800m) area is dominated by subalpine rocky grasslands. Climate on Grammos is of the mountainous type (mean annual temperature 8-12°C and mean annual rainfall 800-2200 mm), while climate on Rodopi is transitional from the sub-Mediterranean type to central European type with a strong continental character (mean annual temperature is 11.4°C, mean annual precipitation 1200 mm). We selected these mountain ranges because of their

considerable conservation value (Mertzanis et al., 2005; Xirouchakis, 2005; Zografou et al., 2009) and because of their similarities in vegetation structure and in low human impact relative to land-use changes; they are characterized by scattered human settlements, where logging, periodic livestock grazing and small-scale cultivations constitute the main activities. However, their climate and geographical position differ: Rodopi is located to the Greek-Bulgarian border (NE) and Grammos is near to the Greek-Albanian border (NW). In addition, the Rodopi Mountain Range has been designated as a National Park from 2009 and a management body consisting of special scientists and representatives of local entities has been in place since then.

Sampling design

We sampled insects hierarchically: we partitioned the study area into four nested spatial levels as ecoregions, elevational zones, habitat types and transects for butterflies and into five spatial levels as ecoregions, elevational zones, habitat types, transects and plots for Orthoptera (**Fig. 1**). The two mountain ranges represented the ecoregions. In each ecoregion, we used four elevation zones (0-500m, 501-1000m, 1001-1500m, 1501-2000m) and three main habitat types according to the dominant habitats accounted in the study system were nested within each elevation zone: agriculture fields (A), grasslands (G) and forests (F). With the exception of the fourth elevational zone and the agriculture habitat type (not available at such high altitudes), each habitat type had two to six replicates (see **Table S1**). Within each replicate we defined one 300m transect for butterfly sampling and four 5x2m plots along this transect (at 0, 100, 200, 300m) for Orthoptera. Overall, the sampling design included 41 transects (164 plots) in Rodopi and 27 transects (104 plots) in Grammos.



Figure 1. (a) The study area is located in the north and north-west of Greece, (b) Ecoregions correspond to two mountainous ranges: the upper is Rodopi mountain (north Greece) and the lower is Grammos mountain (north-west Greece); triangles are the sampling areas, (c) Elevational zones within each ecoregion consist of four scales (0-500m, 501-1000m, 1001-1500m, 1501-2000m), where the four different colours correspond to the four elevational zones ranging from light grey (0-500m) to black colour (1500-2000m), (d) Habitat types within elevational zones are noted as different polygons; each polygon corresponds to one of the 3 habitats: agriculture areas, forests, grasslands, (e) Plots within transects is the smallest sampling unit: along a 300m length transect (for butterflies sampling) we placed 4 plots of 5 x 2m at 0m, 100m, 200m and 300m (for Orthoptera sampling); plots within transects - within habitats - within elevational zones - within ecoregions - within the study area is the spatial hierarchical model we used to determine species diversity patterns in our study (see Materials and Methods section).

Data collection

We sampled butterflies and Orthoptera in the two mountainous regions (Rodopi and Grammos) in two successive years (2012, 2013) respectively.

Butterflies were recorded at 41 sites in Rodopi and 27 in Grammos (see **Table S1**). The minimum distance between sites was 2 ± 5 (*SE*) km so that each transect will effectively represent an independent population. Standardized 300 m long × 5 m wide transects were walked at a steady step for almost 90 min (*SE* ±10) at each site from April to August in Rodopi, while in Grammos samplings started almost a month later in May due to unsuitable weather conditions (Pollard & Yates, 1993). We visited each site 5 times in 2012 (Rodopi) and 4 times in 2013 (Grammos) keeping a constant sampling window of 20 days intervals.

We sampled Orthoptera by visiting 164 and 104 plots of 5x2m in Rodopi and Grammos respectively (4 plots per butterfly transect, see Sampling design). We covered each plot with systematic transects for a standard time period of 20 min caughting all individuals with a net and identifying them in the laboratory using an Orthoptera guide (Willemse, 1985). Sampling was conducted once (July - August) at the peak of Orthoptera adult activity.

Data analysis

Species Accumulation Curve

To assess the adequacy of our sampling, we constructed sample-based accumulation (rarefaction) curves for each group and each ecoregion (2x2curves) and for each ecoregion, each group and each elevational zone (2x2x4 curves) (Gotelli & Colwell, 2001). The accumulation curve is a first measure of the sampling efficiency that relates visually the number of samples (transects) to the accumulative number of species (i.e. species richness) and can be obtained through the rarefaction process which at random resamples i= 1, 2, ...,t sampling units (without replacement) until all sampling units in the community (e.g. ecoregion, elevational zone, insect group) have been accumulated. We compared the patterns obtained by rarefaction and for similar slopes, even if sampling was not exhaustive, ecoregions, elevation zones or insect groups considered to give the same amount of information; therefore their results can be compared. To assess sampling efficiency proportional and to select which communities can be included in our analysis,

we calculated a suite of seven non-parametric species richness estimators (ACE, ICE, CHAO1, CHAO2, Jack1, Jack2, MMRuns) following the example of previous studies (Chiarucci et al., 2003; Wilson et al., 2007). We took the ratio of the observed species richness and the final extrapolated richness for each estimator and then we calculated a mean for the seven measures corresponding to the reference sample (i.e. ecoregion, elevational zones, insect group). The mean value was an estimation of sample coverage. Only sites that satisfied an estimated sample coverage > 0.7 (i.e. >70% sampling effort) were included in the analysis. Both rarefaction analysis and species richness estimator calculations were performed using EstimateS version 9.1.0 (Colwell et al., 2004).

Additive Partitioning of Diversity

As a measure of species diversity, we used the additive partitioning framework where the total γ -diversity is the sum of alpha and beta diversity (within and among sampling units respectively). We decomposed the total diversity into its components (alpha and beta diversity) and we calculated the contribution of each nested spatial scale across to species diversity (butteflies and Orthoptera separately). The null hypothesis, that is butterfly and Orthoptera's diversity is uniformly distributed at different spatial scales, was tested in terms of species richness and of the Shannon-Wiener index (H'). Shannon index is a measure of the confounded effect of richness and abundance (Magurran, 2004).

To assess the spatial hierarchy of species diversity, we pooled all data collected for butterflies and we aggregated them by the four spatial scales: transects, habitat type, elevational zone and ecoregions, resulting with 68, 3, 4 and 2 sampling units respectively. Since Orthoptera were sampled per plot within transects their species pool was aggregated by five spatial scales: plots, transects, habitat type, elevational zone and ecoregions resulting with 248, 67, 3 and 2 sampling units. We calculated the average diversities at each level (alpha), while the differences between them corresponded to beta diversity (Kotliar & Wiens, 1990). Thus beta diversity was measured among plots b_{plot} (for Orthoptera), among transects $b_{transect}$ (for butterflies) and among elevational zones $b_{elevation}$, habitat types $b_{habitat}$ and ecoregions $b_{ecoregion}$ for both groups. Accordingly, alpha diversity was calculated as the mean number of species found per plot (a_{plot}), transect ($a_{transect}$), elevational zone ($a_{elevation}$), habitat type ($a_{habitat}$) and ecoregion ($a_{ecoregion}$). Within this framework, the total γ -diversity is the sum of alpha diversity at the lowest level ($a_{transect}$ or a_{plot}) and beta diversity at all levels (Crist et al., 2003; Lande, 1996) according to the following formula:

 $\gamma_{\text{butterflies}} = a_{\text{transect}} + b_{\text{transect}} + b_{\text{elevation}} + b_{\text{habitat}} + b_{\text{ecoregion}}$

 $\gamma_{\text{Orthoptera}} = a_{\text{plot}} + b_{\text{plot}} + b_{\text{transect}} + b_{\text{elevation}} + b_{\text{habitat}} + b_{\text{ecoregion}}$

Given that both alpha and beta diversity are mean values and therefore expressed in the same units, we were able to assess the contribution of each nested spatial level to the total γ -diversity and thus the significance of each spatial component (Veech et al., 2002). Analysis of diversity were conducted in PARTITION version 3.0 (Veech & Crist, 2009), where alpha and beta estimates were tested through a randomization procedure. We estimated diversity using equal sample weights (species richness) and unequal sample weights (Shannon-Wiener index (H')) (Jost, 2007; Jost et al., 2010). Given that two samples, even taken from the same community, it is unlikely to contain the same number of species in the same abundances, values of beta diversity may be an artefact of the sampling effort (Ribeiro et al., 2008). Thus, we tested the null hypothesis that alpha and beta diversity were biased by sampling effort. We estimated the significance of the observed diversity using the individual-based option, where each individual reassigns to any sample at the lowest level of analysis (Crist et al., 2003). For example, to test for the significance of beta diversity among elevation zones, individuals were randomly placed among elevation zones within each habitat type. We carried out 1000 trials for the randomization process at each spatial level: a null distribution was obtained and was then compared with alpha and beta diversity at each spatial level respectively. We assessed statistical significance at each diversity level by the proportion of null values obtained in the randomizations greater than the observed value (Crist et al., 2003; Gering et al., 2003; Veech et al., 2002). This proportion can be interpreted as a *p*-value as in any other test of significance. Thus, if alpha species richness of butterflies in Level 1 is 40 (P = 0.001), means that 1 out of 1000 randomized datasets had alpha richness greater than 40 (Gering et al., 2003).

We further examined the role of different spatial scales on species richness (S), dividing species pool into two categories according to their abundance-occupancy relationship (Gaston, 1996): rare and common species. We considered rare those species for whom abundance was lower than 0.05% of the abundance of the entire community (i.e. < 5

individuals) and they were present at < 5% of the sampled transects (i.e. < 3 transects) or < 5% of the sampled plots (i.e. < 13 plots). On the contrary, species for whom abundance overcame 0.5% of the abundance of the total community (i.e. > 50 individuals) and they occupied more than 5% of the total sampled transects were considered to be common. Species between these thresholds with intermediate levels of abundance-occupancy were excluded from the analyses (Marini et al., 2012). We further measure how close is our local classification to others that use life history traits such as trophic specialization of butterfly larvae and dispersal ability of Orthoptera (Marini et al., 2012; Reinhardt et al., 2005; Stefanescu et al., 2011). We found a significant dependency between our local classification and the butterfly trophic specialization trait referred by Stefanescu et al. (2011) (Fisher's exact test, P = 0.03, n=105) and a marginally significant relationship with the mobility category for Orthoptera reported in Marini et al. (2012) and Reinhardt (2005) (Fisher's exact test, P = 0.06, n = 47). Note here that tests were conducted only for the common species between our species pool and the one referred in those studies.

To test whether there is a great variation of diversity components (alpha, beta) between the two insect groups (butterflies, Orthoptera), species categories (common, rare) and between the two ecoregions (Grammos, Rodopi) we run General Linear Mixed Models. We used insect groups, species categories and ecoregions as fixed effects, the log-transformed alpha and beta components as response variables and habitats nested within elevational zones as random factor. We run the first model for insect groups and a second for common and rare species within each insect group. Statistical analyses were performed with the software R (R & CoreTeam, 2014) using nlme package.

Results

Accumulation curve and species richness estimators

Accumulation curves for each ecoregion nearly reached an asymptote for both butterfly slopes, while showed little evidence of approaching an asymptote for Orthoptera (**Fig. S1a**). In addition, none of the accumulation curves for elevation zones (**Fig. S1b-e**) reached an asymptote, but their slopes had similar patterns suggesting that even if we did not sample total species richness in any of the communities, our sampling effort gives the same amount of information for each on of them. Sampling effort for each spatial scale
(ecoregion, elevation zone) and group (butterflies, Orthoptera) overcame the 80%, satisfying the criteria of sample coverage (>70%).

Additive partitioning of diversity patterns

We recorded 9918 and 9393 individuals representing 151 and 78 species of butterflies and Orthoptera respectively. The most noticeable result from the additive partitioning analysis was that for both diversity measures, the highest beta component ($b_{ecoregion}$) in the model was always greater than expected by chance (**Table 1**). Also, beta component of diversity among elevational zones ($b_{elevation}$) showed significant larger values than expected by chance for all categories except for the rare species (butterflies and Orthoptera, P > 0.05), while beta diversity among habitats ($b_{habitat}$) was found to have a greater value than expected for Orthoptera (**Table 1**). On the other hand, alpha component was significantly lower than expected by chance for both groups, species categories and spatial scales, although it accounted for a large fraction of the richness of butterfly common species (**Fig. 2a**) and a large proportion of the total butterfly community in terms of the Shannon diversity index (**Fig. 3a**). For butterfly species richness, $b_{elevation}$ and $b_{ecoregion}$ were significantly higher than expected by chance and accounted for 28.94 and 32.24% of the total gamma diversity respectively (**Table 1; Fig. 2a**).

Table 1. Spatial partitioning of species diversity of two insect groups (butterflies,Orthoptera) in two mountainous ranges (Grammos, Rodopi) in Greece. S: species richness,H': Shannon–Wiener Index.

| Group | Level | | S Observed | % | Expected | P | H' Observed | % | Expected | P |
|-------------|---------------------------------|-----------------------|---------------|-------|----------|---|----------------|-------|----------|---|
| Butterflies | a _{transect} | within transects | 30.73 | 19.95 | 58.81 | 1 | 19.97 | 78.38 | 43.59 | 1 |
| | b _{transect} | among transects | 26.41 | 17.15 | 32.61 | 1 | 1.64 | 6.44 | 1.33 | 0 |
| | b _{habitat} | among habitat types | 20.05 | 13.02 | 23.24 | 1 | 1.25 | 4.91 | 1.12 | 0 |
| | b _{elevation} | among elevation zones | 44.57 | 28.94 | 28.48 | 0 | 1.42 | 5.57 | 1.06 | 0 |
| | $\mathbf{b}_{\text{ecoregion}}$ | among ecoregions | 32.24 | 20.94 | 10.86 | 0 | 1.2 | 4.71 | 1.01 | 0 |
| | γ | | 154 | | | | 25.48 | | | |
| Common | a _{transect} | within transects | 22.98 | 44.19 | 42.8 | 1 | 14.55 | 73.93 | 31.25 | 1 |

| | b _{transect} | among transects | 13.36 | 25.69 | 7.64 | 0 | 1.47 | 7.47 | 1.15 | 0 |
|------------|------------------------|-----------------------|-------|-------|-------|---|-------|-------|-------|---|
| | b _{habitat} | among habitat types | 6.53 | 12.56 | 1.53 | 0 | 1.19 | 6.05 | 1.05 | 0 |
| | b _{elevation} | among elevation zones | 6.76 | 13.00 | 0.03 | 0 | 1.3 | 6.61 | 1.02 | 0 |
| | b _{ecoregion} | among ecoregions | 2.37 | 4.56 | 0.02 | 0 | 1.17 | 5.95 | 1 | 0 |
| | γ | | 52 | | | | 19.68 | | | |
| Rare | a _{transect} | within transects | 1.59 | 9.35 | 3.21 | 1 | 1.44 | 16.13 | 2.74 | 1 |
| | b _{transect} | among transects | 0.61 | 3.59 | 0.62 | 1 | 1.27 | 14.22 | 1.25 | 1 |
| | b _{habitat} | among habitat types | 1.71 | 10.06 | 3.11 | 1 | 1.81 | 20.27 | 1.8 | 1 |
| | b _{elevation} | among elevation zones | 6.74 | 39.65 | 6.68 | 1 | 2.68 | 30.01 | 2 | 0 |
| | b _{ecoregion} | among ecoregions | 6.35 | 37.35 | 3.38 | 0 | 1.73 | 19.37 | 1.26 | 0 |
| | γ | | 17 | | | | 8.93 | | | |
| Orthoptera | a _{plot} | within plots | 6.04 | 7.65 | 28.63 | 1 | 3.94 | 34.77 | 16.36 | 1 |
| | b _{plot} | among plots | 4.32 | 5.47 | 16.02 | 1 | 1.39 | 12.27 | 1.32 | 0 |
| | b _{transect} | among transects | 12.23 | 15.48 | 13.49 | 1 | 1.83 | 16.15 | 1.12 | 0 |
| | b _{habitat} | among habitat types | 7.33 | 9.28 | 5.92 | 0 | 1.22 | 10.77 | 1.04 | 0 |
| | b _{elevation} | among elevation zones | 28.34 | 35.87 | 10.86 | 0 | 1.7 | 15.00 | 1.02 | 0 |
| | b _{ecoregion} | among ecoregions | 20.74 | 26.25 | 4.09 | 0 | 1.25 | 11.03 | 1 | 0 |
| | γ | | 79 | | | | 11.33 | | | |
| Common | a _{plot} | within plots | 4.11 | 18.68 | 15.53 | 1 | 2.87 | 29.77 | 9.56 | 1 |
| | b _{plot} | among plots | 2.4 | 10.91 | 4.47 | 1 | 1.30 | 13.49 | 1.19 | 0 |
| | b _{transect} | among transects | 5.27 | 23.95 | 1.48 | 0 | 1.56 | 16.18 | 1.06 | 0 |
| | b _{habitat} | among habitat types | 2.76 | 12.55 | 0.49 | 0 | 1.19 | 12.34 | 1.02 | 0 |
| | b _{elevation} | among elevation zones | 6.46 | 29.36 | 0.03 | 0 | 1.56 | 16.18 | 1.01 | 0 |
| | b _{ecoregion} | among ecoregions | 1 | 4.55 | 0 | 0 | 1.16 | 12.03 | 1 | 0 |
| | γ | | 22 | | | | 9.64 | | | |
| Rare | a _{plot} | within plots | 1.46 | 8.59 | 3.06 | 1 | 1.36 | 13.72 | 2.61 | 1 |
| | b _{plot} | among plots | 0.13 | 0.76 | 0.14 | 1 | 1.06 | 10.70 | 1.05 | 0 |
| | b _{transect} | among transects | 0.61 | 3.59 | 0.62 | 1 | 1.27 | 12.82 | 1.25 | 1 |
| | b _{habitat} | among habitat types | 1.71 | 10.06 | 3.08 | 1 | 1.81 | 18.26 | 1.79 | 1 |
| | b _{elevation} | among elevation zones | 6.74 | 39.65 | 6.67 | 1 | 2.68 | 27.04 | 2.01 | 0 |
| | b _{ecoregion} | among ecoregions | 6.35 | 37.35 | 3.42 | 0 | 1.73 | 17.46 | 1.26 | 0 |
| | | | | | | | | | | |

γ 17 9.91

P-values=0 means that the observed value of alpha or beta diversity is significantly larger than the randomized datasets produced, *P*-values=1 indicates that diversity value of the randomized datasets is significantly larger than that observed after 1000 trials (see Data analysis section), Expected value for S and H' is the mean of null distribution.

For Orthoptera species richness, b_{habitat}, b_{elevation} and b_{ecoregion} were significantly higher than expected by chance and accounted for 9.28, 35.87 and 26.25% of the gamma diversity respectively (Table 1; Fig. 2b). On the contrary, butterfly species richness at smaller spatial scales, i.e. b_{transect}, b_{habitat} were significantly lower than expected by chance and accounted for 17.15 and 13.02% of the gamma diversity (Table 1; Fig. 2a). Accordingly for Orthoptera species richness at b_{plot} and b_{transect} were also significantly lower than expected by chance and accounted for 4.32 and 12.23% of the total gamma diversity (Table 1; Fig. 2b). For the Shannon Index (H'), all four and five scales of beta diversity values for butterflies and Orthoptera respectively, were significantly higher (P < 0.001) than those expected by chance (Table 1; Fig. 3a, b). Between rare and common species we found differences in the percentage of overall richness distributed among levels of the hierarchical model (Table 1; Fig. 2a,b) but differences followed a similar pattern between butterflies and Orthoptera; deviations from expected values were significantly larger for rare species only at the highest spatial level in the model ($b_{ecoregion}$, P < 0.001), whereas deviations from expected values were significantly larger for common species at all higher levels, except at the b_{plot} for Orthoptera (**Table 1**).



Figure 2. Additive partitioning of butterflies (**a**) and Orthoptera (**b**) species richness on four and five spatial scales respectively: plots for Orthoptera only, transects, habitats, elevational zones and ecoregions for both groups. Contributions of alpha and beta components in the total gamma diversity in percentage (%) for butterfly total community, common and rare species (a), Orthoptera total community, common and rare species (b).









Figure Additive partitioning of 3. butterflies (a) and Orthoptera **(b)** Shannon diversity on four and five spatial scales respectively: plots for Orthoptera, transects, habitats, elevational zones and ecoregions for both groups. Contributions in the total gamma diversity explained by alpha and beta components of the entire butterfly Orthoptera (a) and (b) community.

Both models showed significant variability of alpha and beta diversity components between butterflies and orthotpera (first model) and between rare and common species (second model) (**Table 2**). We found higher alpha and beta components for butterflies than for Orthoptera and for common than for rare species. Although no significant effect was revealed for ecoregion, yet the interaction term (i.e. group x ecoregion) was found to strongly influence alpha diversity patterns in the first model. In particular, Grammos ecoregion was found to have higher alpha diversity for Orthoptera and lower for butterflies

than Rodopi ecoregion and a tendency to non-significant lower beta diversity. The residuals of the models were always normally distributed (Shapiro-Wilk normality test, P > 0.05).

Table 2. General linear mixed model results, testing the effects of insect groups (butterflies, Orthoptera: first model), species categories (common, rare: second model) and ecoregions (Grammos, Rodopi) on alpha and beta diversity patterns (see Data analysis for more details on the fixed and random factors).

| | | | F-value | Р |
|-------|-------|-------------------|---------|---------|
| | alpha | group | 352.79 | < 0.001 |
| del | | ecoregion | 2.20 | n.s |
| moe | | group*ecoregion | 8.54 | 0.006 |
| first | beta | group | 1953.26 | < 0.001 |
| | | ecoregion | 0.01 | n.s |
| | | group*ecoregion | 3.19 | n.s |
| | alpha | group | 67.60 | < 0.001 |
| | | species | 221.52 | < 0.001 |
| odel | | ecoregion | 0.24 | n.s |
| id m | | species*ecoregion | 1.96 | n.s |
| secon | beta | group | 84.41 | < 0.001 |
| | | species | 33.08 | < 0.001 |
| | | ecoregion | 0.09 | n.s |
| | | species*ecoregion | 0.01 | n.s |

Discussion

Diversity patterns between ecoregions

For both diversity measures used, we found greater beta diversity than expected by chance between ecoregions ($b_{ecoregion}$) (**Table 1**). This means that both insect groups (butterflies, Orthoptera) and species categories (rare, common) are not randomly distributed between the two mountainous ranges (ecoregions). The observed patterns could be the result of environmental dissimilarities between the two mountains (such as geographical position, climate), differences in species ecological traits such as dispersal ability and resource specialization (Loreau, 2000) or simply because of the different management regime (see Methods section). All these factors are well known to influence composition of butterfly and Orthoptera communities (Schirmel et al., 2010). Our results at this level of the hierarchical model coincide with the "first low of geography" (Tobler, 1970) or the distance decay of similarity hypothesis (Nekola & White, 1999), where species turnover between two communities increases with geographical distance that separates them (Morlon et al., 2008). Thus, for long distances between the studied communities, as the distance between the two ecoregions in our study system (**Fig. 1**), species dispersal activity is limited, resulting to different species pools adapted to local environmental and climatic conditions (Marini et al., 2012). In addition, Grammos is situated at lower latitude compared to Rodopi and its climate reflects a mountainous environment, while Rodopi's climate is marked by a transition from Mediterranean to continental climate; the former climatic transition can also explain why many plant species and vegetation types reach their southernmost distribution in this region (e.g. the Norway spruce forest). Our results agree with previous studies assessing the variation of insect communities composition across broader spatial scales (Ekroos et al., 2010; Gering et al., 2003; Ribeiro et al., 2008; Wu et al., 2010).

Diversity patterns along elevational gradient

Arthropods are well know to shift strongly along elevational gradients (Wettstein & Schmid, 1999) and a possible destruction or loss of these gradients may cause local species extinctions (see Kattan & Beltran, 1999) for an example with birds). Here, we found beta diversity among elevational zones (b_{elevation}) to have a great impact on community composition and species richness. Moreover, species turnover at this level had a considerably larger contribution to the total gamma diversity compared to the highest hierarchical level (b_{ecoregion}), suggesting a high compositional change of species along elevation gradients (see Fig. 2); this was true for all except the rare category of butterfly and Orthoptera species (Table 1). A likely explanation for the observed pattern is that the resources requirements of the common species can be more general and their feeding niches widely distributed along the elevation gradient than those of more specialized to habitat and resource requirements species (Menéndez et al., 2007; Stefanescu et al., 2011). Other studies have documented that trophically unique species occurring at only one or two habitats can suffer a greater extinction risk by habitat loss or change than more generalists species (Mattila et al., 2008; Tscharntke et al., 2012; Warren et al., 2001). In our study system, most of the rare species (84% butterflies, 70% Orthoptera) limit their

distribution to only one elevational zone, increasing thus their sensibility in the light of a possible loss or modification of the area within the appropriate zone. For example if the second elevational zone was lost, 10 of our butterfly species pool (e.g. Apatura ilia, Apatura metis, Hipparchia syriaca) and 6 Orthoptera species (e.g. Omocestus petraeus, Anacridium aegyptium, Omocestus haermorrhoidalis) would have gone extinct from our study system. Furthermore, there are butterflies species migrating from lower to higher altitudes during the summer period as for example Polyommatus coridon (found at the first two elevational zones in May and at the two last zones in August) or Numphalis polychloros (found at the first two zones in May, at all zones in May, at the last two in June and only at the last zone in July). A possible destruction of an elevational zone might cause local extinctions of seasonal migrators too. Undoubtedly, habitat loss is one of the principal drivers of biodiversity loss (Öckinger et al., 2009) but also climate has been identified to provoke insects' population and species richness declines (Tscharntke et al., 2005). It is well established that ongoing climate warming is forcing species to shift their distribution towards higher altitudes or latitudes (Araújo et al., 2011; Parmesan & Yohe, 2003), while species specialized to the mountains move their optimum elevation further upwards to the mountains' tops (Lenoir et al., 2008).

Diversity patterns among habitats, transects and plots

The beta diversity for species richness among transects and habitats for butterflies ($b_{transect}$, $b_{habitat}$) and among plots and transects for Orthoptera (b_{plot} , $b_{transect}$) was not significantly greater than expected by chance. This means that all butterfly assemblages of transects and habitats are subsamples of the same species pool; accordingly all Orthoptera assemblages of plots and transects are subsamples of the same species pool, suggesting an appropriate designation of these sampling units (plots, transects, habitats) at these levels of the hierarchy. However, for the same spatial levels we found Shannon index to have a greater value than expected (**Table 1**). By definition, the Shannon index gives more weight to common species than species richness (Magurran, 2004), suggesting that differentiation of the most abundant (or common) species which, in turn, affect the structure and determine diversity patterns in these communities (Ribeiro et al., 2008). We further found beta diversity among habitats ($b_{habitat}$) to be greater than expected for Orthoptera for both

diversity measures (**Table 1**). A possible explanation for both butterfly and Orthoptera's diversity patterns observed is the environmental variation (Loreau, 2000) mainly referring to specific vegetation composition imposed by each habitat type (Kerr & Packer, 1999). A number of studies have documented the importance of habitat diversity for arthropods' diversity and distribution (Kemp et al., 1990; Kerr & Packer, 1999; Stefanescu et al., 2011) suggesting that different types of habitats support different species assemblages. Given that plots and transects are nested within habitats and each habitat belongs to a different type (agriculture, forest, grassland) the above mentioned differences might be justified.

Common vs rare species

At all spatial levels (except b_{plot} for Orthoptera, see Table 1) beta diversity of common species was higher than expected by chance, whereas for rare species richness was higher only between the higher spatial level (becoregion). The scale-dependence differences in diversity patterns between common and rare species were also supported with the mixed model. Contrary to other insect studies (Davies et al., 1997) it seems that diversity patterns of common species are closer to the patterns of the entire community, probably because they account for a high percentage of the species present in the community (79% Orthoptera, 82% butterflies). On the other hand, strong beta diversity for rare species becomes traceable only between ecoregions, simply because it is less likely to encounter for a significant amount of rare species at the smaller spatial scales. The low species turnover of common Orthoptera species at small spatial scales (b_{plot}) reflects the overall Orthoptera community results: Orthoptera species associated to high species turnover at higher spatial scales rather than small scales such as plots (see Table 1). According to Marrini et al. (2012), the authors suggest that "orthopteran species do not response at finer spatial scales but rather they exploit the whole meadows", which in our study system meadows were the habitats.

Although we found a strong impact of the factor ecoregion in alpha diversity patterns of butterflies and Orthoptera, no significant effect was found when species' ecological identity taken into account (see **Table 2**). In particular, we documented common and rare species to follow similar patterns in Grammos and Rodopi per insect group, which means that the way rare and common species contribute to overall species richness patterns differs between butterflies and Orthoptera but not among ecoregions (Grammos, Rodopi). The

assessment of the group effect at the expense of ecoregion underlines the important role of species' ecological identity in species richness modelling (Bazelet & Samways, 2012; Pearman & Weber, 2007) and reveal a uniform pattern between the ecoregions in terms of their proportion of common *vs.* rare species (see **Table 2**). On the other hand, the interaction between group and ecoregion was found to be an important driver of diversity patterns for the two groups confirming the non random distribution of species across the mountainous ranges. In both cases, either between species categories or species groups, incongruent diversity patterns limit the use of one group as indicator for the other (Lovell et al., 2007; Niemela & Baur, 1998).

Conservation implications

Scale dependent differences in spatial patterns of diversity such as those detected for butterflies and Orthoptera have rarely been observed in Mediterranean ecosystems. Beta diversity at larger scales (ecoregion and elevational zones) proved to have a great influence in both groups, even if our results showed incongruent diversity patterns limiting the surrogate value of butterflies for Orthoptera and *vice versa*,. It is therefore suggested that regional designation of nature reserves should cover the whole range of the elevational gradient within mountainous systems (Kattan et al., 2006).For example, there were cases where regional rare species (e.g. *A. ilia, A. metis*) were recorded only to intermediate elevational zones or species that have been considered near threatened (NT) according to the European Red List of butterflies (Van Swaay et al., 2010) such as *Zerynthia cerisy*, *Chazara brizeis, Hipparchia statilinus* were found only at the first and/or the second elevational zone.

On the other hand, across small and intermediate spatial scales commonness was found to affect the structure and determine diversity patterns in butterfly and Orthoptera communities. Although rare species are more prone to extinction and special attention is usually be paid to their distribution patterns in order to be effectively conserved (Stefanescu et al., 2011), our results showed that common species appeared to be much more indicative in terms of the total diversity assessing also the importance of environmental heterogeneity at finer scales. We therefore suggest maintaining the nested design of transects within habitats throughout the whole study system, as the loss of one or more of the sampling units might severely reduced regional heterogeneity and thus local

diversity. In particular, when designing a regional reserve system for common species we need to incorporate all spatial scales (except b_{plot} for Orthoptera), whereas when rare species is the target group we need to focus at the higher level of the hierarchical model ($b_{ecoregion}$). Due to the great impact of the ecoregion in diversity patterns between butterflies and Orthoptera but not between common and rare species, regional monitoring programs might need to adapt different strategies with respect to the focal organisms (butterflies or orthotpera), whereas a single strategy for both mountains will likely serve for monitoring either common or rare species (Pearman & Weber, 2007).

To effectively protect the biodiversity of these mountainous ecosystems it is crucial to understand how species are distributed across them. Especially for a biodiversity hotspot like Greece (Balletto & Casale, 1991) where monitoring schemes are scarce and species conservation status is problematic, nearly unknown, we need a powerful tool to quantify spatial variation in biodiversity (Veech & Crist, 2009). Additive partitioning is a well used method for montane nature reserves designs elsewhere (Kattan et al., 2006) and results from this study could be useful for the designation of other montane systems throughout Greece as well.

Conclusions

In this chapter we found (i) beta diversity to be significantly higher among elevational zones than between ecoregions and habitat types, (ii) common species to be much more indicative of the total diversity of both groups than rare species, and (iii) the two groups to have a limited congruency in terms of their diversity patterns. As a result, we recommended a regional monitoring plan where all elevational zones will be included with adequate samples from different habitats and both ecoregions, while special attention must be paid to common rather to rare species. This regional monitoring program might need to adapt different strategies with respect to the focal organisms (butterflies or Orthotpera).

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Supplementary Information





Figure S1. (a) Sample-base accumulation curves for both ecoregions (Grammos and Rodopi) and both groups: B=butterflies, G=grasshopers., (b) Sample-base accumulation curve for butterflies per elevation zone in Grammos, (c) Sample-based accumulation curve for Orthoptera per elevational zone in Grammos, (d) Sample-based accumulation curve for Orthoptera per elevation zone in Rodopi, (e) Sample-based accumulation curve for Orthoptera per elevation zone in Rodopi.

Table S1. Sampling design: proportions (%) of Corine cover and transects per elevational zone, habitat type and ecoregions; where Zone 1=0-500m, Zone 2=501-1500m, Zone 3=1001-1501m, Zone 4=1501-2000m. Inside the parenthesis is the actual number of transects (replicates) (see Methods and Materials for details).

| | | Grammos | | Rode | opi |
|--------|-------------|--------------|-----------|--------------|-----------|
| | | Corine cover | Transects | Corine cover | Transects |
| Zone 1 | Agriculture | 40.24 | 33.33 (2) | 51.42 | 38.46 (5) |
| | Forests | 37.8 | 33.33 (2) | 24 | 46.15 (6) |
| | Grasslands | 20.73 | 33.33 (2) | 17.37 | 15.38 (2) |
| Zone 2 | Agriculture | 4.8 | 33.33 (2) | 8.89 | 20 (2) |
| | Forests | 62.66 | 33.33 (2) | 66.06 | 50 (5) |
| | Grasslands | 31.81 | 33.33 (2) | 24.74 | 30 (3) |
| Zone 3 | Agriculture | 1.19 | 25 (2) | 0.38 | 18.18 (2) |
| | Forests | 68.95 | 25 (2) | 79.84 | 27.27 (3) |
| | Grasslands | 29.85 | 50 (4) | 19.64 | 54.54 (6) |
| Zone 4 | Agriculture | 0 | 0 (0) | 0 | 0 (0) |
| | Forests | 43.75 | 28.57 (2) | 57.39 | 28.57 (2) |
| | Grasslands | 56.25 | 71.42 (5) | 42.6 | 71.42 (5) |

Steps for Corine cover calculation: 1) we clipped corine layer (available for the entire Greece) to each ecoregion perimeter, 2) we grouped the corine codes occurring in each ecoregion into five main categories: artificial areas, agriculture areas, forests, grasslands and water (e.g. lakes, rivers etc), 3) we clipped again the dissolved corine layers to the elevational zones and calculated the proportions for the three habitat types used in our study system: agriculture area, forests and grasslands. To test whether there is a consistency between the corine cover and transects we used per habitat type within elevational zones and ecoregions, we run paired t-tests and the resulted large p-value (P = 0.9) suggested that the data are consistent with the null hypothesis, namely the proportions of corine cover and the proportions of the transects we used per habitat type per elevational zone.

| Spatial Level | Alpha diversity | Beta diversity |
|------------------|------------------------------------|--|
| Study area | Diversity in the entire study area | |
| Ecoregion | Diversity of each mountain range | Turnover of species' diversity among mountain ranges |
| Elevational zone | Diversity of each elevational zone | Turnover of species' diversity among elevational zones |
| Habitat type | Diversity of each habitat type | Turnover of species' diversity among habitat types |
| Transect/ plot | Diversity of each transect/ plot | Turnover of species' diversity among transect/ plot |

Figure 1. Hierarchical model of species richness. Total γ -diversity at each spatial level derives from the sum of alpha and beta diversity at the next lower level.

- Chapter 5 -

Does phenology change along altitude? A case study of butterflies from Mediterranean region

Abstract

1. Increasing temperature is a major driver for species earlier emergence in the year. Detecting species' phenological trends as climate is warming in the absence of long-term time series data can be achieved by substituting space for time. As temperature is know to alter from low to high altitudes, elevational gradients are ideal proxies in phenological research.

2. We use butterfly data from two Mediterranean mountainous areas (ecoregions) to test temperate-zone hypothesis regarding altitudinal effects and phenology. Specifically, the mean date of butterfly assemblages and individual species appearances is expected to delay with altitude increase and also the duration of the flight period to be shorter as moving to the upper altitudes. The analyses performed on species, both collectively and individually.

3. We found a 16-day delay in the mean date per km increase in altitude for the whole butterfly species pool and an average of 20-day shift for the 13 individual species tested, when the temperature lapse rate was of 3°C/km. A progressive shortening of the duration of the flight period of 8-days/km was also observed at community but not at species level. A significant differentiation between the two ecoregions emerged regarding the rate of delays but the rate did not seem to be in accordance with the respective temperature lapse rate we recorded per region.

4. Our results agree with previous findings where butterfly community or species appear to delay the time of appearance and shorten the duration of flight period along altitude. Further evidence supported the idea of an adaptive strategy of multivoltine species in the absence of significant decrease of the length of flight period at species level. Future research on the environmental factors forcing common butterfly assemblages (found in ecoregions) to vary significantly on the rate of their delays is recommended.

Keywords: butterflies, climate change, phenology, altitude, Mediterranean

Introduction

It is predicted that climate change will lead to an increase of about 1.5–4.5°C in global mean surface air temperatures in the next century (IPCC, 2007) and an 3.5-7°C by the end of century (2070 - 2099) in the Eastern Mediterranean and the Middle East (Lelieveld et al., 2013). Phenology, the timing of seasonal activities of fauna and flora, has been

identified as an important metric to track changes in the ecology of species in response to climate change. Thus, there is a considerable interest on temporal trends on species phenology and their associations with warmer conditions constitutes the milestone of phenological studies (Parmesan, 2007; Parmesan & Yohe, 2003), with much popularity on butterfly phenological fluctuations (Altermatt, 2012; de Arce Crespo & Gutiérrez, 2011; Forister & Shapiro, 2003; Illán et al., 2012; Stefanescu et al., 2003). Butterflies and herbivores insects in general, are especially sensitive to climatic change because climate can directly influence their rate of growth and the time of emergence from winter diapause (Bale et al., 2002). In addition climate, can influence trophically interacting species (e.g. butterflies and their host-plants) creating phenological mismatches (Bale et al., 2002; Parmesan & Yohe, 2003): if for example butterflies emerge earlier in the year as a response to temperature rise but their host-plants do not, then their trophic interaction is expected to be disrupted and local extinctions to occur (Schweiger et al., 2008).

Forecasts for both butterfly community and individual species suggest a general earlier flight period as the climate warms (Dell et al., 2005; López-Villalta, 2010; Stefanescu et al., 2003; Wilson et al., 2005). However, in most cases, ecological forecasting is determined by looking at high-quality monitoring data collected over an extended time period (Banet & Trexler, 2013; de Arce Crespo & Gutiérrez, 2011). Although projections based on population time series is a commonly used method for the north and central Europe (Altermatt, 2012; Roy & Sparks, 2000; Van Strien et al., 2008), other areas like Mediterranean lack this "privilege" of high quality monitoring data and time series projections are limited (but see Stefanescu et al., 2003). An alternative to study temporal variability in phenology, when no long term data is available, is the space-for-timesubstitution approach, assuming that the spatial relationship between the environmental factor (e.g. altitude) and the response variable (e.g. time of species appearances) can be used as a proxy for the temporal relationship (Banet & Trexler, 2013). Indeed, a common application is to investigate how phenotypic traits change along latitudinal or elevational gradients (de Arce Crespo & Gutiérrez, 2011; Gutiérrez & Menéndez, 1998; Hodkinson, 2005; Merrill et al., 2008). Elevational gradients in particular, considered to be useful temporal proxies because they combine a significant variation in temperature over short horizontal distances (in km) (Körner, 2007) and a minimal variability of day length or photoperiod (Fielding et al., 1999; Hodkinson, 2005). In spite of criticism that this

approach generally ignores factors affecting ecosystem responses (Isaak et al. 2011; La Sorte et al. 2009), there are cases where space for time substitution has proved to produce predictions commensurate with models created from temporal data (Banet & Trexler, 2013; Hodgson et al., 2011; Leingärtner et al., 2014).

Ecological gradients that occur with increasing altitude are often expected to lead species' activity to be shorter, later in the season and more synchronized (Brown & Lomolino, 1998; Hodkinson, 2005). In this context, a number of studies in the temperate zone have already shown that butterflies become active later in the colder and higher altitude areas (de Arce Crespo & Gutiérrez, 2011; Illán et al., 2012; Merrill et al., 2008; Shapiro, 1975). However, there is also empirical evidence of the considerable complex species' phenological patterns along altitudinal gradients from 1920 (Brakefield, 1987; Verity, 1920) that make scientists to be more sceptical on the validity of the observed patterns (de Arce Crespo & Gutiérrez, 2011; Illán et al., 2012). Considering the existing controversy and the future increase of temperature, phenological studies, are of particular importance for better understanding species' phenological processes to climate change.

The present study examines the altitudinal patterns in timing and duration of the flight period of butterflies along an elevation gradient, between ecoregions and across different habitat types. We use data collected from two mountainous areas in north and west Greece (Rodopi and Grammos) during 2012 and 2013 respectively. Both areas are biodiversity hotspots (Xirouchakis, 2005; Zografou et al., 2009) containing a large number of endagered and endemic species (Pamperis & Stavridis, 2009). We tested the hypothesis of species gradual delay in the timing and a progressive shortening of the duration of the flight period as we move to upper altitudes in terms of community and species level. Furthermore, we investigated whether there is a significant differentiation on elevational patterns of butterfly communities between the two mountainous areas (ecoregions) and across the different habitat types.

Materials and Methods

Study area

Our study area consisted of two mountainous regions: Grammos mountain situated in NW Greece (350 km²: long. 20°50', lat. 40°21') and Rodopi mountain-chain situated in NE Greece (1731km²: between 41° 12' and 41° 36' N and 24° and 25° 06' E). Both mountain

ranges include areas protected by the European NATURA 2000 network (GR1320002, GR1140008, GR1140003, GR40002, GR1140001, GR1140004). Vegetation follows the same structure: low elevation (0-500m) is dominated by riparian forests (Salix spp., Populus spp., Platanus orientalis), agriculture fields and human settlements, mid-elevation (500-1000m) is dominated by deciduous broadleaf forests and scrublands (Ouercus coccifera, Q. frainetto, Q. pubescens), grasslands and abandoned agricultures, highelevation (>1000m) is dominated by beech, pine forests (Fagus sylvatica, Pinus nigra), conifer forests (Pinus sylvestris, Abies spp.) and grasslands, and above the treeline area (>1800m) is dominated by subalpine rocky grasslands. Climate in Rodopi is marked by the transition from a Mediterranean to a more or less continental climate (Mavromatis, 1980) (mean annual temperature is 11.4°C, mean annual precipitation 1200 mm), while in Grammos is characterized as humid continental (Korakis, 2002) with a mean annual temperature 8-12°C and mean annual rainfall 800-2200 mm). We selected these mountain ranges because of their considerable conservation value (Mertzanis et al., 2005; Xirouchakis, 2005; Zografou et al., 2009) and because of their similarities in vegetation structure and low impact of human activities; they characterized by scattered human settlements where logging, periodical livestock grazing and small-scale cultivations constitute the main activities. However, their climate and geographical position differs: Rodopi is located to the Greek-Bulgarian border marked by its closed vicinity to the aegean sea, while Grammos has a significant distance from the sea, situated to the Greek-Albanian borders. In addition, Rodopi Mountain Range has been designated as a National Park from 2009 and a management body has been activated since then.

Survey sites were representative of the dominant habitats accounted in the study system (agriculture fields, grasslands and forests) and selected on the basis of elevational gradient in the region (0-500m, 501-1000m, 1001-1500m, 1501-2000m). Excluding agriculture fields from the fourth elevational zone (not available at altitudes >1500m), each habitat type had two to six replicates (see **Table S1**).

Butterfly sampling

Butterflies were recorded at 41 sites in Rodopi and 27 in Grammos (see **Table S1**). The minimum distance between sites was 2 ± 5 (*SE*) km so that each transect will effectively represent an independent population. Standardized 300 m long \times 5 m wide transects were

walked at a steady step for almost 90 min ($SE \pm 10$) at each site from April to August in Rodopi and from May to August in Grammos; samplings started a month later in Grammos due to unsuitable weather conditions (Pollard & Yates, 1993). We visited each site 5 times in 2012 (Rodopi) and 4 times in 2013 (Grammos) keeping a constant sampling window of 20 days intervals.

Phenological descriptors

Timing and duration of flight period were calculated to describe species phenological responses along the altitudinal gradient. The timing of flight period was summarised per transect as the weighted mean flight date (hereafter mean date) according to the following formula:

$$Mean date = \sum \frac{Number of individuals per visit \times Date}{Total number of individuals}$$

Date was estimated in Julian dates (January 1 = 1). The same formula was used when comparisons between ecoregions and across different habitat types were conducted: only common species present at both ecoregions or all the habitats respectively, were considered. Mean date is a widely used descriptor in phenological studies for butterflies, and considered to be more reliable than other phenological measures such as the first day of adult appearance (Van Strien et al., 2008). We also calculated the duration of flight period (hereafter duration) as the standard deviation about mean date (Brakefield, 1987). At community level, we used all recorded species and individuals, while at species level we excluded the species falling into these categories: 1) species with one generation per year (univoltine), 2) recorded in less than three sites (transects) and two records per site, 3) early spring species for which phenology may not be recorded (e.g. Anthocharis cardamines, Callophrys rubi), 4) species overwintering as adults (e.g. Inachis io, Gonepteryx rhamni) and 5) species with summer aestivation (e.g. Maniola jurtina). Information on voltinism, adults wintering and summer aestivation was based on published records (Pamperis & Stavridis, 2009; Tolman & Lewington, 1997). We investigated the relationships of elevational delays with sampling size and elevational delays with elevational range by first calculating the following measures at species level: the number of transects where the species was present, the minimum elevation, the maximum elevation and the range of these two variables (range = maximum - minimum).

Data analysis

To test changes in timing and duration of the flight period considering the whole butterfly community, we carried out linear regressions of the mean date and standard deviation about the mean date against the altitude of each transect, where regression slope was the delay (days/meters). We repeated the same procedure at species level, only for those species satisfying the predefined criteria (13 species).

We investigated intraspecific variability on the magnitude of delays at species level by regressing the elevational delay with the number of transects where the species was present and the elevational delay with species' elevational range. We expected that species present at a higher number of transects or species with wider elevational range would appear to have longer delays elevation compare to those concentrate their distribution in few high altitude transects and thus possibly be associated with short elevational delays in phenology (Alexander & Hilliard, 1969).

We also tested whether butterfly assemblages occur in Rodopi appear to have bigger delays with elevation compared to their counterparts in Grammos (considering common species only) and vice versa, as a result of the different geographic location and thus environmental conditions such as atmospheric pressure, temperature, clear-sky solar radiation (Despland et al., 2012). To investigate the above relationships, we considered the same sampling periods, excluding thus the first sampling conducted in Rodopi (April). We quantified and compared bivariate relationships using Standardised Major Axis analysis (hereafter SMA). SMA is a slope-fitting technique that shows how one variable scales against another: slopes are fitted by minimising the sums of squares of errors in X and Y dimensions synchronously (Domínguez et al., 2012; Warton et al., 2006). Comparisons were conducted in terms of slope differentiation via a permutation test, reflecting different rate of elevational delays or duration of flight periods.

In order to remove the potentially confound effect of canopy cover and different habitat types (Fielding et al., 1999), we tested whether butterfly assemblages that occur in forests appear to have longer delays (steeper slopes) in elevation compared to their counterparts in grasslands or agriculture areas (species present at three habitat types). We expected that different habitat types will have a different impact on butterfly assemblages' elevational delays. For example, forests that are not open and not influence by direct radiation (Scherrer & Körner, 2011) could cause longer delays on species elevation associations. We

performed SMA analysis using the common species between ecoregions and across habitat types.

We used Minitab for conducting the linear regressions and R software (R & CoreTeam, 2014) for SMA analysis, specifically using the SMATR 3 package (Warton et al., 2012) and *ggplot2* library for graphical representation of the SMA results.

Lapse rate

Using a Hobo data logger, we collected temperature data per transect determining thus the change in temperature along the altitudinal gradient in the study area (lapse rate). We first evaluated the seasonally mean temperature per mountain range and then for both mountains as a whole. Logger was set up at the beginning of each transect under full shade cover and it remained there until the end of the sampling (90 min). Measures were taken every minute and a mean value per transect was then estimated. Lapse rate was calculated by regressing the mean temperature on altitude (km) per transect.

Results

Phenological patterns of butterfly community

We found a positive and significant relationship between the mean date and elevation for butterfly populations occurring in both mountains $[R^2 = 0.29, P < 0.001, n = 68;$ mean date (days since 1 January) = 162 + 16.1*elevation (km)]. A delay of butterfly appearances of 16-days for every km increase in elevation and a 85-day interval between the species occur in low and high altitudes is shown in **Figure 1a**. The relationship between the duration of flight period and altitude for butterfly community was also found to be significant $[R^2 = 0.27, P < 0.001, n = 68;$ mean date (days since 1 January) = 37.2 -8.2*elevation (km)]. The negative slope indicates a progressive shortening of the duration of butterfly flight period with increase in altitude of 8-days per km (**Fig. 1b**).



Figure 1. Relationships of (a) mean date (days elapsed from 1 January, 1=1 January) and (b) duration of the flight period (standard deviation about the mean date) with altitude (km). Each dot corresponds to a sampling transect (total number of transects, n = 68).

Phenological patterns of butterfly species

We analysed elevational patterns for 12 species: for eleven of them the relationship between the mean date and elevation had a positive slope and for one a negative slope (**Table 1**). Positive slopes were significant for six species indicating a delay in the flight period with increase in altitude, while non significant relationships emerged for the rest of the species (**Fig. S1**). *Aporia crataegi* had the bigger delay (25-days/km) in terms of its mean date of appearance along the altitude and *Argynnis paphia* the smallest delay (15days/km). The results for the relationships between duration of flight and altitude are shown in **Table 2**: eight species had negative slopes and four positive slopes, but relationships were always non significant. For intraspecific variation in the magnitude of delays, we carried out linear regression considering each species as an independent data point. Both relationships between species elevational delays and the number of sites where the species were present ($R^2 = 0.1\%$, P = 0.95, n = 12) as well as species elevational delay and elevational range ($R^2 = 1\%$, P = 0.76, n=12) were found to be non significant.

Table 1. Results of the linear regressions of mean date (days elapsed since 31 March, 1 = 1 April) against altitude (km) for 12 univoltine species. The species are in alphabetically order and in bold the ones with significant regressions. The number of sites occupied, the minimum and maximum altitudes (m), and the elevational range for each species are also shown.

| Species | Intercept | Slope (days/km) | R (%) | F | Р | Number of sites | Minimum altitude | Maximum altitude | Range (max - min) |
|-------------------------|-----------|--------------------|--------------|--------|--------|--------------------|---------------------|---------------------|-------------------------|
| Aporia crataegi | 146 | 24.9 | 62.4 | 24.92 | <0.001 | 17 | 128.35 | 1516.41 | 1388.06 |
| Argynnis adippe | 186.7 | 1.39 | 0.1 | 0.00 | 0.971 | 6 | 1247.42 | 946.80 | 300.63 |
| Argynnis paphia | 187 | 15.4 | 21.9 | 6.73 | 0.016 | 26 | 128.35 | 1410.25 | 1281.90 |
| Brenthis daphne | 161 | 22.5 | 59.6 | 8.85 | 0.025 | 8 | 127.75 | 1205.10 | 1077.35 |
| Brintesia circe | 172 | 22.6 | 73.9 | 28.32 | <0.001 | 12 | 420.13 | 1638.03 | 1217.90 |
| Coenonympha arcania | 158 | 20.3 | 43 | 3.77 | 0.11 | 7 | 860.00 | 1410.25 | 550.25 |
| Lysandra philippi | 146 | 37.2 | 50.4 | 1.02 | 0.497 | 3 | 1187.75 | 1745.50 | 557.75 |
| Melanargia galathea | 172 | 19.9 | 69.5 | 38.71 | <0.001 | 19 | 422.10 | 1532.00 | 1109.91 |
| Parnassius mnemosyne | 127 | 24.7 | 36.7 | 1.16 | 0.394 | 4 | 1516.41 | 1912.21 | 395.80 |
| Pyronia tithonus | 221 | -8.3 | 6.7 | 0.14 | 0.742 | 4 | 545.75 | 1035.00 | 489.25 |
| Thymelicus lineola | 151 | 35.5 | 88.9 | 8.02 | 0.216 | 3 | 915.13 | 1247.42 | 332.29 |
| Thymelicus sylvestris | 163 | 17.1 | 99.9 | 2930.5 | <0.001 | 4 | 127.75 | 1410.25 | 1282.50 |

Table 2. Results of the linear regressions of duration of flight period (standard deviation about the mean date) against altitude (km) for 12 univoltine species. The species order is alphabetically.

| Species | Intercept | Slope (days/km) | R (%) | F | Р |
|-----------------------|-----------|--------------------|--------------|-------|-------|
| Aporia crataegi | 22 | -4.72 | 8.7 | 1.42 | 0.252 |
| Argunnis paphia | 21.2 | 0.69 | 0.1 | 0.02 | 0.887 |
| Argynnis adippe | 5 | 9.9 | 5.2 | 0.22 | 0.664 |
| Brenthis daphne | 18.1 | 0.88 | 0.4 | 0.02 | 0.887 |
| Brintesia circe | 13.4 | -0.52 | 0.5 | 0.05 | 0.827 |
| Coenonympha arcania | 17.4 | 1.1 | 0.1 | 0.00 | 0.959 |
| Lysandra philippi | 58.6 | -22.3 | 60.5 | 1.53 | 0.433 |
| Melanargia galathea | 21.3 | -4.38 | 9.3 | 1.75 | 0.204 |
| Parnassius mnemosyne | 32.9 | -10.1 | 9.3 | 0.21 | 0.695 |
| Pyronia tithonus | 19.3 | -7.5 | 9.8 | 0.22 | 0.687 |
| Thymelicus lineola | 30.5 | -15.9 | 98.6 | 72.04 | 0.075 |
| Thymelicus sylvestris | 22.5 | -9.34 | 64.3 | 0.20 | 3.6 |

Phenological patterns between ecoregions and across habitats

The relationship between mean date and altitude was found to differ significantly among ecoregions (Test statistic: 6.949, P = 0.007, n = 67) indicating a different rate at which butterfly assemblages delay their appearances along altitude (**Fig. 2**). Both ecoregions had positive slopes, but Rodopi showed a steeper regression slope and therefore a bigger delay of butterfly assemblages of 30-days for every km increase in elevation than Grammos (16-days). On the other hand, although both regressions had a negative slope (Grammos: -9.024, Rodopi: -10.061), no significant differentiation emerged between ecoregions (Test statistic: 0.192, P = 0.669, n = 67), signifying a similar rate at which the duration of flight period changed along altitude. Furthermore, we found no significant differentiation on the rate of delays of butterfly assemblages across the three habitat types (Grammos test statistic: 0.22, P = 0.907, n = 26; Rodopi test statistic: 0.69, P = 0.74, n = 41) suggesting no particular influence of rate of delays provided by a specific type of habitat. Note that for this analysis we considered each ecoregion seperately.



Figure 2. Variation on relationships between mean date and elevation between the two ecoregions. Black dots (and lines) corresponds to Grammos and grey to Rodopi. Only butterfly species present in both ecoregions were considered for the calculation of the mean date. Each dot corresponds to a sampling transect (total number of transects, n=67).

Lapse rate

There was a significant decrease in seasonally mean temperature with altitude: for Rodopi in 2012, seasonally mean temperature decreased by 3°C per km [($R^2 = 0.337$, P < 0.001, n = 41; seasonally mean temperature (°C) = 24.1 - 2.70*altitude(km)] and for Grammos in 2013 by 5°C [($R^2 = 0.63$, P < 0.001, n = 27; seasonally mean temperature (°C) = 26.8 - 4.58*altitude(km)] over the altitudinal gradient of the study system. Considering both the mountainous ranges, we found a significant decline [($R^2 = 0.42$, P < 0.001, n = 68; seasonally mean temperature (°C) = 24.8 - 3.2*altitude(km)] identifying thus a decrease of 3°C for every km increase in elevation.

Discussion

Timing of appearance

For the whole butterfly species pool we found a 16-day delay in the mean date per km increase in altitude. Our findings support previous evidence that species appear later as we move to the upper altitudes in Mediterranean area (de Arce Crespo & Gutiérrez, 2011; Gutiérrez & Menéndez, 1998; Illán et al., 2012; Wilson et al., 2005). Among the species that have appeared significantly earlier, the average shift is 20 days, broadly close to the one reported in California (Forister & Shapiro, 2003) or in Britain (Roy & Sparks, 2000). *Aporia crataegi* had the steeper slope (25 days/km) approaching the one recorded in central Spain (33.08 days/km, (de Arce Crespo & Gutiérrez, 2011); 33.1 days/km, (Merrill et al., 2008) increasing thus the strength of our results and *Thymelicus sylvestris* had the

shallower slope with 17 days/km. Based on a temperature lapse rate of approximately 3°C per 1km elevation increase, our findings suggest that a 1°C decrease in mean seasonal temperature could be associated with a 5-day and a 6-8day phenological delay at community and species level respectively. A similar trend of 3.7-day delays for the entire butterfly community and a 2-8-day delay for individual species has been reported from Spain (de Arce Crespo & Gutiérrez, 2011; Illán et al., 2012).

Although we did found half of individual species analyzed to change significantly their time of appearance along altitude, the rest had shallower and non-significant relationships of the flight period and elevation (Table 1). It has been shown that species occurring at higher elevations in mountains and those flying later in the year show greater phenological synchrony (i.e. shallower slope) than those occurring at lower elevations (Illán et al., 2012). Indeed, the individual species tested for which no significant relationship emerged had a later onset of their flight period beginning at mid July onwards (e.g. Argynnis adippe, Pyronia tithonus) or a mean elevational distribution above 1200m (e.g. Parnassius mnemosyne, Lysandra philippi). We investigated interspecific variability in the magnitude of species delays but no significant association with species' elevational range (P = 0.76) or species sample size (P = 0.9) emerged (Illán et al., 2012). This finding is in accordance with previous studies (de Arce Crespo & Gutiérrez, 2011; Illán et al., 2012) and suggests that some species do not respond to elevational temperature change probably because of a better synchronization of their flight period with increasing altitude. Synchrony in time of emergence across temperature gradients might be a first signal for local adaptation of butterfly species to regional climates (Ayres & Scriber, 1994; Roy & Asher, 2003). Such adaptations can be masked by species specific biology and include a suite of mechanisms such as behavioural and physiogical (e.g. selection of warm microhabitats for egg-laying at high altitudes or selection of greater bare ground cover for larval activity, (Ashton et al., 2009; Merrill et al., 2008; Roy & Asher, 2003) or life history adaptations such as reduced number of instars or generations (Hodkinson, 2005).

Duration of the period of activity

Our results support a 8-day decline in the duration of the flight period of butterfly community along the altitude (**Fig. 1b**) supporting previous studies that also report declines in Spain (de Arce Crespo & Gutiérrez, 2011; Illán et al., 2012). On the contrary, we found no such evidence when analyzing patterns at species level (**Table 2**). Although

there was a tendency for relationships between duration and altitude to generate negative slopes (8 out of the 12) none of them was found to be significant. Consequently the general pattern observed for the whole butterfly assemblage is certainly not influenced by the subset of the individual species tested. An alternative explanation is that multivoltine species complete fewer generations per annum at the higher altitudes as a result of their adaptive strategy to maintain sunchrony with the phenology of their host plants (Tauber et al., 1986). Investigating this alternative, we were able to provide some evidence: we repeated the regression between the duration of flight period and altitude considering only the univoltine species in the analysis but no progressively shortening of the duration of the flight period with altitude was detected in this case (P = 0.13).

Phenological patterns between ecoregions

Troubling from a biological, if not statistical, perspective is our finding that there is a significant differentiation on elevational delays of butterfly assemblages between the two ecoregions (Grammos and Rodopi). In particular, Rodopi showed a bigger delay of 30days for every km increase in elevation compared to 16-days in Grammos, when the temperature lapse rate (decrease) we recorded for Grammos in 2013 (-4.58°C/km) was almost double from the one recorded in Rodopi in 2012 (-2.7°C/km). Indeed, climate in Grammos is of the mountainous type with a mean annual temperature of 10° C and a mean annual precipitation of 1500 mm, when for Rodopi, the respective values are 11.4°C and 1200mm. In addition Rodopi, although at higher latitude, is located in close vicinity to the sea creating a mixture of Mediterranean and continental climate when Grammos is located at the north-west edge of Pindos mountain range with a humid continental climate (Korakis, 2002; Xirouchakis, 2005). A possible explanation for this contradictory pattern, is that species whose activity periods occur earlier in the year show greater advances in timing of activity than those that are active later (Altermatt, 2012; Forister & Shapiro, 2003) leading thus to steeper slopes and bigger delays as the ones observed for Rodopi. However, we excluded data recorded in April from the analysis (first sampling in Rodopi) in order to standardize the length of the sampling periods between ecoregions and therefore we minimized the possibility of early emergence species to influence the relationship between the mean date and altitude. Yet still, a significant differentiation of the rate of delays between the two ecoregions was found.

Furthermore, we looked for abundant species that could be amenable for the observed pattern, influencing disproportionaly altitudinal delays and also species with different contribution due to the later emergence along the altitudinal gradient (de Arce Crespo & Gutiérrez, 2011). However, the three most abundant species in Rodopi region, Polyommatus icarus (407), Coenonympha pamphilus (351) and Colias crocea (303) counting for 21% of the total records, were present at both transects of low and high altitude (127-1745m; 127-1458m and 127-1745m respectively) and had more than one broods covering the whole sampling period (from May to August) suggesting no such effect. Further research in testing the consistency of the pattern observed per ecoregion and also the establishment of permanent meteorological stations within each region may be needed to enlighten our findings. Analyses for relationships between the flight period and the temperature of preceding months (e.g. February and March) would be particularly useful given that higher temperature in those months tend to produce significant advances in species' flight period (Stefanescu et al., 2003). Apart from temperature, other environmental factors such as precipitation, cloud cover or wind velocity are often associated with altitude (Körner, 2007) and further research on these relationships would be desirable.

Predictions from phenological responses

An advantage of studying insects phenology along altitudinal gradients is the use of their responses to the local climatic conditions to predict the effects of future climate change (Fielding et al., 1999). Based on different climatic scenario proposed for the Eastern Mediterranean and the Middle East (EMME), the mean temperature rise will be about 1-3°C in the near-future (2010 - 2039), 3-5°C by mid century (2040 - 2069) and 3.5-7°C by the end of century (2070 - 2099) (Lelieveld et al., 2013). Under the first scenario, butterfly phenology would be advanced by 5–15 days in terms of community and by 6.6–20 days in terms of individual species tested; under the second scenario by 15-25 days and 20-33.3 days respectively and under the third by 17.5-35 days and 23.1-46.2 days. Advanced emergence suggest that climate change has the potential to disrupt trophic interactions and cause phenological mismatches between groups (e.g. butterflies and their host-plants) if one of them (e.g. host-plants) does not react in a similar manner to global change, having

thus important effects on survival and population growth of the other (e.g. butterflies) (Parmesan & Yohe, 2003; Schweiger et al., 2008).

Trading space for time is commonly used to study phenotypic responses of both communities and species across altitudinal gradients and can further our understanding of their biology providing also clues to the likely responses to environmental change (Byars et al., 2007; Riba et al., 2009). However, our findings showed signals of geographic variation on the phenology of butterfly community as well as a great amount of species which had well-synchronized their mean flight date to the altitudinal gradient. Especially when lacking the knowledge of the temporal relation between temperature and phenology and local adaptation is suspected, it is recommended that care must be taken in the use of space-for-time substitutions (La Sorte et al., 2009; Phillimore et al., 2010). Nevertheless, due to lack of long-term time-series data especially in Mediterranean (but see Stefanescu et al., 2003), the limited resources and the increasing interest in documenting and forecasting the ecological impact of climate change, space-for-time substitution is a widely used (e.g. de Arce Crespo & Gutiérrez, 2011; Illán et al., 2012; Leingärtner et al., 2014). In a recent study, monitoring data were used to test whether spatial data can be substituted for temporal data in forecasting models and results supported the validity of the space for time substitution approach and its effectiveness without sacrificing the accuracy of the model's predictions (Banet & Trexler, 2013).

Conclusions

Our study demonstrated that there was a delay in timing of appearance of butterfly community and a shortening on the duration of flight period along the altitudinal gradient. Although delays were found for half of the individual species tested, the other half was found to emerge synchronously along the altitudinal gradient. In addition, signals for a significant differentiation on the rate of delays of butterfly communities between the two ecoregions confirm the great variability of phenological responses. Our results imply that elevational gradients can be important predictors of phenological responses to climate change, especially in the lack of time-dependent records, but they also suggest that further investigation of the underlining mechanisms by which elevation and its attributes influence phenology may aid in gaining a more consistent insight in the study system and better understanding species'responses to current and future climate change.
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Supplementary information

Table S1. Sampling design: proportions (%) of Corine cover and transects per elevational zone, habitat type and ecoregions; where Zone 1=0-500m, Zone 2=501-1500m, Zone 3=1001-1501m, Zone 4=1501-2000m. Inside the parenthesis is the actual number of transects (replicates) (see Methods and Materials for details).

| | | Grammos | | Rodopi | |
|--------|-------------|--------------|-----------|--------------|-----------|
| | | Corine cover | Transects | Corine cover | Transects |
| Zone 1 | Agriculture | 40.24 | 33.33 (2) | 51.42 | 38.46 (5) |
| | Forests | 37.8 | 33.33 (2) | 24 | 46.15 (6) |
| | Grasslands | 20.73 | 33.33 (2) | 17.37 | 15.38 (2) |
| Zone 2 | Agriculture | 4.8 | 33.33 (2) | 8.89 | 20 (2) |
| | Forests | 62.66 | 33.33 (2) | 66.06 | 50 (5) |
| | Grasslands | 31.81 | 33.33 (2) | 24.74 | 30 (3) |
| Zone 3 | Agriculture | 1.19 | 25 (2) | 0.38 | 18.18 (2) |
| | Forests | 68.95 | 25 (2) | 79.84 | 27.27 (3) |
| | Grasslands | 29.85 | 50 (4) | 19.64 | 54.54 (6) |
| Zone 4 | Agriculture | 0 | 0 (0) | 0 | 0 (0) |
| | Forests | 43.75 | 28.57 (2) | 57.39 | 28.57 (2) |
| | Grasslands | 56.25 | 71.42 (5) | 42.6 | 71.42 (5) |

Steps for the calculation of Corine cover

1. We clipped corine layer (available for the entire Greece) to each ecoregion perimeter

2. We grouped the corine codes occurring in each ecoregion into five main categories: artificial areas, agriculture areas, forests, grasslands and water (e.g. lakes, rivers etc

3. We clipped again the dissolved corine layers to the elevational zones and calculated the proportions for the three habitat types used in our study system: agriculture area, forests and grasslands.

To test whether there is a consistency between the corine cover and transects we used per habitat type within elevational zones and ecoregions, we run paired t-tests and the resulted large p-value (P = 0.9) suggested that the data are consistent with the null hypothesis, namely the proportions of corine cover and the proportions of the transects we used per habitat type per elevational zone.



Figure S1. Relationships of mean date (days elapsed from 1 January, 1=1 January) and duration of the flight period (standard deviation about the mean date) with altitude (km) for each individual species tested (12 species). Only significant relationships have been drawn. Each dot corresponds to a sampling transect.

- Chapter 6 -

Synopsis

Even though, it is now well-established that climate change is a major threat to biodiversity (Thomas, 2005), there are still regions where evidence is missing, due to restricted funding, expertise and time resources to monitor. One of these regions is the Mediterranean Basin, a hotpsot of biodiversity (Blondel et al., 2010), where monitoring schemes are largely missing (but see Marini et al., 2012; Stefanescu et al., 2003; Van Strien et al., 2008) and therefore observations of species responses to climate change or provisions for future consequences on species composition, abundances and distribution are simply lacking. The present study has been conducted in Greece (see Figure 1, Chapter 1) and attempts to give answers to current occurring climate-induced changes using butterflies and Orthoptera as model systems. Significant changes in butterfly composition along a 13-year and regional temperature rise (Chapter 2), phenological advancement for both butterflies and Orthoptera (Chapter 3), high butterfly and Orthoptera species turnover along elevational zones (Chapter 4), and significant phenological delay of butterfly community as well as a shortening in the duration of flight as altitude increase (Chapter 5), constitute the milestones of this study.

Part A. Species and communities' responses to climate change: a time dimension

(1) Has mean annual temperature trend significantly increased since the 1990s

(2) Has butterfly community composition and species richness have changed across a thirteen-year period in response to climate warming in this area, given that is largely free of major changes to land use?

Butterfly community composition changed significantly over the 13-year period (1998-2011) in Dadia-Leukimi-Soufli national park (Dadia NP hereafter) in conjunction with regional temperature increase (see Figure 2, chapter 2). Over the same time period, significant changes in the abundance of regionally high- versus low-altitude species were also found. Of course, it is well known that there are changes over all timescales in temperature time-series due to local or regional changes that need not be attributed to a prevailing global-warming trend (Filz et al., 2013). However, the protected status of Dadia NP and the subsequent stability of land use regimes over the last decade (see Introduction, chapter 2), suggested that our results are nonetheless consistent with what

is expected from the global warming interpretation. Adding the fact that our analysis gave no evidence of significant year-to-year variability in butterfly community composition, as well as a significant increase in the butterfly Community Temperature Index, an index based on the thermal associations of species' distributions in Europe (see Figure 3, **chapter 2**), all these findings are potential signals of butterfly species responses to climate change in the Eastern Mediterranean basin. Surprisingly, our results regarding the Community Temperature Index (CTI hereafter), did not agree with the ones observed in Spain (Stefanescu et al., 2011b), but they were consistent with similar patterns of CTI observed in northern Europe (Devictor et al., 2012; van Swaay et al., 2011; Van Swaay et al., 2012). A very interesting finding was that butterfly community in agriculture land changed from hotter (first survey, 1998) to cooler (second survey, 2011) overwhelming the general CTI increase. We attributed this pattern to both the presence of natural hedges and tree lines at field edges as well as to the irrigation system which recently has proved to provide favourable conditions for butterfly communities under Mediterranean climate (González-Estébanez et al., 2011). Therefore, to increase species resilience to climate change, we recommend the conservation of traditional small agricultural plots with hedges and tree lines and the maintenance of artificial "cooler" microhabitats through irrigation system (Asher et al., 2001). We also propose the expansion of the existing reserve's borders towards higher altitudes to effectively accommodate the possible distributional movement of species to upper altitudes and latitudes, as this was partially observed (Hickling et al., 2006; Huntley et al., 2008; Lenoir et al., 2008; Root et al., 2003; Wilson et al., 2007). The documented signals even in this relatively short period of the 13 years, compared to the longer time period that is probably needed to detect changes in species richness or communities in cold ecosystems of north latitudes (Settele et al., 2008), underline the necessity for systematic research hotter, low latitude, Mediterranean ecosystems.

(3) Did phenology change significantly for a period of 13 and 12 years for butterflies and orthopterans respectively (1998-2011: butterflies, 1999-2011: orthopterans)?

(4) Is the rate at which the phenology changed consistent for butterflies and orthopterans?

Based on the signals of species responses to climate change we found in Chapter 2, we further investigated phenological patterns in Dadia NP and how these changed over time,

across different habitats and along environmental gradients (Chapter 3). For this scope, we used a novel approach (Standardized Major Axis technique) in a phenological study. Even though insects undergo phenological change at different rates (Primack et al., 2009), we found that the rate at which the phenology changed was consistent between butterflies and Orthoptera. On the other hand, a concurrent advancement of the mean date of butterfly and orthopterans' emergence over a 13 and a 12 year period respectively, seem to be in accordance with previous findings (Illán et al., 2012; Nufio et al., 2010; Wilson et al., 2005).

(5) Is there a significant effect of habitat type (grasslands, forests and agriculture) on phenological patterns?

(6) Is there a significant effect of habitat-specific variables (microclimatic: temperature, humidity) on species phenology per habitat type?

(7) Whether the phenological patterns of both target groups are congruent vis-a-vis the environmental gradient of canopy cover.

A new insight of butterfly phenological responses was gained, when responses were tested between different habitats (see Table 1, chapter 3) and when habitat-specific climatic variables (temperature and humidity) were taken into consideration (see Table 2, chapter 3). It is concluded that the duration of the flight period can be influenced by the amount of habitat's openness (García-Barros, 2000) and nectar availability until late summer (Zografou et al., 2014). Given that adult butterflies use nectar resources as they become available during the season (Shreeve, 1992; Stefanescu et al., 2009), earlier adult appearance in grasslands and later in forests may be an adaptation to the different flowering periods of plants in each of the two habitats. What is more revealing is the possible adaptation of butterfly species' thermal tolerance, resulting from the different range of microclimatic conditions recorded per habitat. It has been shown that each habitat has a distinct thermal character which probably could shape species' thermal tolerance (Suggitt et al., 2011). However, there is a relative uncertainty of whether the pattern observed is an artefact resulting from differences in the microclimates associated with different habitats or an actual species adaptation. Although we did not find a congruent phenological pattern between the two taxonomic groups along the canopy gradient, we did find butterflies to gradually delay their mean date of appearances as canopy cover increases.

It is concluded that further studies would elucidate to what extent habitats' thermal range can influence species thermal tolerance disentangling the rate of species phenological responses from possible artefacts. Nevertheless, Chapter 3 demonstrated the utility of SMA analysis in phenological studies and assessed the need to incorporate both habitat type and habitat-specific variables that refine species' phenological responses.

Part B. Species and communities' responses to climate change: spatial dimension

As climate alters globally, in Mediterranean Basin, climate also changes along altitudinal gradient in mountainous ecosystems and when travelling from west to east (Blondel et al., 2010). Especially for a biodiversity hotspot like Greece (Balletto & Casale, 1991) where monitoring schemes are scarce and species conservation status problematic, we need a powerful tool to quantify spatial variation in biodiversity across complex systems such as mountains. In chapter 4 we build a hierarchical model (Veech et al., 2002) for two mountainous areas (Rodopi and Grammos) and we defined the species turnover herein (Gering et al., 2003) interpreting also the driving forces for the patterns observed (Marini et al., 2012; Ribeiro et al., 2008). The results from this chapter acted as the springboard for the next chapter, where butterfly phenological responses to climate change were further analyzed and future predictions were estimated (chapter 5).

(1) How diversity patterns of butterfly and Orthoptera are distributed across different spatial scales (ecoregions, elevational zones, habitats, transects/plots)

(2) Is there a consistent pattern between the two insect groups

(3) Whether species assemblages within each group (common, rare species) are responsible for the observed patterns of diversity

Scale dependent differences in spatial patterns of diversity such as those detected for butterflies and Orthoptera in chapter 4 have rarely been observed in Mediterranean ecosystems. Even if our results showed incongruent diversity patterns limiting the surrogate value of butterflies for orthoptera and *vice versa*, beta diversity at larger scales (ecoregion and elevational zones) proved to have a great influence in both groups (**see Table 1, chapter 4**). It is therefore suggested that regional designation of nature reserves should cover the whole range of the elevational gradient within mountainous systems

(Kattan et al., 2006). In addition, our results showed that common species appeared to be much more indicative in terms of the total diversity compared to rare species, although the latest are more prone to extinction and special attention is usually paid to their distribution patterns (Stefanescu et al., 2011a). Due to the great impact of the factor "mountain or ecoregion" in diversity patterns between butterflies and Orthoptera but not between common and rare species, regional monitoring programs might need to adapt different strategies with respect to the focal organisms (butterflies or orthoptera), whereas a single strategy for both mountains will likely serve for monitoring both common or rare species (Pearman & Weber, 2007). Evaluating our results, it is concluded that this study will benefit future research and will serve as useful guide for the monitoring designation of similar montane systems throughout Greece.

(4) Whether butterfly species have a gradual delay in the timing and a progressive shortening on the duration of the flight period as we move to upper altitudes at community and species level

(5) Whether there is a significant differentiation on elevational patterns of butterfly communities between the two mountainous areas and across the different habitat types

Combining the great impact of altitude on species turnover (Chapter 4), the great need for ecological forecasting in the light of further temperature rise and the lack of high quality long-term data, we attempted to substitute space-for-time, assuming that the spatial relationship between the environmental factor (e.g. altitude) and the response variable (e.g. time of species appearances) can be used as a proxy for the temporal relationship (Banet & Trexler, 2013).

For the whole butterfly species pool we found a 16-day delay in the mean date per 1km increase in altitude, while for individual species investigated, the average shift was 20 days, broadly close to the one reported in California (Forister & Shapiro, 2003) or in Britain (Roy & Sparks, 2000). In addition, a progressively shorter flight period (8 days) of butterfly community along the altitude (see **Figure 1b, Chapter 5**) supported previous studies that also reported declines of butterfly's flight period in Spain (de Arce Crespo & Gutiérrez, 2011; Illán et al., 2012). Based on different climatic scenario proposed for the Eastern Mediterranean and the Middle East (EMME), the mean temperature rise will be about 1-3°C in the near-future (2010 - 2039), 3-5°C by mid century (2040 - 2069) and 3.5-

7°C by the end of century (2070 - 2099) (Leingärtner et al., 2014). Under the first scenario, butterfly phenology would be advanced by 5–15 days in terms of community and by 6.6–20 days in terms of individual species tested; under the second scenario by 15-25 days and 20-33.3 days respectively and under the third by 17.5-35 days and 23.1-46.2 days. Constituting space-for-time is well-used technique in phenological studies, when long-term data are unavailable (Illán et al., 2012). Here, we demonstrated that this technique, although seriously criticised (Isaac et al., 2011), can further our understanding of butterflies biology providing also clues to the likely responses to environmental change (Byars et al., 2007; Riba et al., 2009). Of course, some evidence of geographic variation on the phenology of butterfly community which does not seem to agree with the temperature lapse rate we recorded, as well as a possible synchronization of individual species on their mean flight date to the altitudinal gradient may need further investigation.

Although some of our findings raise some more questions that needed to be further investigated in order to fully explain the patterns observed, undoubtedly, this thesis will act as the beginning of upcoming studies that are necessary to forecast future impacts of the likely consequences of extreme climate scenarios anticipating in the Mediterranean Basin and it is likely to affect severely a vulnerable part of our fauna, i.e. insects.

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Publications

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Under review or in preparation

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