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Πληθυσμιακή οικολογία και γενετική εκπροσώπων του γένους *Campanula* σε ορεινούς όγκους της Ελλάδας: το κέντρο και τα άκρα της κατανομής.

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Population ecology and genetics of *Campanula* genus representatives in Greek mountainous regions: The centre and edges of species distributions

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PROLOGUE

This thesis was concluded in the Department of Biological Applications and Technology of the University of Ioannina. Collection of data took place in the areas of Mt. Olympus, Mt. Falakro and Mt. Smolikas. Molecular analysis of data was performed the University of Ioannina Molecular Oncology Lab under Prof. Evangelos Briasoulis

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ΠΕΡΙΛΗΨΗ

Τα πρότυπα διακύμανσης αφθονίας φυτικών ειδών στον χώρο έχουν υπάρξει αντικείμενο εκτεταμένης έρευνας. Οι κατανομές αφθονίας στη βιβλιογραφία είναι ισχυρά συσχετισμένες με περιβαλλοντικές κλίνες όπως η υγρασία και το υψόμετρο. Σύμφωνα με την Υπόθεση Αφθονίας Κέντρου (Abundant Centre Hypothesis – ACH), τα είδη είναι πιο άφθονα στο κέντρο της κατανομής τους – που ορίζεται ως το σημείο που συναντούν τις βέλτιστες συνθήκες για την επιβίωση και την αναπαραγωγή τους κατα μήκος των περιβαλλοντικών αυτών κλινών. Καθώς προχωράμε προς τα άκρα, οι πληθυσμοί γίνονται όλο και μικρότεροι, καθώς οι συνθήκες δεν είναι πλέον ευνοϊκές. Η κατανομή «Αφθονίας Κέντρου» έχει υπάρξει βάση για πολλές υποθέσεις αναφορίκα με οικολογικές και εξελικτικές διεργασίες όπως η διαδικασία των εξαφανίσεων, ο ενδοειδικός και διαειδικός ανταγωνισμός, η ειδοποίηση και η γενετική διαφοροποίηση, οι οποίες καθορίζουν τα πρότυπα παρουσίας και αφθονίας των ειδών στον χώρο.

Έγινε καταγραφή πληθυσμών ειδών *Campanula* (*C. lingulata, C. spatulata, C. rotundifolia*), σε διατομή 74 περίπου χλμ. κατα μήκος της υψομετρικής κατανομής των ειδών, στην περιοχή του Ολύμπου. Η δειγματοληψία πραγματοποιήθηκε κατα την θερινή περίοδο, τα έτη 2012 και 2013. Η παρουσία ειδών χαρτογραφήθηκε σε διαφορετικές χωρικές αναλύσεις που κυμαινόταν απο 10 τ.μ έως ~ 10 τ.χλμ. Συνολικά, έγινε καταγραφή 1.130 και 3.897 ατόμων για την *C. lingulata,* 1.234 και 1.291 για την *C. spatulata* 989 και 659 για την *C. rotundifolia* για το 2012 και 2013 αντοίστιχα. Τα περισσότερα άτομα *C. lingulata* και *C. spatulata* εντοπίστηκαν στην Βορειοανατολική και Νοτιοανατολική πλαγία του βουνού, ενώ παρουσία *C. rotundifolia* παρατηρήθηκε μόνο σε υψηλά υψόμετρα.

Ανάλυση ANCOVA της παρουσίας ειδών ως συνάρτηση της μέσης πλυθησμιακής πυκνότητας σε διαφορετικές χωρικές αναλύσεις έδειξε σημαντικές διαφοροποιήσεις αναφορικά με τα πρότυπα «συσσωμάτωσης», και σχετικά μεταξύ ειδών, καθώς και μεταξύ έτους δειγματοληψίας. Η κατανομή της *C. rotundifolia* παρουσίαζεται περιορισμένη σε σχέση με τις κατανομές της *C. lingulata* και *C. spatulata,* ενώ η *C. spatulata* παρουσιάζεται πιο «συσσωματωμένη» σε σχέση με την *C. lingulata* στις αδρότερες χωρικές αναλύσεις.

Η κατανομή αφθονίας της *C. lingulata* κατα μήκος της υψομετρικής διαβάθμισης παρουσίαζει ενα δικόρυφο πρότυπο (μια κορυφή στα 650 - 750 μ και μια στα 1,100 - 1,300 μ). Εμφανίζεται μια άποτομη πτώση της πλυθησμιακής πυκνότητας στα 1,300 - 1,500 μ, που μπορεί να αποδοθεί στην αλλαγή της περιβάλλουσας βλάστησης. Παρατηρείται κατανομή

«Αφθονίας Κέντρου» για το 2013, αλλα δεν παρατηρείται παρόμοιο πρότυπο για το 2012, οπότε δεν μπορούμε ούτε να απορρίψουμε, ούτε να επιβεβαιώσουμε την ACH κατα μήκος της υψομετρικής διαβάθμισης για το είδος υπό μελέτη.

Η μέση παρουσία ατόμων *C. lingulata* παραμένει σταθερή στις διαφορετικές χωρικές κλίμακες, κατι το οποίο υποδηλώνει κατανομή fractal, ενώ η μέση αλλαγή του αριθμού των ατόμων για το 2012-13 είναι μεγαλύτερη απο την αναμενόμενη.

Ταυτόχρονα, εγίνε συλλογή δειγμάτων πληθυσμών *C. lingulata* πρός μοριακή ανάλυση γενετικού υλικού (DNA) κατα μήκος της υψομετρικής διαβάθμισης του όρους Ολύμπου και όρους Φαλακρού, στα οποία εφαρμόστηκε η τεχνική RAPD (Random Amplification Polymorphic DNA). Τα αποτελέσματα έδειξαν σημαντική διαφοροποίηση μεταξύ των πληθυσμών. Η διαφοροποίηση μεταξύ πλυθησμών (Φ_R) εκτιμήθηκε στο 0.131, τιμή μικρότερη απο τις αναμενόμενες σύμφωνα με την σχετική βιβλιογραφία. Η ενδοπληθυσμιακή διαφοροποίηση (H_O) εκτιμήθηκε στο 0.306 για τον Όλυμπο και και 0.301 για το Φαλακρό. Ηεκτιμήση της H_O είναι μεγαλύτερη συγκριτικά με την σχετική βιβλιογραφία, και ενδεικτική υψηλής γενετικής «υποδομής - στρωμάτωσης» και διαφοροποίησης των πληθυσμών στις περιοχές μελέτης. Παραύτα, τα άτομα που προερχόταν απο τις χαμηλότερες και μέσου ύψους περιοχές της υψομετρικής διαβάθμισης στον Όλυμπο είναι πιο όμοια μεταξύ τους, ενώ τα άτομα των μεγαλύτερων υψομέτρων παρουσιάζουν ομοιότητες με τα άτομα που συλλέχθηκαν απο το Φαλακρό – στοιχείο που ίσως να αποτελεί ένδειξη κάποιας γενετικής διαφοροποίσης σε χαρακτηριστικά που σχετίζονται με την προσαρμογή στην υψομετρική διαβάθμιση.

Η εκτεταμένη παρουσίαστης Υπόθεσης Αφθονίας Κέντρου στην βιβλιογραφία καθώς και η ευρεία χρήση της ως βάση σε οικολογίκες και εξελικτικές θεωρείες είναι ενδεικτική του τρόπου με τον οποίο οι σύγχρονοι ερευνήτες προσεγγίζουν τις κατανομές ειδών στο χώρο, και υποδηλώνει οτι το συγκεκριμένο πρότυπο είναι ευρέως διαδεδομένο σε φυσικούς πλυθησμούς ειδών. Παραύτα, σε αρκετές περιπτώσεις δεν μπορούμε να επιβεβαιώσουμε την οικουμενικότητα του «Άφθονου Κέντρου», καθώς και των μηχανισμών και διεργασιών που διαμορφώνουν τις κατανομές της αφθονίας των είδων στον χώρο με την εμπειρική παρατήρηση. Το κενό αυτό έρχεται να γεμίσει ενα εναλλακτικό θεωρητικό πλαίσιο, στο οποίο παράγοντες που δρούν σε διαφορικές χωρικές κλίμακες με διαφορική ένταση, διαμορφώνουν τις κατανομές των ειδών στο χώρο. Ο «θόρυβος» που προκύπτει απο την διεργασία αυτή δεν μπορεί να αποδωθεί σε έναν συγκεκριμένο παράγοντα που δρά σε μια χωροχρονική κλίμακα, και υπάρχει περίπτωση να υπερκαλύπτει την επίδραση των περιβαλλοντικών παραμέτρων που θα μπορούσαν να μας δώσουν στοιχεία αναφορικά με

πρότυπα κατανομής όπως αυτό του «Άφθονου Κέντρου». Η χρήση εργαλείων της γεωμετρίας των fractal η οποία είναι συνυφασμένη με την έννοια της κλίμακας, αποτελεί μια καλή εναλλακτική προσέγγιση.

SUMMARY

Patterns of spatial variation in abundance for plant species are the focus of extensive investigation. Their abundance distributions have long been associated with environmental gradients, such as moisture or elevation. According to the "abundant centre" hypothesis (ACH), species are more abundant towards the centre of their distribution along environmental gradients, where they meet optimal conditions for their survival and reproduction. As we move toward the edge, their numbers decline, since conditions are no longer favorable. The species' "abundant centre" distribution has been the basis for many speculations on ecological and evolutionary processes — such as extinction, intra/inter-specific competition, speciation and genetic differentiation — that dictate the observed patterns of species' occurrence and abundance across space.

Populations of *Campanula* species (*C. lingulata, C. spatulata, C. rotundifolia*) where recorded along transects of approximately 74 km, across each species' altitudinal range at the area of Mt. Olympus, Greece. Sampling took place during the summer months of 2012 and 2013. The species occurrence was mapped in various spatial resolutions ranging from 10 m to ~10 km grid squares. A total of 1,130 and 3,897 individuals were recorded for *C. lingulata*, 1,234 and 1,291 for *C. spatulata*, and 989 and 659 for *C. rotundifolia*, in 2012 and 2013, respectively. Most individuals were recorded at the NE and SE part of the mountain for *C. lingulata* and *C. spatulata*, while *C. rotundifolia* was only recorded at high elevations.

An ANCOVA analysis of each species occupancy as a function of mean density across different spatial resolutions has shown significant differences in terms of the species' "aggregation" patterns, both relative to each other, and for each year of sampling. *C. rotundifolia* occurrence is more restricted than those of *C. lingulata* and *C. spatulata*, while *C. spatulata* individuals appear significantly more aggregated relative to *C. lingulata* at the coarsest resolutions.

C. lingulata abundance along the altitudinal gradient produces a 2-peak pattern (one at 650 – 750 m, and one at 1,100 – 1,300 m of altitude). There is an abrupt decline in density at 1,300

to 1,500 m, which may be attributed to the change in the surrounding vegetation. An "abundant centre" for the species distribution can be observed in 2013, however no such pattern occurs in 2012, hence the ACH along an altitudinal gradient cannot be verifyied.

Mean presence of *C. lingulata* individuals appears constant across different spatial scales, which is indicative of a fractal-like distribution, while mean population turnover for this species appears greater than expected.

C. lingulata individuals from across the species altitudinal range were collected for molecular DNA analysis. The Random Amplification Polymorphic DNA (RAPD) technique was applied for samples from Mt. Olympus and Mt. Falakro. This showed substantial differentiation of the species' populations. Recorded between-population diversity Φ_{RT} was estimated at 0.131, which is lower than expected from similar studies. Within-population H_O was estimated at 0.306 and 0.301, for Mt. Olympus for Mt. Falakro, respectively. Within-population diversity was higher in comparison to similar studies and indicative of high genetic structure and variability of the specie's populations across the areas of study. Nevertheless, Mt. Olympus individuals from lower and middle parts of their altitudinal range appear more similar, whilst individuals from Olympus's higher altitudes display similarity to Mt. Falakro individuals, suggesting a differentiation of the species' genetic traits along an altitudinal gradient as well.

The persistence of the abundant centre concept in the literature and its ubiquity in ecological and evolutionary theories expresses deeply embedded ideas held by ecologists about how populations should be distributed and suggests that the pattern should be widespread in natural populations. However, every so often, we fail to detect it. The lack of empirical studies that support intuitive notions (such as the ACH) about the mechanisms that generate the observed patterns of abundance in species distributions calls for an alternative conceptual framework. Multiple factors operating at various spatial scales with varying intensity generate the observed patterns of species distributions across space. The generated "disturbance"—which, cannot be readily attributed to a single factor operating at a single spatial scale and temporal dimension— is bound to superimpose the effect of environmental drivers that could provide us with evidence of underlying patterns such as that of the intuitive "abundant centre" of species' abundance distributions. A fractal framework — wherein the factor "scale" is inherent — might constitute a more suitable approach.

A.INTRODUCTION

I. The "abundant centre" hypothesis

1. Observed patterns of variation in abundance

"How environmental conditions and population processes determine the abundance and distribution of species is a central problem in ecology and biogeography" (Brown, 1984).

Patterns of variation in abundance for many species and of a variety of taxa have been the focus of extensive investigation. Numerous theoretical frameworks, within which researchers have been attempting to explain the observed patterns of species' abundance, have been proposed. One of the most prominent ones, proposed by Brown (1984), is the "abundant centre" hypothesis (ACH). Within this framework that essentially links the observed abundance and spatial distribution of a species to its ecological requirements through variable mechanisms that shape species' dynamics, Brown managed to establish a theory which has shaped the scientific thought ever since, by providing background to investigations regarding many aspects of species' ecological and evolutionary attributes.

Brown based a lot of his observations on Whittaker's (1956, 1960, 1965) empirical studies of vegetation, across environmental gradients of moisture and elevation. He noted that, although individual species attain different maximum densities in different parts of the environmental gradients, abundances of most species decline relatively gradually and symmetrically with increasing distance in either direction from their peaks. The species' abundance in response to environmental gradients resemble Gaussian distributions, since Whittaker routinely fitted his data on plant distributions with normal curves. Nevertheless they do differ highly significantly from random or uniform distributions, exhibit a strong central tendency and are neither highly skewed nor strongly leptokurtic or platykurtic (Brown, 1984). Such patterns have not only been recorded to occur across environmental gradients for plants, but for other species as well. Notably, Field & Robb (1970) noted similar patterns for invertebrates within gradients of intertidal exposure in the northern Gulf of California. In other words, Brown inferred that species tend to decline in abundance as we move along a gradient from the species' distribution "centre", in a gradual fashion.

The "abundant centre" pattern for local population densities seems to extend beyond environmental gradients, to species' geographic ranges, with a gradual decline from the distribution centre towards the species' geographic range limits. Such patterns have been

noted for birds with data from censuses from the North American Breeding Bird Survey (roadside, count-based survey by volunteers, which began in 1966), when density is plotted as a function of distance along four transects through the widest part of the species' range, in the four major compass directions (Brown, 1984).

Brown (1984) also notes that rapid changes in density within a species' range are sometimes associated with abrupt discontinuities in the physical environment (bodies of water, transition between different soil types). In addition, when suitable habitat occurs in isolated patches, there are multiple modes in the distribution of abundance over space (Brown, 1984). On a sufficiently small scale, the environment of most organisms is patchy, so population density should exhibit a multimodal distribution over space. Hengeveld (1989) suggests that such distributions of densities occur at local scales as at regional ones. Thus, the abundance structures of the geographical ranges of species can be visualized as a series of abundance peaks, with the magnitudes of these peaks declining towards the range periphery (Gaston *et al*., 1997). Examples of discontinuous variation in abundance and precipitous declines in population density at range boundaries appear to be associated to an abrupt change in a single environmental variable, either a physical factor or the population density of an intensively interacting species of competitor, predator or prey (competitive exclusion) (Brown, 1984).

Finally, Brown (1984) observed that species that have the highest local population densities also tend to inhabit a greater proportion of sample sites within a region and to have wider geographic ranges; conversely species that are always rare also have restricted spatial distributions. He also noted a highly significant positive correlation between average density within a site, and the number of different local sites where the species was found.

2. The formulation of a theoretical background for the underlying mechanisms.

In an effort to provide a theoretical framework for the observed patterns of variation in abundance across space, Brown (1984) proposed the "abundant centre" hypothesis (ACH), which was based on three fundamental assumptions.

- ***Ecological requirements of species.***

Brown assumes that combinations of many physical and biotic variables that are required for survival and reproduction of its individuals determine the abundance and distribution of each

species. These requirements define the dimensions of Hutchinson's (1957) multidimensional niche for each species. Variations in population density of a species over space are assumed to reflect the probability density distribution of the required combinations of environmental variables (Brown, 1984)

The fact that so many empirical distributions resemble normal curves might be due to the fact that the normal probability density function is the limit distribution of a sum of random variables (Brown, 1984).

- ***Spatial variation in the environment.***

The spatial variation in the environment has both stochastic and deterministic components. Some sets of variables are distributed independently of each other and there is a significant degree of apparently random local variation. Environmental variation is also autocorrelated so that the probability of sites having similar combinations of environmental variables is an inverse function of the distance between them (Brown, 1984).

From the first two assumptions follows that population density should be highest near the center of a species range and should decline toward the boundaries. For each species there should be one most favorable site, where population density should be greatest, because the combination of environmental variables most closely corresponds to the requirements of the species. If spatial variation in the environment is autocorrelated, then with increasing distance from this site, the environment will become progressively more different, niche requirements of the species will be met less frequently, and abundance will decline. There will be a decreasing number of local sites where individuals can occur at all, and even within these patches population densities will tend to be lower because resources are scarce and/or approach the limits that can be tolerated. In other words, the exact form of spatial variation in abundance will depend on the number and kind of environmental factors that comprise the niche and on the spatial pattern of variation of these variables (Brown, 1984).

- ***Ecological requirements of closely related species.***

This refers to the extent to which species vary in their requirements. Closely related, ecologically similar species differ substantially in only one or a very small number of niche dimensions. This differentiation reflects evolutionary constraints on morphology, physiology and behavior as a result of relatively recent descent from a common ancestor (Brown, 1984).

Brown notes that in order to account for the general relationship between abundance and distribution, it is necessary to understand not only the multidimensional nature of the niche but also the spatial variation in the dynamics of population growth and regulation. Grinnell (1922) and Wiens & Rocenberry (1981) suggested that the geographic distributions of bird populations may represent a dynamic equilibrium between the export of emigrants from source areas, where birth rates exceed death rates, and the importation of these individuals into sink areas — usually regions at the periphery of the range where these continual immigration sustains local populations whose death rates exceed birth rates (Brown, 1984). Hengeveld (1993) highlighted the need to consider the dynamic aspects of abundance distributions as well. He deemed that abundant centre patterns would arise in response to risk surfaces that identify threats to individual survival among and between generations in different parts of the range, while Lennon *et al.* (1997) demonstrated that an abundant centre could arise as metapopulations respond to extinction gradients.

3. The "abundant centre" and contemporary scientific thought

The abundant centre distribution has widely been used as the basis for hypotheses about ecological and evolutionary processes involving the edge dynamics of populations. Such hypotheses directly address many fundamental issues in ecology and evolution, such as how genes flow between populations, as well as applied ecological issues, such as how populations will respond to climate change and what populations should be the focus of limited conservation resources (Sagarin & Gaynes, 2002).

i) Population dynamics and extinction potential

Brussard, in his 1984 study, discussed the observed geographic patterns of *Drosophila* sp. populations along dominant environmental gradients in terms of their evolutionary and adaptation potential under the different selection regimes in core and peripheral habitats.

Populations within the central part of the gradient curve should be able to inhabit a variety of ecotopes. The densities reached in these different ecotopes will reflect their relative favorableness at any one time (Brussard, 1984). There will be high colonization rates in less favorable habitats within such zone, with the resulting population reaching occasionally high densities. However, these populations are transient, and extinction of ecologically marginal populations in geographically central localities might explain their lack of local adaptation as in Moore *et al*. (1979). Effective populations in the centre of the range should also be high. Furthermore, selection for intra-specific competition or saturation selection (as in Whittaker & Goodman, 1979) is expected to be high. Wallace (1985) suggested that adaptations to these habitats should reflect specialization for competitive ability (Brussard, 1984).

In intermediate areas, average population size is lower and fluctuates more. Although populations are permanent, fluctuations in numbers related to variation in the physical environment will be common and pronounced (Brussard, 1984). As in Whittaker & Goodman (1979), the prevalent selective regime is exploitation selection, characterized by a flexibility of demographic strategies. There are frequent episodes of selection for fast increase, followed by selection favoring those traits that dampen population decline through resistance to unfavorable conditions. Populations here are generalists with respect to adaptation to various densities and to different levels of environmental stress (Brussard, 1984).

Populations inhabiting the ecologically marginal areas of the gradient will be even smaller and fluctuate more. Demographic peaks and troughs will be produced by changes in the physical environment, since even relatively small changes in some critical parameter can have important demographic consequences on populations living close to their tolerance limits. Short periods of favorable environmental conditions can result in population explosions, but numbers are bound to decline rapidly, once conditions return to normal. The greatest selective pressures under such condition would be toward evolving means of surviving unfavorable conditions, as in the adversity selection of Whittaker & Goodman (1979) (Brussard, 1984). Whittaker & Goodman also suggested that the infrequency of opportunities for rapid increase would also select against “r characteristics”, whereas, most

individuals resulting from population eruptions would be expended in long-distance dispersal gambits (Brussard, 1984).

Curnutt *et al*. (1996) analyzed time series survey data of nine species of sparrows in North America. They argue that temporal variability is related to spatial variation in abundance, thus processes that generate patterns of temporal variability must necessarily generate characteristic spatial patters. They find that their empirical study suggests a mechanistic interpretation of such patterns in terms of variation in population stability across the species' geographic range.

Curnutt *et al.* (1996) concluded that core areas, with typically high abundances are relatively persistent through time, while populations in peripheral areas are more likely to become extinct due to rarity, and larger relative variability in local abundances. They suggest that this pattern of variability might occur due to source-sink dynamics. Core populations act as sources from which excess individuals move to peripheral sinks in hopes of finding better resources. As a result, the core appears to have more stable populations than the periphery, where population is governed largely by migration rather than reproduction and survival (Williams *et al*., 2003). However, they argue that if variability increased evenly in core and peripheral populations through time, the edges would soon become locally extinct. They find that the biggest increase in variability occurs in high abundance, core areas (Curnutt *et al*., 1996).

Williams *et al*. (2003) tried to determine the potential factors affecting population variability across species' ranges. To address this issue, they examined spatially subdivided long-term seasonal time series data for 3 small avian game species in Kansas. They argue that population variation is driven by the interaction between environmental variability and density dependence (Ives, 1995; Turchin, 1995; Williams *et al*., 2003). They also suggest that the relative importance of density-dependent vs. density-independent processes may vary throughout a population's range (Lawton, 1996; Williams *et al*., 2003).

They found that the greater population variability toward the periphery of the species' ranges is largely the result of greater variability in density-independent per-capita growth rates. In other words, populations in the periphery experience greater fluctuations in response to environmental factors, since peripheral regions tend to be more variable (Mehlman, 1997; Nantel & Gagnon, 1999; Whittaker, 1971; Williams *et al*., 2003). Such fluctuations might make peripheral populations more likely to go extinct (Williams *et al*., 2003).

Enquist *et al*. (1995) used data of mollusk species in the Gulf of California, whose shells occurred in recent and Pleistocene deposits. The turnover between Pleistocene and recent samples was measured as the number of species showing local extinction/colonization events versus the number of species that were present in both samples. They found that turnover was most likely to have occurred in species near the edges of their ranges (Enquist *et al*., 1995).

According to the ACH, populations along the periphery of the range will be more fragmented, and as a result are less likely to receive immigrants from other populations. Consequently, a population's probability of extinction is directly correlated with its variability and inversely correlated with density and immigration rate (MacArthur & Wilson, 1967; Pimm *et al.*, 1988; Tracy & George, 1992; Brown & Kodric-Brown, 1977; Channell & Lomolino, 2000a). This has led to the prediction that, when a species becomes endangered, its geographical range should contract inwards, with the core populations persisting until the final stages of decline (Lawton, 1995; Brown *et al*., 1995). However, Channell & Lomolino in their study (2000a), where they analyzed range contraction for 245 species from a broad range of taxonomic groups and geographical regions, did not support the above predictions, as most species persisted in the periphery of their historical range (Channell & Lomolino, 2000a). They suggest that the range contraction of species better fits on the expectations of the 'contagion hypothesis', which is derived from the declining species paradigm. This hypothesis emphasizes the geographical dynamics of the extinction factors in determining where populations should persist (Lomolino & Channell, 1995; Channel & Lomolino, 2000b). The 'contagion hypothesis' holds that those populations that are last to be impacted by the extinction force will persist the longest (Channell & Lomolino, 2000b).

ii) Gene flow and genetic drift as opposing factors to local adaptation

How an abundant centre distribution is manifested in the amount and partitioning of genetic diversity among populations across the range has been the subject of a long-standing and largely unresolved debate (Carson, 1959; Soule, 1973; Antonovics, 1976; Brussard, 1984; Hoffman & Blows, 1994; Lesica & Allendorf, 1995; Barton, 2001; Eckert *et al.*, 2008)

The simplest extension of the abundant centre model suggests that two key genetic parameters, effective population size and the rate of gene flow, should be highest at the

range centre and lowest at range margins. As a result, geographically peripheral populations should exhibit lower genetic diversity and higher genetic differentiation than central populations (Eckert *et al.*, 2008).

Eckert *et al.* (2008) surveyed 134 studies representing 113 species that tested for a decline in population diversity and an increase in differentiation amongst populations towards their range margins. They concluded that on average, this pattern persists. However, in most cases they assessed, the difference in genetic diversity between central and peripheral population was not large and the mechanisms that generated such pattern were not clear (Kunin *et al*., 2009).

Another unresolved issue that arises is that species exhibit evolutionary stable limits to their geographical distributions (Eckert *et al.*, 2008). Their spatially restricted ranges are a result of populations not being able to become established beyond their range, since they have negative growth rates in these new habitats. However, it is clear that species can adapt to inhospitable conditions over longer time periods, otherwise there would be no life on land, no mammal in the ocean and only a few species on oceanic islands (Bridle & Vines, 2007).

Bridle & Vines (2007), argue that smaller and fragmented populations at a species range margins are a result of a species failure to adapt to local conditions. They cite two contrasting but not mutually exclusive explanations for this phenomenon:

i) *If the range edge is highly fragmented, Allee effects, genetic drift and the low rate of mutational input into marginal populations might limit the availability of locally beneficial alleles, preventing adaptation and, therefore, range expansion (Bridle & Vines, 2007).*

Allee (1949) observed that many animal and plant species suffer a decrease of the per capita rate of increase as their populations reach small sizes or low densities. Under such conditions, the rate of increase can reach zero or even negative values, because of a decrease in reproduction and/or survival when conspecific individuals are not numerous enough (Courchamp *et al.*, 1999). Allee effects might occur due to genetic inbreeding and a loss of heterozygosity that might lead to decreased fitness, demographic stochasticity — including including sex ratio fluctuations— and lack of facilitation amongst conspecific individuals.

The strength of genetic drift is inversely related to a population's effective size (N_e), which is determined largely by a population's abundance and variability (Vucetich *et al.*, 1997). Thus, large-scale spatial patterns in abundance and variability would likely generate important spatial patterns in N_e (Vucetich & Waite, 2003). Vucetich & Waite (2003) predicted spatial patterns in N_e across the geographic distribution of six grassland bird species. They used point-count data obtained from the North American Breeding Bird Survey (analyzed by Curnutt *et al*. (1996)) and the relationship between abundance, variation in abundance and N_e as is approximated by Crow & Kimura (1970) **(Eq.1),**

$$N_e = N / (1 + CV^2) \quad (1)$$

where N represents average abundance and CV is the coefficient of variation in abundance over time. They find that effective population size (N_e) could be 2 to 30 times greater near the core of the species' range than near the edge of the range, thus, the rate of genetic drift may be 2 to 30 times greater near the edge of the range. However, they find that edge populations may exhibit levels of genetic diversity that fall within a continuum from much lower to much higher than expected, due to variability in spatial patterns of migration.

i) *If populations at the margins remain connected to large well-adapted central populations, the continual immigration of these locally deleterious alleles could swamp the establishment of locally adaptive alleles, thus maintaining negative population growth and again preventing expansion (Bridle & Vines, 2007).*

Mayr (1963) emphasized that gene flow is an essential mechanism that maintains genetic and phenotypic homogeneity within a species. However, genetic homogeneity induced by gene flow might result in local maladaptation due to spatial variation of natural selection (Alleuaume-Benharira *et al.*, 2006). In other words, local adaptation describes the adequacy between the phenotypes and the local environment. In this context, natural selection, which increases the frequency of locally adapted genes, interacts with gene flow, which introduces potentially maladapted genes that have been selected elsewhere (Lenormand, 2002; Alleuaume-Benharira *et al.*, 2006).

Antonovics (1976) investigated the hypothesis that gene flow from a parent population acts to prevent genetic differentiation and hence range expansion across the ecological boundary. He differentiates between physically small marginal populations (peripheral isolates) and

marginal populations that show a reduction in density (ecotonal populations). He argues that gene flow into a peripheral isolate would be largely dependent on its degree of isolation. In an ecotonal population, however, a lower density of marginal individuals would increase the swamping effect of gene flow. He argues that gene flow will have drastically different effects depending on whether the genes concerned are effectively neutral, advantageous or mildly deleterious in the population into which they migrate. A neutral gene will migrate at a slow rate. An advantageous gene however, will not only spread in the local population, but will migrate and spread into other populations as well. He states that the dispersal rates in this case may be highly effective and rapid; therefore, advantageous genes will be readily disseminated throughout that species range.

He finally demonstrates through a simulation of gene flow with pollen from wind-pollinated plants from central populations, towards ecotonal and isolated marginal populations, relative to their distance from the centre. The ratio of central/total pollen received would be a measure of gene flow from the central population into the marginal population. He argues that gene flow will be very high if the density of the individuals in the marginal population declines rapidly. He finds that whereas dispersal from the central habitat falls off rapidly into the ecotonal and marginal areas, gene flow is actually greater in the marginal population than in the ecotonal. Thus, the swamping effects of gene flow can be very real and substantial (Antonovics, 1976).

Garcia-Ramos & Kirkpatrick (1997), assuming an abundant centre distribution of density, describe a quantitative genetics model for a species continuously distributed through space along some environmental gradient, which generates clinal selection on a phenotypic character. Such demographic asymmetries lead to a net flow of individuals from core populations toward the margins. They show that asymmetric gene flow can result in phenotypic clines at the scale of the species' range deviating from optimal values. Increased migration then generates higher maladaptation in peripheral populations (Alleaume-Benharira *et al*., 2006).

Much discussion on why evolution fails at range margins hinges on determining how much gene flow is necessary to maintain adaptive potential at the margins without swamping local adaptation (Bridle & Vines, 2007). Holt & Keitt (2005) argue that whether or not gene flow amongst natural populations primarily restricts range limits or facilitates range expansion, is a question yet to be answered, since gene flow via dispersal from numerically abundant central populations may ‘swamp’ adaptation to marginal conditions (Mayr, 1963; Antonovics, 1976; Kirkpatrick & Barton, 1997). Yet, it can also facilitate local adaptive

evolution if genetic variation is limited in local populations (Bradshaw, 1991) and gene flow permits enhancement of local pools of variation (Gomulkiewicz *et al.,* 1999; Barton, 2001; Holt & Keitt, 2005). Furthermore, theoretical studies investigating the effect of drift on the evolution of gene frequency along environmental gradients with constant density of individuals (Hastings & Rohlf, 1974; Felsenstein, 1975; Slatkin & Maruyama, 1975; Nagylaki, 1978) have shown that gene flow, rather than canceling the effect of selection, could help mitigate the effect of drift and maintain smooth clines along such environmental gradients (Alleaume-Benharira *et al*., 2006).

Both gene swamping and genetic drift have an effect on small peripheral populations. They reduce their genetic variability, impede their response to selection and can lead to the fixation of locally maladapted genotypes. However, gene flow may result in the local fixation of a genotype that deviates from the local optimum, but at least this genotype was positively selected somewhere else in the range. Genetic drift, on the other hand is a random process that may result in the local fixation of a genotype that may be deleterious everywhere in the range of the species. Furthermore, gene flow has directional effect and will leave a consistent signature across generations in the local adaptation of peripheral populations as long as the environmental gradient does not vary too much in time. On the contrary, drift effects are not directional (Alleaume-Benharira *et al.*, 2006).

Alleaume-Benharira *et al.* (2006), used individual-based stochastic simulations and analytical deterministic predictions to investigate the interaction between drift, natural selection and gene flow on the patterns of local adaptation across a fragmented species' range under clinally varying selection, where migration between populations followed a stepping stone pattern and density decreased from the centre to the periphery of the species' range. They found that in the presence of genetic drift, increasing gene flow might attenuate fitness heterogeneity within the range, resulting in higher total fitness. In addition, positive effects of gene flow were detected when the environmental gradient was moderate, peripheral populations were small, mutation rates high, and very low migration rates among adjacent populations (genetic rescue effect) (Alleaume-Benharira *et al.*, 2006). They hypothesized that, in addition to Barton's (2001) assumption that migration counterbalanced the negative effects of gene swamping, moderate effects of migration helped restore genetic variability eroded by drift. However, their model failed to predict the 'genetic rescue effect' for shallow environmental gradients. Migration mechanisms in this respect, could not replenish genetic variation eroded by selection (Alleaume-Benharira *et al*., 2006).

iii) Speciation

Brown's work (1984) challenged contemporary notions about the ecological consequences of the trade-off between specialization and generalization implied by the saying "jack of all trades, master of none". If this were true, specialists with narrow tolerances should be more efficient in exploiting a more limited range of resources, and hence should have more restricted distributions but higher local abundances than generalists. However, Brown noted that there is a very general tendency for species with restricted ranges to be rare, whereas more widespread species attain higher local population densities. There is data to suggest a lack of trade-offs amongst different niche dimensions. Species that can tolerate wide variation in one factor also tend to be tolerant of other factors, and hence to be both locally abundant and spatially widespread (Brown, 1984). This notion suggests a wide distribution of evolutionary success amongst species. If the definition of success of a species is the probability of leaving descendants over evolutionary time, then, in general the abundant, widespread species must be more successful than the rare restricted ones (Brown, 1984).

Central populations of widespread, abundant species would seem relatively resistant to rapid, directional evolutionary change, since little improvement in ecological performance is likely. Furthermore, such species are relatively continuously distributed over a variety of local environments, so there is little opportunity for spatial isolation to facilitate genetic differentiation for locally adapted populations. Most of the selection will be stabilizing selection that tends to maintain the generalized adaptations. In contrast, peripheral populations of the same widespread species, will tend not only to be rare, but also restricted to isolated patches of suitable habitat. If this spatial isolation reduces gene flow sufficiently, these populations can respond to directional selection, adapt to local conditions and eventually differentiate. Such newly formed species could increase substantially in abundance and distribution, if the environment changes so as to favor forms with their special adaptations, or if they are able to evolve to increase their share of limited resources, by increasing their ability to compete with ancestral and other closely related species (Brown, 1984).

iv) The effect of intra- and inter-specific competition

According to Brown (1984), populations of species are often found in a greater variety of habitat types near the middle of their geographic range, since the geographic abundance patterns of species reflect the probability density distributions of the species required environmental resources and biotic interactions. Based to such assumption, Hall et *al*. (1992)

argue that the observed patterns of distribution and abundance of plant and animal species within space and time are related directly to species-specific energy costs and gains in response to the many environmental or resource gradients (Hall *et al*., 1992).

They infer that individuals near the centre of the collective distribution will enjoy a high-energy profit, which will allow a large net gain of energy, growth and reproductive success. Individuals on the margins of their physiological, territorial, or nutritional range with respect to a gradient will be unable to generate a sufficient energy profit to produce population growth. Competition might be important at the margins of the range, since it raises the cost of resource acquisition and creates more of an impact on individual energy gain in locations on the gradient where the net gain is small (Hall *et al.,* 1992).

Darwin (1859) suggested that species in the northern hemisphere are more likely to be limited by competition at their southern border and by abiotic factors at their northern one. If this is generally the case, one would predict that parapatric margins often consist of a species at its environmental limit and a second, more environmentally tolerant species that is excluded from the range of the first species by competition, parasitism or predation (Bridle & Vines, 2007).

In the classic conception, species are excluded from ranges of other species because they compete for resources, although predation or parasitism can have similar effects. Range expansion requires adaptation to condition at or just beyond the range edge, which in this case includes the presence of other species. Once a viable population can be maintained in the other species' range, the species can coexist in sympatry. This might require evolving to exploit a different ecological niche (ecological character displacement) or the development of new predator avoidance mechanisms. In either case, the margin might be maintained either because such adaptation is prevented by low population densities or because gene flow from populations away from the range edge that never encounter the competitor (Bridle & Vines, 2007).

Case & Taper (2005) show that interspecific competition can greatly expand the impact of gene flow as a factor limiting species' ranges (Holt & Keitt, 2005). They extended the Kirkpatrick and Barton model to include the presence of a competing species, and found that range margins formed at shallower environmental gradients in the presence of a competitor compared with the range margins that were formed in the absence.. However, when disruptive selection due to competition was stronger than stabilizing selection towards the environmental optimum or when the environmental gradient itself was flat, the species

became sufficiently different to maintain a viable population at the other's range. This divergence enabled the species to spread into full sympatry, eradicating their shared range edge (Bridle & Vines, 2007).

The importance of competition in maintaining range boundaries is a contentious topic, as many parapatric margins also coincide with transition between environments, making it difficult to determine whether competition or environmental selection is maintaining the border. However, these two scenarios can be distinguished with reciprocal transplant experiments, whereby the survival of both species is measured on their non-native side of the ecotone (Bridle & Vines, 2007).

v) Climate change

There is now ample evidence that modern climate change is reshuffling the geographic distributions of plant and animal species world-wide (Hampe & Petit, 2004; Parmesan & Yohe, 2003). The dynamics of those populations that inhabit the latitudinal margins of the distribution range are likely to be critically important in determining species' responses to expected climate change (e.g. Thomas *et al*., 2001; Iverson *et al*., 2004; Travis & Dytham, 2004; Hampe & Petit, 2004).

According to Brown's (1984) view of the ACH, the position of a site within a species' range is a surrogate for the environmental suitability of that site, and it might be expected that site position would be correlated with any change in abundance induced by environmental change, assuming that the same determinants of the geographic range are responsible for the varying abundance "topography" within the range. (Brown, 1984; Brown *et al*., 1995; Mehlman, 1997). For example, if closeness to the edge of range indicates a worse environment for a species, it would be predicted that if environmental suitability declines, then relative abundance change should be greatest toward the range margins (Mehlman, 1997).

Mehlman (1997) used range-wide information before and after an environmental perturbation in order to examine the geographic pattern of abundance change caused by broad-scale environmental change. He used data acquired from the North American Breeding Bird Survey through a series of harsh winters in the late 1970s, in order to document the changes in abundance across the geographic ranges of three passerine bird species. His results suggested that when the climate changed radically for the worse over a large area, then the sites closest to the edge were more at risk, while sites toward the centre of the range were comparatively insulated. However, he noted that the exact consequences of climate change would depend on each species and its interaction with climate. He concluded that negative effects of climate change would be greatest at peripheral parts of the range, producing a general contraction toward the formerly highest abundance portions of the species' range, and conversely, in the event of beneficial effects, the most radical changes in abundance would be expected at sites of low abundance in general, and sites closest to the edge of the range, in particular (Mehlman, 1997).

Safriel *et al*. (1994), argue that the relative persistence under novel changes, such as those associated with Global Climate Change (GCC), is determined by micro-evolutionary mechanisms that operate differently depending on the size and spatial patterns of these

populations, and their interaction with the environment. Population persistence is enhanced through the the occurrence of genetic combinations for resistance to stress, and/or through having a wide range of tolerance that is somewhat wider than the currently prevalent range of conditions and covers further deterioration likely to result from GCC (Safriel *et al*., 1994). Assuming an "abundant centre" scenario, core and peripheral populations will differ in respect to these two features. Accordingly, peripheral populations will persist either better or worse than core populations, or no differences are to be expected (Safriel *et al*., 1994).

Since the core is environmentally more favorable, it harbors dense and contiguous populations, whereas peripheral populations are small and isolated (Mayr, 1965; Lewontin, 1974; Safriel *et al.*, 1994). Environmental favorableness is expressed in the number of types of exploitable ecological niches, and is, thus, greater in core than periphery (da Cunha & Dobzhansky, 1954; Safriel *et al*., 1994). In addition, populations at the core are highly heterozygous and heterotic. Thus, the same genotypes can perform better in the variety of niches available in the core, and the load of producing less fit homozygotes is balanced by the large size of these populations and their high degree of outcrossing (Carson, 1959; Safriel *et al*., 1994). To conclude, core populations are expected to undergo balancing selection and therefore they maintain high additive genetic variance, whereas peripheral ones are smaller and isolated and have lower additive variance. This is called the "Carson" hypothesis and implies that under GCC core populations are more likely to respond to the novel selection pressure and persist, than peripheral ones (Safriel *et al*., 1994).

The Fisher hypothesis (Fisher, 1930a,b) predicts that if the environment of core populations is perceived stable, genetic additive variance and heritability are low. At the periphery, the environment is fluctuating, which induces fluctuating selection and, therefore, additive variance and heritability are larger than in the core. Thus, as favorableness and predictability decrease from the core to periphery, selection changes from one for high average fitness, to one promoting genetic flexibility (Brussard, 1984). In the periphery, many genotypes are maintained, each adapted to cope with a specific environmental state. GCC is expected to make some climatic states more common than they are now; thus the frequency of genotypes that can cope with these states will increase, while others may perish. In the core, on the other hand, genotypes adapted to rarely occurring environmental states may not be maintained (Safriel *et al*., 1994).

Furthermore, small and isolated populations are subject to strong random evolutionary forces, such as drift, inbreeding and founder effect (Holt, 1990; Heywood, 1991; Safriel *et al*., 1994). Even though a random mutation is less likely to occur when the number of individuals

is small, when it does, it represents a relatively large proportion of the population and will be more likely to increase to fixation due to genetic drift. Under these conditions, in the periphery, the within-population genetic variability will be low, but the between-population genetic variability will be high. This is why peripheries are considered sites of much genetic innovation (Mayr, 1965). At least some of the innovations are likely to be resistant to GCC-induced changes (Safriel *et al*., 1994).

Concluding, no differences in persistence between core and peripheral populations may occur if gene flow from the core is stronger than selection in the periphery. In the case of peripheral populations being replenished by a steady stream of immigrants from a more favorable portion of the species range, peripheral populations merely act as "sinks", while core ones act as "sources". In such a case of virtual equality of polymorphisms between core and periphery, no differences in persistence under GCC are expected (Safriel *et al*., 1994).

Although the utility of the "centre-periphery hypothesis" at a local to regional scale is generally accepted, recent empirical work has challenged its significance at broad geographical scales (Channell & Lomolino, 2000a; Sagarin & Gaines, 2002a,b; Vucetich & Waite, 2003; Hampe & Petit, 2004), In particular, phylogeographic surveys show that range wide patterns of population genetic diversity are usually shaped by past climate-driven range dynamics (Hewitt, 2000,2004; Hampe & Petit, 2004) rather than by demo-genetic stochasticity per se, as proposed in the centre-periphery model (Hampe & Petit, 2004).

vi) Conservation

Assuming the spatial pattern arising from the ACH, peripheral populations have been portrayed as being of low conservation priority, because they are more vulnerable to extinction, or high, because of their potentially unique genetic characteristics (Vucetich & Waite, 2003).

Brown *et al*. (1995) suggest that the highly aggregated distribution across the landscape and within the geographic range of a species should be considered in establishing biological reserves and managing ecosystems to maintain or restore biological reserves. Their analysis of data of common passerine species from the Breeding Bird Survey, suggested that more than 50% of all individuals were concentrated in a small proportion of the sites the species occurred. They argue that it is important to identify the "hotspots", where species are most abundant and to design reserves that protect such hotspots (Shoener, 1987; Brown *et al*., 1995). Their results also suggested that the hotspots of different species were positively

associated, thus such sites should be given high priority for protection as nature reserves (Brown *et al*., 1995).

Griffith *et al*. (1989) report the percentage of success of intentional introductions of native birds and mammals to the wild. The percentage of successful introductions of the 198 species (134 birds and 64 mammals) was 78% when the location of release was within the core of their historical range, while the percentage of success for introductions in the periphery or outside was 48%. A follow-up study by Wolf *et al*. (1996) obtained similar results.

Lesica & Allendorf (1995) argue, on the other hand, that the long-term conservation of species is likely to depend upon the protection of genetically distinct peripheral populations (Shreeve *et al*., 1996; Smith & Theberge, 1966). Such populations are expected to diverge from central populations as a result of the interwoven effects of isolation, genetic drift and natural selection. They suggest that conservation of such populations may be beneficial to the protection of the evolutionary process and the environmental systems that are likely to generate future evolutionary diversity (Lesica & Allendorf, 1995).

In regard to species responses to GCC, Safriel *et al*. (1994) speculate that under the Carson hypothesis (see above), core populations are more likely to respond to the novel selection pressure than the peripheral ones (Safriel *et al*., 1994). Assuming the Fisher hypothesis, though, peripheries are considered sites of much genetic innovation (Mayr, 1965). Thus, at least some of the innovations are likely to be resistant to GCC-induced changes (Safriel *et al*., 1994)

4. Assessing the validity of the "abundant centre" hypothesis

Sagarin & Gaines (2002a) reviewed the contemporary literature of direct and indirect testing of the ACH, and quantified the percentage of studies that support it, based on the original authors' data and interpretations.

They classified the methods for directly testing the validity of the ACH into three categories:

i) Analyzing complete or partial transects of density along a path through the range.

ii) Measuring correlation coefficients between abundance and distance from the range edge or centre.

iii) Comparing densities found among varying numbers of 'range classes' defined by their distance from the centre or edge.

Their key finding was that only 39% of the direct tests supported an abundant centre distribution. The very few empirical studies that have estimated demographic parameters from across entire geographical ranges provide even weaker support for such “abundant centre” pattern (see Gaston, 2003; Sagarin *et al*., 2006; Samis & Eckert, 2007).

Sagarin and Gaynes (2002) summarized the limiting factors throughout the relevant literature. These are:

- Limited spatial coverage of the studies with spatial coverage being a function of number of sampled sites).

- Limited geographical, taxonomic, and ecological extent; most studies were conducted for European and North American terrestrial species of avian vertebrates.

- Under-sampling the range edge.

- Most studies tested the “abundant centre” indirectly. Hypotheses to explain geographical scale patterns are based on a wide range of theoretical frameworks and thus may propose multiple explanations for the same pattern or may contradict one another (Gaston & Blackburn, 1999; Sagarin & Gaines, 2002a) — Opposite outcomes, such as genetic differentiation due to bottlenecks and genetic drift, or alternatively, genetic swamping from central populations may be attributed to an underlying “abundant centre” distribution, thus indirect testing may lead to inconclusive results relative to the validity of the ACH.

- Partial sampling (e.g. include only one range edge or part of the species’ range).

- The fact that many researchers performed linear regression analysis that compared abundance to continuous variables such as latitude or range positions. This may prove

problematic in cases where sampling is not completely uniform across the explanatory variables' range.

Sagarin & Gaynes (2002a) highlighted the need for spatially explicit information, collected throughout the species' range, in order to adequately characterize the patterns of abundance when either directly or indirectly testing the ACH. However, they limited their analysis on empirical studies that focused on intra-specific variation over the species' geographical distribution, thus excluding studies over altitudinal gradients or local environmental clines.

II. Occupancy – Abundance relationships

1. Intra- and inter-specific occupancy – abundance relationships and the "abundant centre" hypothesis.

"*...distribution and abundance are but the obverse and reverse aspects of the same problem" (Andrewartha & Birch, 1954).*

The abundance and the spatial distribution of species tend to be linked, such that species declining in abundance often tend also to show declines in the number of sites they occupy, while species increasing in abundance tend also to be increasing in occupancy. Therefore, intraspecific occupancy-abundance relationships tend to be positive (Gaston *et al*., 2000) [see also: Observed patterns of variation in abundance (Section I.1 above)—Species that have the highest local population densities tend to inhabit a greater proportion of sample sites within a region and to have wider geographic ranges; conversely species that are always rare also have restricted spatial distributions].

Positive intraspecific occupancy-abundance relationships, or evidence suggestive of such relationships, have been documented in a number of studies, across a variety of habitats and spatial resolutions. These include investigations of plants (Boecken & Shachak, 1998), butterflies (Pollard *et al.*, 1995; van Swaay, 1995), fish (Winters & Wheeler, 1985; Crecco & Overholtz, 1990; MacCall, 1990; Rose & Leggett, 1991; Swain & Sinclair, 1994) and birds (Gibbons *et al*., 1993; Fuller *et al.*, 1995; Venier & Fahrig, 1998; Telleria & Santos, 1999; Gaston *et al.,* 2000a).

The existence of such positive occupancy-abundance patterns, both in an intra- and an inter-specific context, has motivated a search for a general explanation. A number of mechanisms have been proposed, embracing sampling artefacts, species attributes and population dynamics (Gaston *et al.*, 1997). Gaston *et al*. (1997) made an appraisal of such mechanisms, their assumptions and associated predictions. They focused on closely related ecologically similar species that more or less occupied the same geographical extent since, according to Brown (1984) and Gaston (1994), this is where abundance-range size relationships tend to be the strongest [see Section I.2 above: Ecologically similar species differ substantially in only one or a very small number of niche dimensions and spatial variation in the environment tends to be autocorrelated].

Positive interspecific occupancy-abundance relationships could partially arise directly as a result of the study area's positioning along the species' "abundant centre" distributions. Species closer to the edges of their ranges might have a smaller range size in the study area

in two ways. First, their range might only penetrate a relatively small part of the study area. Second, not only do abundances decline towards range limits, but occurrence also becomes patchier. Then, a species closer to the edge of its range might be widely dispersed through a study area, but occupy a relatively small proportion of it. In either case, the species for which the centres of their geographical ranges overlapped the study area would occur at relatively high abundance and be widely distributed, whilst those for which only their range edge overlapped the study area would occur at relatively low abundances and would be restricted in occurrence (Gaston *et al*., 1997).

Such a hypothesis leads to certain predictions. Firstly, a positive interspecific abundance-range size relationship will not exist when based on measures of the entire geographical ranges of species and their average abundances across those ranges. However, Bock in his 1984 study on North American winter landbirds, where their winter ranges were largely (over 75%) confined to the area for which good abundance data were available, still recovered a positive abundance range size relationship (Gaston *et al*., 1997). Secondly, those species in an assemblage that are locally rare or occupy a small range size will tend, on average, to be nearer the edge of their geographical range. Indeed, Hengeveld & Haeck (1982) have shown that for several assemblages, there was an increase in the numbers of individuals or numbers of grid squares occupied by species for which an area was more central to their geographical range (Gaston *et al*., 1997). However, the matter becomes more complicated when the spatial scale of the study is taken into account. As Gaston in his 1994 study noted, it is difficult to distinguish the effect of proximity to the range edge from the effect of self-similarity in abundances and range sizes for a species with a small geographic range at the edge of its distribution. Thirdly, interspecific abundance-range size relationships may tend to be lower triangular, such that widely distributed species may have either high or low densities, while geographically restricted species can only have low densities. This prediction follows from the observation that species, which are close to the edge of their distribution and hence have low abundances, may nonetheless be quite widespread in a study area. However, such a pattern might be expected for a variety of other reasons (Gaston *et al*., 1997).

Brown's hypothesis (1984) went a step further from a range-position explanation in interpreting the observed positive interspecific occupancy-abundance relationships. Brown's hypothesis or "resource (niche) breath" hypothesis, according to Gaston e*t al.* (1997), is based on Brown's assumptions, as in [I.2]. Species that have broad environmental tolerances are able to use a wide range of resources; in so doing, they achieve high local densities and will be able to survive in more places and hence over a larger area; in this case, the 'jack-of-

all-trades' is master of all [see: I.3.iii—speciation]. Those that have a narrow environmental tolerance are able to use only a narrow range of resources and will be unable to attain either high local densities or extensive distributions; the specialist is never very successful (Gaston *et al*., 1997). A fundamental assumption of such a hypothesis is that more abundant and widespread species have an ability to use a broader range of resources. However, even though range size is likely to increase with "resource" or "niche breadth", there is no obvious reason why an ability to exploit a range of resources and hence occur more widely should enable species to attain a greater local abundance (Kouki & Hayrinen, 1991; Hanski *et al*., 1993; Gaston *et al*., 1997). Nonetheless, a positive relationship between "niche breath" and abundance is assumed by many models of species abundance distributions (e.g. Sugihara, 1980; Kolasa, 1989; Tokeshi, 1990; Gaston *et al*., 1997).

Another explanation for a positive interspecific abundance-range size relationship, often conflated with "resource breadth", is based on resource usage. Species that are locally abundant and widespread utilize resources that are locally abundant and widespread, whilst those species that are locally rare and restricted in occurrence utilize resources with similar relative levels of abundance and distribution (Hanski *et al*., 1993; Gaston, 1994; Gaston *et al*., 1997). While this mechanism escapes the difficulty of explaining why environmental generalists should attain higher local abundances, it necessitates that locally abundant resources are also widely distributed (Gaston *et al*., 1997).

Indeed, this hypothesis makes intuitive sense for host-specialist consumers. If the distribution of the host plant of a specialist herbivore is widespread and abundant, the consumer is bound to be abundant and widespread as well (Gaston *et al*., 1997). On the matter of resources utilized by other groups of species, authors have claimed that such hypothesis seems to be the case. Fuller (1982) argues that the most abundant and widespread species of breeding birds on saltmarshes in Britain are those which utilize the typical and common features of the marshes, while the least abundant and poorly distributed species are those which are restricted by their preference for special habitats. (Gaston *et al.*, 1997). Further indication of a relationship between resource availability and local abundance is provided from studies of niche pattern, which document the correlation between niche position and local abundance. A large niche position means that a species occurs in habitats characterized by extreme values compared with the mean value of all habitats in the sample, and whilst some studies find no, or a weak positive, relationship with local abundance (Mac Nally, 1989; Rogovin *et al*., 1991; Shenbrot *et al*., 1991), others find negative relationships (Seagle & McCracken, 1986; Robey *et al*., 1987; Urban & Smith, 1989; Shenbrot, 1992;Gaston *et al*., 1997). Blackburn *et al*. (1996), note in their study of British birds with fast development

and high abundances which they related to resource availability, that this idea is consistent with Brown's hypothesis if niche breadth is related to the amount of resource available to a species. Therefore, its predictions are similar. However, the resource availability hypothesis can explain the same patterns more parsimoniously, because it need make no assumptions about variation in niche breadth (Gaston *et al*., 1997).

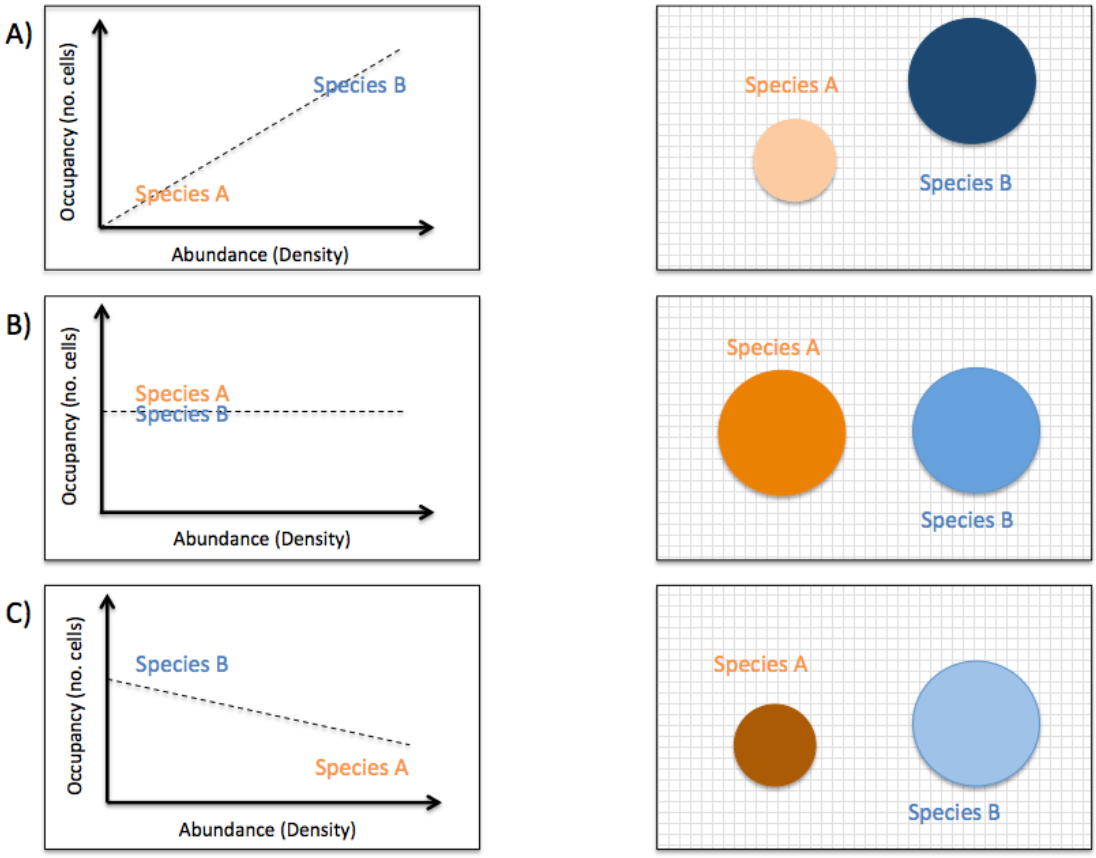


Figure 1: Occupancy-abundance relationships. A) A positive inter-specific occupancy-abundance relationship. Species A is occupying a smaller proportion of the grid relative to species B, and has a lower density of individuals; B) A case of no correlation between occupancy (no. of grid cells) and abundance (density of individuals). Species A and B occupy the same number of grid cells, and have the same density of individuals; C) A case of negative correlation between occupancy and abundance. While species A is occupying a smaller proportion of the grid, it displays higher density relative to species B.

2. The need for a statistical/spatial distribution approach

Positive intra- and inter-specific positive occupancy-abundance relationships have been widely documented, however, the efforts to determine why such pattern occurs, both within and out the ACH context, have been met with limited success [see II.1, (Gaston, *et al*., 1997; He e*t al*., 2002)]. Theory and empirical evidence strongly suggest that positive occupancy-abundance relationships result from the action of several mechanisms, and that in different systems these vary in their relative importance (Holt *et al*., 2002). Furthermore, macroecological patterns are increasingly seen as being best understood as the net outcome of several processes that pull in essentially the same direction (Gaston, 2000; Gaston & Blackburn, 2000; Lawton, 2000; Holt *et al*., 2002).

Several statistical occupancy-abundance and spatial distribution models have been proposed so as to address the implications of the relationship between occupancy and abundance by quantifying the observed patterns in the absence of a comprehensive general descriptive model. Most of the models found in literature were originally developed for other purposes and were empirical in nature (Nachman, 1981; Wright, 1991; Gaston, 1994; Hanski & Gyllenberg, 1997; Leitner & Rosenzweig, 1997; He *et al*., 2002). In the following, p is the probability of occurrence of a species in a sample unit, and μ is the mean local density of the species.

1. The simplest model is derived from the Poisson distribution and is assuming that individuals are randomly and independently distributed in space (Wright, 1991).

$$p = 1 - e^{-\mu} \tag{2}$$

2. The negative binomial distribution model describes species that are aggregated in space (Evans, 1953; Boswell & Patil, 1970; Wright, 1991); k is a positive aggregation parameter (however He & Gaston, (2000a) describe a positive binomial distribution model which corresponds to a regular distribution of organisms), with small k representing strong aggregation and large k random distribution.

$$p = 1 - \left(1 + \frac{\mu}{k}\right)^{-k} \tag{3}$$

3. The Nachman (1981) model of occupancy, where α is a positive parameter and β is a positive scale parameter that determines the shape and curvature of p versus μ curve.

$$p = 1 - e^{-\alpha\mu^{\beta}} \quad (4)$$

4. The power occupancy-abundance model of Leitner & Rosenzweig (1997), which is used in modeling species area curves in terms of range size or occupancy; α is positive and β is a scale parameter.

$$p = \alpha\mu^{\beta} \quad (5)$$

5. In the same context, the Hanski & Gyllenberg (1997) logistic model; $\alpha=e^{\alpha}$ and $\beta=b$, positive parameters.

$$p = \frac{1}{1+e^{-a-b\ln(\mu)}} \; or \; p = \frac{a\mu^{\beta}}{1+a\mu^{\beta}} \quad (6)$$

6. He & Gaston (2000a) developed a model that unified the Poisson, the Nachman and the logistics models, under a mathematical framework, by outlining the links between them and the NBD model (He *et al.*, 2002). Each of the aforementioned models constitutes, in fact, a special case of the model (Eq.7) they developed as below.

$$p = 1 - \left(1 + \frac{a\mu^{\beta}}{k}\right)^{-k} \quad (7)$$

α is a positive parameter, β a scale parameter, and k a negative or positive binomial distribution parameter.

7. Following Hanski (1994; 1997), when a metapopulation in steady state, the occupancy probability can be expressed as (Eq.8),

$$p = \frac{C}{C + E} \quad (8)$$

where C is the colonization rate of empty sites and E is the extinction rate of extant populations, with C being an increasing function, and E a decreasing function of population density (μ) (Gilpin & Diamond, 1976; Hanski & Gyllenberg, 1997; He *et al*., 2002). He & Gaston (2000) expressed Hanski's (1994) occupancy probability as a function of colonization/extinction rates and population density defined as constants ($C=a\mu^{b}$, and $E=c\mu^{-d}$, where *a, b, c and d* are constants) as frequently cited in literature. From there, they derived their general model (Eq.7), where $\alpha=a/c$ and $\beta=b+d$.

He *et al*. (2002) argue that one plausible interpretation of the mostly empirical models described above can be found from metapopulation dynamics. Their model relates spatial aggregation and population density with rates of colonization and extinction of a metapopulation, thus providing a plausible and realistic biological interpretation for observed patterns (see Tilman *et al.*, 1997, species aggregation is associated with poor colonization rates).

They conclude that the aforementioned models manage to point out the way that occupancy depends on density and distribution, capture the positive intra-/inter-specific relationship between abundance and occupancy of species and can be interpreted in a plausible realistic metapopulation dynamics context. They state, however, that the interpretation of occupancy-abundance data and their models is subject to the sampling scale used. If resolution (or grain size) changes, both abundance and occupancy of a species will change, thus it is very likely that the model that best fits the observed data will change as well (He *et al*., 2002).

3. On the matter of scale

Multiple factors, operating across a hierarchy of spatial and temporal scales, shape species distributions (Levin, 1992). However, little is known about how the determinants of the distributions of single species vary across spatial scales (Mackey & Lindenmayer, 2001; Pearson & Dawson, 2003; Guissan & Thuiller, 2005), due to lack of a theoretical framework connecting these scale – variant effects (Hortal *et al*., 2010).

In such an attempt, Soberon (2010) developed a theoretical framework that merges the Grinnelian and Eltonian views of the niche (abiotic, and biotic respectively; Soberon, 2007; Soberon & Nakamura, 2009), in a single formal mathematical definition of three important elements: the abiotic factors that affect the net growth rate of populations, the biotic interactions that may affect fitness in a regulatory manner, and the effects of the spatial movements of individuals (Soberon, 2007; see also: Pulliam, 2000; Hortal *et al*., 2010) .

Soberon (2010) argues that abiotic conditions (or schenopoetic factors) are fundamental at large scales. They determine the shape and size of species distributions at continental or regional scales, while their effect becomes negligible in site scales. Such factors are responsible of a number of processes affecting species distributions, including physiological

constraints, responses to climatic and habitat gradients, active habitat selection and range shifts in response to changes in climate and/or habitat. Climate can constrain the ranges of species at large scales (i.e. from global to regional), while habitat-related variables seem to operate at landscape and local scales (Pearson & Dawson, 2003; Thuiller *et al*., 2004; Hortal *et al*., 2010). The mechanisms by which different factors influence species distributions may vary with scale as well (see Kriticos & Leriche, 2010 – scale dependent predictions of models assessing the areas climatically suitable for two insect pests) (Hortal *et al*., 2010).

On the other hand, the influence of biotic factors is often negligible at continental scales, but becomes progressively more important as scale decreases. Many bionomic processes can affect species distributions, but their effects are often complex and do not have significant effects at scales larger than point or site (Hortal *et al*., 2010). At large scales, only extreme (mainly trophic) specialists will have distributions constrained by the bionomic part of their niches (Araujo & Luoto, 2007; Cornelissen & Stiling, 2009; Hortal *et al*., 2010). Such restriction scales down to the site scale. For example, viable populations of a silver-spotted skipper butterfly studied by Wilson *et al*. (2010) are unavoidably limited to the dry grasslands where its host plant, sheep's fescue grass, occurs (Hortal *et al*., 2010).

While Soberon (2007, 2010) and Soberon & Peterson (2005) present all movement-related factors within a single group to understand species distributions throughout all scales, Hortal *et al*. (2010) differentiate between biogeographic factors and those related to occupancy dynamics. Biogeographic factors have a significant effect on the large-scale processes that determine the size and shape of species distribution ranges. However, accounting for these factors is not straightforward because their precise effects on the distributions of individual species are often the subject of debate, or simply unknown (Hortal *et al*., 2010).

Occupancy dynamics are the output of a series of processes affecting the demography of populations and the movements of their individuals, including metapopulation dynamics, small-distance dispersal and localized disturbances, among others (Hortal *et al*., 2010). At scales smaller than the landscape, these processes can determine the observed degree of aggregation of populations within the geographic range of the species (Cabeza *et al*. 2010, Wilson *et al*. 2010) or of individuals within a locality (Bell *et al*. 2010; Nachman & Borregaard, 2010) (Hortal *et al.*, 2010).

The form of the intra- and inter-specific occupancy-abundance relationships may depend not only on the underlying ecological mechanisms but also on species' occupancy dynamics quantitative expression (Brown 1984; Hanski *et al.* 1993, Gaston 1994a, 1994b, 1996, 1998;

He & Gaston, 2000b; Hortal *et al*., 2010). Different measures of occupancy and abundance, as well as sampling scales, tend to be employed in regard to species' distributions. It remains unclear how this affects the qualitative and/or quantitative patterns observed, and perhaps also their ecological interpretation (He & Gaston, 2000b). Furthermore, describing the spatial aggregation of individuals — which, according to Hui et *al.* (2010) and Proches et *al*. (2010) is scale dependent — is difficult and counter-intuitive (Hui et *al*., 2010; Hortal et *al*., 2010). In addition, although spatial scale is clearly an important determinant of occupancy patterns, the relationship between occupancy and spatial scale (measured as grain or window size) remains difficult to predict (He & Gaston 2000a; McGeoch & Gaston 2002; Hui et *al*., 2006).

Nevertheless, in order to describe species distributions and understand the effect of different factors that shape them, it is necessary to explicitly consider the effect of scale (Hui *et al*. 2010; Kriticos & Leriche, 2010; Hortal *et al*., 2010).

4. A fractal approach

The use of fractal geometry in ecology is not new. There is evidence that many environmental phenomena (e.g. mountains, coastlines, rivers, clouds) have fractal properties (Mandelbrot, 1977; Rodriguez-Iturbe & Rinaldo, 1997; Lennon *et al*., 2002) as well as evidence that some individual species have approximately self-similar distributions across scales (Williamson & Lawton, 1991; Kunin, 1998; Lennon *et al*., 2002). Fractal spatial structure has also informed part of theories for biodiversity patterns (Ritchie & Olff, 1999; Lennon *et al.*, 2002). Kunin (1998) deemed that where distributions are approximately fractal, scale-area curves are approximately linear, with a slope of **1-Db/2** [Db box-counting dimensions of the distribution]. As the fractal dimension measures the propensity of a pattern to fill space, the slope of a scale-area curve measures the degree to which a species' population fills its geographical range: the steeper the slope, the sparser the distribution. The slope and height of a linear scale area curve should encapsulate species abundance information across a broad range of spatial scales, providing a scale-independent description of abundance (Kunin, 1998).

At the root of variation in the relationship between occupancy and spatial scale is the manner in which species' aggregation patterns change over distance (Moloney *et al.,* 1992; He *et al*., 2002; He & Gaston, 2003; He & Hubbell, 2003; Hui *et al*., 2006), with aggregation determined by a combination of species biology, behavior, abundance and environmental heterogeneity (Nachman 1981; Taylor *et al.,* 1983; Levin, 1992; Dungan *et al*., 2002; Perry *et al*., 2002; Hortal *et al.*, 2010, Hui *et al.*, 2006).

Kunin (1998) and He & Gaston (2000) went on to demonstrate that it is possible to predict fine scale abundance from information gathered at coarser scales. This notion can be developed as a fractal model of species distributions (Kunin, 1998; Harte *et al*., 1999), or as a negative binomial model with a constant cross-scale aggregation parameter (He & Gaston 2000; Kunin *et al*., 2000). Both those types of models assume that aggregation parametres of each species' distribution display consistent, predictable properties across scales. For example, even if species' distributions were in general twice as aggregated at fine scales as at coarse scales, good fine-scale predictions could be made so long as the relative behavior of species remained constant: the most aggregated species at one scale being the most aggregated at other scales as well (Hartley *et al.*, 2004).

Fractal models are based on the scaling pattern of occupancy (Kunin, 1998; Hartley & Kunin, 2003; Hui *et al.*, 2007), which describes how adjacent occupied grid cells merge with increasing grain (i.e., the percolation process of presence records across spatial scales (see Hui & McGeoch, 2007), reflecting the scale dependence of species range size (e.g., Kunin, 1998; Hurlbert & Jetz, 2007). These models have the potential to predict species abundance by extrapolating the occupancy-scale relationship down to a scale fine enough to encompass only a single individual (Hartley & Kunin, 2003; Hui *et al*., 2009). Negative binomial models on the other hand, can predict species abundance by presupposing a specific probability distribution of population density or by assuming that particular population dynamics underlie a species distribution (He & Gaston, 2000).

III. The study areas

1. Mount Olympus

Mt Olympus is the highest mountain in Greece and is located at the border between Thessaly and Macedonia. Its highest peak (Mitikas) rises up to 2,917 m. It covers an area of 56,000 ha in total (Dafis, 1989).

Olympus has been declared as a National Park since 1938. The core of the park is located on the eastern side of the mountain in an area of about 4,000 hectares, and the peripheral zone of the National Park extends to about 24,000 hectares, in total. In 1981 the national park was designated a biosphere reserve by UNESCO. The area is included in the 79/409/EEC Birds Directive and in the Natura 2000 network (92/43/EEC Habitats Directive).

i) Geomorphology

Olympus' consists mainly of dolomitic limestone and marble. Most of the eastern slopes' substrate, between 500 and 2,000 m, consist mainly of dolomitic limestone of the Upper Triassic period. The Mitikas complex (2,000 m and above) consists of dolomitic limestone of the Jurassic period. The West and most of the northern slopes (between 1,200 and 2,000) consist of dolomitic limestone of the lower Eocene or the Cretaceous period. There occurs a zone of Gneiss in the western and southern slopes, between 700 and 1,200 m. Gneiss may occur locally, at the northern slopes of the mountain. Flysch occurs at the northwest part of the mountain, over the area of Petra village, at around 600 to 1,200 m. Loose conglomerates form the substrate of the eastern and northern foot of the mountain (Strid, 1980; Dafis, 1989; Theodoropoulos *et al*., 2011).

Olympus' topography is characterized by steep slopes and deep ravines and valleys, mainly in the eastern and northern sides of the mountain. Precipitation is drained deep within the substrate, and as a result, there are no surface water reserves or springs above 1,100 m altitude during the summer months, even though precipitation levels are sufficiently high (Theodoropoulos *et al*., 2011).

ii) Climate

Olympus' climate can be described as Mediterranean, with continental influences. In lower elevations the climate is typically Mediterranean (hot and dry in summer, while cold and rainy in winter). Temperature varies in the winter from -20 ^{o}C to 10 ^{o}C and in the summer from 0 ^{o}C to 20 ^{o}C, while winds are almost an everyday occurrence. Generally, the temperature falls by 0.5 ^{o}C per 100 m of altitude. The coastal northeast slopes of Olympus receive more rain than the continental northwest. The hottest month is August, while the coldest is February. The mountain's highest zone (over 2,000 m) is snowcapped from November to May-June. The average annual precipitation ranges from 110 cm to 180 cm, half of which is snow during the winter months. (Management agency of Olympus national Park, webpage)

The occurrence of the limestone aggravates the drought phenomena by increasing maximum temperatures during the summer. The eastern slopes of the mountain are influenced by the presence of the sea, and as a result, the climate appears to be more oceanic relative to the western slopes, where climate is continental. Slopes and solar exposure contribute to the local differentiation of climatic conditions (Strid, 1980).

iii) Vegetation

Vegetation can be discerned in four well-defined zones: Mediterranean vegetation (*Quercetalia ilicis*), Zone of beech, fir and mediterranean mountain conifers (*Fagetalia*), Boreal conifers (*Vaccinio-Piceetalia*), and Alpine meadows (*Astragalo-Acantholimonetalia* or *Daphno-Festucetalia*). There exists a deciduous oak vegetation zone (*Quercetalia pubescentis*) as well, though it is not clearly defined. (Dafis, 1973; Horvat *et al*. 1974; Mavromatis, 1980; Quézel & Barbero, 1985; Athanasiadis, 1986; Habeck & Reif, 1994; Zagas *et al*. 2002; Bohn *et al*., 2000/2003; Theodoropoulos *et al*., 2011).

1. Mediterranean Vegetation (Quercetalia ilicis)

Maquis vegetation occurs at the eastern (and to a lesser degree at the northern and northeastern) slopes of the mountain, at 200 – 800 m altitude. It extends for approximately 750 ha and includes evergreen and deciduous shrubs that will often reach 2 – 4 m, in height (Theodoropoulos *et al*., 2011).

- Evergreen species: *Quercus ilex*, *Fraxinus ornus*, *Quercus coccifera*, *Arbutus andrachne, Arbutus unedo, Phillyrea latifolia, Juniperus oxycedrus, Erica arborea, Laurus nobilis*, etc.

- Deciduous species: *Acer monspessulanum, A. campestre, Cercis siliquastrum, Pistacia terebinthus, Cotinus coggygria, Ostrya carpinifolia, Carpinus orientalis, Cornus mas*, etc.

There exist 12 species of orchids in the herbaceous subfloor, with the most common being *Orchis quadripunctata, O. morio* subsp. *picta* and *Anacamptis pyramidalis*. *Platanus orientalis* azonic vegetation may occur close to waterfronts at 270 – 450 m altitude as well (Theodoropoulos *et al*., 2011).

2. Zone of beech, fir and mediterranean mountain conifers (Fagetalia)

- Deciduous oak vegetation zone (Quercetalia pubescentis)

Mediterranean vegetation is gradually succeeded by *Pinus nigra* ecosystems (*P. nigra* subsp. *nigra* var. *caramanica* = *P. nigra* subsp. *pallasiana*). *P. nigra* dominates arid ridges and rocky slopes of the eastern and northern side of the mountain, at 500 – 1700 m altitude, covering approximately 6,800 ha. Deciduous vegetation may occur at places where moisture

conditions are favorable. From 1600 m and above, *P. nigra* is gradually replaced by *Pinus heldreichii*. *P. nigra* and *Abies x borisii-regis* individuals may occur within maquis vegetation, from 400 m and above, at the northern and eastern slopes of the mountain (Theodoropoulos *et al*., 2011).

The forest shrubbery subfloor is comprised of maquis vegetation such as *Quercus coccifera, Juniperus oxycedrus, Arbutus andrachne and Phillyrea latifolia* at lower altitudes. A widespread characteristic species is *Staehelina uniflosculosa.* Other shrubs that frequently occur are *Rhus coriaria* and *Coronilla emerus*, while *Genista radiate* is quite common at the upper part of the *P. nigra* distribution (Theodoropoulos *et al*., 2011).

Common species of the herbaceous subfloor are *Astragalus monspessulanus, Chamaecytisus polytrichus, Campanula lingulata, Clinopodium vulgare, Dorycnium hirsutum, D. pentaphyllum, Ferulago sylvatica, Festuca valesiaca, Geranium sanguineum, Helianthemum nummularium, Inula oculus-christi*, *Origanum vulgare, Scutellaria rubicunda, Sesleria robusta, Teucrium chamaedrys, Thalictrum minus* subsp. *olympicum, Trifolium alpestre,* and other (Theodoropoulos *et al*., 2011).

Other species that may occur are *Achillea ageratifolia, Anthericum liliago, Centaurea graeca, C. grbavacensis, Cnidium silaifolium, Genista sakellariadis, Laser trilobum, Laserpitium siler* subsp. *garganicum, Phlomis samia, Silene oligantha, Pulsatilla halleri, Saxifraga scardica, S. grisebachii, , Salvia ringens, Satureja montana, Scorzonera hispanica, Sedum ochroleucum, S. sartorianum, S. dasyphyllum* and other (Theodoropoulos *et al*., 2011).

Along the road network (between 700-1300 m), *Carlina acanthifolia, Carduus thoermeri, Cirsium candelabrum, Ptilostemon afer, Verbascum eriophorum, V. phlomoides* frequently occur (Theodoropoulos *et al*., 2011).

The ***mediterranean deciduous oak vegetation zone*** (*Quercetalia pubescentis*) is not fully developed. However individual oak trees may occur within patches of *P. nigra*. There exists a *Quercus pubescens* forest at the northern slope of the Stream Ziliana (Xirolakos) (600-700 m), which extents to approximately 120 ha. *Quercus pubescens* may occur in mixed patches with *P. nigra* over the village Petra (500-800 m) as well. *Quercus petraea* subsp. *medwediewii* (*= Q. dalechampii*) (600-1100 m) and *Castanea sativa* occur sporadically (Theodoropoulos *et al*., 2011). *Abies x borisii-regis* may occur in small mixed clusters with *P. nigra* and P. *heldreichii* at lower attitudes. The total occupied area of the species is 130 ha. Locally, it may occur in up to 2,000 m altitude at the northeastern side of the mountain. Small clusters of *Fagus sylvatica*

s.l. may occur as well, where conditions are favorable. The total area occupied by the species is approximately 1,130 ha.

Common shrubs to be found in this vegetation zone belong to *Buxus sempervirens, Cotoneaster nebrodensis, Daphne laureola, Euonymus latifolius and Hedera helix*.

The herbaceous subfloor's most common grass species are *Bromus benekenii, Festuca drymeja, Melica uniflora, Milium effusum, Poa nemoralis* etc., the fern species *Phyllitis scolopendrium* and *Polystichum aculeatum, and the perennial herbs Actaea spicata, Arabis turrita, Calamintha grandiflora, Cardamine bulbifera, Circaea lutetiana, Coralloriza trifida, Cyclamen hederifolium, Epipogium aphyllum, Galium odoratum, G. rotundifolium, Lathyrus venetus, L. laxiflorus, L. grandiflorus, Lilium chalcedonicum, Melittis melissophyllum, Mercurialis ovata, Orthilia secunda, Potentilla micrantha, Salvia glutinosa, Saxifraga rotundifolia, Stachys sylvatica, Symphytum bulbosu, and others* (Theodoropoulos *et al*., 2011).

3.Boreal coniferous (Vaccinio-Piceetalia)

This vegetation zone occurs solely on high mountains of Northern Greece. The characteristic forest species are *P. sylvestris*, *P. heldreichii*, and *Picea abies* (Athanasiadis, 1986).

Within the limits of the National park at the location "Prionia", *P. heldreichii* occurs sporadically from 700 m to 1,400 m, where it replaces *P. nigra* gradually up to 1,700 m, before it becomes the dominant forest species. The forest becomes scarcer above 2,000 m and forms the tree line, at approximately 2,500 m altitude. Small shrubbery forms of *P. heldreichii* may occur sporadically up to 2,700 m in altitude (Theodoropoulos *et al*., 2011). *P. heldreichii* occurs in loose clusters at the eastern and northeastern side of the mountain, between 1,500 and 2,000 m altitude. It occupies an area of approximately 3,500 ha.

Common species of the shrubbery sub-floor are *Buxus sempervirens* (up to 2,100 m)*, Cotoneaster integerrimus, Daphne laureola*, *D. mezereum Genista radiata* (1,300- 1,900 m) and *Juniperus communis* subsp. *nana.*

Common grass species: *Bromus cappadocicus* subsp. *lacmonicus, Festuca varia*, *Polystichum lonchitis, Sesleria robusta.* Perennial herbs: *Euphorbia amygdaloides* subsp. *heldreichii, Gentiana asclepiadea, Pedicularis brachyodonta, Prenanthes purpurea, Saxifraga rotundifolia, Senecio hyrcinicus* subsp. *expansus* and other. Orchids: *Coeloglossum viride,*

Gymnadenia conopsea, Orchis pallen and other.

4. Alpine meadow (Astragalo-Acantholimonetalia or Daphno-Festucetalia)

Above the Boreal coniferous zone, there occurs an extensive alpine zone that covers approximately 5.200 ha. It comprises of a mosaic of alpine meadows, whose species composition depends on the topography, the slope and exposure of the ground. There occur over 150 species, from which, approximately half are Balkan Endemics (12 are local endemics).

Olympus Endemics are: *Achillea ambrosiaca, Alyssum handelii, Asperula muscosa, Aubrieta thessala, Campanula oreadum, Centaurea incompleta, C. litochorea, C. transiens, Cephalaria tenuiloba, Cerastium theophrasti, Coincya nivalis* (*=Rhynchosinapis nivalis*), *Erysimum olympicum, Festuca olympica, Genista sakellariadis, Jankaea heldreichii, Ligusticum olympicum, Melampyrum ciliatum, Potentilla deorum, Silene oligantha* subsp. *oligantha, Viola pseudograeca, Viola striis-notata* (Tutin *et al.* 1968-1980, 1993; Erben, 1985; Strid, 1986; Strid & Tan, 1991, 1997, 2002; Theodoropoulos *et al*., 2011).

2. Mount Falakro

Mt. Falakro is located in eastern Macedonia (northeastern Greece), northwest of the city of Drama. It covers an area of 96,000 ha. It highest peak is Profitis Ilias, at 2,232 m.

It belongs to the crystalline mass of Rodopi and consists mainly of marble. Gneiss and granite prevail at the eastern slopes of the mountain. Schist substrate occurs in a small area at the southern slope of the mountain (Tsiftsis *et al*., 2006).

i) Climate

The climate of Falakro can be characterized as transitional between the mediterranean and continental type, and it has a relatively short dry period (less than two months) during the summer. The mean annual temperature is 10.6 ^{o}C and the annual precipitation is 758 mm on average (Petermann, 1999; Tsiftsis *et al*., 2006). At the highest altitudes of the mountain the climate becomes continental, without a dry period during the summer (Tsiftsis *et al*., 2006).

ii) Vegetation

The vegetation of Mt. Falakro is differentiated according to altitude, substrate and physiography. The lower altitudes of the southern slopes of the mountain are covered by pseudo-maquis, in which *Quercus coccifera* and *Carpinus orientalis* dominate (upon calcareous substrates). At the northeastern slopes, deciduous oak forests (*Quercus frainetto*, *Q. petraea* ssp. *medwediewii*) replace the pseudo-maquis vegetation. *Pinus nigra* ssp. *nigra* forests occur above the pseudo-maquis, from the altitude of about 800 m (calcareous substrates), while stands of *Fagus sylvatica* s.l. occur inside the *Pinus nigra* ssp. *nigra* forests in the northeastern, moister slopes, of gneiss or granite. Mountainous and subalpine grasslands occupy a large part of the mountain above the timberline, of *Pinus nigra* ssp. *nigra* (S and W slopes) and *Fagus sylvatica* s.l. (SE, NE, E, N slopes) (Tsiftsis *et al*., 2006), which is formed from 1,200 to 1,800 m.

IV. *Campanula* species

1. Family Campanulaceae – Genus *Campanula*

Campanula L. is the largest genus of the family of Campanulaceae with c. 350–500 taxa inhabiting a wide range of habitats, including meadows, woodland-edges, moorlands, and cliffs, as well as steppe and mountainous habitats in the Northern hemisphere (Fedorov, 1957; Kovacic, 2004; Roquet *et al*., 2008).

Considerable variation in morphology, carpology (Kolakovsky, 1986), palynology (Dunbar, 1975; Dunbar & Wallentinus, 1976) and karyology (Gadella, 1964; Contadriopoulos, 1984) is found within the genus. Most representatives are annual to perennial herbs, with pentamerous flowers. The corolla is often campanulate or infundibuliform, tubular, rotate or any of several other peculiar forms. The anthers are free or occasionally connate around the style. The filaments have generally expanded bases (triangular) that form a dome over the nectariferous disk. *Campanula* has a characteristic stylar type of secondary pollen presentation, well described and discussed in the literature (Shetler, 1979; Yeo, 1993). The ovary is usually tri- or pentalocular, with the same number of stigmatic lobes. The capsule dehisces by pores of valves (Roquet *et al*., 2008).

The circumscription of *Campanula* is difficult, and the infra-generic classification has been highly controversial. Many approaches to *Campanula* taxonomy have been geographically limited and usually based on a few morphological characters. The main early treatments of

Campanula were the works of De Candolle (1830, 1839) and Boissier (1875), which resulted in quite different classifications. Fedorov's work (1957) was restricted to former USSR and Dambolds's work (1976) included only Europe and Turkey. Other important works were by Hayek (1925, 1931) for the Balkans, Quézel (1953) for North Africa, Shetler (1963) for North America and Oganessian (1995) for Caucasus, but all of them were limited by a narrow geographical scope (Roquet *et al*., 2008).

Gadella (1964) and Contadriopoulos (1984) attempted to infer phylogenetic relationships by combining cytology and morphology. However, *Campanula* presents a great variety of base chromosome number, even within the taxa of the Mediterranean basin alone. The most common number is x=17.

Pollen studies *in Campanula* were made by Dunbar (1975) and Dunbar & Wallentinus (1976). However, those characters were insufficient to separate *Campanula* from allied genera, such as *Adenophora*, *Asyneuma* Griseb. & Schenk, *Edraianthus* A. DC., *Jasione* L., *Phyteuma* L., *Roella* L., *Symphyandra*, and *Wahlenbergia* Schrad. ex Roth. Carpological studies by Kolakovsky (1986) did not serve to clarify the relationships between these taxa neither. Works dealing with the seeds (Geslot, 1980; Shetler & Morin, 1986) also found high similarity among them (Roquet *et al*., 2008); a light requirement for germination, constitutes a collective characteristic of the family (Koutsovoulou *et al.,* 2014). Finally, Shulkina *et al*. (2003), in a work of growth and seedling morphology, suggested that *Campanula* is a heterogeneous group that should be revised, and that similarities in Campanulaceae due to convergent evolution occur in reproductive and vegetative structures (Roquet *et al*., 2008).

Many studies have also remarked on the role of reproductive systems and pollinator service and behavior in the plasticity or evolution of flower shape (Shetler 1982; McCall & Primack, 1992; Maad & Armbruster, 2005; Maad *et al*. 2006; Pérez *et al*. 2006; Roquet *et al*., 2005). Pollinator composition is related to the corolla shape and varies from unspecialized taxa, such as Diptera (Syrphidae and Muscidae), small bees and Xylocopa, for rotate corollas, while broad and deep-campanulate corollas are mainly visited by more specialized taxa, such as bumblebees and large solitary bees (McCall & Primack, 1992; Bingham & Orthner, 1998; Blionis & Vokou, 2001; Al-Zein & Musselman, 2004; Schlindwein *et al*., 2005; Roquet *et al*., 2008).

Recently, phylogenetic relationships within the family have been explored by means of analysis of ITS-DNA sequences (Eddie, 1997; Eddie *et al*., 2003; Park *et al*., 2006; Roquet *et al*., 2008) and cpDNA rearrangements (Cosner *et al*., 2004; Roquet *et al*., 2008; Haberle *et al*.,

2009; Mansion *et al*., 2012). Results from such studies suggest that the family is divided into two groups: the taxa related to *Campanula*, which have porate pollen grains, and the remaining genera, which have colporate or colpate grains (*Campanumoea* Blume, *Canarina*, *Codonopsis* Wall., *Cyananthus* Wall. ex Benth., *Leptocodon* Lem., and *Platycodon* A. DC.) (Roquet *et al*., 2008; Haberle *et al*., 2009).

i) Campanula lingulata

C. lingulata Waldst. & Kit, is a biennial hemicryptophyte. Its geographical distribution extends to the Balkans and S. Italy. Its altitudinal distribution on Mt Olympus ranges from 200 to 1,700 m. *C. lingulata* is the most common species from the genus *Campanula* on Mt Olympus.

The genus on Mt. Olympus has been extensively studied by Blionis (2002), Blionis & Vokou (2001; 2002; 2005), Vokou *et al.* (2002). These studies established distributional, population, phenological, pollination and morphometric patterns. According to Blionis *et al*. (2001), *C. lingulata* flowers, at the lower altitudes, from late spring (mid-May) to early summer (early June), whereas it flowers from early June to mid to late July for middle to high elevations.

C. lingulata *Image: http://www.freenatureimages.eu*

C. lingulata is hairy and usually bears many offshoots, which can be decumbent, ascending or sometimes erect, ranging from 15 to 30 cm in length. The basal leaves are spatula-shaped and with an obtuse serration. The leaves of the shoot are epiphytic, anti-bladed to oblong, and with a slightly obtuse serration. The blossoms grow in apical heads, supported by oval lanceolate leaf-shaped bracts and sometimes also by oligoanthus axillary bunches. The blossoms are epiphytic. The bud features elongated lanceolate serrations with oval, upwards-curved appendices between them. The corolla is tubular to narrowly bell-shaped.

ii) Campanula spatulata

C. spatulata Sibth. & Sm. is the only species of *Campanula* on Mt. Olympus with a root tuber. It is divided in two subspecies, the lower *spruneriana,* which ranges from 400 to 1,100 m in altitude, and the upland *spatulata*, which ranges from 1,700 to 2,500 m. According to Blionis *et al*. (2001), at the lower altitudes, *C. spatulata* flowers from late spring (mid-May) to early summer (early June), whereas it flowers from mid-July to early September at high elevations. It is perennial and endemic to the southern Balkan Peninsula.

C. spatulata *Image: http//www. http://koinotopia.gr*

C. spatulata usually produces many thin, ascending to erect offshoots, 15-45 cm in length, with 1-5 blossoms. The basal leaves are spatular to angular, with a more or less blunt serration. The leaves of the shoot are oblong to narrowly lanceolate and epiphytic. The serrations of the bud are linearly lanceolate to acicular, at least twice longer than the ovary. The corolla is bell-shaped, 15-30 cm in length. The capsule is cylindrical to narrowly inverse conical, 6-10 mm in length. Two subspecies are found on Mount Olympus. The subspecies *spruneriana* (Hampe) Hayek is found at low altitudes (400-1100 m). It features flowering shoots with 2-5 blossoms, corolla 25-30 mm in length, and bud lobes, about five times longer

than the ovary. The subspecies *spatulata* is found at higher altitudes of 1,700 to 2,000 m. It has shorter flowering shoots with 1 blossom, and shorter and darker colored corolla and bud lobes. Hartvig (1991) mentions that the distinction between the two subspecies is "somewhat arbitrary." A third subspecies has been described, ssp. *filicaulis* (Halascy), which is found only in Crete (Fedorov & Kovanda 1976, Hartvig 1991; Blionis, 2002).

The species *C. spatulata* belongs to the European endemic species with chromosomal number 2n=20, which according to Contandriopoulos (1984) "appear as new elements, comparable to other *Campanula* species."

iii) Campanula rotundifolia

C. rotundifolia is found at high altitudes of 1,200 to 2,700 m on Mt. Olympus, where it flowers from mid-July to early September. It is represented by sleek, perennial individuals, with sparse creeping rhizome and thin, ascending to erect flowering shoots, 10-12 cm in length. The basal leaves have a long stem and feature a widely oval lamella, which is cardioid at its base, serrated, and is often absent during flowering. The leaves of the shoot are linear and compact. 1-3 blossoms grow per flowering shoot. The flowering buds are erect. The serrations of the bud are linearly lanceolate, 7-10 cm in length. The corolla is bell-shaped, 17-24 mm in length. The ovary is smooth. According to Hartvig (1991), it extends to Europe, northwest Africa, and temperate and boreal parts of northern Asia and North America. Fedorov & Kovanda (1976) and Strid (1980) regard the Greek plants as belonging to the separate species *C. albanica* Witasek (spreading in the southern and western parts of the Balkan Peninsula). However, Hartvig (1991) includes them (along with other species, such as *C. velebitica* or *C. hellenica*), in the aforementioned diverse species (Blionis, 2002).

C. rotundifolia
Image: www.wikipedia.org

V. Study objectives

This study aims toward the development of a conceptual framework regarding the spatial patterns of species distributions that incorporates the effect of factors and processes that shape them, which operate at various spatial scales, in an ecologically meaningful way. More specifically, we search for the relationship between occupancy and abundance for *Campanula* species. For *C. lingulata*, in particular, we search for an abundant centre along an altitudinal gradient and for genetic variability within and among populations.

To achieve this goal, the specific study objectives are the following:

- To record the presence of *C. lingulata, C. spatulata (*ssp. *spatulata,* ssp. *spruneriana)* and *C. rotundifolia* individuals on Mt. Olympus and map their occurrence at various spatial resolutions.

- To explore the form of the abundance-occupancy relationships across different spatial resolutions for the *Campanula* species that occur (and co-occur in part of their altitudinal range) in the mountainous region of Mt. Olympus.

- To investigate the "abundant centre" hypothesis in a spatially explicit context, by evaluating the observed patterns of variation for *C. lingulata* abundance in space and time, along an elevation gradient.

- To assess the patterns of genetic differentiation for *C. lingulata* populations that are located throughout the species altitudinal range on Mt. Olympus and Mt. Falakro, by use of random neutral genomic markers (RAPDs—random amplification of polymorphic DNA).

- To discuss the development of a model for species distributions in space, which would incorporate the variable effect of factors operating across various spatial scales, by superimposing a fractal-like "disturbance" on species' observed distribution patterns.

B. METHODS

I. Sampling – Data processing

1. Sampling

Sampling on Mt. Olympus was carried out during each species' flowering season, since the plants are easier to identify when in bloom. Accordingly, the flowering season was divided in 3 sampling periods. The first covered the lower (~200 m) to middle elevations (~1,000 m), where *C. lingulata* and *C. spatulata* ssp. *spruneriana* individuals where recorded. This sampling period started at mid-May, and ended at mid-June. The second sampling period covered middle to high elevations (~1,300 m), where *C. lingulata* and *C. spatulata* ssp. *spruneriana* individuals occur. It began from mid-June and ended at mid-July. The final sampling period covered higher elevations (~2,500 m); it began in mid-July and ended in late August. *C. spatulata* ssp. *spatulata* and *C. rotundifolia* individuals were recorded. Two full surveys for the region were carried out, in 2012 and 2013. The two surveys were identical in terms of time that they were conducted and routes followed.

An initial appraisal of the study area took place during the summer months of 2011. Various roads and paths on and around the mountain were assessed with accessibility, positioning, directionality, length, elevation, habitat heterogeneity, and presence of individuals of *Campanula* species being the criteria for the final route selection.

Sampling was carried out along transects consisting of existing roads and paths on and around Olympus. The paths that were surveyed are shown in Fig.2. The routes that were on the Road network (National/Rural) were traversed by car, with a steady very low speed, while the routes that were on paths on foot, all by the same researcher. Individuals that occurred along the road network were further visited on foot. The total surveyed route length was 74 km.

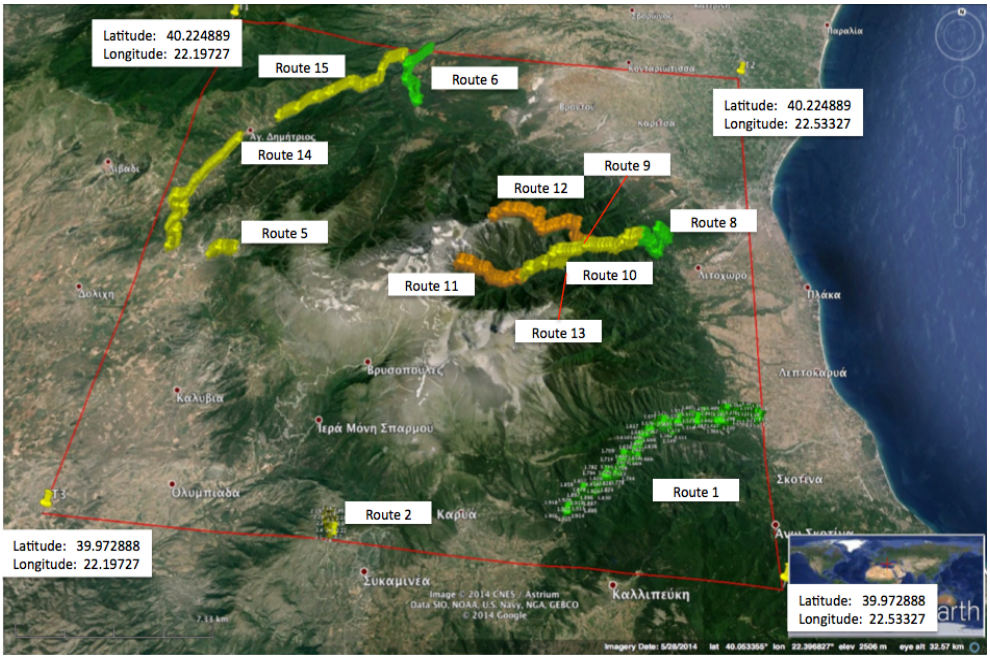
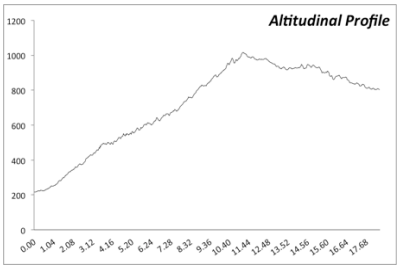


Figure 2: Sampling area of Mt. Olympus with the surveyed routes. Green markers correspond to routes sampled during the first, yellow markers correspond to routes samples during the second, and orange markers, to routes sampled during the final sampling period (*Image from Google Earth*).

Route 1: Leptokarya - Karya



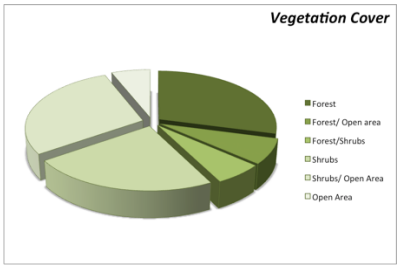


Figure 3: Route 1 — Altitudinal profile and Vegetation cover (*Image from Google earth*)

Route 1 traverses the southeastern part of the mountain. Its direction is from the East (town of Leptokarya) towards the village of Karya (to the southwest). Its total length is 18.38 km. Altitude varies from 216 to 1017 m. Mean altitude is 711 m.

Route 1 was surveyed during the first and the second sampling period. The dominant forest species is *P. nigra*. Maquis vegetation is present at lower altitudes. Along this route, the main human activities are: residential, tourism infrastructure (Leptokarya), agriculture, grazing and beekeeping (Karya).

Route 2: Rural Road Network (Karya - Petra) – Kria Vrisi settlement

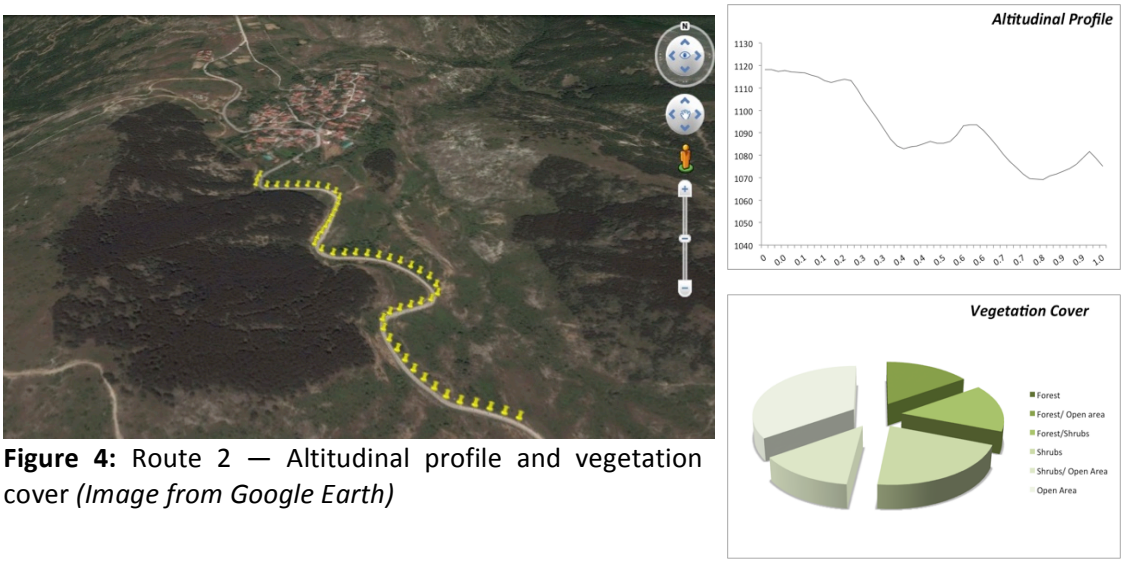


Figure 4: Route 2 — Altitudinal profile and vegetation cover *(Image from Google Earth)*

Route 2 is at the southern side of the mountain. Its direction is South (Rural Network) to North (village of Kria Vrisi). Its total length is 1.2 km. Altitude varies from 1,069 to 1,118 m. Mean altitude is 1,092 m. Route 2 was surveyed during the second sampling period.. The dominant species of the forest patch is *P. nigra*. Along this route, the main human activities are agriculture, livestock raising and grazing.

Routes 3 and 4 were located along the rural road network that connects Karya settlement, which is located at the southern part of the mountain, to the settlement of Petra in the North. However, few to no *Campanula* individuals were observed, and thus they were excluded from the study. To note, a ski resort owned by the Greek army at the area of Vrisopoules (1, 850 m altitude) operates in the area.

Route 5: Rural road network (Karya - Petra) – Kokkinopilos village

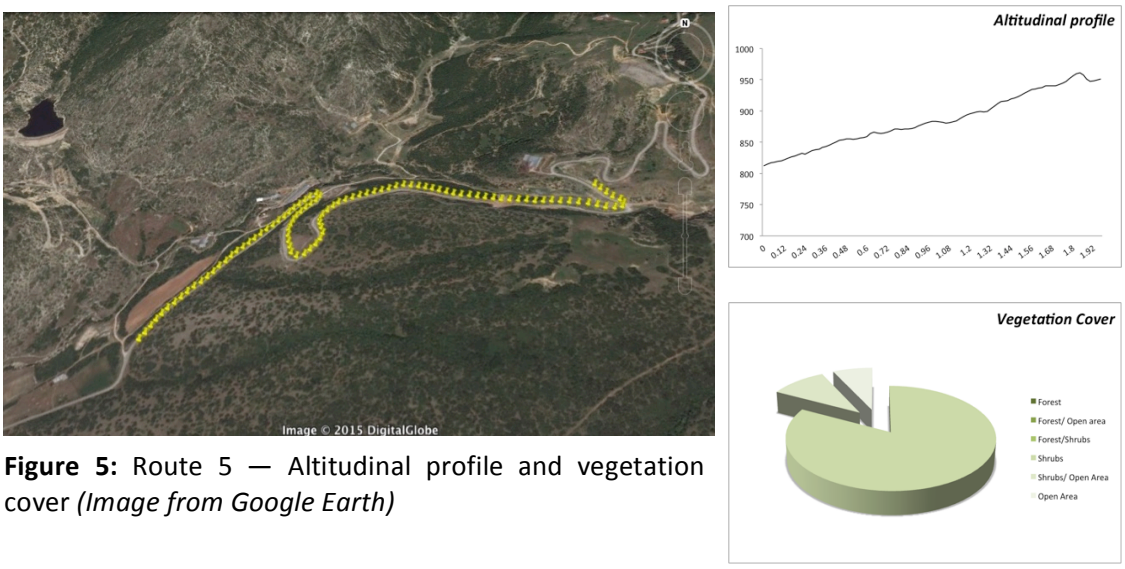
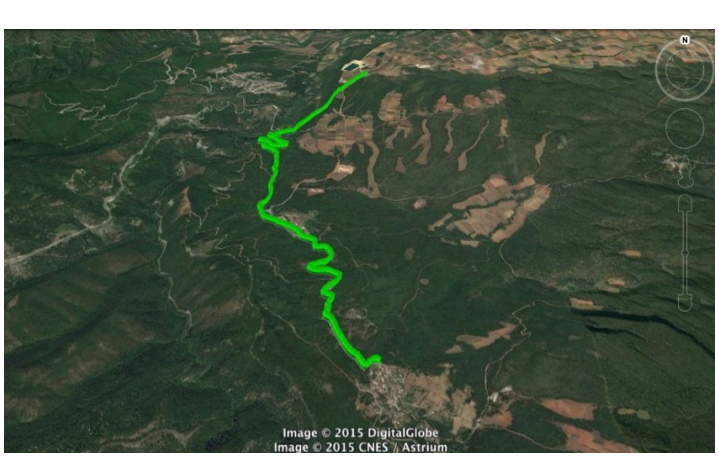
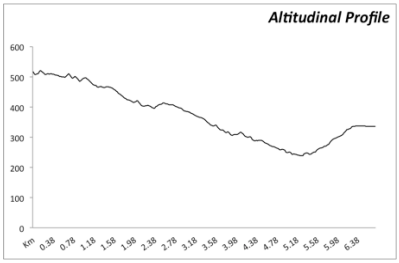


Figure 5: Route 5 — Altitudinal profile and vegetation cover *(Image from Google Earth)*

Route 5 is on the western part of the mountain. Its direction is West (Road Network) to East (Kokkinopilos village). Its total length is 1.9 km. Altitude varies from 812 to 961 m. Mean altitude is 885 m. The vegetation consists mainly of shrubs. Along this route, the main human activities are farming, livestock raising and grazing.

Route 6: Petra settlement - National road network (Petra-Katerini)





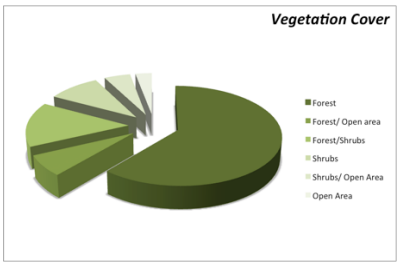


Figure 6: Route 6 — Altitudinal profile and vegetation cover *(Image from Google Earth)*

Route 6 is at the northern part of the mountain. Its direction is from South (Petra village) towards the National road network (to Katerini). Its total length is 6.74 km. Altitude varies from 238 to 520 m. Mean altitude is 370 m. It was surveyed during the first sampling season. The area is forested, with dominant species being *P. nigra.* Other species that were observed were *Quercus pubescens*, at higher altitudes, and *Platanus orientalis*, at the low ones. Along this route, the main human activities are grazing, livestock raising and farming nearby Petra village.

Route 7 is located at the northern part of the mountain as well (town of Dion). However no individuals were observed during the initial appraisal of the area, so it wasn't included in the study.

Route 8: Litochoro - Stavros refuge

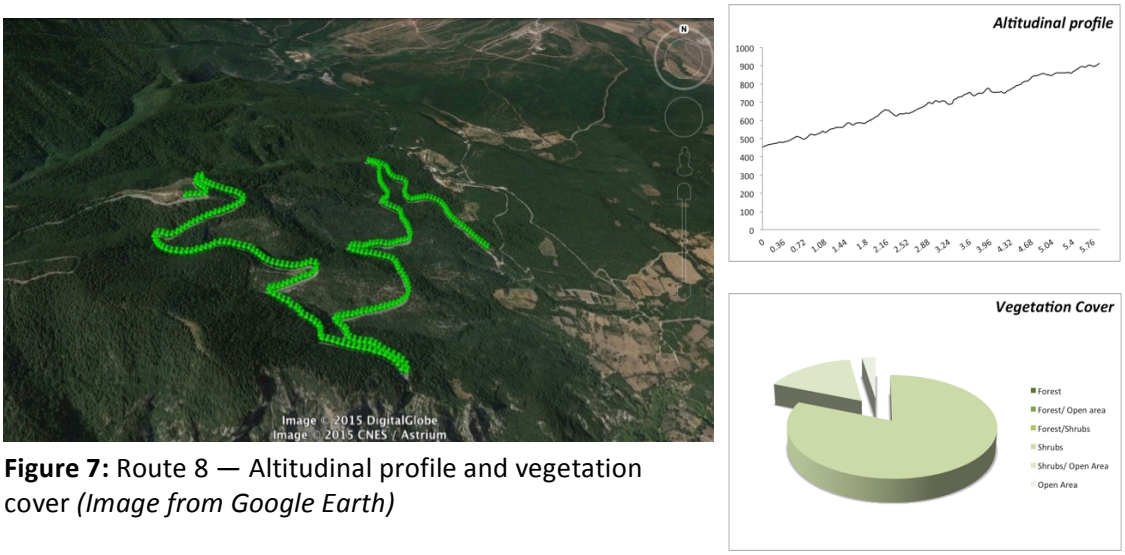
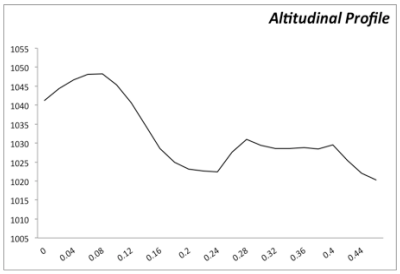


Figure 7: Route 8 — Altitudinal profile and vegetation cover *(Image from Google Earth)*

Route 8 is at the northeastern part of the mountain. Its direction is from the east (town of Litochoro) toward the west (Stavros refuge). The total surveyed length was 6.74 km. Altitude varies from 238 to 520 m, with mean altitude being 370 m. It was surveyed during the first sampling period. It is located within the National Park limits, and human activities are strictly regulated at the area. The area of Litochoro has a significant tourist infrastructure. Hiking and other related activities occur. The vegetation is typically Mediterranean, with the dominant species being *Quercus ilex*. Along this route, the main human activities are hiking and related activities.

To note, populations of *C. lingulata* and *C. spatulata* spp. *spruneriana* were observed in and around the town of Litochoro. However, road constructions took place during the sampling period, disturbing the area, so the few recorded individuals in the area were excluded from further analysis.

Route 9: Observation Point Karia



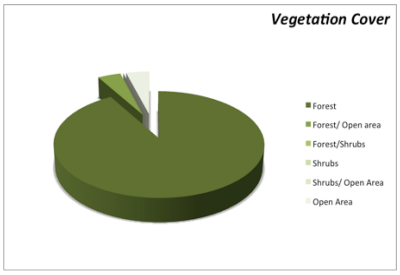
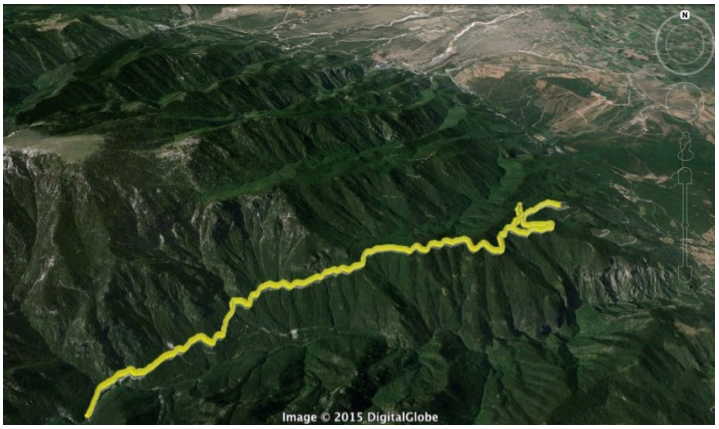
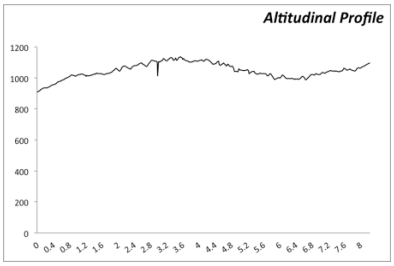


Figure 8:Route 9 — Altitudinal profile and vegetation cover *(Image from Google earth)*

Route 9 is a small path within the area of the National park (direction South to North), which starts from an observation point directly above Stavros refuge. Its total length is 0.46 km. Altitude varies from 1,020 to 1,048 m. It is forested (*P. nigra* forest).

Route 10: Stavros Refuge - Prionia





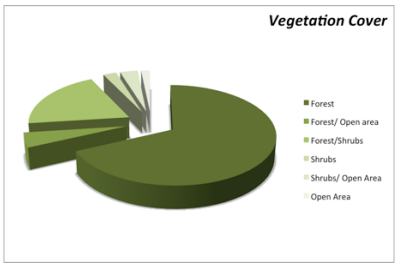
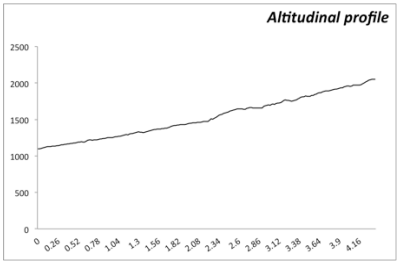


Figure 9: Route 10 — Altitudinal profile and vegetation cover *(Image from Google Earth)*

Route 10 is within the area of the National park, at the northeastern part of the mountain. Its direction is east (Stavros Refuge) to West (Prionia), and was surveyed during the second sampling period. Its total length is 8.22 km, and altitude varies from 909 to 1,136 m. Mean altitude is 1,046 m. Vegetation gradually changes from Mediterranean to a *P. nigra* forest.

Route 11: Prionia - Zolotas Refuge path



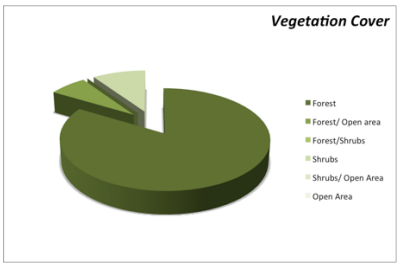
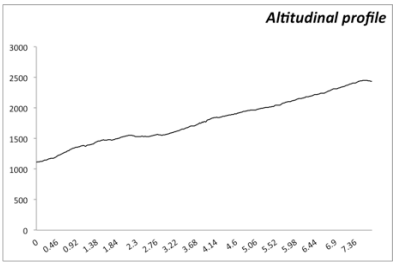


Figure 10: Route 11 — Altitudinal profile and vegetation cover *(Image from Google Earth)*

Route 11 follows a path located within the National park. Its direction is from East (Prionia) to West (Zolotas refuge). Its total length is 4.36 km. Altitude ranges from 1,095 to 2,053 m. Mean altitude is 1,530 m. It is a well-traversed path, which crosses *P. nigra* and Boreal coniferous forests and also the alpine meadows.

Route 12: Petrostrougga - Oropedio Mouson path



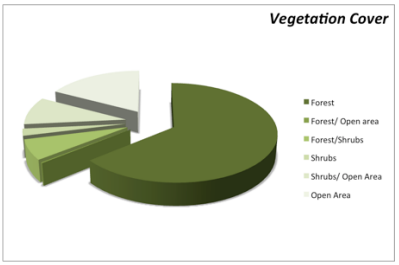


Figure 11: Route 12 — Altitudinal profile and vegetation cover *(Image from Google Earth)*

Route 12 follows another path within the National Park. Its direction is from Southeast (Road to Prionia, beginniing of the path) to Northwest (Oropedio Mouson). Its length is 7.74 km, and the altitude ranges from 1,113 to 2449 m. Mean altitude is 1,789 m. It is a frequently crossed path, which traverses the *P. nigra* and the Boreal coniferous forest and also the alpine meadows. A large part of the route is within the alpine vegetation zone, where *C. spatulata* ssp. *spatulata* and *C. rotundifolia* individuals were recorded.

Route 13: Agios Dionisios monastery

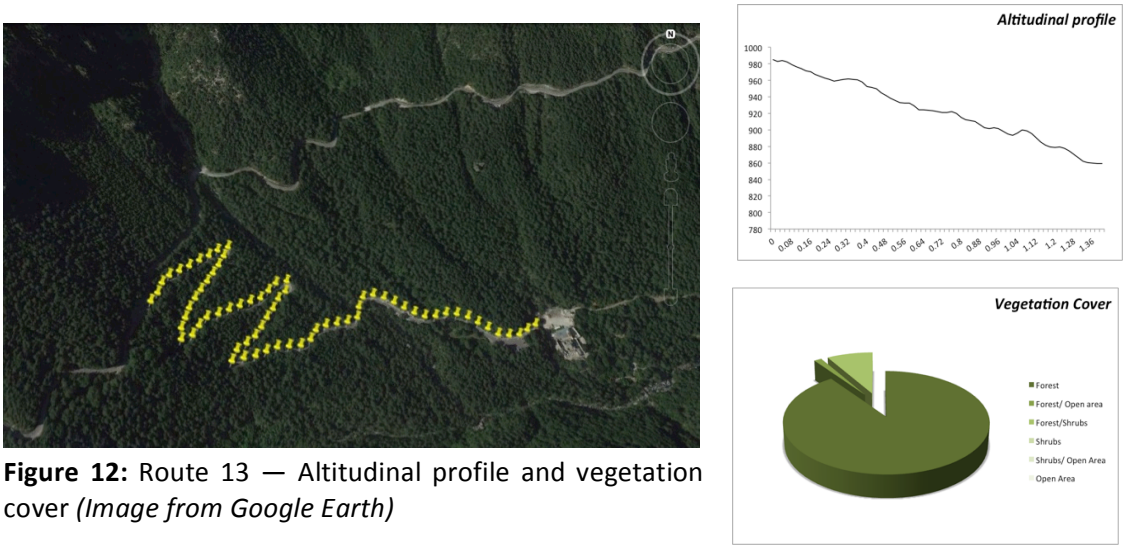
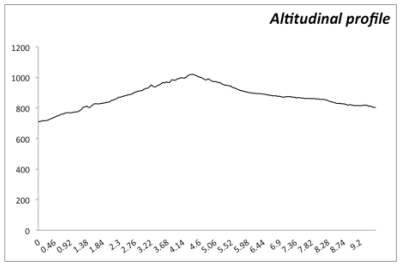


Figure 12: Route 13 — Altitudinal profile and vegetation cover *(Image from Google Earth)*

Route 13 is within the limits of the National park. Its direction is from West (Road to Prionia) to East (Agios Dionisios monastery). Its total length is 1.4 km. Its altitude varies little, from 959.1 to 984.8 m. Mean altitude is 923 m. It is a forested area (*P. nigra* forest), and it is very close to the Enipeas' riverbank. Other species that characterize the area are *Abies x borisii-regis*, *Fagus sylvatica* s.l., *Quercus pubescens*, *Ulmus glabra* as well as *Prunus cerasifera*, *Juglans regia and Taxus baccata* (Theodoropoulos *et al.,* 2011).

Route 14: National Road Network (SW) (Katerinis - Ellasonas dir: SouthWest)



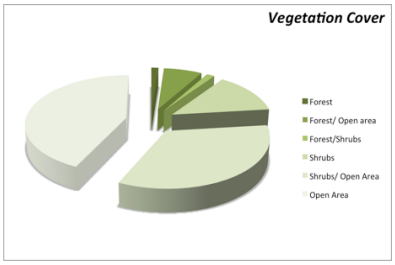


Figure 13: Route 14 — Altitudinal profile and vegetation cover *(Image from Google earth)*

Route 14 traverses the western part of the mountain. Its direction is from South to North, along the National Road network. Its total length is 9.46 km. Its altitude varies from 710 to 1,020 m. Mean altitude is 875 m. It goes through an agricultural landscape. The remaining vegetation is mostly Mediterranean. Human presence and activities have severely altered the characteristics of the area.

Route 15: National Road Network (NE) (Katerinis - Ellasonas dir: NorthEast)

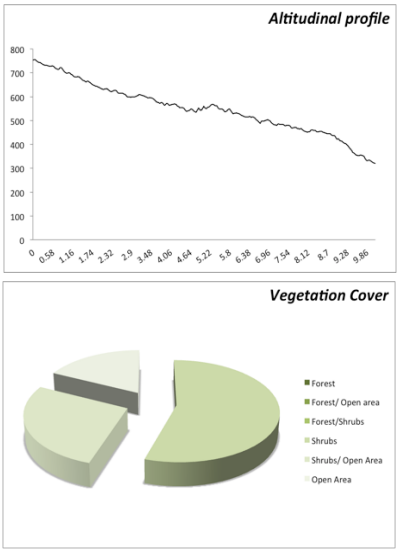


Figure 14: Route 15 — Altitudinal profile and vegetation cover *(Image from Google Earth)*

Route 15 traverses the western side of the mountain as well. Its direction is from Southwest to Northeast, along the National road network. Its total length is 10.1 km. Its altitude varies from 319 to 755 m. Mean altitude is 548 m. The vegetation is typically Mediterranean.

There have been 3 visits on Mt. Falakro, one each sampling season, during the year 2012. Presence of *C. lingulata, C. spatulata* and *C. rotundifolia* was recorded, and samples of *C. lingulata* individuals, from the areas shown in Fig 16 were collected for molecular analysis.

There have been 3 visits on Mt. Smolikas as well, one each sampling season, during 2013. However no *Campanula* individuals were observed in the area.

2. Climate, vegetation and sampling effort

The type of vegetation of the surroundings of each route was noted in 20 m intervals along each sampled route, as seen in Google Earth [see *placemark*s on Figures 2-14 (B.I.1); each placemark corresponds to a set of decimal coordinates], and was afterwards compared with field observations. Each *placemark* was then assigned to one of the following categories. closed forest (70%-100%), open forest (30%-70%), woodland (10%-30%), closed-scrub (70%-

100%), open-scrub (30%-70%), open shrubland (<10%), based on the percentage foliage cover of the tallest plant layer (Australian National Botanical garden,webpage). The altitude in meters above the sea level was noted as well (Figs 3-14; Figs 23-25).

Climate variables were obtained from http://www.worldclim.org/ (WorldClim – Global Climate Data) online database. The decimal coordinates of each *placemark* where utilized to extract information on Mean Temperature and Mean Precipitation per Month, from raster layers which were generated through interpolation of average monthly climate data from weather stations on a 30 arc-second resolution grid (often referred to as "1 km^2" resolution) [see: Hijmans *et al.*, (2005) for extrapolation methods]. The highest resolution available was utilized [30 arc-seconds) ~ 1 km^2]. Mean temperature and precipitation were averaged for the number of placemarks for each route. (Figs 20-22). The command lines used in order to extract information from the database are given in Appendix I.

Effort was defined as the number of *placemarks* for each route. The distance between each of the *placemarks* is 20 m. 10 *placemarks* correspond to a 200-m transect, 100 *placemarks* to a 2000-m transect, etc (Fig 26).

3. Mapping the species distribution at different spatial scales

All individuals in bloom within 20 m of the surveyed route on either side were recorded and their position was estimated with a hand-held GPS device (eTrex Vista HCx). The coordinates were stored in decimal degrees, in order to facilitate further calculations. Data processing was performed in R statistical and programming environment, version 3.13 (R Development core team, 2008).

A set of eight decimal coordinate values can define a quadrangle on the Earth's surface, wherein all the observed individuals are included (Fig 2). Twelve nested grids at different scale resolutions were produced to overlay the surveyed area. In order to produce the finest grid, with 10 m × 10 m cells, 10 m intervals in the WE axis were assigned by adding 0.00012 decimal points to each preceding longitude coordinate value, starting from the SW point of the square a total of 3,072 times (Fig 2). Likewise, 10 m intervals were assigned on the SN axis, by adding 0.00009 decimal points to each preceding latitude coordinate value. In order to produce 20 × 20 m grids, 20 m intervals were assigned on the WE and SN axes by adding

0.00024 and 0.00018 in longitude and latitude, respectively, 1,536 times for each, and so on. In converting between meters and decimal points the curvature of the Earth was ignored.

To sum up, for each resolution, a longitude and latitude increment to be added to the corresponding preceding coordinate value was calculated. Starting from the SW corner of the sampled area, each successive coordinate value for longitude and latitude from this point on defined a different successive interval on the WE and SN axes. After *n* intervals on each axis were established for each resolution, an $n \times n$ two-dimensional grid or matrix for each scale was obtained. Twelve matrices, one for each resolution were produced in total, as given in Table 1. Thus, each cell at each scale contains 2 × 2 sub-cells from the next scale. The exception to this rule is the coarsest scale, which is divided into 3 × 3 cells (Table 1).

Table 1: Cell numbers, cell side lengths and Longitude and Latitude increments for each spatial scale.

Scale ID	*No of rows or columns*	Cell side length *(m)*	*Longitude interval (d. d)*	*Latitude interval (d. d)*	*Area covered per cell (m^2)*
0	1	30720	-	-	943,718,400
1	3	10240	0.12288	0.09216	104,857,600
2	6	5120	0.06144	0.04608	26,214,400
3	12	2560	0.03072	0.02304	6,563,600
4	24	1240	0.01536	0.01152	1,537,600
5	48	640	0.00768	0.00576	409,600
6	96	320	0.00384	0.00288	102,400
7	192	160	0.00192	0.00144	25,600
8	384	80	0.00096	0.00072	6,400
9	768	40	0.00048	0.00036	1,600
10	1536	20	0.00024	0.00018	400
11	3072	10	0.00012	0.00009	100

Every observation of the surveyed area was placed in each grid, by comparing the coordinate values of the observations with the values that marked the intervals in each spatial scale, and assigning them to corresponding interval and row or column of the matrix. Rows indicated position of each point relative to the WE axis, and columns its position relative to SN axis. Each pair of indices, thus combined, denotes an individual's placement within each of the twelve grids. See Appendix II for a detailed description of the command lines.

4. Occupancy-abundance estimates at different spatial scales.

In order to visualize the species' abundance in space, two Locator Variables that indicated the position of each individual on row/column of a matrix, which corresponded to the SN and WE direction, accordingly, where utilized (See Appendix II; Fig 27).

Occupancy refers to the number of occupied squares for each spatial scale. Spatial scale is not perceived as defined in Table 1[B.II.2]. Spatial scale 1 now refers to the finest (10 × 10 m) resolution, spatial scale 2 to the 20 × 20 m resolution, and so on.

Abundance refers to the number of individuals of every species in each occupied square in every spatial scale. Occupancy as a function of mean abundance (mean value of abundances on occupied cells) is shown in Fig 32a. Occupancy and mean abundance at a log-log scale is shown in Fig 32b. Occupancy as a function of mean density (mean abundance/relative grid size) is shown in Fig 32c. Relative grid size was calculated as the size of the grid (N × N) divided by 1/9 (N × N for the spatial scale 1 as defined in B.II.2).

A linear model of occupancy as a function of mean density was applied (ModN). It aims to describe the relative aggregation of the species. A species was considered more "aggregated" relative to the others for a given average density (over grid), if within a spatial scale it occupied a smaller number of grid squares, or if it occupied the same number of squares or less in a finer resolution. Furthermore, an ANCOVA analysis of variance was performed as a function of the factors "species" (*C. lingulata*, *C. spatulata*, *C. rotundifolia*) and "year" (2012,2013), in order to interpret the residual model variance in terms of differences in occupancy amongst species (ModNS), amongst years of sampling (ModNSY), and amongst combination of both (ModNSYa). The summary of the models and a graphic description of the residuals' behavior is given in section C.I.4 of the Results.

For a detailed description of the command lines used, see appendix III.

5. The use of environmental gradients in modeling species distributions

Species abundance distributions have long been associated with environmental gradients (Merriam, 1894; Shelford, 1911; Andrewartha & Birch, 1954; Kendeigh, 1974; Cox & Moore, 1985; Sagarin & Gaynes, 2002; Sexton *et al.*, 2009) and the importance of climate to explain animal and plant distributions was recognized early on (Humboldt & Bonpland, 1807; de

Candolle, 1855). Climate in combination with other environmental factors has been much used to explain the main vegetation patterns around the world (e.g. Salisbury, 1926; Cain, 1944; Good, 1953; Holdridge, 1967; McArthur, 1972; Box, 1981; Stott, 1981; Walter, 1985; Woodward, 1987; Ellenberg, 1988). The quantification of such species-environment relationships represents the core of predictive geographical modeling in ecology (Guissan & Zimmerman, 2000). Sophisticated statistical treatments have gradually supplanted visual or verbal associations (e.g., Arntzen & Themudo, 2008), and the advent of environmental niche modeling has rapidly increased the number of correlative studies over the past decades (Sexton *et al.*, 2009).

The success of such predictive species modeling depends crucially on our knowledge of the physical environment (Huntley *et al.*, 2004; Austin, 2007). Environmental predictors are usually selected on the basis of availability and our confidence that such variables show correlations with species distributions so as to act as surrogates for more fundamental variables (Austin, 2007). Elevation could be conceived as one such surrogate predictor. Mountains' steep environmental gradients provide researchers with the opportunity to explore the species' response to gradual changes in their environment over short spatial distances (Körner, 2007).

As noted in the Introduction [I.4], Sagarin & Gaines (2002a) performed a systematic examination of the validity of the ACH, based on empirical studies. However, their analysis involved studies of intra-specific variation over the species' geographical ranges, excluding studies along altitudinal gradients and environmental clines.

Assuming that altitude is an approximation to the species' multidimensional environmental niche, there exists an "optimal" point along the altitudinal gradient where the species attain maximum density, and that the "centre" of the species' distribution is the central part of their altitudinal range, an assessment of the hypothesis at hand was attempted. *C. lingulata* from Mt. Olympus was used for this analysis, since it is the most common and widespread of the *Campanula* species examined in this study.

Mean abundance along an elevation (from the sea level) gradient at a resolution of 20 x 20 m was estimated. Descriptive statistics for the distribution of individuals across their entire altitudinal range for 2012 and 2013, respectively, and for each elevation class are given in Table 6. Descriptive statistics in both cases refer to a dataset generated as described in the section below [B.I.5.i]. Based on said dataset, an ANOVA analysis (one way) with elevation class as a differentiating factor was performed as well (Tables 7-12, Fig 38-43). Finally, kernel

density probability curves for expected density within each elevation class were constructed (Fig 44). All analyses were performed in R statistical and programming environment, version 3.13 (R Development core team, 2008).

i) Population density and kernel density Probability density estimates per elevation class

The altitudinal range of the *observations* (see [B.I.3]) was divided in 14 elevation classes of 100 to 200 m change in altitude. The elevation classes were defined as such in order to account for uneven sampling within each elevation zone. The final elevation class included all *C. lingulata* individuals above 2,100 m (See Fig 37a), since this is well above the species' established altitudinal range (Blionis, 2001), and few individuals were observed.

Each *placemark* (see [B.I.2]) was assigned to an elevation class according to its elevation. Then, each placemark was placed in the 1,536 × 1,536 (20 m × 20 m) matrix [see Table 1].
Each value ID that corresponded to a cell that containing a *placemark* was considered sampled. Thus, each sampled square in this grid has an ID tag that corresponds to its elevation class and abundance, which is the number of individuals observed in that square.

Effort was defined as the number of 20 m length intervals (or no. of *placemarks*) that were traversed within each elevation class. The correction of sampling effort for uneven sampling within each elevation class was made by multiplying abundance in each class with the total number of 20 m intervals divided by the effort invested iin the corresponding elevation class (in number of 20 m intervals) (Fig 37c).

$$Corrected\ Abundance_i = No.of\ individuals_i \times \frac{Overal\ sampling\ effort\ (no.placemarks)}{Effort\ invested\ in\ class\ i (no.placemarks)}$$

where *i* is elevalation class.

- Mean Population density (No. of individuals in 400 m^2 – 20 x 20 m) was calculated as the mean value of 100 sets of 100 randomly selected (with replacement) sampled squares per elevation class

- Descriptive Statistics (*mean, st. deviation, minimum value, 1st quartile, median, 3rd quartile, maximum value*) were estimated based on the mean values of 100 sets of observations, for both the entire altitudinal range and within each altitudinal class for both years of sampling

- Analysis of variance (ANOVA) with elevation class as explanatory variable was performed as a linear model for log-transformed data, since the original dataset did not satisfy the assumptions of the normality (Fig. 38-43). The featured post-hoc trial is Tukey's range test, which is used in order to define the means that are significantly different from each other, in conjunction with an ANOVA analysis.

- A probability density function was constructed. Gaussian Kernel density estimation was used as a smoothing factor. Kernel density estimation is a non-parametric way to estimate the probability density function of a random variable. Each probability density function corresponds to the probability of acquiring a given number of individuals in a 20 x 20 m quadrat (or a given population density) in each elevation class (Fig 44). A detailed description of the command lines is given in Appendix IV.

6. Mean presence and mean population turnover at various spatial resolutions

An important aspect of species' spatial distributions is the fashion by which species' presence/occupancy patterns change across different scales and instances. In order to illustrate the presence and turnover dynamics of *C. lingulata* across its range, mean presence and mean population turnover were investigated across different spatial scales.

An observation that is located within a cell at a given resolution may be located in four different positions or sub squares in the next (finer) scale relative to its centre.
Consider the numbers of occupied squares in two successive resolutions (spatial scales). Suppose in the coarse resolution k that n_k squares are occupied and that in the finer resolution k+1, the number of occupied squares is n_{k+1}. Mean presence p_k was defined as:

$$p_k = \frac{n_{k+1}}{4n_k} \quad , \quad k > 0. \tag{9}$$

Since each cell can be divided into four sub cells, one of which must be occupied, it is clear that p_{k+1} must lie between 1 and ¼. The one exception is for p_0, since there are nine sub cells for the first level, $p_0=n_1/9$ (presence in the overall study surface is equal to 1).
Each square we find occupied is considered observed at a given resolution. If a sub-square is not occupied or not sampled, we exclude it when we calculate mean presence for the next resolution (Fig 15).

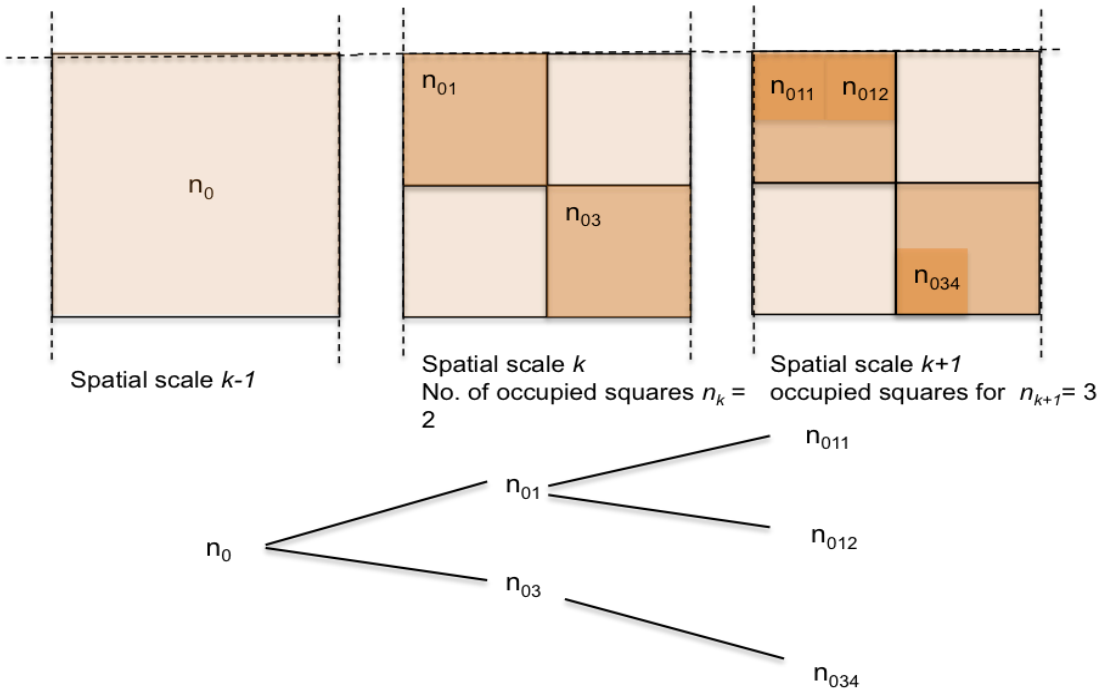


Figure 15: Illustation for mean presence estimates at multiple spatial scales. 3 consecutive spatial resolutions (*k-1, k, k+1*) are featured. The squares that are denoted as n_0, n_{01}…n_{034} represent the grid cells that contain observations.n_0 is initially divided in 4 squares, two of which (n_{01}, n_{03}) contain observations. Square n_{02} and square n_{04} do not contain any observations. Square n_{01} is then subdivided in another 4 squares 2 of which (n_{011} and n_{012}) contain observations. n_{013}, and n_{014} are subsequently removed from further calculations

While for *k*=1, mean presence is estimated as:

$$p_1 = \frac{\frac{2}{4} + \frac{1}{4}}{2} = 0.375$$

where n_k is the total number of occupied squares in spatial scale *k*, in this case, the occupied squares n_{01} and $n_{03.}$ And n_{k+1} are the squares n_{011}, n_{012}, n_{034}.

Mean turnover was calculated as the proportion of the sub-squares that have changed state between the two years of sampling, divided by the number of squares that have changed state at a given resolution. Unoccupied squares that occurred in, at times, the coarsest resolution, were excluded from the calculations. In Figure 15, spatial scale *k* cells with no

name value were excluded. Likewise, they were excluded in spatial scale k+1. If a cell doesn't contain observations in spatial scale k, it will not contain observations in spatial scale *k+1* (finer) as well (Fig 15). A description of the command lines used to calculated mean presence and mean change of state is given in the Appendix IV.

7. Box counting dimension

The Box counting method for estimating fractal dimension *γ* of the species distributions was applied. D_{sp} is calculated as the slope of the number of occupied grid cells and relative to the coarser scale cell size at a log-log axis. Fractal Dimension D_{sp} was corrected for the sampling process as in Halley *et al*. (2004). The distributions' fractal dimension is perceived as the intersection of a fractal object with the fractal dimension D_{sp} with the fractal dimension of a transect D_t, which equals to 1 - upon which the sampling occurred, minus the fractal dimension of the plane, D_p which is equal to 2.

$$\gamma = |D_{sp} + D_t - D_p| \qquad \gamma = |D_{sp} - 1| \tag{10}$$

II. Molecular analysis

1. Measuring genetic diversity and differentiation

Measurement and characterization of genetic diversity have always been a primary concern in population and evolutionary genetic studies, because genetic variability is the foundation for survival, adaptation and evolution (Nevo & Beiles, 1989; Semagn *et al*., 2001). Knowledge of genetic relationships among individuals and populations can be useful to offer evidence of the evolutionary forces shaping natural populations, to choose populations *in situ* or *ex situ* conservation programs, and to assist the selection of parents for breeding purposes (Thornman *et al*., 1994; Semagn *et al*., 2001).

The study of genetic diversity and structure of species of interest has been greatly facilitated by the availability of a number of DNA-based markers (Semagn *et al*., 2001). One such DNA-based method is the RAPD technique. The RAPD technique or Random Amplification of Polymorphic DNA involves a type of PCR (Polymerase Chain Reaction), where random DNA segments are amplified. It involves the use of several arbitrary short primers (usually 10-mers) and a large template of genomic DNA. The primers are bound to corresponding

segments in the genomic DNA, and are amplified through PCR cycles, resulting in semi-unique genetic profiles.

The use of the polymerase chain reaction in generating random amplified polymorphic DNA (RAPD) has already proven valuable in the construction of genetic maps (Quiros *et al*., 1991; Klein-Lankhorst *et al*., 1991; Giovannoni *et al*., 1991; Reiter *et al*., 1992; Rieseberg *et al*., 1992), systematics (Hilu, 1995; Bartish *et al*., 1999*),* the production of genetic markers linked to specific phenotypic traits (Mulcahy *et al*., 1992; Paran *et al*., 1991; Martin *et al*., 1991; Michelmore *et al*., 1991), parentage determination (Welsh *et al*., 1991), clone identification (Wilde *et al*., 1992; Smith *et al*., 1992), population dynamics (Arnold *et al*., 1990; Fritsch & Rieseberg, 1992) (Fritch, 1993), and assessment of gene flow between species, via studies of hybrid progeny (Arnold *et al*, 1991; Smith *et al*., 1996; Daehler & Strong, 1997; Ayres *et al*., 1999; De Greef & Triest, 1999; Kuehn *et al*, 1999; Neuffer *et al.,* 1999; Rieseberg & Linder, 1999; Randell, 2000; Caraway *et al*., 2001). Other studies involve the effects of mutagens on plants (Erdem & Oldakey, 2004).

RAPD markers have been considered as suitable characters for genetic analysis since they allow the examination of accumulated genetic differences that are important at various taxonomic levels. Additionally, since RAPDs are randomly distributed over the entire genome, extensive amount of polymorphisms can be detected (Aagard *et al.*, 1998), therefore, they can detect low levels of genetic variability. RAPD technique is a method of choice for studying genetic diversity for species where there is little or no molecular data (Nybom, 2004), as it does not require sequence information for the target species. Furthermore, it is especially suited for studying large number of samples as it is relatively simple, fast and cheap (Geleta *et al.*, 2007).

Molecular markers such as RAPDs are neutral characters with no known phenotypic effects and, therefore, they constitute excellent tools for studying genetic variability of natural populations. Semagne *et al*. (2000) suggest, however, that part of the RAPD polymorphism could be adaptive and responsive to environmental selection. They obtained significant correlation between population means from RAPDs and environmental variables and strong associations with eco-geographical variables, in a multiple regression analysis of *Phytolacca dodecandra* individuals sampled across an altitudinal gradient.

A major drawback for all methods that rely on unspecific primers and produce multilocus band patterns (i.e. RAPD, AFLP and ISSR) is the fact that the investigated loci are biallelic (a band is present or absent), and that attempts to distinguish heterozygotes from

homozygotes on band intensity have not proven feasible. Consequently, the DNA bands must be treated as dominantly inherited markers (Nybom, 2004). Other technical difficulties that may arise concern the poor reproducibility of RAPD markers, in comparison with other methods such as AFLP (Amplified Fragment Length Polymorphism) and ISSR (Inter-Simple Sequence Repeats), which can however be avoided through improved laboratory techniques and band scoring procedures (Skroch & Nienhuis 1995; Weising *et al*. 1995; Nybom, 2004).

In order to approximate the genetic diversity and differentiation amongst and within *C. lingulata* populations of Mt. Olympus and Mt. Falakro, a RAPD analysis was performed.

2. Location populations for DNA extraction

Three Individual samples from 6 sites on and around Mt. Olympus at varying altitudes were collected for DNA extraction. The areas ranged from 356 m (Petra area) to 1,980 m in elevation (Zolotas area) (Fig 16).

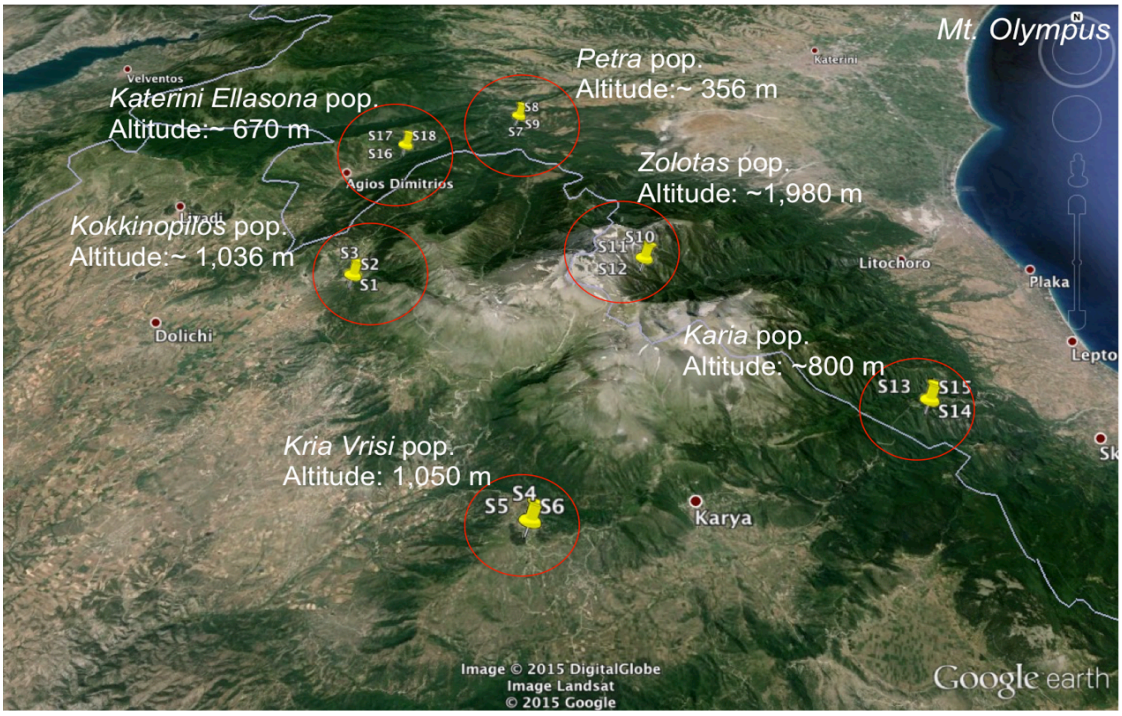


Figure 16: Samples of *C. lingulata* individuals for molecular analysis, from Mt. Olympus area.

Three individuals from 2 sites on Mt. Falakro, at 490 m and 1,300 m were collected for DNA extraction (Fig 17).

DNA was extracted with Mackeray-Nagel Nucleospin^{R} Plant II Genomic DNA extraction kit from plants. 20 to 50 mg of plant tissue were homogenized with 400 µl PL1 (buffer solution) and incubated at 65 ^{o}C with 10 µl of RNAase. The samples were centrifuged for 2 min at 11,000 g, and 400 µl PC buffer was added. The samples were moved to the provided collection tubes, and then were centrifuged again at 11,000 g for 1 minute. The flow-through was discarded and 400 µl of PW1 was added (1st wash). The samples were centrifuged again (11,000 g, 1 min), the flow-through was discarded, and 700 µl were added (2nd wash). The flow-through was discarded (Centrifuge 11,000 g, 1 min) and 200 µl of PW2 were added for the third and final wash. The samples were centrifuged again, for 2 minutes at 11,000 g and the flow-through was again discarded. The final steps involved the elusion of the DNA with 50 µl buffer PE (incubated at 65 ^{o}C), which was placed at the membrane of the provided collection tubes twice resulting in 100 µl of extracted genomic DNA solution. Electrophoresis confirmed DNA presence for each sample.

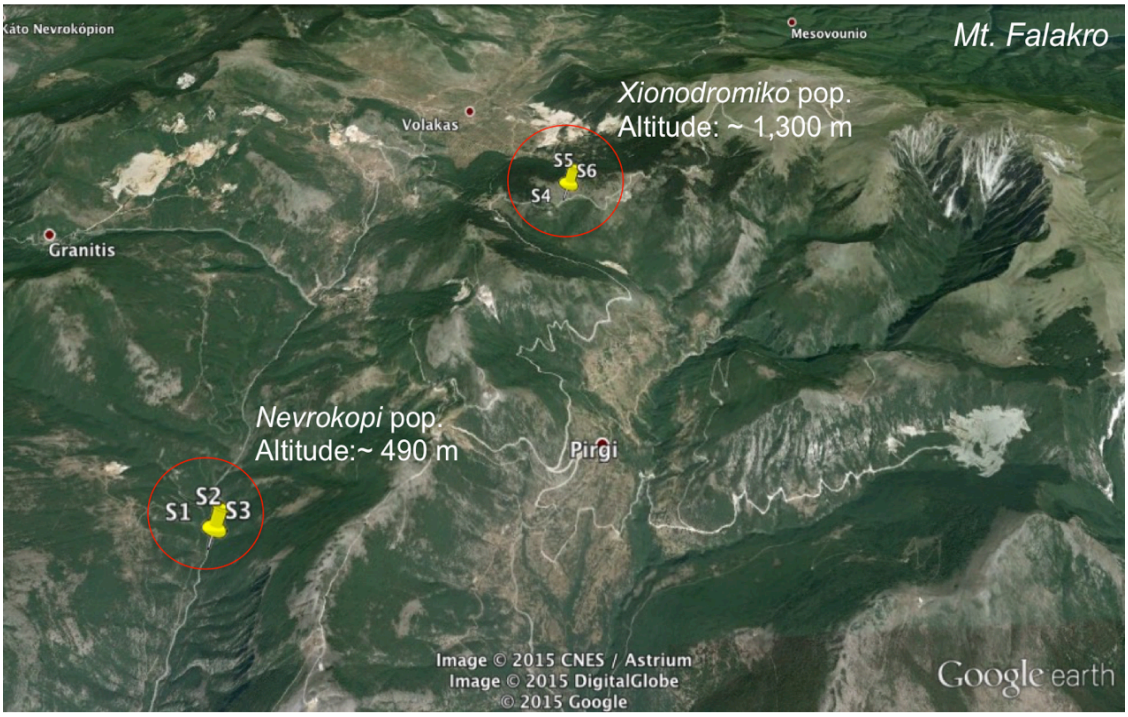


Figure 17: Samples of *C. lingulata* individuals for molecular analysis, from Mt. Falakro area.

3. Primer selection

The selection of the arbitrary primers was made on the basis of criteria of number of detected polymorphisms from across the relative literature (no. of generated bands). Several studies were assessed in terms of the numbers of yielded generated bands (loci) and the number of unique polymorphic bands from an array of species. A PCR with a set of 20 primers was applied to 4 samples randomly chosen from across the *C. lingulata* populations. The 10 primers that yielded the largest number of generated bands on 4 randomly selected samples were chosen for further study. A table with the primers utilized is given in Table 13 of the Results.

4. Polymerase Chain Reaction (PCR)

PCR was performed for the 20 individuals in question. A master mix was prepared for 22 individuals in order to account for errors was initially prepared with:

864.6 µl H_2O
110 µl of *Buffer 10x*
22 µl dNTPs
44 µl genomic DNA

47.3 µl from the master mix was then divided into 20 PCR tubes. 2.5 µl of each selected arbitrary primer in 1:10 concentration was added in each tube. 0.2 µl of Kappa *Taq* Polymerase were added. Each PCR tube contained a total of 50 µl of the following:

39.5 µl H_2O
10.5 µl Buffer
25 mM $MgCl_2$ (from Buffer)
10 mM dNTPs
2.5 µl Primer
2 µl DNA
0.2 µl Taq Kappa Polymerase,

The PCR cycle specifications were defined from across the literature, as follows:

95^oC for 3 minutes
95^oC for 30 sec X 35 cycles
35^oC for 30 sec X 35 cycles
72^oC for 1 min X 35 cycles
72^o C for 5 min
4^oC o/n

The PCR protocol has duration of 1:50 min. A total of 280 individual reactions were performed. In order to visualize the banding profile of each primer, electrophoresis was performed with 5 µl of PCR product and with 1 µl of electrophoresis loading buffer. The agaroze gel for 20 samples (1 primer per gel for a total of 10 primers) was prepared with 1 g agaroze, 100 ml TB (Buffer), and 5 ml of BrEth, which is binding to the DNA in order for it to

be visible under UV light. The agaroze gels were then examined under UV light and were photographed in order to be scored.

i) Scoring

20 *C. lingulata* samples [9(1)…9, see Fig 19] from the populations of Olympus and Falakro were run for 10 primers each (A9, Fig 18). A banding profile for each primer was then generated**.** Each unique generated band profile-locus (dotted lines – Fig 19) was treated as a binary independent variable with the values 0 for absence and 1 for presence of each locus for each individual sample.

The samples that did not produce any loci (bands) in a banding profile were excluded from further analysis for the primer used. A total of 7 samples did not produce any loci for any of the primers, and was excluded from further analysis as well. A generated band was considered polymorphic if it did not occur across the entire population. The number of unique generated bands, the number of common and polymorphic bands for each primer and Primers' Resolve Power are given in Table 13 of the Results. Resolve power of each primer was calculated after Eq. 11.

$$R_p = \sum Ib_i \text{ where } Ib_i = 1 - (2 \times |0.5 - p_i|) \quad (11)$$

p*i* referring to the observed frequency of each locus.

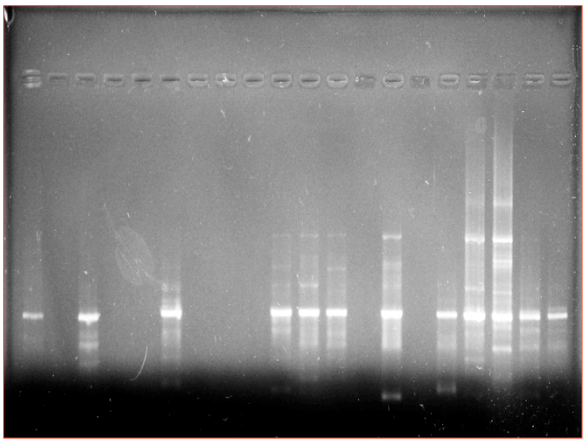


Figure 18: Agaroze gel electrophoresis of the RAPD banding pattern (primer A9) for *C. lingulata* individuals from Olympus and Falakro.

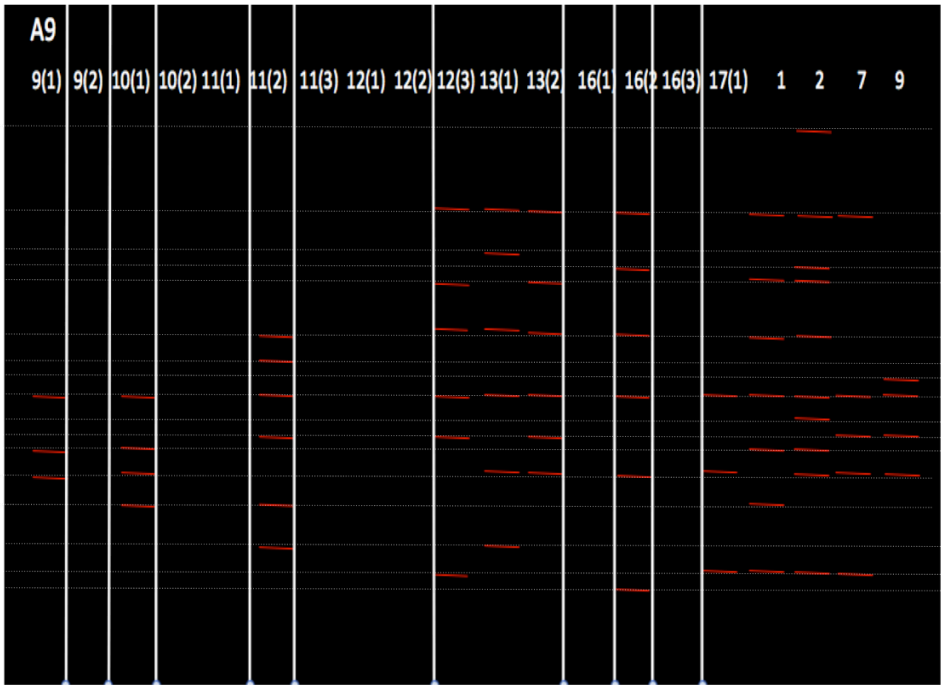


Figure 19: Banding pattern for A9 primer. Distinguishable loci are marked with white dotted lines, while alleles are marked red for presence, for each individual.

Iii) Diversity and differentiation indices

Shannon's Index of genetic diversity (H_0) (Eq. 12) was used to determine genetic diversity within populations of Olympus and Falakro mountains. Samples that did not produce clear band patterns were excluded. Seven individuals from Olympus and 4 from Falakro are featured below.

$$H_0 = -\frac{1}{m}\sum p_i ln p_i \tag{12}$$

where p_i is the frequency of presence or absence of a RAPD band in a population and m is the number of loci. Average Diversity over both populations (H_{pop}) was calculated after Eq. 13.

$$H_{pop} = \frac{1}{n}\sum H_0 \tag{13}$$

where n equals to the number of populations. Mean diversity at species level was calculated after Eq. 14.

$$H_{sp} = -\frac{1}{m}\sum p_s ln p_s \quad (14)$$

where m is the total number of loci and p_s is the frequency of presence or absence of a RAPD band in all populations.

The Diversity index (DI) measures the expected heterozygosity for both populations. It was calculated according to Wier (1996) after Eq. 15.

$$DI = 1 - \frac{1}{L}\sum_{l}\sum_{i} p_i^2 \quad (15)$$

The results are shown in Table 13.

For dominantly inherited DNA markers, genetic differentiation among populations is often estimated with G_{ST} according to Nei (1973). When there are two alleles at a locus, as in dominant DNA marker analyses, this G_{ST} is identical to Wright's F_{ST} (Nei, 1973) and seems to produce robust data that are relatively insensitive to assumptions about Hardy-Weinberg equilibrium, heterozygosity and levels of inbreeding (Arafeh *et al*., 2002) (Nybom, 2004). With two populations and two alleles, G_{ST} ranges from 0.0 to 1.0, as expected, with 0 representing no differences in allele frequencies between two populations and 1.0 indicating that the two populations are fixed for alternate alleles. A multi-locus approach, AMOVA (analysis of molecular variance) is nowadays even more widely used than G_{ST} for the partitioning of genetic variability (Excoffier *et al*. 1992; Nybom, 2004). Values for Nei's G_{ST} and for the AMOVA-derived Φ_{PT} (which is analogous to F_{ST}) are usually very similar when calculated on the same data set (Nybom & Bartish 2000; Nybom, 2004).

Nybom & Bartish (2000) reviewed 108 RAPD based studies on wild plant materials. Differentiation indices derived from RAPD techniques were compared with allozyme data as reported by (Hamrick & Godt, 1989). Allozymes are variant forms of an enzyme that are coded by different alleles at the same loci. The grand mean for RAPD-derived within-population gene diversity was 0.214 (Nybom, 2004), which is rather close to the allozyme-derived H_{SP} = 0.230.

Average among-population diversity was 0.35 (AMOVA Φ_{PT}) or 0.29 (G_{ST}) for the RAPD-based studies, and 0.22 (G_{ST}) for the allozyme-based. As previously verified with allozyme data, RAPD markers showed that long-lived, outcrossing, late successional taxa retain most of their

genetic variability within populations. By contrast, annual, selfing and/or early successional taxa allocate more of their genetic variability among populations. Estimates for among- and within-population diversity, respectively, proved to be negatively correlated, as previously reported for allozyme data (Nybom, 2004)

The only major discrepancy between allozymes and RAPD markers concerns geographical range; within-population diversity was strongly affected by distributional range of the investigated species in the allozyme data but not in the RAPD data. Moreover, RAPD-based values for among-population diversity increased with increasing distributional range. For allozymes, the opposite association has been reported (Hamrick & Godt 1996); as well as a lack of association whatsoever (Gitzendanner & Soltis, 2000; Nybom, 2004).

In addition, an AMOVA analysis was performed in GenAlEx 6.502 for the 2 populations (Olympus, Falakro) (Table 15, Fig 49).

iii) UPGMA (cluster) analysis

An UPGMA (complete linkage) cluster analysis with 1000 bootstrap repetitions for binary data was performed in R, in order to visualize the differences amongst the populations of Olympus and Falakro. Seven out of 20 samples did not yield any loci for the selected primers. The dendrogram is featured in Fig. 50 of the Results. In addition, a PcoA analysis for the samples that produced clear banding patterns (7 from Olympus and 4 from Falakro, as above) was performed in GenAlEx 6.502 and is featured in Fig 51.

C. RESULTS

I. DATA PROCESSING

1. Overview of the results

Information on the surrounding vegetation, bioclimatic variables and invested effort are illustrated in section C.I.2 of the results. Occupancy-Abundance relationships across different spatial scales are featured in section C.I. 3 - 4, Mean abundance (density) along an altitudinal gradient is presented in section C.I.5; Mean presence and population turnover, at section C.I.6; Molecular analysis in C.II.1 - 3.

2. Climate, Vegetation and sampling effort

In Figs 20-22, given are the mean, minimum and maximum temperature and precipitation per route and per month, for the sampled areas.

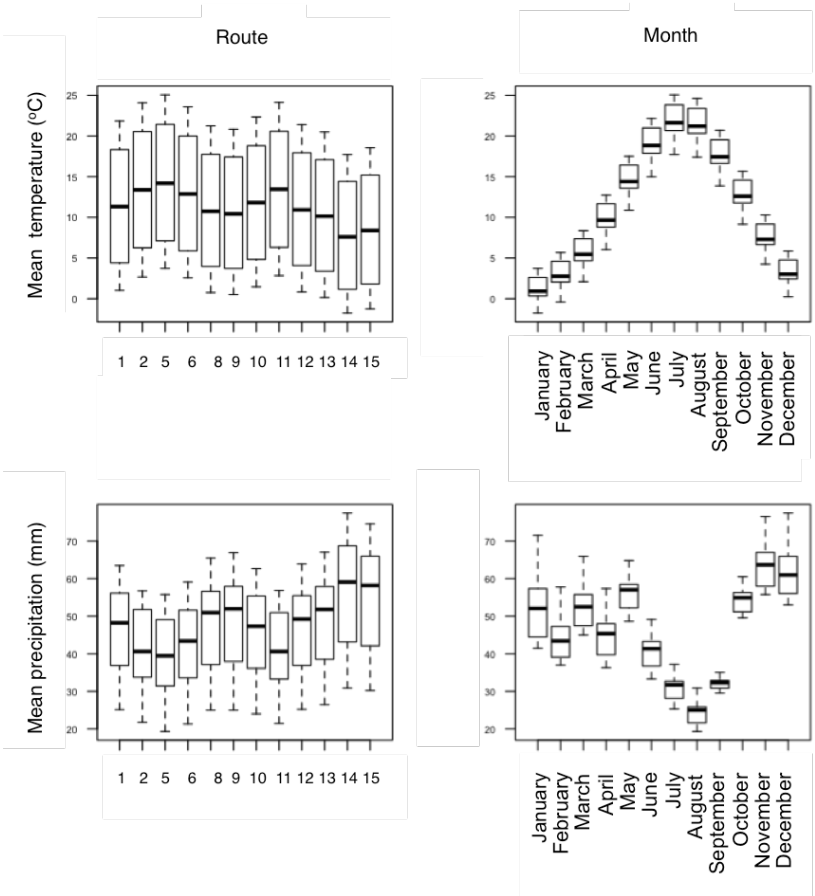


Figure 20: Mean temperature and precipitation for all routes, and each calendar month, as extracted from worldclim.org.

Routes 14 and 15, which traverse the western side of the mountain seem to have the lowest temperatures and the greater precipitation. Routes 2, 5 and 1, located at the south, northwest, and northeast of the mountain, respectively, seem to have the highest temperatures and to receive the least amount of rainfall. The hottest months are July and August, and the coldest is January. The driest month is August, while precipitation maximizes during the month of November.

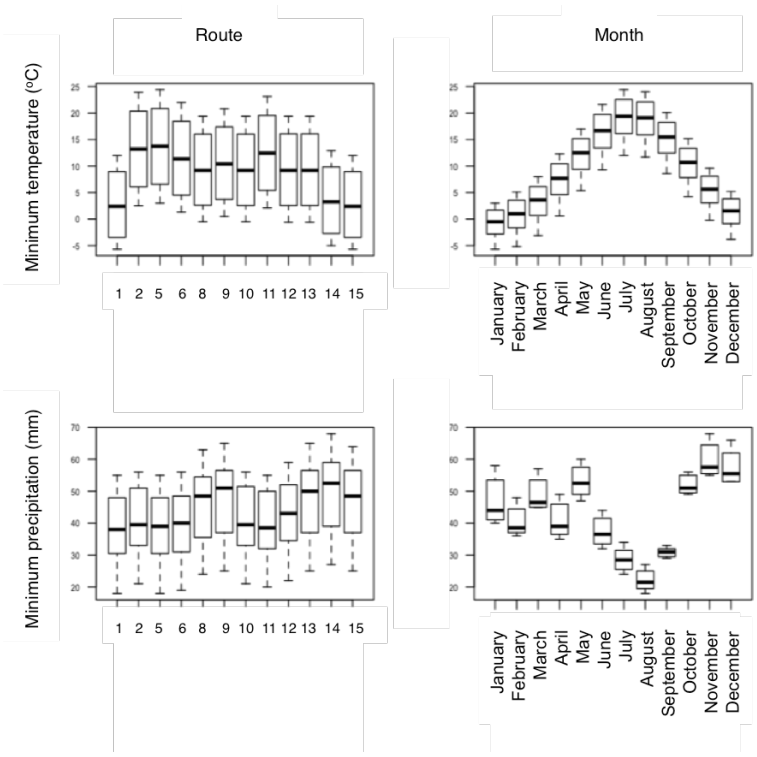


Figure 22: Minimum temperature and precipitation for all routes, and each calendar month, as extracted from worldclim.org.

Minimum temperatures can reach -5 ^{o}C during the coldest months, at routes 1 (SE side), 14 and 15. The lowest recorded temperature during the summer months is ~12 degrees and was recorded for routes 1 and 15.

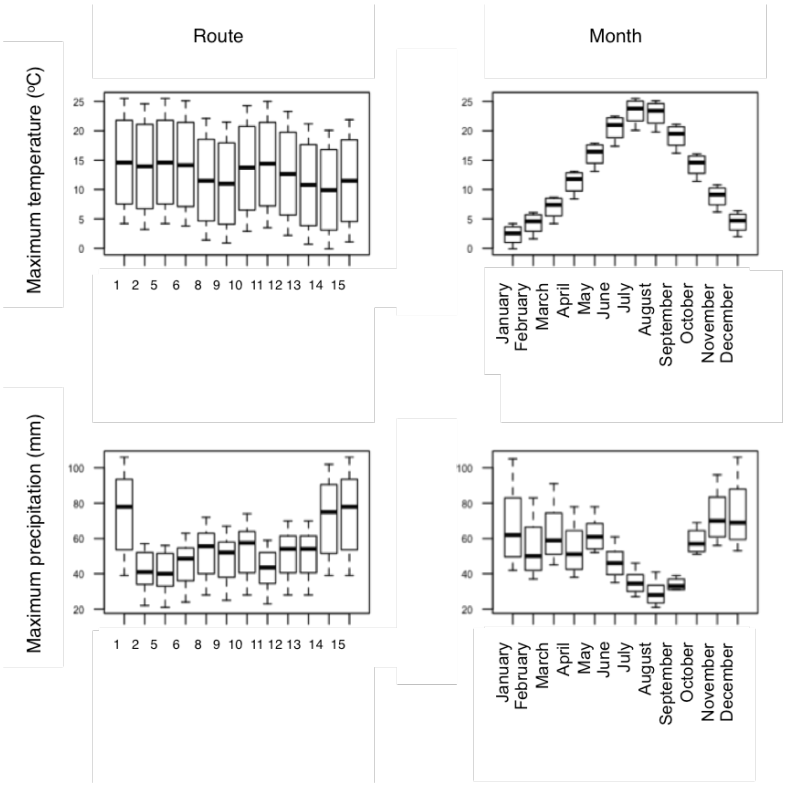


Figure 22: Maximum temperature and precipitation for all routes, and each calendar month, as extracted from worldclim.org.

The highset summer temperatures were recorded for routes 1, 5, 6 and 11, at ~25 ^{o}C. The maximum amount of recorded rainfall is ~80 mm, for routes 1, 14 and 15.

The type of vegetation for each sampled route relative to altitude is shown in Figs 23-25.

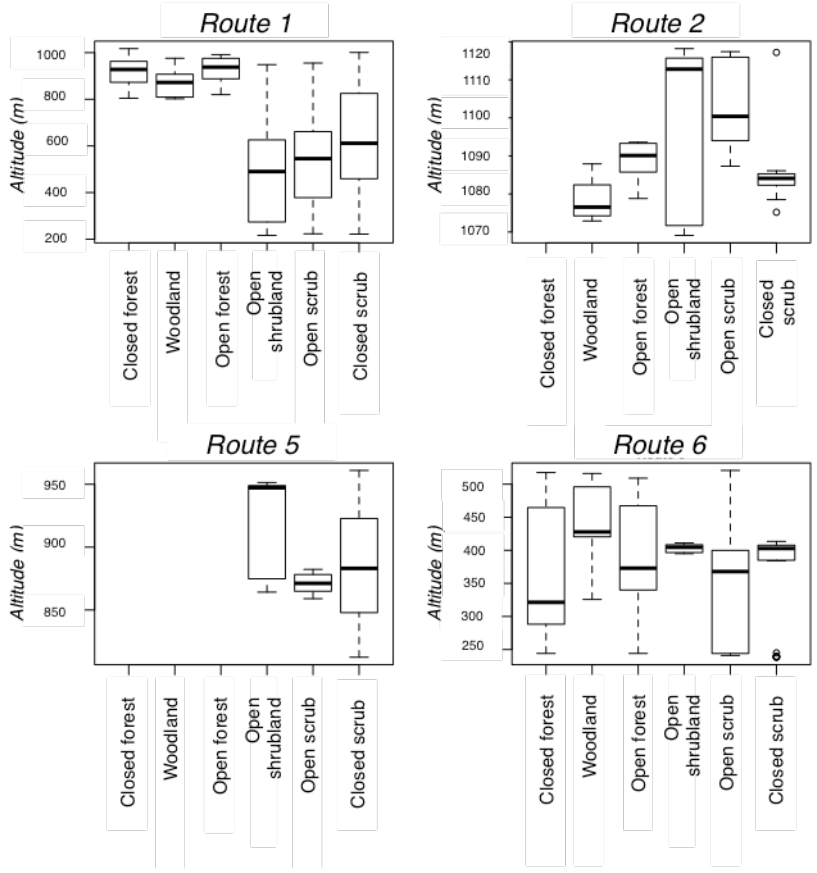


Figure 23: Vegetation cover at the different elevation of for the Routes 1, 2, 5 and 6.

Route 1 extends from 200 m to approximately 1,100 m of altitude. Mediterranean vegetation occurs up to 800 m of altitude. Open shrubland occurs up to 600 m, while more dense vegetation occurs from 800 m and above. Route 2 is extends from 1,050 to approximately 1,130 m. Vegetation is generally sparse, with open areas occupying most of the area surrounding the route. Route 5 extends from 750 to ~1,000 m; it is surrounded by sparse Mediterranean vegetation. Route 6 is mostly forested, with dense vegetation across its range, from 250 to approximately 630 m of altitude. Patches of Mediterranean vegetation occur up to 400 m.

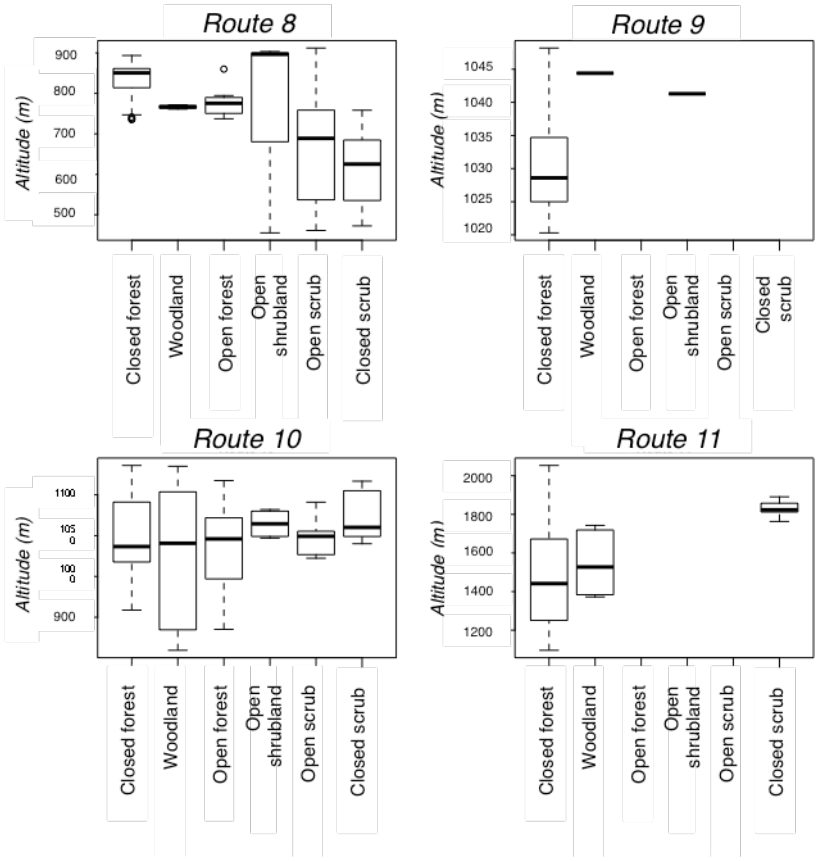


Figure 24: Vegetation cover relative to altitude for Routes 8, 9, 10 and 11.

Route 8 extends from ~450 to ~900 m. Open areas with Mediterranean vegetation occur across its range. From 700 m and above, forested patches occur as well. Route 9 extends from 1,020 to 1,240 m and is mostly forested. Route 10 is mostly forested, too; it ranges from ~850 to 1,200 m of altitude. Patches of Mediterranean vegetation occur all across its range. Route 11 is another forested area, which extends from 1,100 to approximately 2,100 m.

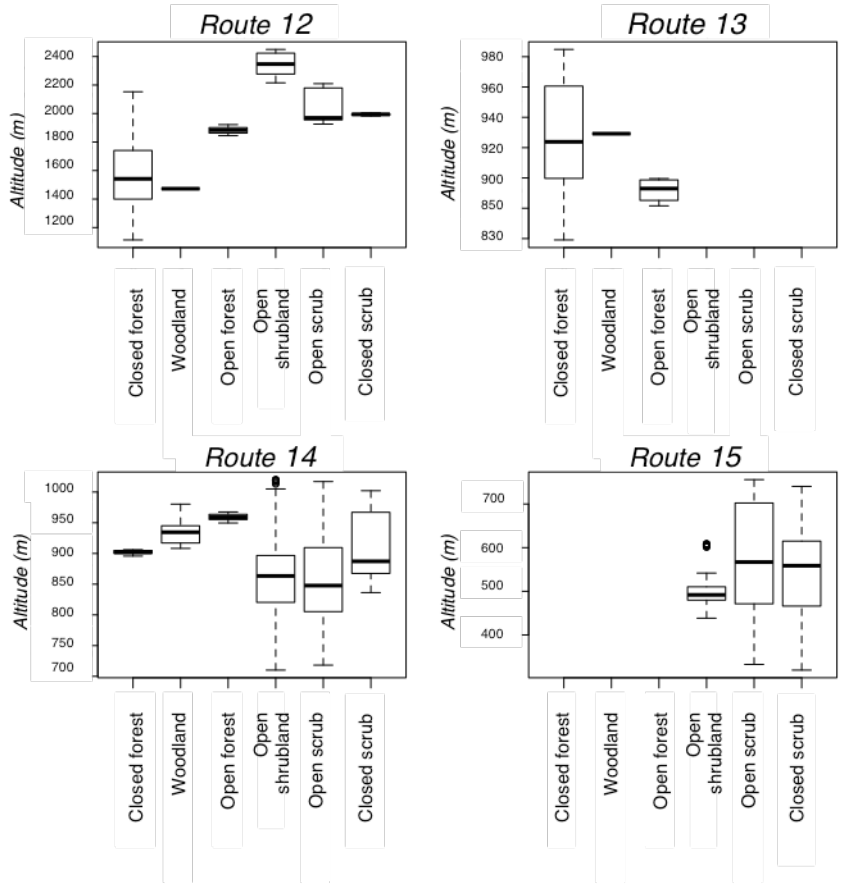


Figure 25: Vegetation cover relative to altitude for Routes 12,13,14 and 15.

Route 12 extends from ~ 1,100 to ~2,500 m of altitude. It is surrounded by forests up to ~1,700 m, where the forest is gradually replaced by shrubs of the boreal vegetation zone. Alpine meadows occur from 2,200 m and above. Route 13 is a forested area that extends from 860 m to 960 m in altitude. Route 14 is mostly covered by Mediterranean vegetation and reaches up to ~950 m. Forested patches occur as well at 900 to ~1,000 m. Route 15 is also surrounded by mediterranean vegetation, from 400 to 700 m.

Sampling took place in all vegetation types. In Fig 26, the height of bars indicates the sampling effort per route, and how this effort was divided in the different vegetation types within route. The sampling effort equals to the length (in meters) of each route/vegetation zone.

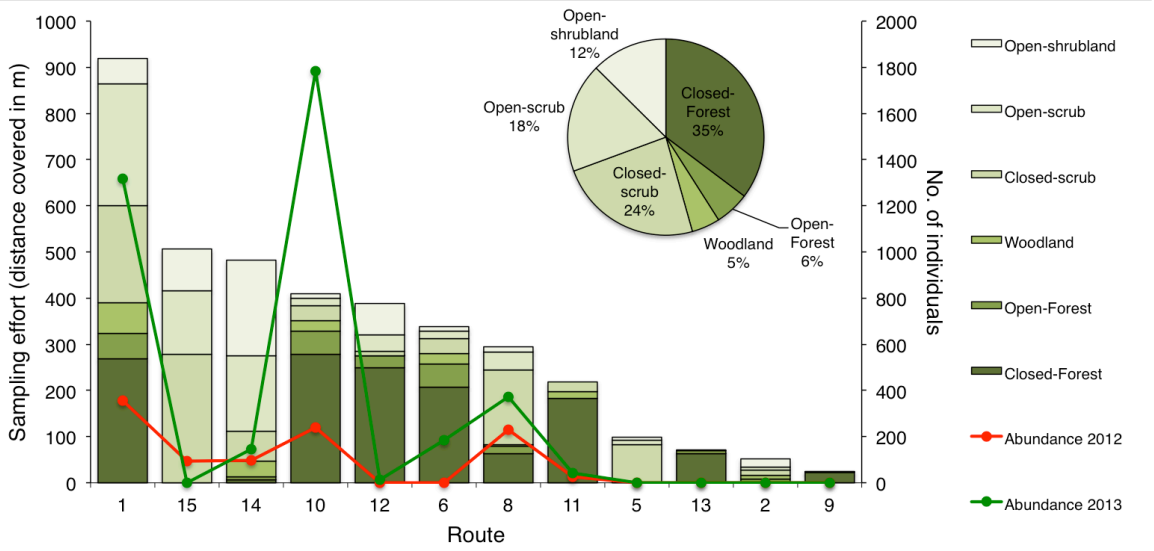


Figure 26: Sampling effort per route. The color-coded bars correspond to the type of vegetation of the surroundings. X-axis corresponds to the different routes, while Y-axis to the number of placemarks per type of vegetation (proportional). The lines correspond to the number of individuals recorded in each vegetation type.

3. Mapping the species on the surveyed surface.

A total of 1,130 and 3,897 individuals were recorded for *C. lingulata*, 1,234 and 1,291 for *C. spatulata* and 989 and 659 for *C. rotundifolia*, in 2012 and 2013, respectively.

The recorded individuals are illustrated in Fig 27, in 11 spatial resolutions, from the coarsest (spatial scale 1) to the finest (spatial resolution 11), as in Table 1 of the Methods section. Data in red refer to records of presence of each species in 2012, while data in green refer to records of presence for 2012.

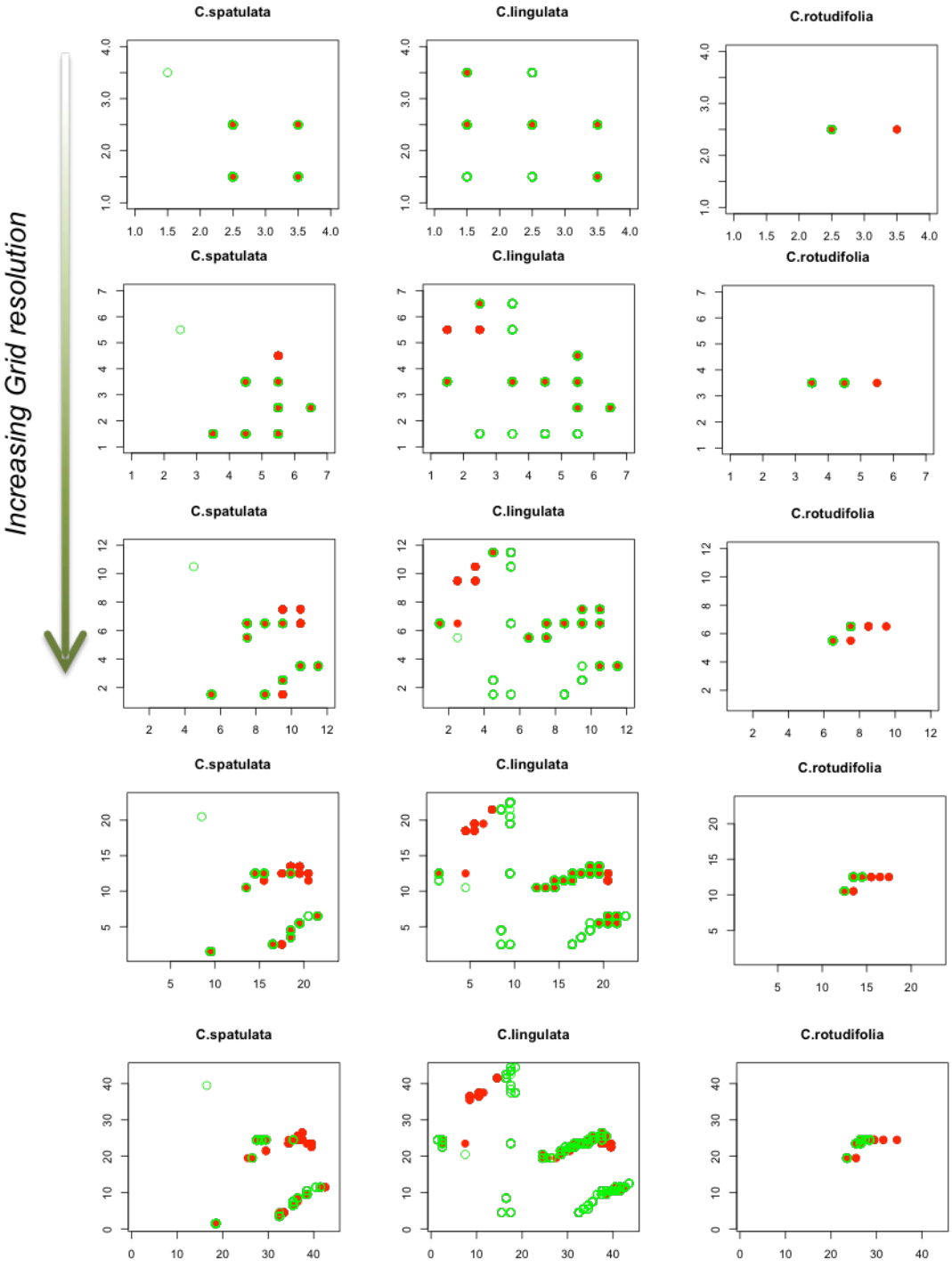


Figure **27:** Mapping of *C. lingulata, C.spatulata, C. rotundifolia*, in 11 spatial resolutions for 2012 (red) and 2013 (green).

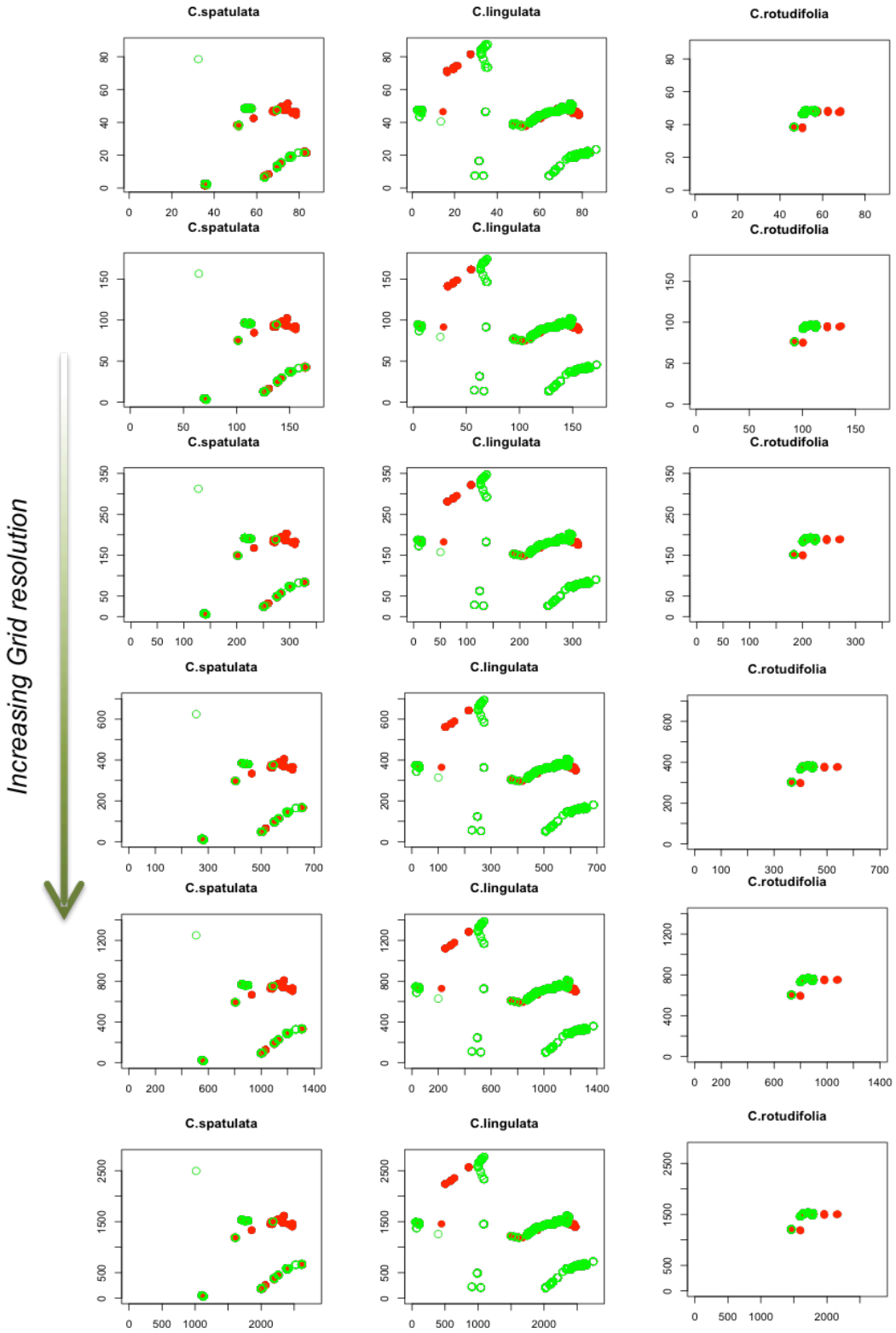


Figure 27 (cont.): Mapping of *C. lingulata, C.spatulata, C. rotundifolia*, in 11 spatial resolutions for 2012 (red) and 2013 (green).

C. lingulata occupies the largest portion of the grid for both years of sampling. *C. spatulata* is mainly found in the SE part of the mountain, whereas *C. rotundifolia* can only be found at the highest altitudes, thus occupying the smallest portion of the grid, at all resolutions. The majority of observations were recorded in the SE part of the mountain, for *C. lingulata* and *C. spatulata,* mostly in Routes 1, 8, and 10 (see Fig 2, Fig 27). Routes 8 and 10 are within the limits of the designated National Park. While individuals were observed in Route 15 for 2012, no individuals were recorded for 2013. Likewise, while individuals were observed in Route 6 for 2013, no individuals had been recorded for 2012. This is fairly evident at the coarser resolutions. Such discrepancy might occur due to variations in climatic conditions during the years of sampling, which may have either advanced or postponed flowering and recording of the individuals (only flowering individuals were recorded).

4. Abundance and Occupancy

Abundance of *C. lingulata* and *C. spatulata*, for both years, for two of the sampled routes is featured in Figs 28-31, in two resolutions (20 x 20 m and 60 x 60 m). *C. lingulata* seems to be increasing in number of individuals and occupancy in both routes. This is evident in both resolutions. *C. spatulata*, on the other hand, is recorded in fewer squares in route 1, even though its abundance has increased from 2012 to 2013 within this route. In route 8, no individuals were observed in 2013. This, once again, does not necessarily mean that the species is absent. It is probable that the individuals weren't in bloom when sampling took place, due to variations in climatic conditions between the two years of sampling. *C. lingulata* and *C. spatulata* do not seem to occupy the same space, and form distinguishable patches of individuals. This is not directly observable when examining the distribution at coarser resolutions, as in Fig 27. To note, *C. lingulata* seems to be expanding towards lower altitudes for 2013 (See route 6 and 14 in Fig 27), while *C. spatulata* appears to be contracting (See route 8 in Fig 29)

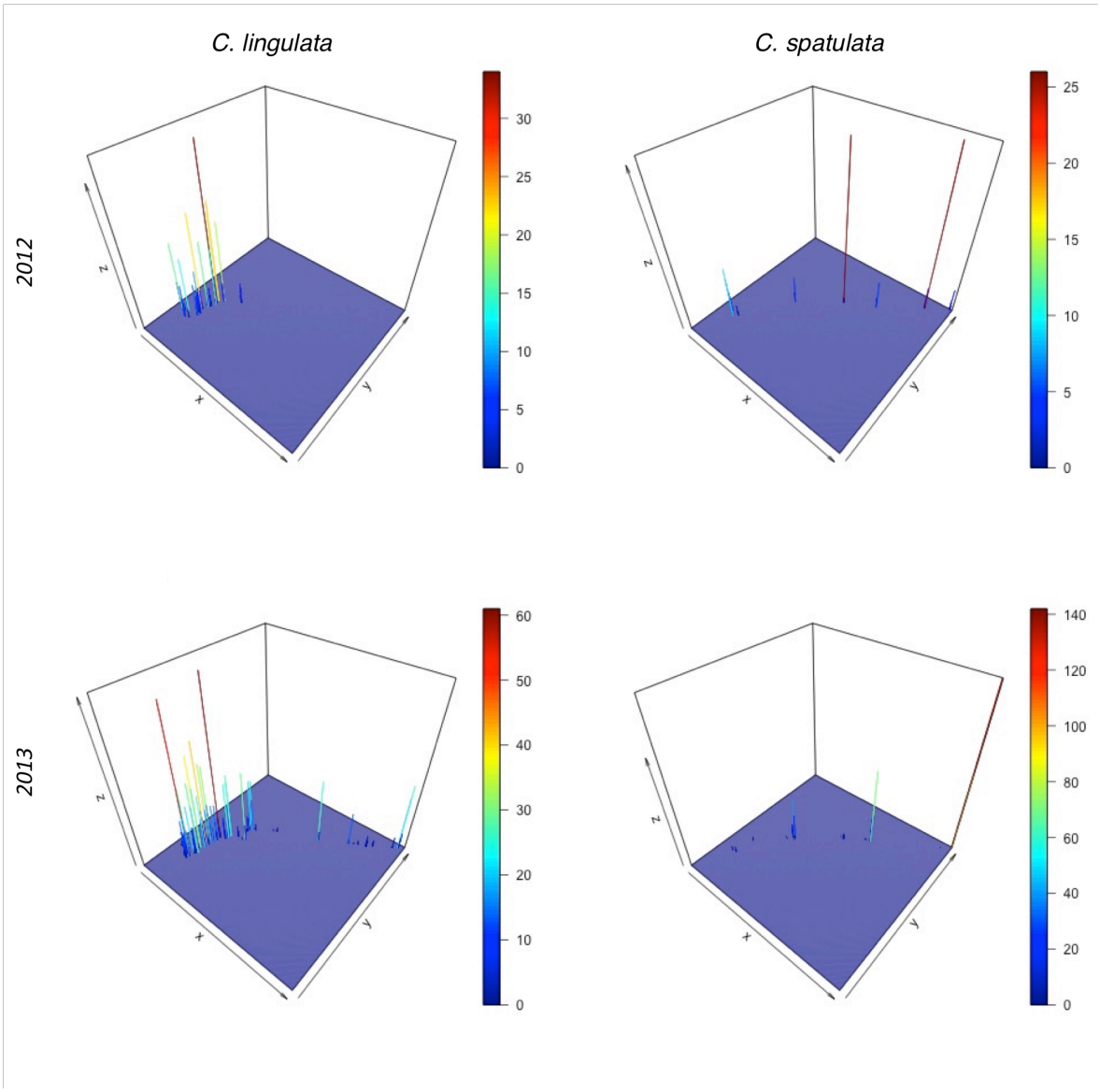


Figure 28: Abundance for *C. lingulata* and) *C. spatulata*, in route 1, in a 20 x 20 m resolution. The species are featured in space, with the height and the color of the bars indicating the species abundance in each sampled square. The number of individuals in each case is given in the bars next to each illustration.

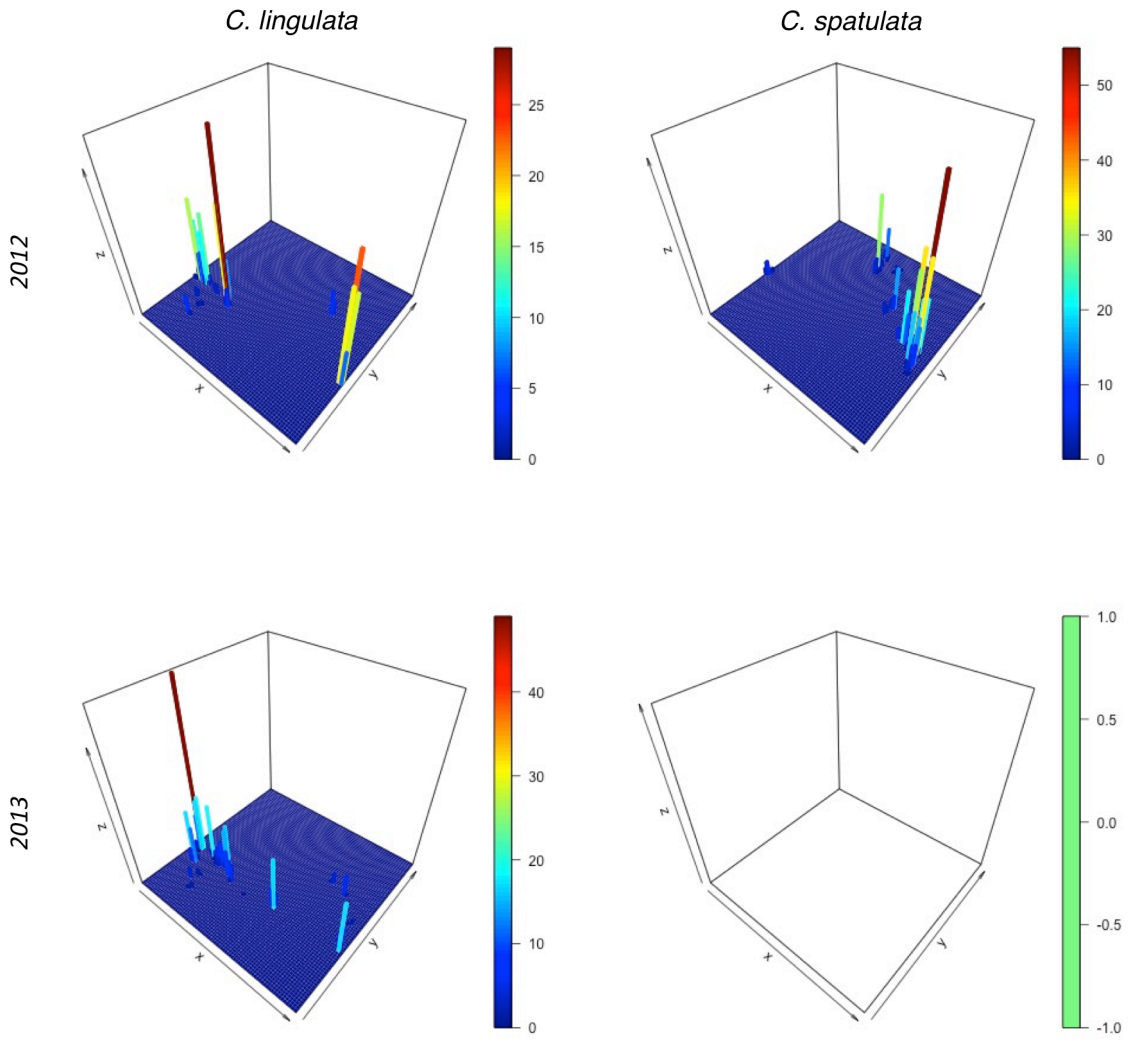


Figure 29: Abundance for *C. lingulata* and *C. spatulata* in route 8, in a 20 x 20 m resolution. The species are featured in space, with the height and the color of the bars indicating the species abundance in each sampled square. The number of individuals in each case is given in the bars next to each illustration.

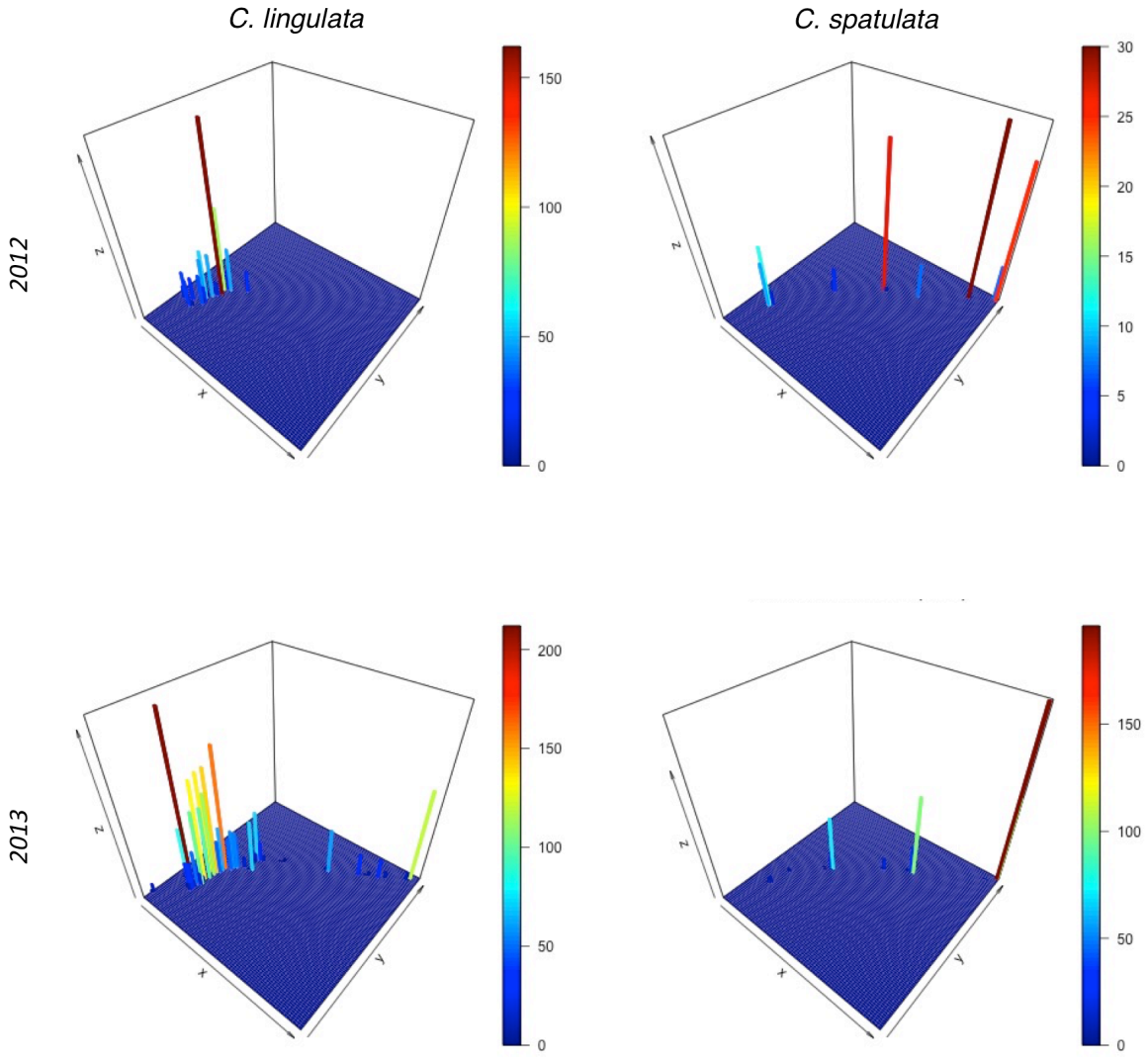


Figure 30: Abundance *C. lingulata* and *C. spatulata*, in route 1, in a 60 x 60 m resolution. The species are featured in space, with the height and the color of the bars indicating the species abundance in each sampled square. The number of individuals in each case is given in the bars next to each illustration.

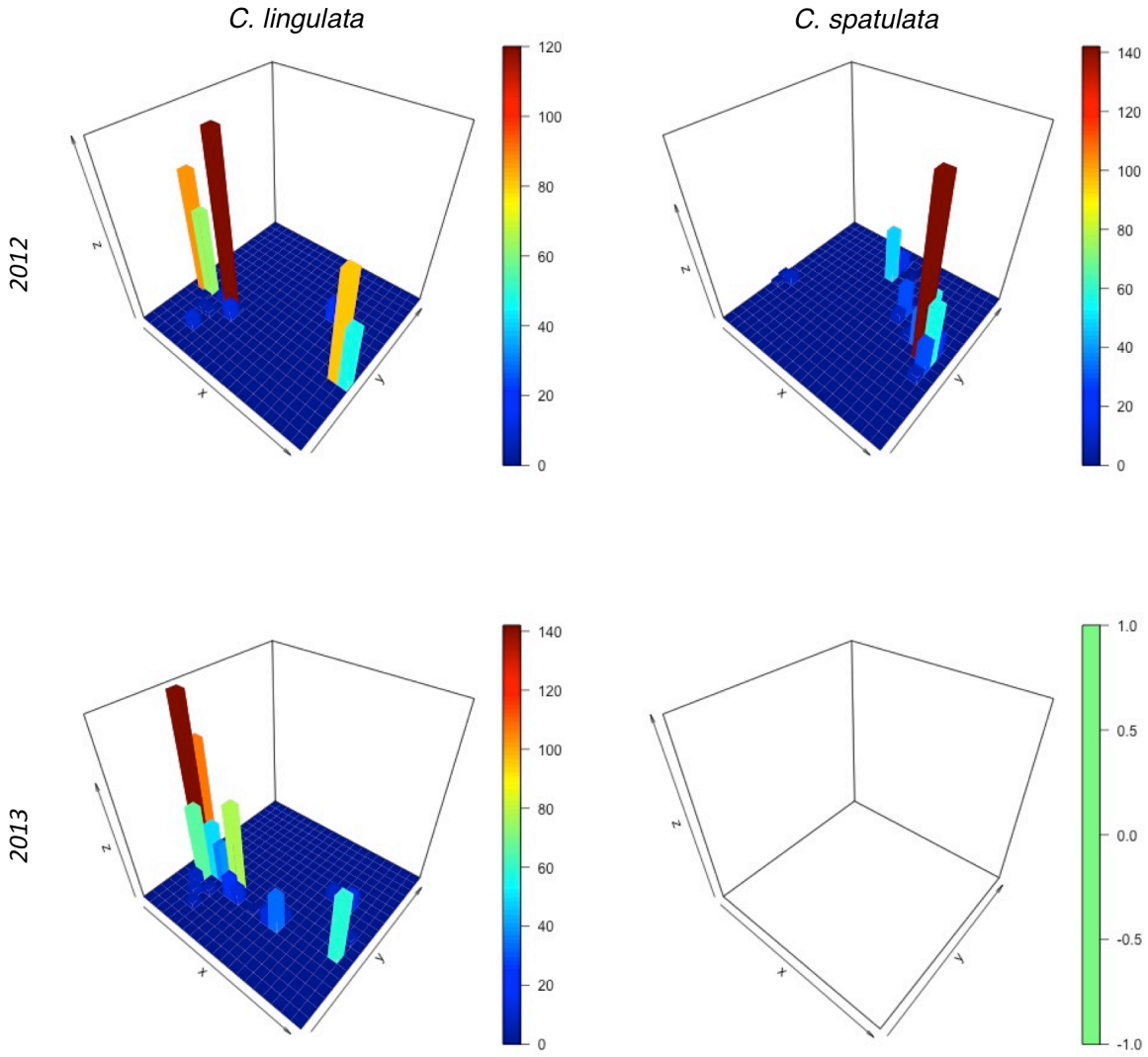


Figure 31: Abundance for a) *C. lingulata* and b) *C. spatulata*, in route 8, in a 60 x 60 m resolution. The species are featured in space, with the height and the color of the bars indicating the species abundance in each sampled square. The number of individuals in each case is given in the bars next to each illustration.

In a positive occupancy-abundance relationship, occupancy is a function of the number of Individuals (mean occupancy or mean density). Occupancy as a function of the number of individuals of *C. lingulata*, *C. spatulata* and *C. rotundifolia* within the study area across different spatial resolutions is given in Figure 32.

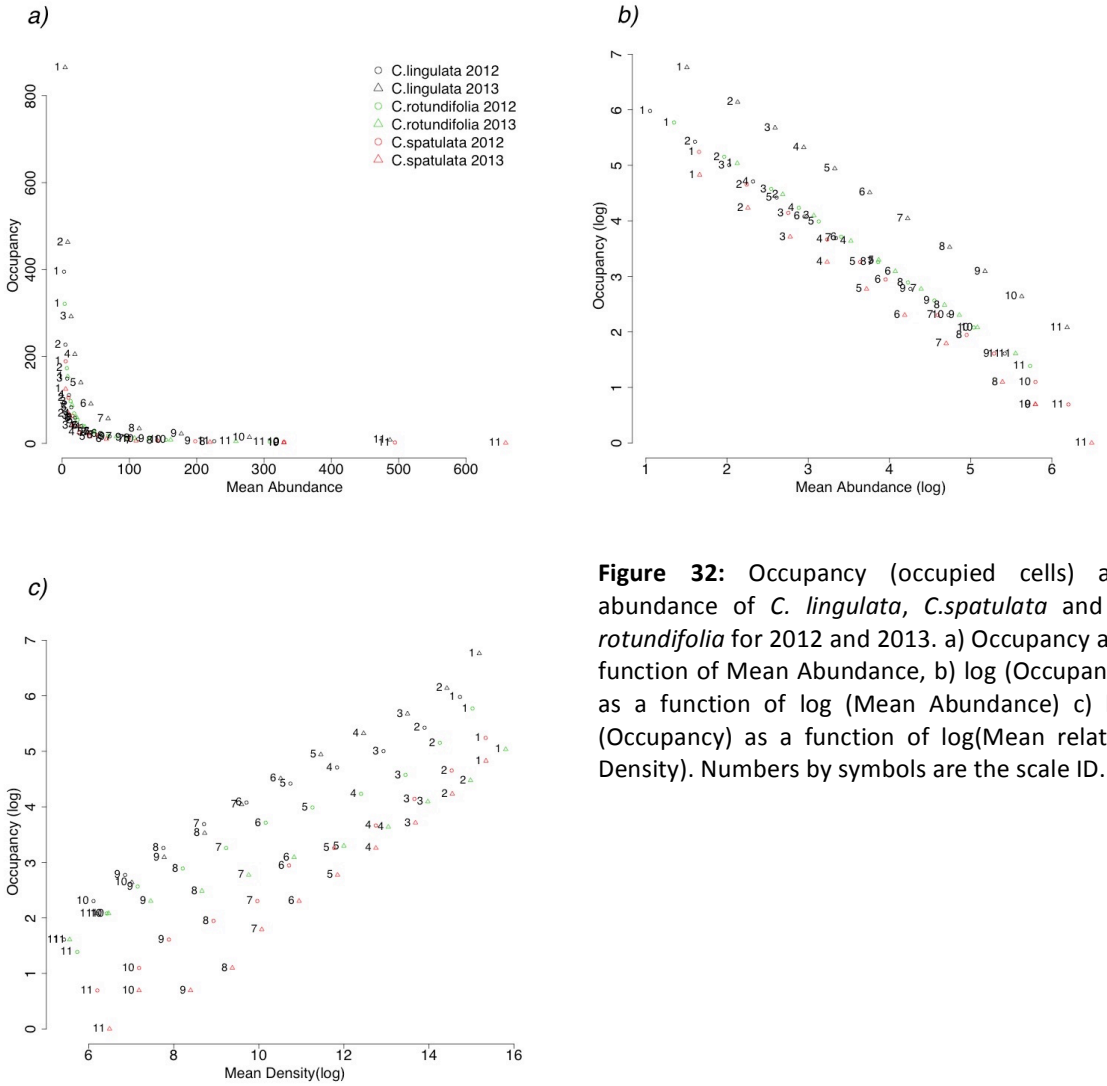


Figure 32: Occupancy (occupied cells) and abundance of *C. lingulata*, *C.spatulata* and *C. rotundifolia* for 2012 and 2013. a) Occupancy as a function of Mean Abundance, b) log (Occupancy) as a function of log (Mean Abundance) c) log (Occupancy) as a function of log(Mean relative Density). Numbers by symbols are the scale ID.

At coarser resolutions we expect to find more individuals in each subdivision of the grid. Indeed occupancy is a decreasing function of mean abundance as we move from finer to coarser resolutions (Fig 32b). Occupancy is an increasing function of density (which is defined as the mean no. of individuals divided by the relative grid size) as we move from coarser to finer resolutions (Fig 32c).

For ModN (log (occupancy) as a function of log(mean relative density), linear regression yields statistically significant results (Pr <2e-16 ***) with both intercept and slope different from zero (Table 2). Model's adjusted R-squared equals 0.672, indicating a good fit. There are departures from normality (see Normal QQ plot, Fig 33), which seem to be related with differences between the species.

Table 2: R syntax and outputs for linear regression of log(Occupancy) as a function of log(Mean relative density) (ModN).

Call:
lm(formula = log(Occupancy) ~ log(Mean.A_Box)

Residuals:

	Min	*1Q*	*Median*	*3Q*	*Max*
	-1.8144	-0.6872	0.1384	0.7135	1.5028

Coefficients:

	Estimate	*Std. Error*	*t value*	*Pr(>|t|)*
(Intercept)	-0.8978	0.3849	-2.332	0.0228 *
log(Mean.A_Box)	0.4056	0.0350	11.581	<2e-16 ***

*Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1*

Residual standard error: 0.8671 on 64 degrees of freedom
Multiple R-squared: 0.677, Adjusted R-squared: 0.6719
F-statistic: 134.1 on 1 and 64 DF, p-value: < 2.2e-16

The models' (ModN) fitted equation is:

*log(Occupancy)= -0.898- 0.406*log(Mean relative density)* (per unit difference in the predictor).

Residual behavior is given in Figure 33, along with the models' fitted and residual values.

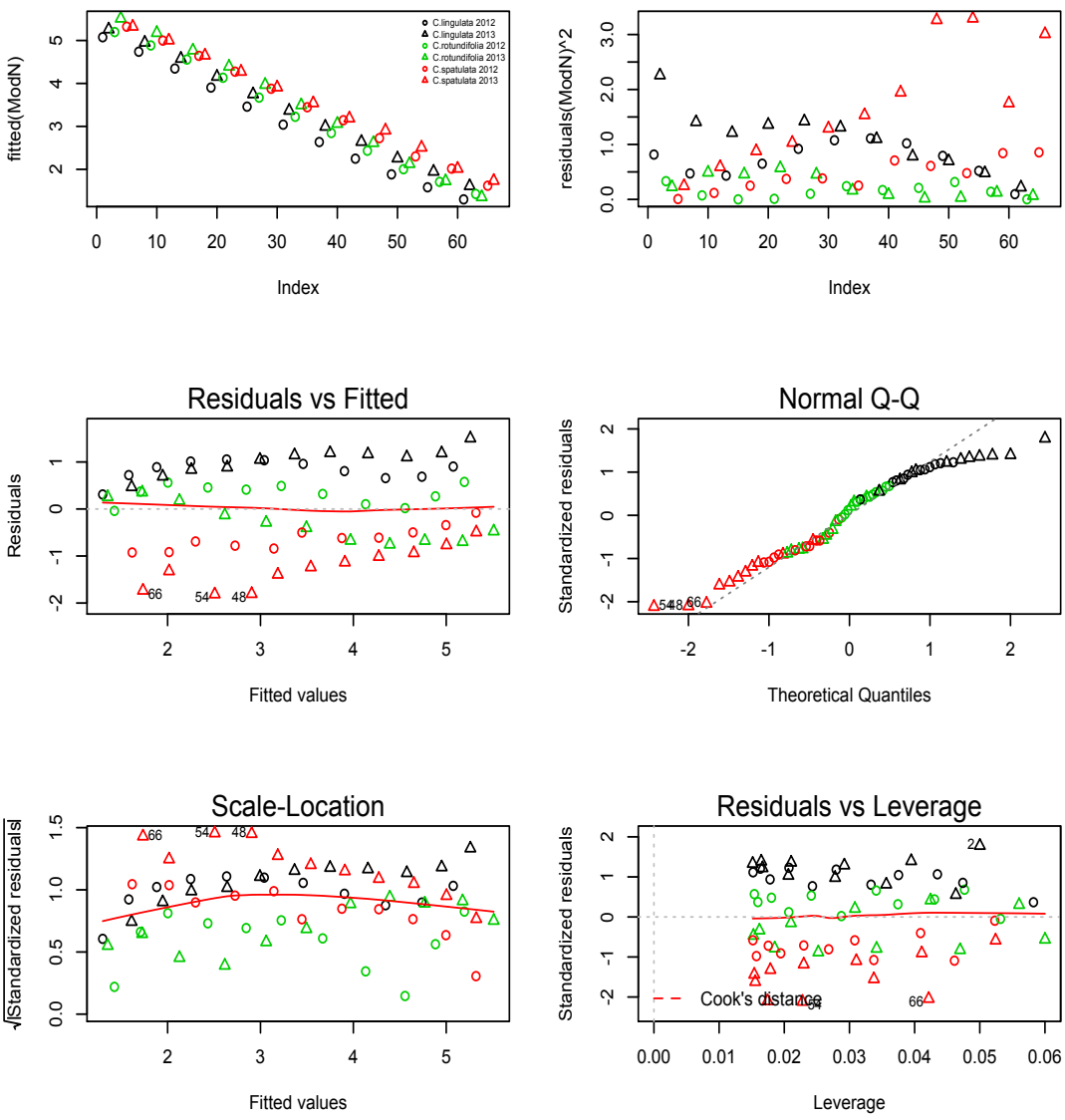


Figure 33:. Each panel is a graphic evaluation on whether the model is satisfying the assumptions for linear regression regarding departures from normality, homogeneity of variances and extreme values. Graphical output (from R's summary (lm) output, see Table 2) for the data for all species in both years. *(Top left)* Fitted model. These are straight lines, this being a linear regression. *(Top right)* Residuals as a function of *x*. In a good fit residuals are small. *(Middle left)* Residuals as a function of fitted values. For homoscedasticity, these should evenly dispersed around the line of the model. *(Middle right)* Normal probability plot. Straight lines indicate normality. *(Bottom left)* Root of standardized residuals as a function of fitted values. In a good fit these should evenly dispersed around the line of the model. *(Bottom right)* Standardized residuals as a function of leverage. Extreme values are those who exceed Cook's distance thresold.

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The largest deviations from the model occur for *C. lingulata* at fine scales (see sq. residuals plot Fig 33), which appears to be *less aggregated* than anticipated from the fitted model, for 2013 (Fig 32c). *C. spatulata,* on the other hand, appears to be *more aggregated* than expected at the coarser resolutions (sq. residuals plot Fig 33, Fig 32c).
ModNS is describing log (occupancy) as a function of log (mean density) and species (Table 3). Mean occupancy as a function of mean density is bound to differentiate for each species. We anticipate differences in the slopes and intercepts of the fitted equations.

Table 3: ANCOVA (Analysis of covariance) table of log(Occupancy) as a function of log(relative Density) and species (ModNS)

Call:
*lm(formula = log(Occupancy) ~ log(Mean.A_Box) * dataset$Species)*

Residuals:

	Min	*1Q*	*Median*	*3Q*	*Max*
	-0.72840	-0.27691	0.03881	0.21280	0.85442

Coefficients:

	Estimate	*Std. Error*	*t value*	*Pr(>|t|)*
(Intercept)	-0.4858	0.2635	-1.843	0.0702 .
log(Mean.A_Box)	0.4552	0.0247	18.452	< 2e-16 ***
dataset$SpeciesCR	-2.4065	0.3921	-6.138	7.22e-08 ***
dataset$SpeciesCS	0.2759	0.3659	0.754	0.4538
log(Mean.A_Box):dataset$SpeciesCR	0.0481	0.0357	1.348	0.1826
log(Mean.A_Box):dataset$SpeciesCS	-0.1139	0.0337	-3.376	0.0013 **

*Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1*

Residual standard error: 0.3477 on 60 degrees of freedom
Multiple R-squared: 0.9513, Adjusted R-squared: 0.9472
F-statistic: 234.4 on 5 and 60 DF, p-value: < 2.2e-16

The models' fitted equations are:

*log(Occupancy)= -0.486+ 0.45518*log(Mean relative density)* for *C. lingulata*
*log(Occupancy)=-2.892 +0.503 *log(Mean relative density)* for *C. rotundifolia*
*log(Occupancy)=-0.209 +0.342*log(Mean relative density) for C.spatulata*

In this case as well, the models' (ModNS) fitted trends yield statistically significant results (Pr = < 2.2e-16 ***), with a much larger fit, compared to ModN (Adjusted R sq. = 0.9472), and the model seems to satisfy the distribution assumptions in terms of residual behavior (see Fig 34).

The model also yields significant differences for *C. rotundifolia* (compared to *C. lingulata*) intercept (Pr = 7.22e-08 ***), which makes sense, since *C. rotundifolia* occupies a much lower percentage of the grid surface. However no significant differences in their respective slopes (Pr= 0.1826) were detected, meaning they could be modeled with a single slope. *C. spatulata* on the other hand, shows no significant differences in intercept (Pr= 0.4538), but the slope that fits the observations is significantly lower than that of *C. lingulata* (Pr = 0.0013 **).

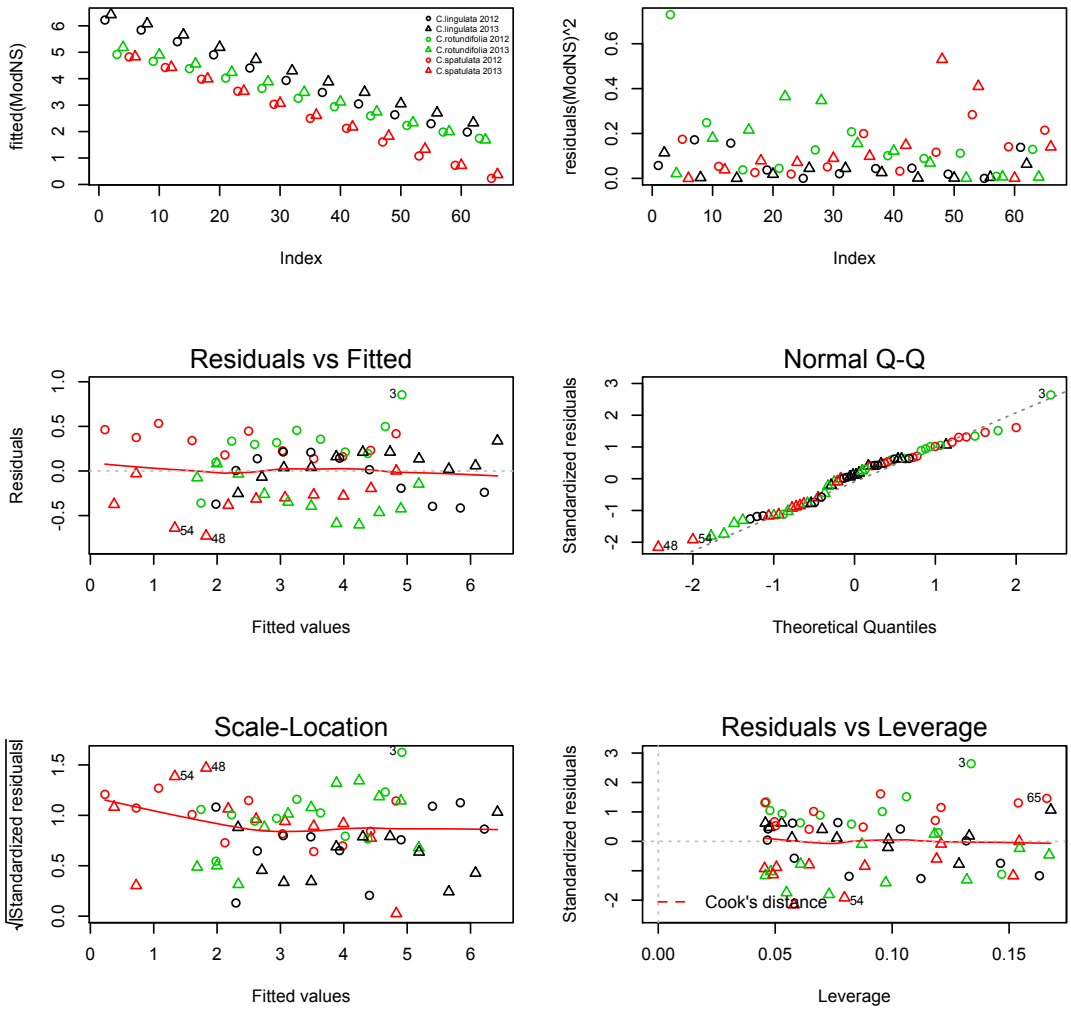


Figure 34: Graphic illustration of (ModNS) fitted and residual values (See Fig 33).

As suspected, the species do not display the same aggregation patterns, since ANCOVA gave significant differences for the factor "species". *C. rotundifolia* seems to differentiate from the model's predictions in fine resolutions (*more aggregated* - see Fig 32c, Fig 34) but, as noted above, this difference is not significant. *C. spatulata* trend, however, displays significant deviations from the model at the coarser resolutions (*more aggregated*— see Fig 32c, Fig 34, sq. residuals plot)

ModNSY is describing log (occupancy) as a function of log (mean density), species, and year of sampling (Table 4). The fitted trends are expected to differentiate for both the factor species and the factor year. We should expect different slopes for each species, which will have a different intercept for each year of sampling.

Table 4: ANCOVA (Analysis of covariance) table of log(Occupancy) as a function of log(relative Density), species, and year of sampling (ModNSY)

Call:
*lm(formula = log(Occupancy) ~ log(Mean.A_Box) * dataset$Species + dataset$Year)*

Residuals:					
	Min	*1Q*	*Median*	*3Q*	*Max*
	-0.6208	-0.1604	0.0181	0.1952	0.6565
Coefficients:					
	Estimate	*Std. Error*	*t value*	*Pr(>|t|)*	
(Intercept)	-0.3816	0.2248	-1.698	0.094817 .	
log(Mean.A_Box)	0.4625	0.0210	22.024	< 2e-16 ***	
dataset$SpeciesCR	-2.3495	0.3331	-7.053	2.20e-09 ***	
dataset$SpeciesCS	0.3078	0.3108	0.990	0.326020	
dataset$Year2013	-0.3589	0.0730	-4.920	7.29e-06 ***	
log(Mean.A_Box):dataset$SpeciesCR	0.04244	0.03032	1.400	0.166784	
log(Mean.A_Box):dataset$SpeciesCS	-0.11716	0.02867	-4.087	0.000134 ***	

*Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1*

Residual standard error: 0.2953 on 59 degrees of freedom
Multiple R-squared: 0.9655, Adjusted R-squared: 0.962
F-statistic: 274.9 on 6 and 59 DF, p-value: < 2.2e-16

The models' fitted equations are:

*log(Occupancy)= -0.382 + 0.463 *log(Mean relative density)* for *C. lingulata* (2012)
*log(Occupancy)= -0.740 + 0.463 *log(Mean relative density)* for *C. lingulata* (2013)
*log(Occupancy)=-2.73 +0.504 *log(Mean relative density)* for *C. rotundifolia* (2012)
*log(Occupancy)=-3.08 +0.504 *log(Mean relative density)* for *C. rotundifolia* (2013)
*log(Occupancy)=-0.074 +0.345*log(Mean relative density) for C.spatulata* (2012)
*log(Occupancy)=-0.432 +0.345*log(Mean relative density) for C.spatulata* (2013)

ANCOVA yielded significant results, with a slight increase in goodness of fit (Adjusted R-sq.= 0.962), and behaves slightly better regarding the model's assumptions (Fig 34). The difference in intercept between years is significant (Pr =7.29e-06***). In all three cases, the

populations behave as above; ModNS is describing log (occupancy) as a function of log (mean density) species (Table 3). in terms of aggregation (modNS).

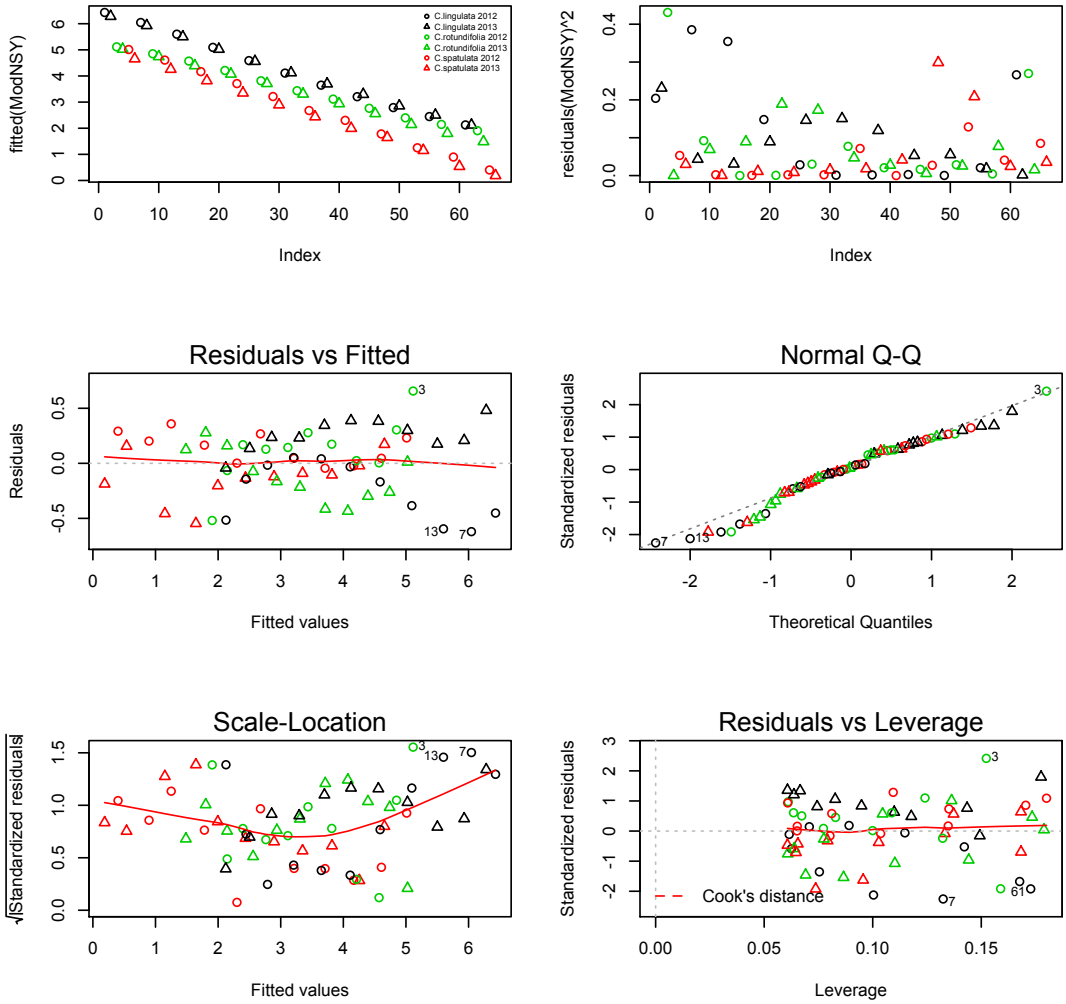


Figure 35: Graphic illustration of (ModNSY) fitted and residual values (See Fig. 33).

ModNSYa is describing log (occupancy) as a function of log (mean density), species, and year of sampling as well (Table 5). However, in this case we should expect different slopes and intercepts for each species, which will depend on the year of sampling.

Table 5: ANCOVA (Analysis of covariance) table of log(Occupancy) as a function of log(relative Density), species, and year of sampling (ModNSYa).

Call:
*lm(formula = log(Occupancy) ~ log(Mean.A_Box) * dataset$Species * dataset$Year)*

Residuals:

	Min	*1Q*	*Median*	*3Q*	*Max*
	-0.46042	-0.09367	-0.00097	0.12257	0.40779

Coefficients:

	Estimate	*Std. Error*	*t value*	*Pr(>|t|)*
(Intercept)	-0.1916	0.1920	-0.998	0.32283
log(Mean.A_Box)	0.4172	0.0186	22.475	< 2e-16 ***
dataset$SpeciesCR	-2.129	0.2897	-7.348	1.12e-09 ***
dataset$SpeciesCS	-0.3723	0.2756	-1.351	0.18243
dataset$Year2013	-0.5829	0.2880	-2.024	0.04798 *
log(Mean.A_Box):dataset$SpeciesCR	0.0627	0.0268	2.339	0.02307 *
log(Mean.A_Box):dataset$SpeciesCS	-0.0128	0.0261	-0.491	0.62563
log(Mean.A_Box):dataset$Year2013	0.0728	0.0269	2.704	0.00914 **
dataset$SpeciesCR:dataset$Year2013	-0.6518	0.4264	-1.528	0.13226
dataset$SpeciesCS:dataset$Year2013	1.0863	0.3987	2.725	0.00865 **
log(Mean.A_Box):dataset$SpeciesCR:dataset$Year2013	-0.0181	0.0388	-0.467	0.64206
log(Mean.A_Box):dataset$SpeciesCS:dataset$Year2013	-0.17729	0.03678	-4.820	1.21e-05 ***

*Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1*

Residual standard error: 0.1881 on 54 degrees of freedom
Multiple R-squared: 0.9872, Adjusted R-squared: 0.9846
F-statistic: 377.9 on 11 and 54 DF, p-value: < 2.2e-16

The models' fitted equations are:

*log(Occupancy)= -0.19159 + 0.41720 *log(Mean relative density)* for *C. lingulata* (2012)
*log(Occupancy)= -0.773 + 0.489*log(Mean relative density)* for *C. lingulata* (2013)
*log(Occupancy)=-2.32 +0.479 *log(Mean relative density)* for *C. rotundifolia* (2012)
*log(Occupancy)=-0.842 +0.399*log(Mean relative density)* for *C. rotundifolia* (2013)
*log(Occupancy)=-0.563 +0.405*log(Mean relative density) for C.spatulata* (2012)
*log(Occupancy)=-0.889 +0.240*log(Mean relative density) for C.spatulata* (2013)

This model (ModNSYa) fits linear regressions to all species for both years of sampling, but the generated slopes for each species do differentiate between 2012 and 2013. It yielded significant results (*p*-value < 2.2e-16), and best describes the variation of the data (Adjusted R-sq.=0.984), while satisfying the model's assumptions (Fig 35). Interestingly enough, the slope of *C. spatulata* for 2012 does not differentiate from the slope of *C. lingulata* for the same year (*Pr* =0.62563). This changes for 2013, where both *C. spatulata* intercept (*Pr*=0.00865**) and slope (*Pr* =1.21e-05 ***) differentiate from the corresponding ones for *C. lingulata* 2012, while *C. lingulata* intercept and slope of 2012 differentiate from intercept (*Pr*=0.04798*) and slope for 2013 (*Pr* =0.00914 **). A significant difference in the slope of *C. rotundifolia* for 2012 is detected as well (*Pr*= 0.02307*), plus a difference in intercept, which was evident before. This is indicative of a very dynamic interaction between species abundance and occupancy for the two years of sampling.

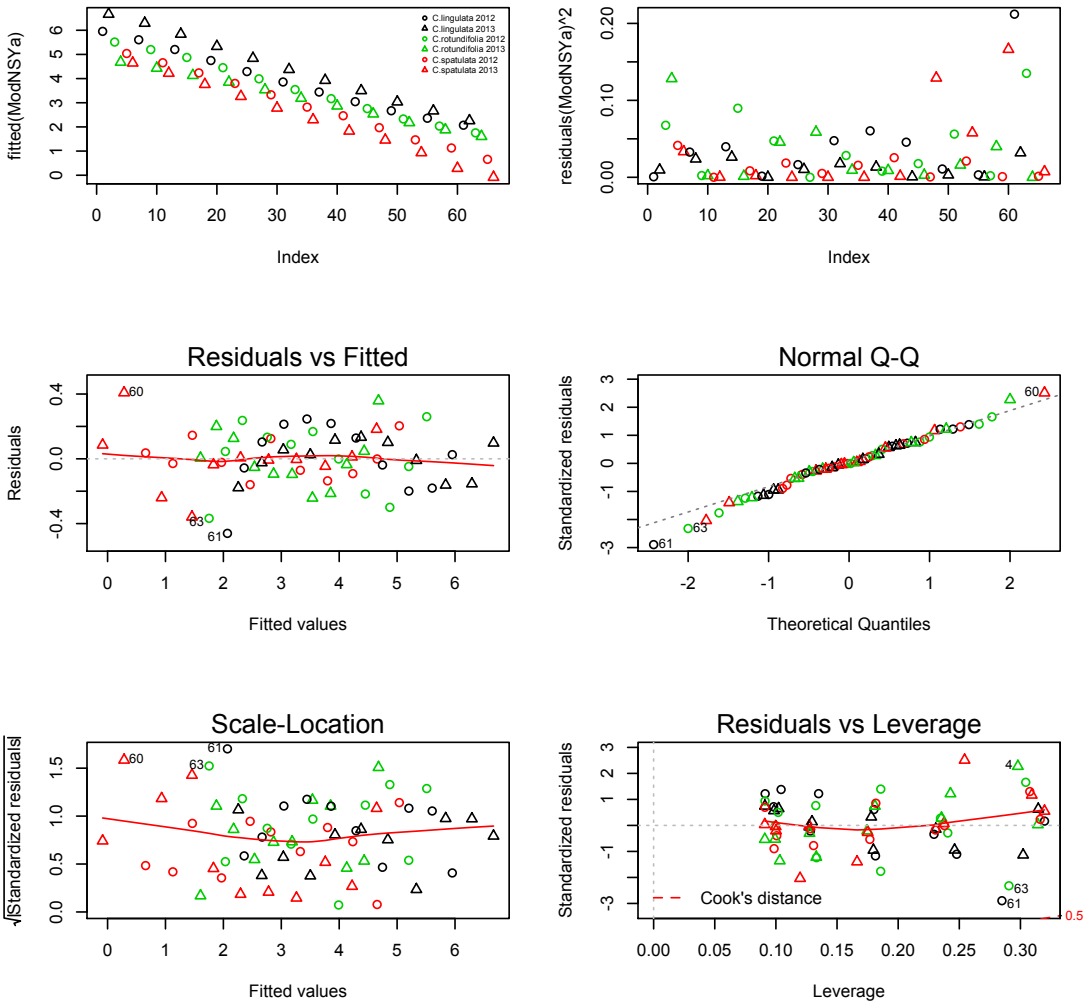


Figure 36: Graphic illustration of (ModNSYa) fitted and residual values (See Fig 33).

5. Abundance and Kernel density probability curves per elevation class

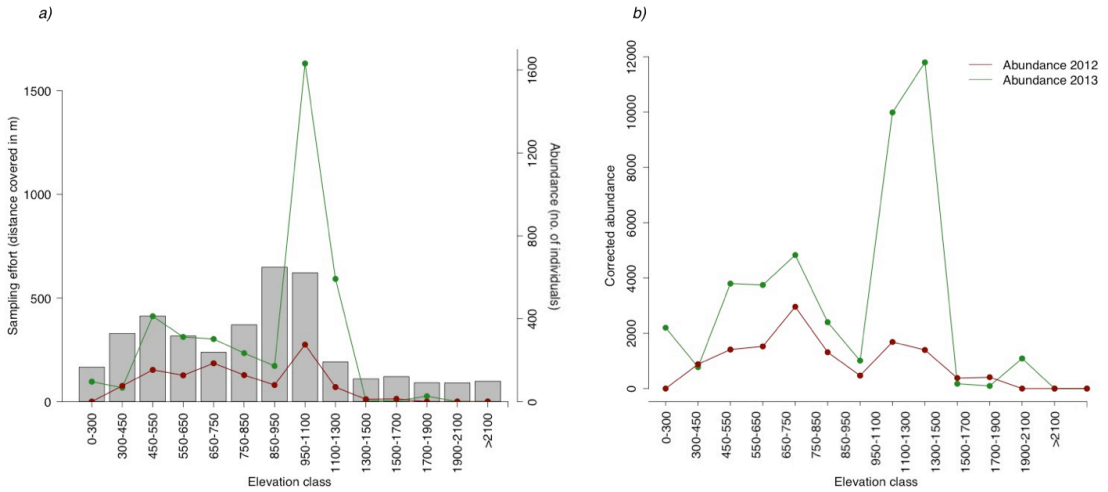


Figure 37: a) Invested effort and no of individuals: Invested effort per elevation, no. of individuals in each elevation class for *C. lingulata* for 2012 (red line) and 2013 (green line). b) Corrected abundance for unveven effort invested in each elevation class for 2012 (red line) and 2013 (green line).

As noted with the raw data (Fig 37a), *C. lingulata* displays two peaks of abundance across its elevation range. One can be detected at elevations of 450-650 m for both 2012 (red) and 2013 (green), while the other can de placed at much higher altitudes of approximately 900 to 1,300 m, which could be considered toward the upper edge of the species altitudinal range, for 2013.

The two-peak abundance pattern is followed by a sudden drop around 1,300 -1,500 m for both years of sampling. This might be attributed to the dense forested areas in paths 11 and 12 (see Figs 24-25).

Table 6: Descriptive Statistics of *C. lingulata* abundance across its altitudinal gradient on Mt. Olympos, in a 20 x 20 m scale.

Year			Elevation Class									
2012		*Range*	*0 - 300 m*	*300 -450 m*	*450 -550 m*	*550 -650 m*	*650 -750 m*	*750 -850 m*	*850- 950 m*	*950-1100 m*	*1100-1300 m*	*1300- 1500 m*
	Mean	0.310	0.000	0.243	0.321	0.436	0.768	0.334	0.293	0.244	0.365	0.100
	St. Deviation	0.278	0.000	0.151	0.162	0.272	0.307	0.269	0.229	0.126	0.176	0.084
	Minimum Value	0.000	0.000	0.040	0.020	0.030	0.180	0.020	0.000	0.050	0.010	0.000
	1st Quantile	0.100	0.000	0.120	0.198	0.228	0.555	0.148	0.088	0.150	0.258	0.030
	Median	0.250	0.000	0.220	0.300	0.380	0.760	0.270	0.255	0.200	0.340	0.080
	2nd Quantile	0.450	0.000	0.310	0.420	0.593	0.923	0.473	0.453	0.320	0.480	0.160
	Maximum Value	1.610	0.000	0.730	0.850	1.390	1.610	1.240	1.010	0.610	0.760	0.330
2013												
	Mean	0.943	0.557	0.227	0.937	0.990	1.031	0.709	0.501	1.582	2.844	0.047
	St. Deviation	0.892	0.318	0.133	0.380	0.428	0.431	0.356	0.322	0.562	1.024	0.034
	Minimum Value	0.000	0.090	0.040	0.320	0.190	0.190	0.050	0.040	0.330	0.590	0.000
	1st Quartile	0.300	0.258	0.110	0.630	0.638	0.718	0.458	0.268	1.208	2.180	0.020
	Median	0.740	0.545	0.225	0.895	0.965	0.970	0.685	0.395	1.575	2.765	0.040
	2nd Quartile	1.243	0.798	0.300	1.213	1.260	1.285	0.910	0.670	1.933	3.555	0.060
	Maximum Value	5.470	1.400	0.740	1.900	2.260	2.360	2.020	1.510	3.350	5.470	0.160

Table 6 contains descriptive statistics for mean abundance (density) of *C. lingulata* individuals at each elevation class, in a 20 x 20 m square, for 2012 and 2013. Maximum density for 2012 is recorded at the 650 - 750 m elevation class (0.768 individuals) and at the 1,100 - 1,300 m, for 2012 (2.844). The largest standard deviation is recorded for the same classes as well (0.307 and 1.024 respectively). No individuals have been recorded at lower altitudes (0 to 300 m) for 2012. The sudden drop in individual density is at the same altitudes as before (1,300 to 1,500 m elevation class Table 6.). Only 10 elevation classes are shown, since no

individuals were recorded at higher altitudes. Mean density for 2012 and 2013 is illustrated in Figs 38-39, for raw and log transformed values respectively.

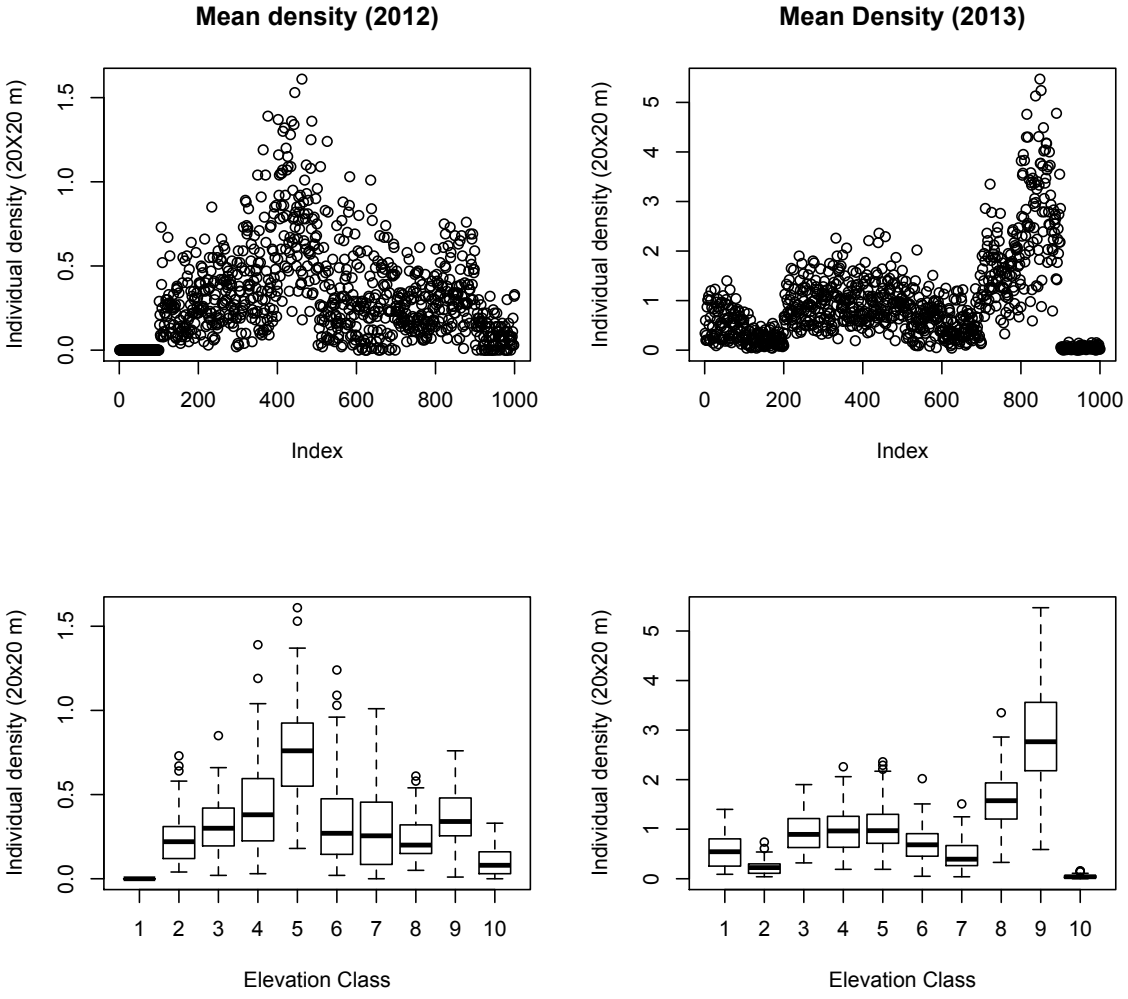


Figure 38: Mean density for 2012 and 2013, in 20 x 20 m squares, for 10 elevation classes.

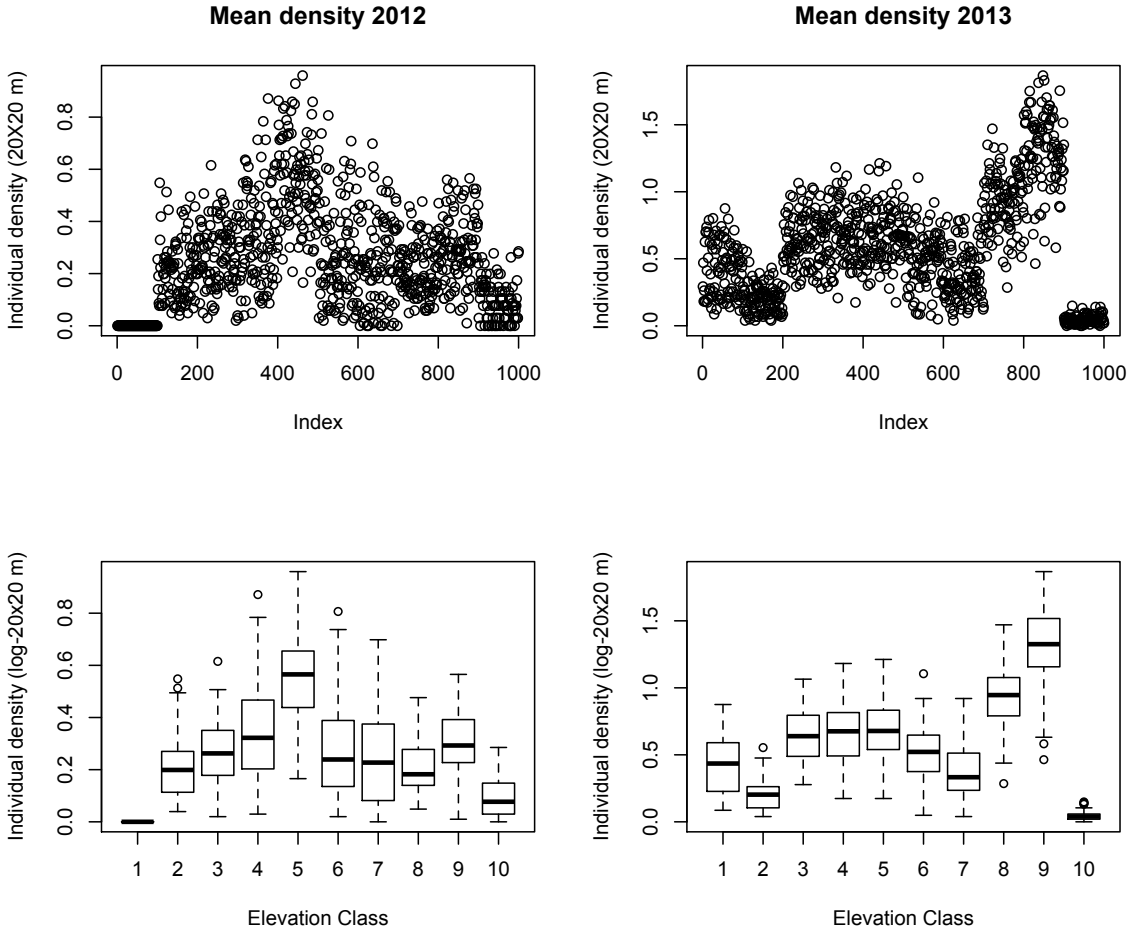


Figure 39: Log mean density for 2012 and 2013 in 20 x 20 m squares, for 10 elevation classes.

Table 7: Linear model of log (Mean density) as a function of elevation class for 2012.

Call:
lm(formula = Mean.Density.Log2012 ~ Elevation.class.

Residuals:

	Min	*1Q*	*Median*	*3Q*	*Max*
	-0.38987	-0.08793	0.00000	0.07232	0.53622

Coefficients:

	Estimate	*Std. Error*	*t value*	*Pr(>|t|)*
(Intercept)	-9.314e-15	1.365e-02	0.000	1
Elevation.class2	2.104e-01	1.931e-02	10.895	< 2e-16 ***
Elevation.class3	2.707e-01	1.931e-02	14.018	< 2e-16 ***
Elevation.class4	3.452e-01	1.931e-02	17.876	< 2e-16 ***
Elevation.class5	5.554e-01	1.931e-02	28.762	< 2e-16 ***
Elevation.class6	2.703e-01	1.931e-02	13.996	< 2e-16 ***
Elevation.class7	2.421e-01	1.931e-02	12.538	< 2e-16 ***
Elevation.class8	2.131e-01	1.931e-02	11.034	< 2e-16 ***
Elevation.class9	3.028e-01	1.931e-02	15.684	< 2e-16 ***
Elevation.class10	9.258e-02	1.931e-02	4.794	1.88e-06 ***

*Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1*

Residual standard error: 0.1365 on 990 degrees of freedom
Multiple R-squared: 0.5153,
F-statistic: 117 on 9 and 990 DF, p-value: < 2.2e-16

Table 8: ANOVA-derived F value for 2012.

	Df	*Sum Sq*	*Mean Sq*	*F value*	*Pr(>F)*
Elevation.class	9	19.622	2.180	116.95	< 2.2e-16 ***
Residuals	990	18.456	0.019		

*Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1*

Table 9: Post Hoc Test—Tukey's multiple comparisons of means (non-significant differences only) for log (Mean Density) for 2012

	Tukey multiple comparisons of means 95 % family-wise confidence level			
	diff	*lwr*	*upr*	*p adj*
3-2	0.060	-0.0009	0.1215	0.0577
6-2	0.060	-0.0014	0.1211	0.0615
7-2	0.032	-0.0295	0.0929	0.826
8-2	0.003	-0.0585	0.0639	1.000
4-3	0.074	0.0133	0.1357	0.005
6-3	-0.0004	-0.0616	0.0608	1.000
7-3	-0.028	-0.0898	0.0327	0.900
8-3	-0.058	-0.1188	0.0036	0.0854
9-3	0.032	-0.029	0.0934	0.8141
9-4	-0.042	-0.103	0.0189	0.4629
7-6	-0.028	-0.0893	0.0331	0.9083
8-6	-0.057	-0.1184	0.004	0.0906
9-6	0.032	-0.0286	0.0938	0.8022
8-7	-0.029	-0.0903	0.0322	0.8905
9-7	0.061	-0.0004	0.1220	0.0539

ANOVA showed significant differentiations (*p*-value < 2.2e-16 ***) between elevation classes for 2012, with Multiple R squared=0.5153. The elevation classes that do not differentiate from each other are given in Table 9**.** Elevation class 2 (300-450 m) does not differentiate from Elevation class 3 (450 to 550 m) in mean density. The same holds true for elevation classes 6 to 9 (750 to 1300 m). These elevation classes do not differentiate among each other as well, leaving only elevation class 5 mean Density (0.768 Individuals), at 650 to 750 m elevation, to differentiate from the others.

Table 10: Linear model of log (Mean Density) as a function of elevation class for 2013.

Call:
lm(formula = Mean.Density.Log2013 ~ Elevation.class)

Residuals:

	Min	*1Q*	*Median*	*3Q*	*Max*
	-0.84529	-0.12451	-0.00634	0.12275	0.59096

Coefficients:

	Estimate	*Std. Error*	*t value*	*Pr(>|t|)*
(Intercept)	0.423	0.0197	21.402	< 2e-16 ***
Elevation.class2	-0.224	0.0279	-8.008	3.25e-15 ***
Elevation.class3	0.220	0.0279	7.863	9.80e-15 ***
Elevation.class4	0.243	0.0279	8.708	< 2e-16 ***
Elevation.class5	0.264	0.0279	9.484	< 2e-16 ***
Elevation.class6	0.092	0.0279	3.280	0.00107 **
Elevation.class7	-0.037	0.0279	-1.330	0.18382
Elevation.class8	0.502	0.0279	17.977	< 2e-16 ***
Elevation.class9	0.886	0.0279	31.734	< 2e-16 ***
Elevation.class10	-0.377	0.0279	-13.494	< 2e-16 ***

*Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1*

Residual standard error: 0.1975 on 990 degrees of freedom
Multiple R-squared: 0.7519,
F-statistic: 333.4 on 9 and 990 DF, p-value: < 2.2e-16

Table 11: ANOVA derived –F value for 2013.

	Df	*Sum Sq*	*Mean Sq*	*F value*	*Pr(>F)*
Elevation.class	9	117.059	13.007	333.45	< 2.2e-16 ***
Residuals	990	38.616	0.039		

Table 12: Post Hoc Test - Tukey's multiple comparisons of means (non significant differences only) for log (Mean Density) for 2012.

	Tukey multiple comparisons of means			
	95 % family-wise confidence level			
	diff	*lwr*	*upr*	*p adj*
6-1	0.092	0.003	0.180	0.036
7-1	-0.037	-0.126	0.051	0.947
4-3	0.024	-0.065	0.112	0.998
5-3	0.045	-0.043	0.134	0.838
5-4	0.022	-0.067	0.110	0.998

ANOVA showed significant differentiations (*p*-value- < 2.2e-16 ***) between elevation classes for 2013 as well, with Multiple R squared=0.7519. The elevation classes that do not differentiate from each other are given in Table 12. The only elevation classes that do no differentiate from each other are 3, 4 and 5, from 450 to 750 m. Both the models' fitted values and residuals for 2012 and 2013 are given in Figs. 40-41, whereas diagnostic plots are given in Figs 41 - 42, respectively. Probability density plots of mean Density for 2012 and 2013 are featured in Fig 44.

.

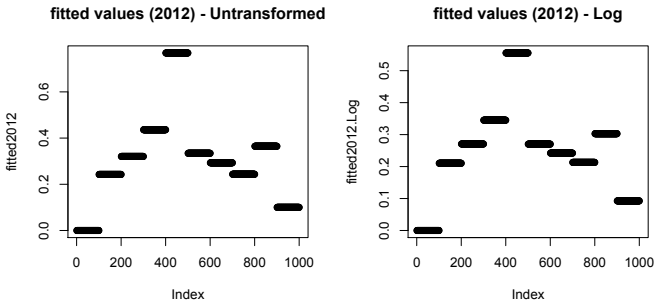
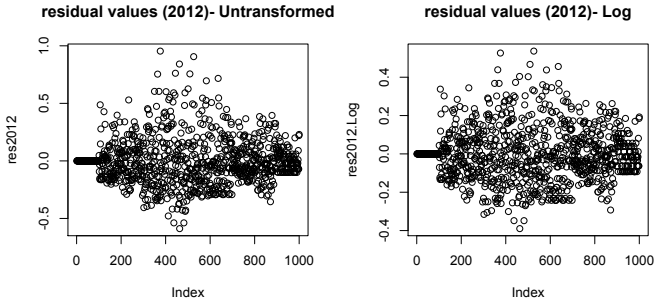


Figure 40: Fitted and residual values for non-transformed and log transformed Mean Density, for 2012



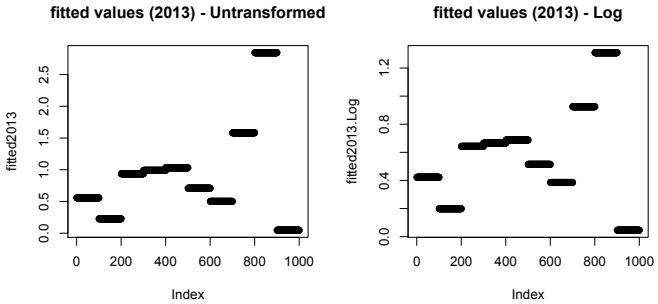
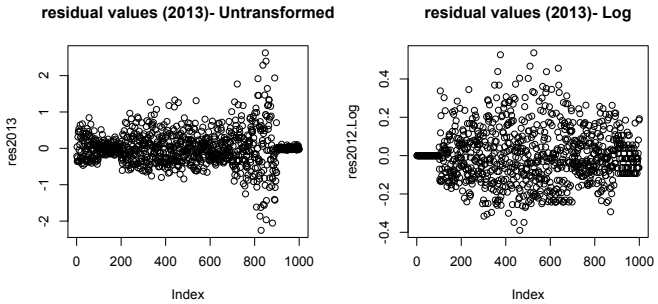


Figure 41: Fitted and residual values for non-transformed and log transformed Mean density, for 2013



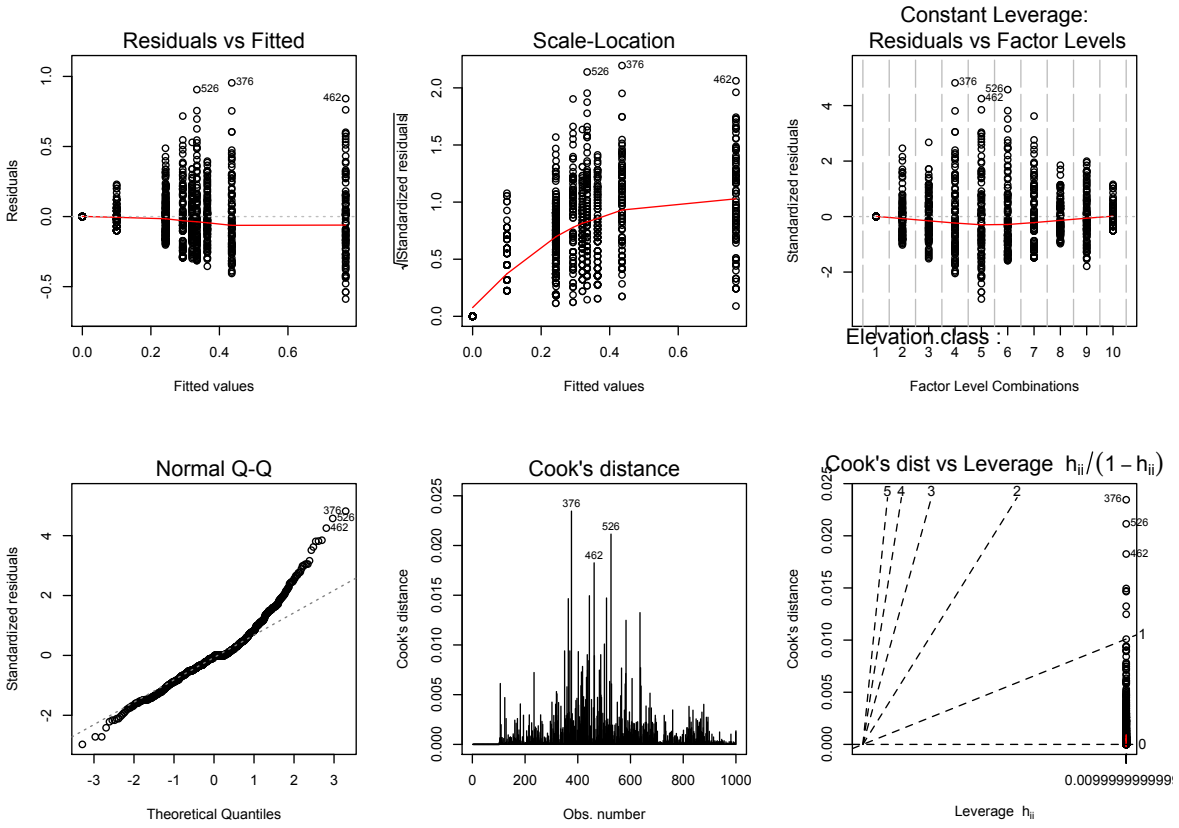


Figure 42: Diagnostic plots for linear model in Table 10 of log(Mean density) in 2012 as a function of elevation class. The top three panels and lower left panel have the same interpretation as in Fig 33. The lower middle and right panel give information on outliers.

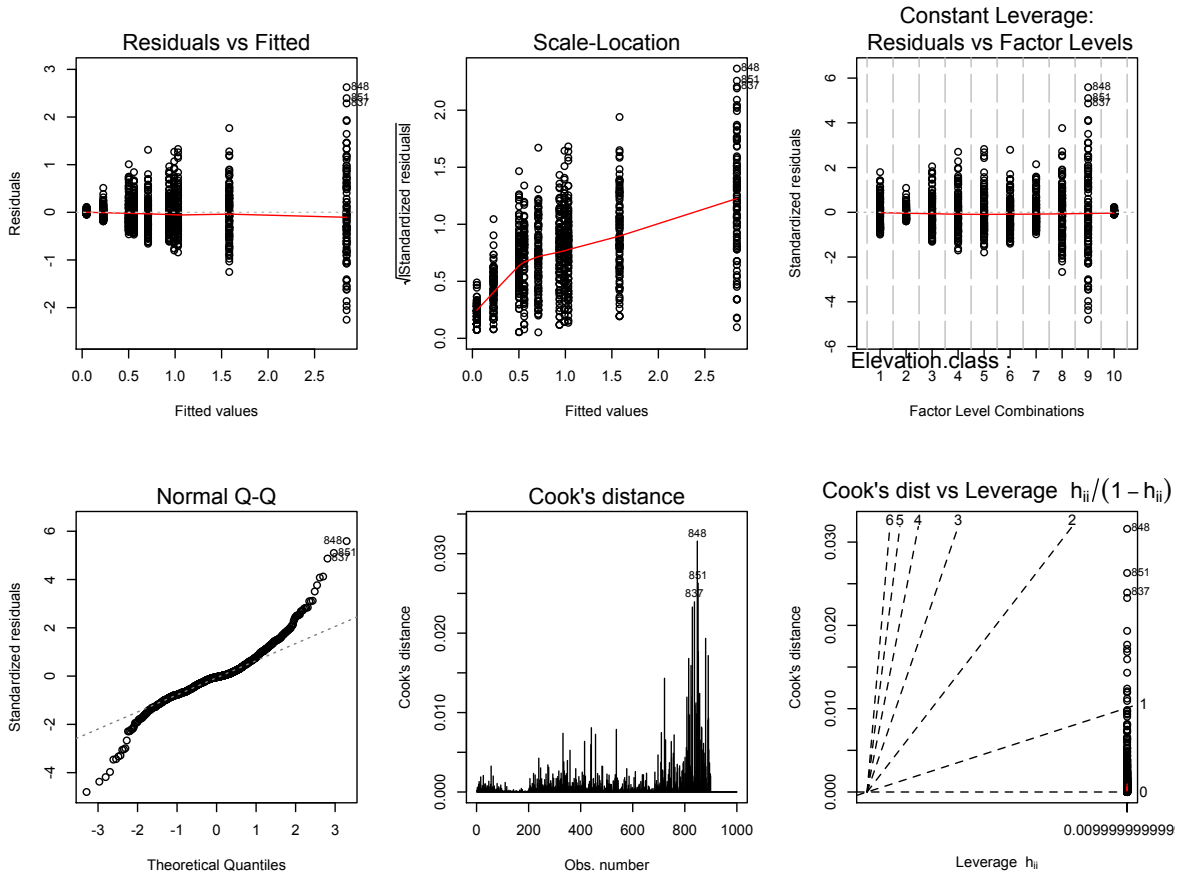


Figure 43: Diagnostics plots for linear model of log(Mean density), in 2013 as a function of elevation class (See Fig 42).

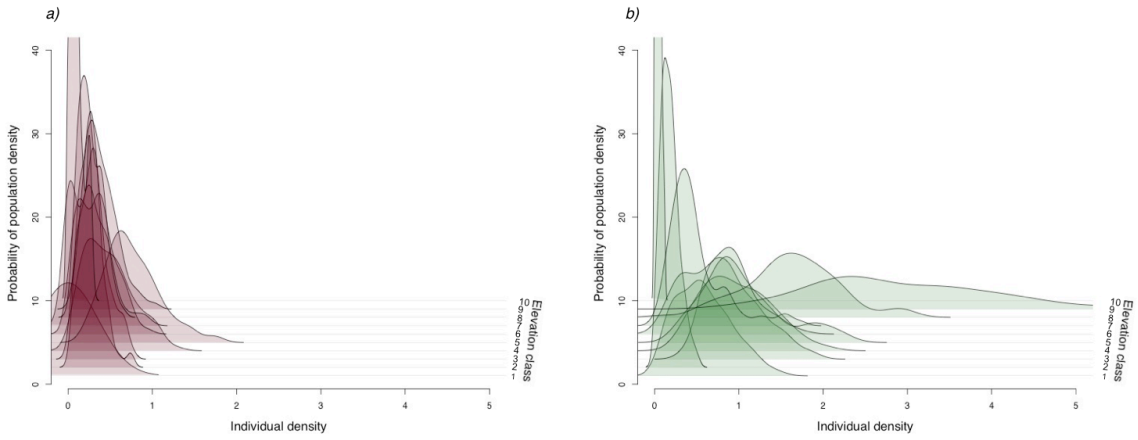


Figure 44: Probability density functions of the number of individuals in the altitudinal range of 300-1,300 m for 2012 (a), and 2013 (b). Each distribution corresponds to an elevation class. X-axis describes the individual density, while y-axis the probability of acquiring each individual density if we sample randomly within each elevation class.

As seen in Fig 44, the probabilities of acquiring a given density of individuals at each elevation class is pretty homogeneous for 2012, thus, not providing a distinguishable centre. The only class that differentiated from the others was elevation class 5 (650 to 750 m). However it is possible to acquire such densities at other elevation classes as well, since the probability curves overlap. Elevation class 9 (1,100 to 1,300 m) from 2013, on the other hand, is pretty distinguishable from the others. The probability curve of acquiring the given higher densities of individuals does not overlap with other elevation classes; hence we could speculate that this elevation class is " an abundant centre" for the species distribution along its altitudinal gradient. Nevertheless, the observed abundance pattern does not repeat itself for both years of sampling, thus failing to confirm the ACH along an altitudinal gradient.

6. Mean presence and mean turnover across different spatial scales

Mean presence of *C. lingulata* individuals for 2012 and 2013, and mean population turnover between the two years of study is illustrated in Figs 45-47. The upper and lower binomial proportion confidence intervals, with the assumption that *p* (p-value for presence) follows a normal distribution for a = 5% (significance level), are depicted as well.

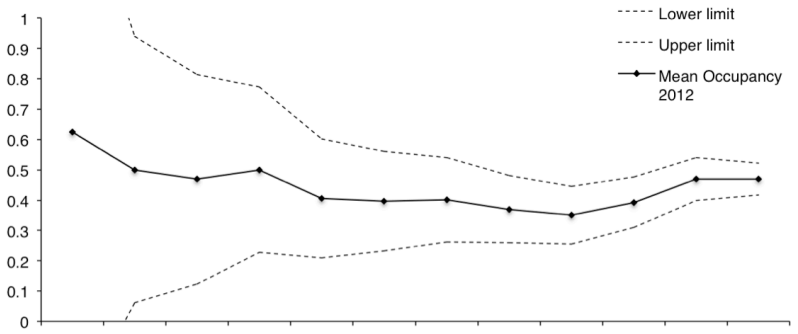


Figure 46: Mean presence (occupancy) for *C. lingulata* for 2012 across different spatial scales.

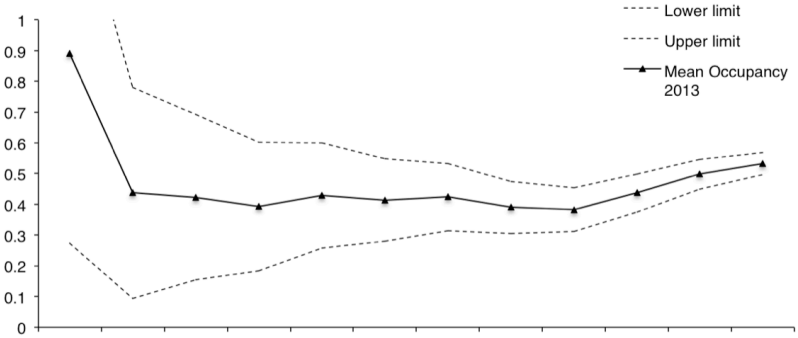


Figure 47: Mean presence (occupancy) for *C. lingulata* for 2013 across different spatial scales.

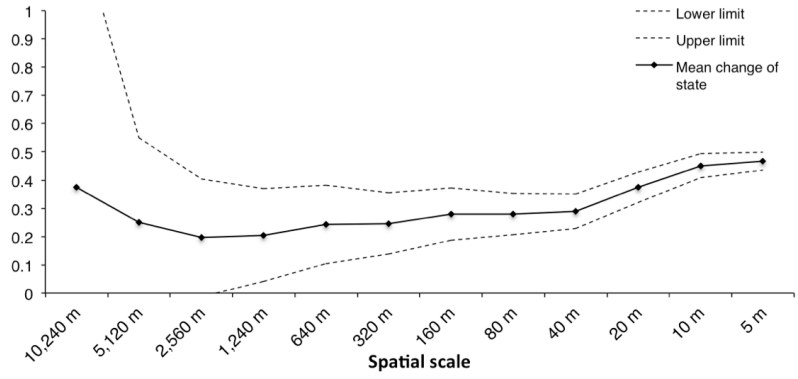


Figure 48: Mean population turnoverfor *C. lingulata* between 2012 and 2013 across different spatial scales.

Mean individual presence for *C. lingulata* appears relatively constant across spatial scales ranging from 5 m to 10,240 m side quadrats, for both years of study— a property that is indicative of an underlying fractal "disturbance" on the species' distribution in space.

The plant flowers once, at the second year of its life cycle, in the purposes of this study, however, is treated as annual. Regarding the mean change of state (turnover), it is anticipated that at very coarse scales (many kilometers, the overall surface) there will not be any substantive population turnover for occupied cells as the species is generally present in the area (mean population turnover close to 0), and its not likely to change state. But at the scale of a single individual, it should be equal to 1, since each position at an individuals' level cannot be occupied if it was occupied the year before (a given space cannot be occupied by flowering individuals for both successive years). We find that the observed turnover is larger than anticipated in the coarser resolutions, considering the spatial scales under question.

7. Box Counting Dimension

The box counting fractal dimension is given in Fig 48:

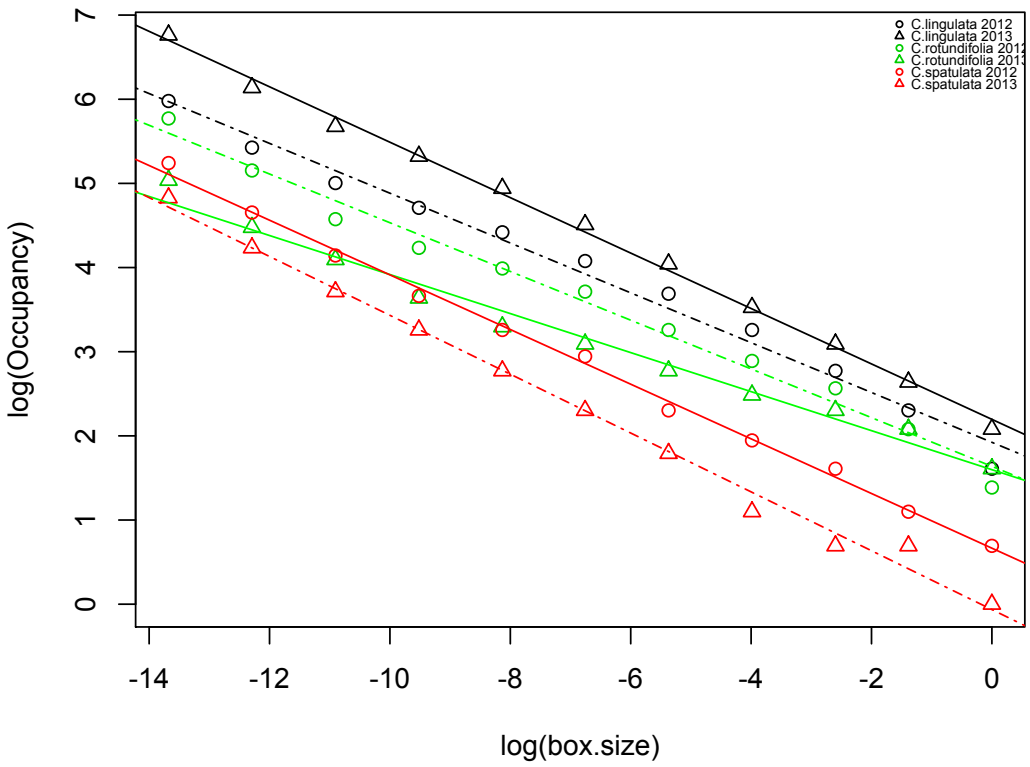


Figure 48: Box counting Fractal dimension of *C. lingulata, C. rotundifolia* and *C. spatulata* for both years of study.

$$\gamma_{cl12} = |D_{cl12} - 1| = |-0.295 - 1| = 1.295$$

$$\gamma_{cl13} = |D_{cl13} - 1| = |-0.329 - 1| = 1.329$$

$$\gamma_{cs12} = |D_{cs12} - 1| = |-0.289 - 1| = 1.289$$

$$\gamma_{cs13} = |D_{cs13} - 1| = |-0.231 - 1| = 1.231$$

$$\gamma_{cr12} = |D_{cr12} - 1| = |-0.324 - 1| = 1.324$$

$$\gamma_{cr13} = |D_{cr13} - 1| = |-0.349 - 1| = 1.349$$

Above are the fractal dimensions of the species' distributions, as intersections of fractal objects with a transect. D_{sp} is the slope of log (Occupancy) as a function of log-box size for each species and both years of study.

II. Molecular Analysis

1.Scoring

As noted in Table 13, a very high percentage of the selected primers yielded polymorphic loci. The total number of yielded bands was 131, with an average of 13.1 bands per marker. 129 out of 131 loci were polymorphic. The Resolving power of each marker varied from 3.66 to 8.8, with an average of 6.62, and the average H_o per primer was 0.29.

Table 13: Random sequence 10-mers for RAPD analysis. The number of yielded and polymorphic loci, along with each primer's resolving power, and the observed per primer Shannon diversity Index

a/a	Sequence	No. of samples	No of Polymorphic bands	No. of yielded bands	H_o	R_p
OPA -4	AATCGGGCTG	11	15	15	0.323	8
OPA -9	GGGTAACGCC	12	17	16	0.267	7.5
OPA -20	GTTGCGATCC	7	14	15	0.276	8.286
OPC -4	CCGCATCTAC	7	16	16	0.283	7.143
OPD -20	ACCCGGTCAC	6	6	7	0.278	3.666
OPF -6	GGGAATTCGG	8	11	11	0.292	5.25
OPF -20	GGTCTAGAGG	5	10	11	0.255	5.2
OPG -4	AGCGTGTCTG	5	15	15	0.335	8.8
OPH -9	TGTAGCTGGG	10	15	15	0.300	6.6
OPM -3	GGTGGTCAAG	11	10	10	0.299	5.818
TOTAL			**129**	**131**		

2.Differentiation indexes

As noted in Table 14, the grand mean of H_{SP} generated by RAPD markers for within population diversity is close to 0.21. The observed heterozygosity for both populations is greater than expected, Indicative of a great variability within the samples that were arbitrarily assigned into two populations, corresponding to the mountains under investigation (Olympus and Falakro).

Table 14: Observed Heterozygosity, Mean Heterozygosity, Mean Diversity at species level and Diversity index for populations/regions of Mt. Olympus and Falakro.

Population	H_O	H_{POP}	H_{SP}	*DI*
Olympus	0.306	-	-	-
Falakro	0.301	-	-	-
Sum	-	0.303	0.291	0.966

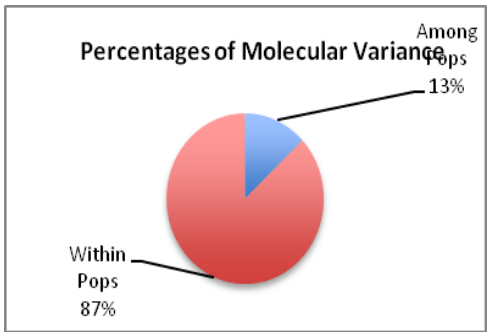


Figure 49: Genetic differentiation within and among populations for Mt. Olympus and Mt. Falakro (AMOVA output).

Table 15: Analysis of molecular variance (AMOVA) within and among Mt. Olympus and Mt. Falakro populations/regions.

As illustrated in Fig 49 of AMOVA, the largest percentage of variability lies within populations (87%) while only 13% corresponds to variability between the regions of Olympus and Falakro. Estimated between population diversity Φ_{PT} is lower than expected (0.131**)** (Table 15)**,** however statistically significant (*p*-value = 0.04)**.** As noted above, the number of statistically valid samples were few, thus such results should be considered with caution. The primers that were selected revealed great differentiation between individuals taken from different parts of Olympus and Falakro, which is indicative of a genetic substructure within the arbitrarily assigned populations/regions.

3. UPGMA analysis

As illustrated in Fig 50, individuals of lower altitudes from Olympus form a distinct clade, which is differentiated from individuals of Mt. Falakro. Individuals, however, which were found at Olympus's higher altitudes (ZOLOTAS-refuge samples) differentiate from individuals from lower altitudes, and group together with individuals of Falakro mountain. This pattern seems to repeat itself in the PcoA (Fig 50). In the PcoA, however (1st and 2nd coordinate

axes), individuals from the ZOLOTAS-reguge site seem to differentiate from Falakro individuals as well. The observed pattern does not contradict the results of AMOVA. The differentiation between the two regions is clear; ZOLOTAS-refuge samples, however, seem to greatly increase differentiation within Olympus population. XIONODROMIKO 3 sample seems to affect Falakro within population differentiation with the same manner.

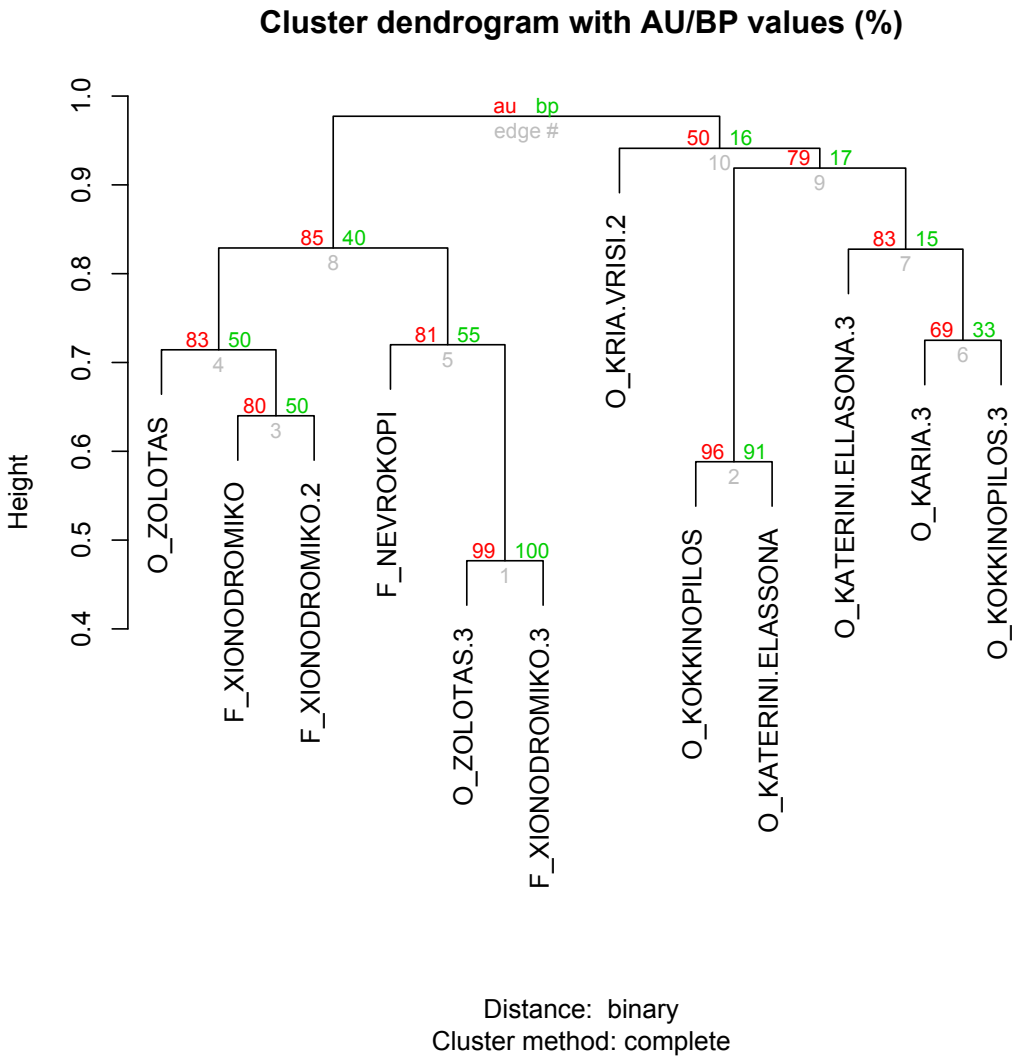


Figure 50: UPGMA dendrogram for 1000 bootstrap repetitions (full linkage) for individuals of *C. lingulata* populations from Olympus and Falakro mountains.

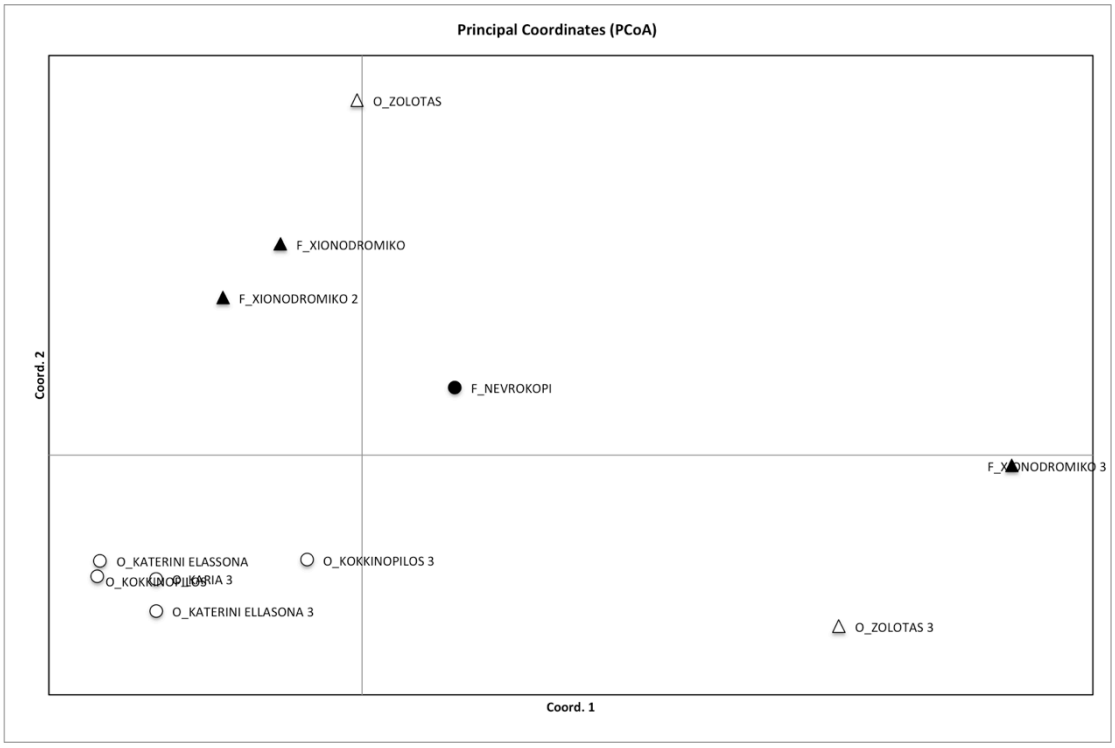


Figure 51: Principal Coordinate Analysis of samples from Olympus and Falakro, treated with RAPD markers.

D.DISCUSSION

I. A Review of the study's findings

A total of 1,130 and 3,897 individuals were recorded for *C. lingulata*, 1,234 and 1,291 for *C.spatulata* and 989 and 659 for *C. rotundifolia*, in 2012 and 2013, respectively. Most of observations were recorded at the NE and SE part of the mountain for *C. lingulata* and *C. spatulata*, while *C. rotundifolia* was only recorded at high elevations within the National Park (Figs 3-14). While *C. lingulata* and *C. spatulata* species seem to co-occur at coarser resolutions, they do in fact form distinct patches of individuals within distances of hundreds of meters (Figs 28-30). *C. lingulata* range appears relatively unstable during the two years of study, since there appears to be a great population turnover, evident at coarser resolutions.

All species display statistically significant differences, as shown by the ANCOVA analysis of species Occupancy as a function of mean density across different spatial scales (ModN, ModSN, ModSNY, ModSNYa – section C.I.4), both relative to each other, and for each year of sampling. *C. rotundifolia* differentiates from *C. lingulata* in terms of the models' intercepts, indicating that its occurrence is more restricted than for the other two species. *C. spatulata* individuals appear significantly more "aggregated" relative to *C. lingulata* at coarser resolutions. Each year of study generated a significantly different pattern of occupancy as a function of abundance for all species.

C. lingulata abundance along an altitudinal gradient produces a 2 peak pattern, (one at 650 - 750 m, and one at 1,100 - 1,300 m) (Fig 37). There seems to be an abrupt decline in density at the 1,300 - 1,500 m elevation class, which may be attributed to the change in the surrounding vegetation (Figs 24-25). An "abundant centre" for the species distribution may be observed for 2013 (Fig 44); however no such pattern occurs for 2012, hence, the ACH along an altitudinal gradient cannot be upheld for the given dataset.

Mean presence of *C. lingulata* individuals appears constant across different spatial scales, a property indicative of an underlying fractal distribution (or disturbance). Mean population turnover for this species, as mentioned earlier, appears greater than expected as well. The Fractal dimensions of the distribution for each species and year of study are: for *C. lingulata,* 1.295 (2012) and 1.329 (2013); for *C. spatulata*, 1.289 (2012) and 1.231 (2013); and for *C. rotundifolia,* 1.324 (2012) and 1.349 (2013).

There seems to be a substantial differentiation at the molecular level of *C. lingulata* species from Mt. Olympus and Mt. Falakro (Fig 49). Recorded between-population diversity Φ_{RT} was estimated at 0.131, which is lower than expected from similar studies. Within population H_O was estimated at 0.306 for Mt. Olympus and 0.301 for Mt. Falakro, which is higher than expected. Increased H_O may be indicative of a genetic population substructure. Nevertheless, Mt. Olympus individuals appear grouped together with PcoA analysis (Fig 51), with the exception of individuals from Olympus's higher altitudes, which appear closer to Mt. Falakro individuals (Fig 50).

II. Occupancy-Abundance Relationships

Fractal models assume that species aggregation parametres have some predictable scaling properties. Hartley *et al*. (2004) combined data from 16 contrasting (relative to aspects of their life cycles) plant species in Britain across a wide range of ecologically relevant spatial scales spanning 6 orders of magnitude (1 m to 100 km), in order to investigate whether the level of clustering of the species' distributions displays such consistent properties, which would allow relatively accurate predictions of abundance from coarser resolutions. They also investigated the ways the distributions' Box counting dimension — which encompasses information about aggregation — for plants with different life traits change and/or correlate to each other, to what extent, and at which level. They detected a breakdown in cross-scale correlations at a 0.5 km scale, which they attributed to differences in observed patterns, due to non-overlapping sets of processes operating at local/regional scales.

Relative aggregation of a species can be expressed by species' occupancy as a function of abundance or density. In this study, relative aggregation of the *Campanula* species was approximated as the slope of a log (Occupancy) as a function of log (Mean Density) [Which is the mean abundance of each species over the number of occupied cells, then divided by the relative size (to the coarsest resolution) of each grid square], for each spatial resolution.

To note, variations in abundance patterns within each species, were not taken into account at this point. The aim was to investigate whether species from the *Campanula* genus that co-occur along an altitudinal gradient, with *C. lingulata* (biennial) and *C. spatulata* (perennial) "sharing" altitudes from ~300 to ~1,500 m, and *C. rotundifolia* occurring at higher altitudes - thus occupying less space - differentiate in terms of aggregation in space, across the spatial resolutions under question, for the two years of sampling. As expected, the intercept of the

slope for *C. lingulata*, differentiated from the intercept of *C. rotundifolia*, but not for *C. spatulata*. Significant differences were detected for *C. spatulata* slope, which indicated differences in aggregation patterns for the two species of lower altitudes. *C. spatulata* slope was shallower, indicating that the species was more aggregated than each model predicted (relative to *C. lingulata*) for coarser spatial resolutions. This difference was accentuated in 2013, were the number of *C. lingulata* individuals approximately doubled. Such differences might be attributed to the differences in species' life traits, or be indicative of a mechanism of "competitive exclusion" which influences the two species' occupancy dynamics.

III. *C. lingulata* distribution and the "abundant centre hypothesis"

C. lingulata abundance along an altitudinal gradient produces a two-peak pattern (one at 650 - 750 m, and one at 1,100 - 1,300 m) and displays an abrupt decline in density at the 1,300 - 1,500 m elevation class.

The absence of a smooth distribution limit might be related to an abrupt change of an environmental variable of the species multidimensional niche, as is the abrupt change in vegetation that is observed at higher elevations. In addition, the observed discontinuities in the specie's abundance patterns might be explained by variations in habitat suitability. In this study's case, altitude is perceived as a surrogate variable for climatic conditions. Assuming that it "summarizes" the effect of abiotic factors, it is regarded as an approximation of the species' multidimensional niche. Even though Brown (1984) assumes that the factors that define a species optimal niche are spatially autocorrelated, he described distributions of species that exhibit two or more peaks in abundance throughout space. This, according to his theory, should occur when suitable habitat is found in isolated patches (Brown, 1984). Therefore, the observed two-peak abundance pattern across the altitudinal gradient, might be the combined result of altitude with gradients of other environmental factors that cannot be approximated by the gradual change in elevation, yet contribute to the formulation of the observed abundance patterns.

Spatial variance, as a spatially implicit measure, has proven insufficient to describe patterns in the physical distribution of individuals across space (see Hurlbert, 1990; Hui *et al.*, 2006). Therefore, the observed abundance patterns along an environmental gradient should be interpreted in the context of the landscape's spatial features. As noted before, the highest abundance is recorded at 1,100 - 1,300 m. Much of the effort invested in these elevations is within the National Park limits (Routes 8 and 10), where human activities are regulated, as opposed to the total absence of individuals in Routes 3 and 4, where farming, grazing and

other human activities take place. In addition, factors such as light exposure, which is greater in open areas, as in roads, compared to densely forested areas, such as along the Routes 11 and 12, might correlate to the species presence or absence from certain elevation classes. Indeed, few to no individuals were recorded in densely forested, high elevation classes, where the upper distribution limit should occur.

Sampling and availability of data is also bound to introduce an error to the results. Sampling design is opportunistic. It reflects the availability of road networks that cover the extent of the study area, thus introduces an error attributed to roadside bias. The majority of individuals, as in many studies of similar nature, was recorded alongside the roads. In their studies of factors affecting the performance of predictive bioclimatic modeling, Kadman *et al*. (2004), however, concluded that roadside bias had much less impact compared to the bias introduced by climatic factors. Thus, one could speculate that altitude is a candidate factor that is bound to produce patterns of abundance that reflect the suitability of the species' habitat with a certain degree of confidence.

According to the ACH, higher probabilities of large abundances at the altitudinal "centre" of the species distribution would be expected. Once more, as in the majority of real situations, the study's findings support Sagarin & Gaynes's (2002) argument, that the intuitive notion of an "abundant centre" of a species distribution is rather difficult to be upheld when put under empirical scrutiny. The hypothesis of an "abundant centre" along an altitudinal gradient doesn't seem to hold since the pattern of abundance for both years of sampling doesn't seem to coincide - while the species seems most abundant at elevations that could be considered "centre" of the species altitudinal range for 2013, the observed pattern in 2012 is rather homogeneous. Therefore, in light of such results, we cannot assume that the prevailing ecological mechanism behind *Campanula lingulata* abundance distribution patterns on Mt. Olympus is its positioning relative to an altitudinal gradient and – to an extent — the species' optimal requirements for the given spatial resolution. The study's findings cannot confirm, yet do not contradict, the ACH.

III. From mechanism to model

As stated earlier, it has long been established that species distributions are often significantly associated with aspects of climate. However, disentangling direct and indirect effects and pinpointing climatic features of relevance to organisms still pose considerable challenges (Kearney & Porter, 2009; Sexton *et al.*, 2009).

Species distribution models attempt to provide detailed predictions of distributions by relating presence or abundance of species to environmental predictors. There is now a plethora of methods for modeling species' distributions that vary in how they model the distribution of the response, select relevant predictor variables, define fitted functions for each variable, weigh variable contributions and allow for interactions, in order to predict geographic patterns of occurrence (Guissan & Zimmerman, 2000; Burgman *et al*., 2005; Wintle & Bardos, 2006; Elith *et al*., 2014). Each of these methods' components is bound to introduce an error to the interpretation of each models' outcome, and each excess predictor variable is bound to compromise its performance and applicability.

So, before moving to predictions, how are we to tell with certainty which predictor variables and to what degree in which spatial scale, can explain the observed patterns? Where do we draw the line between cause and effect, and the unavoidable environmental noise? How do we deal with variations of predictor variables in time?

The persistence of the abundant centre concept in the literature and its ubiquity in ecological and evolutionary theories expresses deeply embedded ideas held by ecologists about how populations should be distributed and suggests that the pattern should be widespread in natural populations. However, every so often, we fail to detect it (see Sagarin & Gaynes, 2002). Given the statistical and geometric methods currently available to ecologists, few direct tests can validate this fundamental assumption. The question yet remains. How do we describe species distributions and abundances, if not in light of the mechanisms that generate them?

There is little doubt that multiple factors, operating across a hierarchy of spatial and temporal scales, shape species distributions (Levin, 1992). Theory and empirical evidence strongly suggest that positive occupancy-abundance relationships result from the action of several mechanisms, and that in different systems these vary in their relative importance (Holt *et al*., 2002). Yet, little is known about how the determinants of the distributions of a single species vary across spatial scales (Mackey & Lindenmayer, 2001; Pearson & Dawson, 2003; Guissan & Thuiller, 2005).

Indeed, this study's findings have highlighted the fact that different aspects of the species' distributions are evident in different spatial resolutions. At larger scales, *C. lingulata* and *C. spatulata* seem to occupy the same space (Fig. 27). If we consider, however, the observed abundance patterns within a smaller portion of the grid at finer resolutions (see Figs 28-30),

this doesn't seem to be the case. *C. lingulata* and *C. spatulata* may occur at the same altitudes but their populations form distinct, separate patches, within hundreds of meters from each other. On the other hand, slight variations in bioclimatic conditions, such as those recorded for the different routes of the study may produce different patterns of occupancy (abundance) for each species in response. This would be only evident at coarser resolutions. In addition, such aggregation patterns might only be detected when investigating a range of scales, and interactions between species.

Finally, information regarding population turnover may be misinterpreted if only the spatial dimension of a distribution is considered. Variations in temperature, precipitation, other factors - and combinations thereof - between the years of study, may have delayed or hastened the flowering season of the respective populations, thus producing an "artefactual" greater population turnover – which, in fact, is probably indicative of the species' dynamics in response to variations in bioclimatic conditions in time.

Macroecological patterns are increasingly seen as being best understood as the net outcome of several processes that pull in essentially the same direction (Gaston 2000, Gaston & Blackburn, 2000; Lawton, 2000; Holt *et al*., 2002). On that basis, *statistical* abundance - occupancy and spatial distribution models have been proposed, in order to address the implications of abundance-occupancy relationships and quantify the observed abundance-occupancy patterns. Such models generally consider species' abundance, occupancy and distribution aggregation parameters across one or several spatial scales. Most of those, however, fail to incorporate the effect of scale in their interpretation of the outcome in an ecological and evolutionary context.

The lack of empirical studies that support intuitive notions (such as the ACH) about the mechanisms that generate the observed patterns of abundance in species distributions calls for an alternative conceptual framework. Such a framework, within which we should revisit the way we perceive deeply rooted ecological and evolutionary concepts, should incorporate the effect of scale, since the effect of various factors operating at various spatial scales with varying intensity, is shaping species distributions. The generated "disturbance"—which cannot be readily attributed to a single factor operating at a single spatial scale and temporal dimension — is bound to superimpose the effect of environmental drivers that could provide us with evidence of underlying patterns, such as that of the intuitive "abundant centre" of species' abundance distributions. A fractal framework — wherein the factor "scale" is inherent — might constitute a more suitable approach.

Given multiple instances of a species' distributions, we might have been able to discern a distribution "centre", where the species meets its optimal conditions. However, such a hypothesis can't be readily put under scrutiny — as is the case in all macroecology studies. We cannot replicate the effect of the various factors that shape species distributions since they act in all spatial scales and have a temporal dimension. Thus, we cannot acquire a statistically valid sample. Nevertheless, in such studies, simulations have proven to be valuable tools (Kallimanis *et al*., 2002). We should be able to investigate such hypothesis, by building a spatially explicit fractal "disturbance" model where each position within the species' geographic range has probability of occurrence relative to its distance from one or multiple hypothetical centres.

In such model, a fractal disturbance is superimposed to and "abundant centre" distribution in space, where the probability of occurance is increased when one is moving from the edges toward the centre of the species distribution. Occupancy is a function of the population density or abundance, and is given by different occupancy-abundance models (Poisson, negative binomial, Nachman, etc.(Fig 52). However, such attempt lies beyond the objectives of this study.

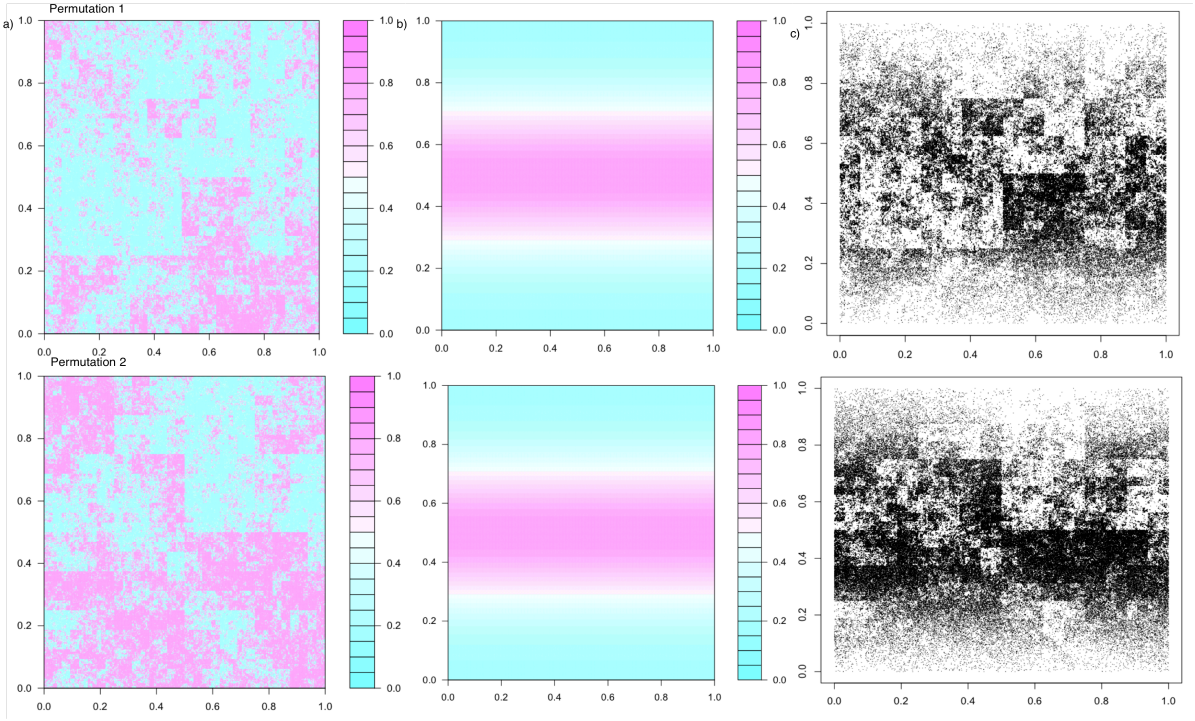


Figure 52: Random permutations of a fractal disturbance-"abundant centre" model in 11 spatial scales superimposed on a Poisson occupancy-abundance model. a) Probability of occurance without

an underlying “abundant centre distribution, b) Probabillity of occurance without a fractal disturbance, c) Probability of occurance of an “abundant centre” with fractal-like disturbance.

IV. Molecular Genetics

Landscape genetics aim to describe and interpret the patterns of genetic variation across the landscape, in terms of the biological processes that created them (Manel *et al.,* 2003; Latta, 2006). Thus, while there is little doubt that modern genetic methods have opened important new avenues of investigation into natural populations, we are still unable to directly read the ‘genetic signatures’ of processes we might wish to study (Whitlock & MacCauley, 1999; Slatkin, 2001; Latta, 2006), since the same spatial pattern can be generated by a number of different processes (Latta, 2006).

If genetic variation, for example, is found to be uniformly distributed across the landscape, we might assume that this pattern arises either because current migration is exchanging genes among sites or because barriers to migration are too recent to be detected. On the other hand, if strong regional differentiation is observed, this could reflect either restricted migration or the differential adaptation of genotypes to different habitats (Latta, 2006).

In recent years, there has been a growing interest in using the contrasts between different genetic marker types to infer process from pattern (e.g., Ennos *et al*., 1999; Black *et al*., 2001; Vitalis *et al*., 2001; Merila & Crnokrak, 2001; McKay & Latta, 2002; Storz, 2005;Latta, 2006). By contrasting the uniparentally and biparentally inherited markers (nuclear genome vs. organelles, such as chloroplast or mitochondrial DNA, for example), we might infer the relative dispersal abilities and genetic spatial structure of female vs. male individuals of populations, and distinguish patterns such as pollen-mediated gene flow in plant taxa.

Migration and drift affect all loci within the nuclear genome, equally. Nevertheless, mutation rates among loci, and selection, is likely to be highly specific to particular loci and traits, as well as to particular environments (Latta, 2006).

Selection affects genetic variation by increasing the frequency of the favored allele(s) or genotype(s) (i.e., those that increase the survival and reproduction of the individuals that carry them). Thus, it affects spatial patterns in cases where different alleles or genotypes are favored in different locations. Furthermore, selection is generally expected to act on expressed loci while non-coding loci are expected to most reliably reflect migration-drift

equilibrium (Holderegger, 2006; Latta, 2006). Non-coding neutral loci might, of course, be linked to expressed loci, which are targets to selection (Latta, 2006).

Mutation introduces new variation into populations, and thus, by increasing the amount of variation within populations, decreases the proportion of the total variation that occurs between populations (Hedrick 1999; Latta, 2006). In order to incorporate mutations in their inference models, have focused on contrasts between microsatellite (which exhibit high, vs. allozyme data which exhibit very low mutation rates).

RAPD markers are random markers, which bind onto 10-mer sequences (sequences of 10 DNA bases) of a species nuclear DNA. They are more likely to bind to non-coding DNA thus reflecting the migration-drift equilibrium rather than diversifying selection in genetic variation patterns they produce. However, studies, such as that of Semagne *et al*., (2000), suggest that part of the RAPD polymorphism could be adaptive and responsive to environmental selection.

The results of this study were based on few individual samples, thus, they should be interpreted with caution. Nevertheless, the relatively large number of polymorphic loci suggests that the selection of 10-mer primers was relevant. High within-population differentiation might be indicative of a genetic sub-structure, both across space and along an elevation gradient that the PcoA/UPGMA analysis could not “pick up”, due to the small number of individuals per location/altitude. Furthermore, loci-specific nuclear markers might provide insights on whether there are selection/adaptation forces at work for populations located at high altitudes, such as those at the Zolotas refuge – which appear to differentiate from the other Olympus individuals. In addition, comparison of microsatellite markers of Falakro and Olympus individuals might provide some insights for mutation rates and the low between-population diversity that was observed. In any case, the development of species-specific molecular markers is the next step in describing the observed patterns of genetic variation, and the processes that generated them.

V. Further research

The main focus of this study has been the link between aspects of spatial distribution of species that could provide insight to their generating mechanisms. A review of various empirical approaches and statistical models as of yet, along with the interpretation of

observed abundance patterns in an ecological/evolutionary context has proven that the current framework within which most of them have been developed is insufficient, when it comes to associating cause and effect (linking observed occupancy-abundance patterns with generating processes).

The development of a null model that assumes that environmental stochasticity (noise) is best approached by a fractal distribution might help link the various species' distributions and underlying patterns of variation to their generating mechanisms. Such model should effectively remove the variance that is associated with each explanatory parameter, when perceived operating at different spatial scales, from the observed occupancy-abundance patterns.

This research has highlighted various aspects of the species population traits in response to their environment (biotic and abiotic), in an ecological and evolutionary context. Nevertheless, as expected from studies of this caliber, further question have arisen. Such questions - should we approach them - should increase our knowledge of the species' response to abiotic and biotic factors that are bound to shape their distribution in space and along environmental gradients, clarify the species' population and metapopulation dynamics, provide insights of the species' genetic variation patterns and associated processes, and finally, help predict the species' response to a rapidly changing landscape.

The correlative study of inter-specific abundance-occupancy dynamics of species of closely related taxa, which co-occur along and an elevation gradient in relatively small spatial scales, such as *C. lingulata* and *C. spatulata*, in similar, montane systems (and differentiate in a parameter in question), may provide insights about the variation in the observed occupancy-abundance patterns in space, as well as evidence of the relative importance of associated generated mechanisms (see variation in response to variable climate conditions, competitive exclusion mechanisms, etc.) at different spatial scales and in time.

The study of ecologically similar species that are bound to differentiate in only few dimensions of their multidimensional ecological niche may provide us with insights on the species' response to their abiotic environment in an evolutionary context as well. Adaptation and/or speciation processes and associated genetic population profiles, in response to a gradual or abrupt change in one such dimension, may help clarify the relative importance of environmental factors in shaping species' distributions in space and time. Phylogenetic studies of such species may help quantify the genetic distance associated with selection/adaptation mechanisms relative to changes in environmental gradients/dimensions

of species' niches. Furthermore, a comparative study of molecular data of such species, with the use of markers that differentiate in terms of hereditability, on sensitivity in "picking up" differences which are due to processes such as selection, mutation, dispersal or genetic drift might help clarify the genetic substructure within populations, as a result of differential dispersal mechanisms, selection regimes, migration processes, source-sink dynamics, or current/past barriers in gene flow.

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F. APPENDICES

1. Appendix I – Extraction of bioclimatic variables from worldclim.org

```
##This script was downloaded from https://gist.github.com/kgturner/6643334
#Load required libraries.
library(rgdal)
library(raster)
library(foreach)



#Read names of all files in directory into a list
#from http://stackoverflow.com/questions/5319839/read-multiple-csv-files-into-separate-data-frames
filenames <- list.files(path="~/Desktop/RClim")

#Load all geoTIFF files
for(i in filenames){
filepath <- file.path("~/Desktop/RClim",i)
assign(i, raster(filepath))
}
#check that all files loaded properly by raster
#from http://stackoverflow.com/questions/15387727/use-object-names-as-list-names-in-r
list <- mget(filenames, envir=globalenv())

for(i in list){
if (hasValues(i)==FALSE){
print(i,"hasValues error")
}
if (inMemory(i)==TRUE){
print(i, "inMemory error")
}
else{
print("All checked out!")
}
}
sam<-read.csv("RouteVeg.csv",header=TRUE,sep=" ")
length(sam[,1])


sam2<-as.factor(seq(1:length(sam$longitude)))
samf<-as.data.frame(cbind(sam$longitude,sam$latitude,sam2))
row.names(samf)<-samf$sam2
samf$sam2<-as.factor(samf$sam2)
#load location coordinates as SpatialPoints
for(i in samf$sam2){
assign(i,SpatialPoints(as.matrix(t(c(samf[i,1],samf[i,2])))))
}

#check that SpatialPoints load correctly from geoTIFFs
```

```
poplist <- mget(levels(samf$sam2), envir=globalenv())

tiffvector <- unlist(list)

#make climate data table
climate <- foreach(p=poplist, .combine='rbind') %:%
foreach(t=tiffvector, .combine='cbind') %do%{
myValue<-extract(t, p)
}

#tidy table
popnames <- sort(as.character(samf$sam2))
clim <- as.data.frame(climate, row.names=popnames)
colnames(clim) <- filenames
write.table(clim,"meanP.csv")
```

2. Appendix II – Mapping the species on the surveyed surface at different spatial scales

```
##Import Datasets with observations

CL12<-read.csv("CL2012.csv",header=TRUE,sep=",");head(CL12)  # C. lingulata  observations from 2012
CL13<-read.csv("CL2013.csv",header=TRUE,sep=",");head(CL13)  # C. lingulata observations from 2013
CS12<-read.csv("Cs2012.csv",header=TRUE,sep=",");head(CS12)  # C. spatulata observations from 2012
CS13<-read.csv("Cs2013.csv",header=TRUE,sep=",");head(CS13)  # C. spatulata observations from 2013
CR12<-read.csv("CR2012.csv",header=TRUE,sep=",");head(CR12)  # C.rotundifolia observation from 2012
CR13<-read.csv("CR2013.csv",header=TRUE,sep=",");head(CR13)  # C.rotundifolia observation from 2012


Total<-as.data.frame(rbind(CL12,CL13,CS12,CS13,CR12,CR13))   #Combined Dataset
names(Total)
attach(Total);length(No.)

##Define the SW corner of the surveyed surface

SpointE<-22.197278   # Starting point West

SpointN<-39.972888  # Starting point South.

## Define resolution (spatial scale) of the Grid

Level<- _   # Import level from 1 (finer) to 11 (coarser)

dir.create(paste(Level))  ##Create working directory
path<-paste(getwd(),Level,sep="/")  # Path of working directory



lcount<-c(2800,1400,700,350,175,88,44,22,11,6,3)

# lcount values correspond to a division of the total surface in Lcount x Lcount (10X10, 20X20, 40X40, 80X80 ##,160X160, 320X320, 640X640,
1280X1280, 2560X2560, ###5120X5120, 10240X10240 m) squares, which ###constitute each subsequently coarser (Level 11) resolution

incE<-c(0.00012,0.00024,0.00048,0.00096,0.00192,0.00384,0.00768,0.01536,0.03072,0.06144,0.12288)
```

```
# Coordinate units increment that corresponds to 10,20,40,80,160,320,640,1280,2560,5120,10240 m when ##moving from West to East.

incN<-c(0.00009,0.00018,0.00036,0.00072,0.00144,0.00288,0.00576,0.01152,0.02304,0.04608,0.09216)

# Coordinate units increment that correspond to 10,20,40,80,160,320,640,1280,2560,5120,10240 m when moving from South to North.


Lcount<-lcount[Level]
# No. of columns for a Location Matrices, This variable takes its values from lcount (Grid's dimensions n).



IncE<-incE[Level]            #Takes values from incE
IncN<-incN[Level]            #Takes values from incN

##Define the coordinate columns of the dataset
CoorE<-as.numeric(coordinates.E)
CoorN<-as.numeric(coordinates.N)

nrows<-length(No.)    #Length of the dataset, equal to the number of observations


#Fix The Criteria, meaning the coordinate values that correspond to equally spaced margins along each ##direction.Crit_1 corresponds to W->E direction, Crit_3 corresponds to the S->N direction.

Crit_1<-numeric(Lcount)
for(i in 1:Lcount)
            {
             Crit_1[1]<-SpointE
             Crit_1[i+1]<-Crit_1[i]+IncE
            }

Crit_3<-numeric(Lcount)
for(i in 1:Lcount)
            {
             Crit_3[1]<-SpointN
             Crit_3[i+1]<-Crit_3[i]+IncN
            }


## Create the first Location Matrix. A matrix where each observation placed on WE axis, denoted by 1 (presence) ### or 0 (absence). nrows corresponds to the number of observed individuals ,while Lcount to the number of ####margins within the WE axis.

MatrixWE<-matrix(0,nrows,Lcount)

# Create a Vector that holds the cell no. of the WE matrix were each observation is held. Its length would be equal to ##the number of observations

LocatorWE<-numeric(nrows)

#Assign each observation within a defined margin on W-E axis. If an observation is located on a boundary, then its considered within the eastmost grid square.

CounterA<-matrix(1:Lcount,1,Lcount)
            for(k in 1:nrows)
                        {
```

```
                for(i in 1:Lcount)

                        {
                            if (coordinates.E[k]<=Crit_1[i+1] & coordinates.E[k]>Crit_1[i])
                            {

                            MatrixWE[k,i]=1
                            LocatorWE[k]<-CounterA[i]


                            }else{

                            MatrixWE[k,i]=0
                               }

                        }
                }
```

#Create a second Location matrix where each observation placed on is placed on SN axis.

```
MatrixSN<-matrix(0,nrows,Lcount)
```

#Vector that holds the cell no. of the SN matrix were each observation is held. Both these values, denote the ##positioning of each observation on the WE and SN axes. In combination they denote the positioning of an ###observation on space/ cell value of a matrix.

```
LocatorSN<-numeric(nrows)
```

#Assign each observation within a defined margin on S-N axis. If an observation is located on a boundary, then its considered within the southmost grid square.

```
        for(k in 1:nrows)
                {
                for(i in 1:Lcount)

                        {
                            if (coordinates.N[k]<=Crit_3[i+1] & coordinates.N[k]>Crit_3[i])
                             {

                            MatrixSN[k,i]=1
                            LocatorSN[k]<-CounterA[i]
                             }else{

                            MatrixSN[k,i]=0
                                }

                        }
                }
#Save presence in WE and SN matrices to specified file
########################   Optional

WEpresence<-write.table(MatrixWE,file=paste(path,"WEpresence.csv",sep="/"),sep=",")
NSpresence<-write.table(MatrixSN,file=paste(path,"NSpresence.csv",sep="/"),sep=",")
```

```
#########################

#Create single digit ID, which is a combination of the SN and WE Locators.


SN<-as.matrix(LocatorSN)
WE<-as.matrix(LocatorWE)
write.table(SN,file=paste(path,"SN.csv",sep="/"),sep=",")
write.table(WE,file=paste(path,"WE.csv",sep="/"),sep=",")
No.Obs<-length(WE)
ID<-numeric(No.Obs)

for(i in 1:No.Obs)
  {
            ID[i]<-Lcount*(SN[i]-1)+WE[i]    # Algorithm used to create a matrix of increasing order (1,2,3,4,5,…etc.)
  }

write.table(ID,file=paste(path,"ID.csv",sep="/"),sep=",")      #Save ID as a .csv file
TotalLoc<-as.data.frame(cbind(Total,ID,LocatorSN,LocatorWE));names(TotalLoc)    #Combine the dataframe of ##observations with both
locators and ID
#Plot the each species and year.

CL12P<-subset(TotalLoc,TotalLoc[,5]=="CL" & TotalLoc[,6]==2012);length(CL12P[,1])
CL13P<-subset(TotalLoc,TotalLoc[,5]=="CL" & TotalLoc[,6]==2013);length(CL13P[,1])
CS12P<-subset(TotalLoc,TotalLoc[,5]=="CS" & TotalLoc[,6]==2012);length(CS12P[,1])
CS13P<-subset(TotalLoc,TotalLoc[,5]=="CS" & TotalLoc[,6]==2013);length(CS13P[,1])
CR12P<-subset(TotalLoc,TotalLoc[,5]=="CR" & TotalLoc[,6]==2012);length(CR12P[,1])
CR13P<-subset(TotalLoc,TotalLoc[,5]=="CR" & TotalLoc[,6]==2013);length(CR13P[,1])

par(mfrow=c(2,2))
plot(CS12P$LocatorWE+0.5,CS12P$LocatorSN+0.5,add=TRUE,xlim=c(1,Lcount+1),ylim=c(1,Lcount+1),pch=16, cex=1.5,col="red",
main="C.spatulata",xlab="",ylab="")
points(CS13P$LocatorWE+0.5,CS13P$LocatorSN+0.5,cex=1.5,col="green")

plot(CL12P$LocatorWE+0.5,CL12P$LocatorSN+0.5,xlim=c(1,Lcount+1),ylim=c(1,Lcount+1),pch=16,cex=1.5,col="red",main="C.lingulata",xlab
="",ylab="")
points(CL13P$LocatorWE+0.5,CL13P$LocatorSN+0.5,cex=1.5,col="green")

plot(CR12P$LocatorWE+0.5,CR12P$LocatorSN+0.5,xlim=c(1,Lcount+1),ylim=c(1,Lcount+1),pch=16,cex=1.5,col="red",main="C.rotudifolia",xl
ab="",ylab="")
points(CR13P$LocatorWE+0.5,CR13P$LocatorSN+0.5,cex=1.5,col="green")
```

3. Appendix III – Abundance and Occupancy

```
#Visualization of abundance in space
## Plot abundance of individuals in selected resolution

Level<-2
lcount<-c(2800,1400,700,350,175,88,44,22,11,6,3)
Lcount<-lcount[Level]

## Create working directory
path<-getwd()
```

```
nwd<-paste(path,Level,sep='/')
setwd(nwd)

##Import Locators (WE and SN) for Abundance data 2013 and 2012  (species C. Lingulata)

LWE13<-as.data.frame(read.csv("LocatorWE_S3.csv",sep=","));head(LWE13)
LSN13<-as.data.frame(read.csv("LocatorSN_S3.csv",sep=","));head(LSN13)
LWE12<-as.data.frame(read.csv("LocatorWE_S2.csv",sep=","));head(LWE12)
LSN12<-as.data.frame(read.csv("LocatorSN_S2.csv",sep=","));head(LSN12)

# Length of Locator Variables, equal to the number of observations of C.lingulata #individuals

no.obs13<-length(LWE13[,1]);no.obs13
no.obs12<-length(LWE12[,1]);no.obs12


##Import route Margins  (locations on the matrices thatinclude all placemarks of a route.

RMarg<-as.data.frame(read.csv("RouteMarg.csv"),sep=",");RMarg


## Create matrices for both years, with dimensions equal to Lcount.

MatrixA12<-matrix(0,Lcount,Lcount)
MatrixA13<-matrix(0,Lcount,Lcount)

## Sums up the abundance for 2013
for (t in 1:no.obs13)
{
        i<-LSN13[t,1]
        j<-LWE13[t,1]
        MatrixA13[i,j]<-MatrixA13[i,j]+1
        }

# Sums up the abundance for 2012
for (t in 1:no.obs12)
{
        i<-LSN12[t,1]
        j<-LWE12[t,1]
        MatrixA12[i,j]<-MatrixA12[i,j]+1
        }

##plot

require(plot3D)   #load plot3D package

AbM13_1<-MatrixA13[RMarg[1,4]:RMarg[1,3],RMarg[1,2]:RMarg[1,1]]
jpeg(file=paste(getwd(),'ABM13_1.jpg',sep="/"))
hist3D(z=AbM13_1,main="Abundance Route 1 (2013)")
dev.off()
AbM12_1<-MatrixA12[RMarg[1,4]:RMarg[1,3],RMarg[1,2]:RMarg[1,1]]
jpeg(file=paste(getwd(),'ABM12_1.jpg',sep="/"))
hist3D(z=AbM12_1,main="Abundance Route 1 (2012)")
dev.off()
AbM13_8<-MatrixA13[RMarg[5,4]:RMarg[5,3],RMarg[5,2]:RMarg[5,1]]
jpeg(file=paste(getwd(),'ABM13_8.jpg',sep="/"))
```

```
hist3D(z=AbM13_8,main="Abundance Route 8 (2013)")
dev.off()
AbM12_8<-MatrixA12[RMarg[5,4]:RMarg[5,3],RMarg[5,2]:RMarg[5,1]]
jpeg(file=paste(getwd(),'ABM12_8.jpg',sep="/"))
hist3D(z=AbM12_8,main="Abundance Route 8 (2012)")
dev.off()
```

Descriptive statistics for Abundance and occupancy for all observations in both years of sampling this script is run 11 times, one each for each spatial scale.

#Import Datasets with observations

```
CL12<-read.csv("CL2012.csv",header=TRUE,sep=",");head(CL12)
CL13<-read.csv("CL2013.csv",header=TRUE,sep=",");head(CL13)
CS12<-read.csv("Cs2012.csv",header=TRUE,sep=",");head(CS12)
CS13<-read.csv("Cs2013.csv",header=TRUE,sep=",");head(CS13)
CR12<-read.csv("CR2012.csv",header=TRUE,sep=",");head(CR12)
CR13<-read.csv("CR2013.csv",header=TRUE,sep=",");head(CR13)
ID<-read.csv("ID.csv",header=TRUE,sep=",");head(ID)

Total<-as.data.frame(rbind(CL12,CL13,CS12,CS13,CR12,CR13))  # Combine dataset of observations with ID as in Appendix II
TotalID<-cbind(Total,ID)
names(TotalID)
```

#Create variables of observations based on the species and year of sampling, contains data from the spatial scale in question

```
CL12P<-subset(TotalID,TotalID[,5]=="CL" & TotalID[,6]==2012);length(CL12P[,1])
CL13P<-subset(TotalID,TotalID[,5]=="CL" & TotalID[,6]==2013);length(CL13P[,1])
CS12P<-subset(TotalID,TotalID[,5]=="CS" & TotalID[,6]==2012);length(CS12P[,1])
CS13P<-subset(TotalID,TotalID[,5]=="CS" & TotalID[,6]==2013);length(CS13P[,1])
CR12P<-subset(TotalID,TotalID[,5]=="CR" & TotalID[,6]==2012);length(CR12P[,1])
CR13P<-subset(TotalID,TotalID[,5]=="CR" & TotalID[,6]==2013);length(CR13P[,1])
```

##Calculate abundance from each subset of observations, by counting the frequency of the unique no. ID

```
AbCL12<-as.matrix(table(CL12P[,8]));AbCL12
AbCL13<-as.matrix(table(CL13P[,8]));AbCL13
AbCS12<-as.matrix(table(CS12P[,8]));AbCS12
AbCS13<-as.matrix(table(CS13P[,8]));AbCS13
AbCR12<-as.matrix(table(CR12P[,8]));AbCR12
AbCR13<-as.matrix(table(CR13P[,8]));AbCR13
```

Calculate occupancy as the length of the Abundance variables. The no. of unique ID cells that contain observations.

```
Oc.AbCL12<-length(AbCL12)
Oc.AbCL13<-length(AbCL13)
Oc.AbCS12<-length(AbCS12)
Oc.AbCS13<-length(AbCS13)
Oc.AbCR12<-length(AbCR12)
Oc.AbCR13<-length(AbCR13)
```

#Calculate Mean Abundance and Occupancy

```
OCUPANCY1<-rbind(Oc.AbCL12,Oc.AbCL13,Oc.AbCS12,Oc.AbCS13,Oc.AbCR12,Oc.AbCR13)
MEAN.ABUNDANCE1<-rbind(mean(AbCL12),mean(AbCL13),mean(AbCS12),mean(AbCS13),mean(AbCR12),mean(AbCR13))
SD1<-rbind(sd(AbCL12),sd(AbCL13),sd(AbCS12),sd(AbCS13),sd(AbCR12),sd(AbCR13))
```

##Save data frame for observations regarding the spatial scale in question (in this case sp. Scale 1 10 x10 m)

```
sp1<-as.data.frame(cbind(OCUPANCY1,MEAN.ABUNDANCE1,SD1,MEAN.ABUNDANCE_SD1,rep(1,6)))
names(sp1)<-c("Occupancy","Mean.Abundance","Standard.Deviation","Spatial.Scale")
write.csv(sp1,"Abundance_occupancy.csv")
```

Plotting and Linear Modelling
 # Import data containing Mean Abundance and occupancy estimates for each spatial scale

```
a1<-read.csv("Abundance_occupancy.csv",header=TRUE,sep=",");a1
a2<-read.csv("Abundance_occupancy2.csv",header=TRUE,sep=",");a2
a3<-read.csv("Abundance_occupancy3.csv",header=TRUE,sep=",");a3
a4<-read.csv("Abundance_occupancy4.csv",header=TRUE,sep=",");a4
a5<-read.csv("Abundance_occupancy5.csv",header=TRUE,sep=",");a5
a6<-read.csv("Abundance_occupancy6.csv",header=TRUE,sep=",");a6
a7<-read.csv("Abundance_occupancy7.csv",header=TRUE,sep=",");a7
a8<-read.csv("Abundance_occupancy8.csv",header=TRUE,sep=",");a8
a9<-read.csv("Abundance_occupancy9.csv",header=TRUE,sep=",");a9
a10<-read.csv("Abundance_occupancy10.csv",header=TRUE,sep=",");a10
a11<-read.csv("Abundance_occupancy11.csv",header=TRUE,sep=",");a11
```

#Combine dataset

```
Species<-c("CL","CL","CS","CS","CR","CR")
Year<-c("2012","2013","2012","2013","2012","2013")
total<-rbind(a1,a2,a3,a4,a5,a6,a7,a8,a9,a10,a11)
dataset<-as.data.frame(cbind(total,Species,Year))
```

```
attach(dataset)
names(total)
```

Plot Occupancy as a function of Abundance, log(Occupancy) as a function of log(Mean Abundance), for every species in both years of sampling

```
plot(Occupancy~Mean.Abundance,pch=as.integer(dataset$Year),col=as.integer(dataset$Species),xlab="Mean
Abundance",ylab="Occupancy")
legend("topright",legend=c("C.lingulata 2012","C.lingulata 2013","C.rotundifolia 2012","C.rotundifolia 2013","C.spatulata
2012","C.spatulata 2013"),pch=dataset$Year,col=dataset$Species,bty="n",cex=0.5)
with(dataset[,2:7], text(Occupancy~Mean.Abundance, labels = dataset$Spatial.Scale, pos = 2,cex=0.5))
plot(log(Occupancy)~log(Mean.Abundance),pch=as.integer(dataset$Year),col=as.integer(dataset$Species),xlab="Mean Abundance
(log)",ylab="Occupancy (log)")
legend("topright",legend=c("C.lingulata 2012","C.lingulata 2013","C.rotundifolia 2012","C.rotundifolia 2013","C.spatulata
2012","C.spatulata 2013"),pch=dataset$Year,col=dataset$Species,bty="n",cex=0.5)
with(dataset[,2:7], text(log(Occupancy)~log(Mean.Abundance), labels = dataset$Spatial.Scale, pos = 2,cex=0.5))
```

 # Create the variable that holds the relative quadrat surface (grain size)

```
box.size<-c(rep(1.14796E-06
,6),rep(4.59184E-06,6),rep(1.83673E-05,6),rep(7.34694E-05,6),rep(0.000293878
,6),rep(0.00116219,6),rep(0.00464876,6),rep(0.018595041,6),rep(0.074380165,6),rep(0.25,6),rep(1,6))
```

 # Calculate Mean Abundance as a function of relative quadrat surface

```
Mean.A_Box<-Mean.Abundance/box.size
```

```
#Plot log(Occupancy) as a function of  log(Mean Density)

plot(log(Occupancy)~log(Mean.A_Box),pch=as.integer(dataset$Year),col=as.integer(dataset$Species),xlab="Mean
Density(log)",ylab="Occupancy (log)")
legend("topright",legend=c("C.lingulata 2012","C.lingulata 2013","C.rotundifolia 2012","C.rotundifolia 2013","C.spatulata
2012","C.spatulata 2013"),pch=dataset$Year,col=dataset$Species,bty="n",cex=0.5)
with(dataset[,2:7], text(log(Occupancy)~log(Mean.A_Box), labels = dataset$Spatial.Scale, pos = 2,cex=0.5))
## Linear Model of Occupancy as a function of Mean Density

ModN<-lm(log(Occupancy)~log(Mean.A_Box))

summary(ModN)

## Plot fitted, residual values and Residual diagnostics graphs

 par(mfrow=c(3,2))

plot(fitted(ModN),pch=as.integer(dataset$Year),col=as.integer(dataset$Species))
legend("topright",legend=c("C.lingulata 2012","C.lingulata 2013","C.rotundifolia 2012","C.rotundifolia 2013","C.spatulata
2012","C.spatulata 2013"),pch=dataset$Year,col=dataset$Species,bty="n",cex=0.5)
plot(residuals(ModN)^2,pch=as.integer(dataset$Year),col=as.integer(dataset$Species))
plot(ModN,pch=as.integer(dataset$Year),col=as.integer(dataset$Species))

## Linear Model of Occupancy as a function of Mean Density and species

ModNS<-lm(log(Occupancy)~log(Mean.A_Box)+dataset$Species)

summary(ModNS)

## Plot fitted, residual values and Residual diagnostics graphs

par(mfrow=c(3,2))

plot(fitted(ModNS),pch=as.integer(dataset$Year),col=as.integer(dataset$Species))
legend("topright",legend=c("C.lingulata 2012","C.lingulata 2013","C.rotundifolia 2012","C.rotundifolia 2013","C.spatulata
2012","C.spatulata 2013"),pch=dataset$Year,col=dataset$Species,bty="n",cex=0.5)
plot(residuals(ModNS)^2,,pch=as.integer(dataset$Year),col=as.integer(dataset$Species))
plot(ModNS,pch=as.integer(dataset$Year),col=as.integer(dataset$Species))

## Linear Model of Occupancy as a function of Mean Density, species and year of sampling

ModNSY<-lm(log(Occupancy)~log(Mean.A_Box)+dataset$Species+dataset$Year)

summary(ModNSY)

## Plot fitted, residual values and Residual diagnostics graphs

par(mfrow=c(3,2))

plot(fitted(ModNSY),pch=as.integer(dataset$Year),col=as.integer(dataset$Species))
legend("topright",legend=c("C.lingulata 2012","C.lingulata 2013","C.rotundifolia 2012","C.rotundifolia 2013","C.spatulata
2012","C.spatulata 2013"),pch=dataset$Year,col=dataset$Species,bty="n",cex=0.5)
plot(residuals(ModNSY)^2,pch=as.integer(dataset$Year),col=as.integer(dataset$Species))
plot(ModNSY,pch=as.integer(dataset$Year),col=as.integer(dataset$Species))
```

Linear Model of Occupancy as a function of Mean Density, species, year of sampling and their interactions

```
ModNSYa<-lm(log(Occupancy)~log(Mean.A_Box)+dataset$Species*dataset$Year)

summary(ModNSYa)
```

Plot fitted, residual values and Residual diagnostics graphs

```
par(mfrow=c(3,2))

plot(fitted(ModNSYa),pch=as.integer(dataset$Year),col=as.integer(dataset$Species))
legend("topright",legend=c("C.lingulata 2012","C.lingulata 2013","C.rotundifolia 2012","C.rotundifolia 2013","C.spatulata
2012","C.spatulata 2013"),pch=dataset$Year,col=dataset$Species,bty="n",cex=0.5)
plot(residuals(ModNSYa)^2,pch=as.integer(dataset$Year),col=as.integer(dataset$Species))
plot(ModNSYa,pch=as.integer(dataset$Year),col=as.integer(dataset$Species))
```

4. Appendix IV – Mean presence and Mean population turnover

#For Mean presence at each spatial scale

#Input data in for C. lingulata species (2012) in csv form.

```
data<-read.csv("Cl12.csv",header=TRUE,sep=";")
names(data)
dim(data)
```

 # Define the coordinates of the species within the dataset

```
CoorE<-data[,2]
CoorN<-data[,3]
```

 # Create objects - variables

```
nrows<-length(CoorE)  #No of rows for Location Matrix (see above),equal to the number of observations

SpointE<-22.197278
SpointN<-39.972888 # SW starting point of the grid
```

 # No. of columns for Location Matrix,This variable takes its values from lcount as above. It defines the spatial resolution

```
Lcount<-3

IncE<-0.12288  #Takes values from incE (as above)

IncN<-0.09216 # Takes values from incN (as above)
```

#Create vectors-Criteria

```
#Fixed points vector, where each value is IncE units larger than the previous. The total number of
##values should be equal to Lcount,starting point equal to SpointE.This vector should hold  fixed points in a trajectory
### from West(starting point of overall surface), to East.
```

```
Crit_1<-numeric(Lcount)
for(i in 1:Lcount)
                {
                 Crit_1[1]<-SpointE
                 Crit_1[i+1]<-Crit_1[i]+IncE
                }
#Midpoints vector.  It will be used as criterion which should help define where each observation on a square is.it should be a
## vector of length Lcount where the first value is equal to (Crit_1(1)+Crit_1(2))/2, and the rest are an addition of IncE units.

Crit_2<-numeric(Lcount)
for(i in 1:Lcount)
               {
                Crit_2[1]<-(Crit_1[1]+Crit_1[2])/2
                Crit_2[i+1]<-Crit_2[i]+IncE
               }

crit_2<-Crit_2[-(length(Crit_2))]

   #Fixed points vector, where each value is IncN units larger than the previous the total number of values should be equal to ##Lcount,
  starting point equal to SpointN. This vector should hold fixed points in a trajectory
### from South(starting point of overall surface), to North.


Crit_3<-numeric(Lcount)
for(i in 1:Lcount)
                {
                 Crit_3[1]<-SpointN
                 Crit_3[i+1]<-Crit_3[i]+IncN
                }

#Midpoints vector.Will be used as criterion which should help define where each observation on a square is.it should be a
## vector of length Lcount where the first value is equal to (Crit_3(1)+Crit_3(2))/2, and the rest are an addition of IncN units

Crit_4<-numeric(Lcount)
for(i in 1:Lcount)
               {
                Crit_4[1]<-(Crit_3[1]+Crit_3[2])/2
                Crit_4[i+1]<-Crit_4[i]+IncN
               }
crit_4<-Crit_4[-(length(Crit_4))]

#Create variables that will hold the observations' position within an axis of a matrix relative to the midpoint vector criterion, for WE matrix

Loc1<-matrix(0,nrows,Lcount)  # Holds observations which are at the left side of each square, at each column
Loc2<-matrix(0,nrows,Lcount)  # Holds observations which are at the right side of each square at each column
Loc3<-matrix(0,nrows,Lcount)  #combines Loc1 and Loc2 vectors into a single matrix.


# Create a 2D matrix of nrows X Lcount (MatrixWE) where each observation is located on bracket (of a line defined by the fixed points in each
  trajectory), and a vector ,that should hold the value of the grid bracket that the observation is placed within as above.

MatrixWE<-matrix(0,nrows,Lcount)


for(k in 1:nrows)
  {
```

```
  for(i in 1:Lcount)
    {
     if(CoorE[k]<=Crit_1[i+1] & CoorE[k]>Crit_1[i] & CoorE[k]>crit_2[i] )  # Runs the loop described above, with the midpoints
  ##added as a criterion
       {
       Loc1[k,i]<-2
       }else{
            Loc1[k,i]=0
           }

    }
  }


for(k in 1:nrows)
  {
  for(i in 1:Lcount)
    {
     if(CoorE[k]<=Crit_1[i+1] & CoorE[k]>Crit_1[i] & CoorE[k]<crit_2[i] )
       {
       Loc2[k,i]<-1
       }else{
            Loc2[k,i]=0
           }

    }
  }

for(k in 1:nrows)
  {
  for(i in 1:Lcount)
    {
    Loc3[k,i]<-Loc1[k,i]+Loc2[k,i]
    }
  }

LocWE<-rowSums(Loc3) # create a single vector out of a matrix. Each observation is located within the left or right side of the square

Loc4<-matrix(0,nrows,Lcount) # Holds observations which are at the upper side of each square, at each row
Loc5<-matrix(0,nrows,Lcount) # Holds observations which are at the lower side of each square at each row
LocNS<-rowSums(Loc6)  #combines Loc4 and Loc5 vectors into a single matrix.



for(k in 1:nrows)
  {
  for(i in 1:Lcount)
    {
     if(CoorN[k]<=Crit_3[i+1] & CoorN[k]>Crit_3[i] & CoorN[k]>crit_4[i] )
       {
       Loc4[k,i]<-1
       }else{
            Loc4[k,i]=0
           }

    }
```

```
  }

for(k in 1:nrows)
  {
  for(i in 1:Lcount)
    {
     if(CoorN[k]<=Crit_3[i+1] & CoorN[k]>Crit_3[i] & CoorN[k]<crit_4[i] )
      {
      Loc5[k,i]<-2
      }else{
           Loc5[k,i]=0
          }

    }
  }

Loc6<-matrix(0,nrows,Lcount)
for(k in 1:nrows)
  {
  for(i in 1:Lcount)
    {
    Loc6[k,i]<-Loc4[k,i]+Loc5[k,i]
    }
  }

LocGrid<-read.csv("IDUp",header=TRUE,sep=",")  # Import single digit ID locator for each observation (as above)
LocG<-as.factor(LocGrid$x)   ## Define the variable a factor, with ID values as factor levels



LocOV<-numeric(nrows)  # single variable that will hold the positioning of each observation with a single notation. a is upper left, b is upper
  right, c is lower left, d  is lower right

a<-(LocWE==1) & (LocNS==1)  # Define the positions based on the combined locators (LocWE,LocNS) vectors
b<-(LocWE==1) & (LocNS==2)
c<-(LocWE==2) & (LocNS==1)
d<-(LocWE==2) & (LocNS==2)


for(k in 1:nrows)
  {
          if(a[k])
            {
            LocOV[k]<-"a"
            }
            if(b[k])
              {
              LocOV[k]<-"b"
              }
              if(c[k])
                {
                LocOV[k]<-"c"
                }
                if(d[k])
                  {
                  LocOV[k]<-"d"
```

```
            }
  }


#Calculate mean.

Cat<-cbind(LocOV,LocGrid)
MT<-as.matrix(table(Cat))  # Indicates the positioning of observations within each square
dim(MT)
dim<-as.vector(dim(MT))
nr<-dim[1]
nc<-dim[2]

MT1<-matrix(0,nr,nc) # if an observation is located within one of the four sub-squares 1, if absent 0 for each grid cell
for(i in 1:nr)
  {
        for(j in 1:nc)
          {
                if(MT[i,j]>0)
                  {
                  MT1[i,j]=1
                  }else{
                          MT1[i,j]=0
                     }
          }
  }

freqMT<-colSums(MT1) # sums up the number of occupied sub-squares
meanF<-mean(freqMT)  # mean number of occupied sub-squares.

#For Mean Turnover,
##Define location of observations for both years of study.


triala<-data.frame(MT)
total<-merge(trial,triala,by.x=c("x","LocOV"),by.y=c("x","LocOVa"),all.x=TRUE,all.y=TRUE) # merge both  MT datasets (2012 –
## 2013) by the ID vector
all$Freq.x<-as.numeric("Freq.x") # refers to the MT table above
Freq1<-total$Freq.x
all$Freq.y<-as.numeric("Freq.y")
Freq2<-total$Freq.y
all$x<-as.numeric("x")

# if an observation is present within a sub-square it is denoted by 1 if not 0  (MT variable above)
Freq3<-ifelse(Freq1>0,1,Freq1) # Presence for 2013

Freq4<-ifelse(Freq2>0,1,Freq2) # Presence for 2012

Table<-cbind(Freq3,Freq4) # Combine them to a single table


Freq5<-abs(Freq3-Freq4)  # Calculate the absolute difference In presence for both years
Tr<-cbind(total$x,Freq5,Freq3,Freq4)
Count<-as.numeric(levels(total$x))
Abs<-tapply(Freq5,total$x,sum)
Abs[is.na(Abs)]<-4
```

```
F13<-tapply(Freq3,total$x,sum)
F12<-tapply(Freq4,total$x,sum)
F13[is.na(F13)]<-0
F12[is.na(F12)]<-0
Absf<-abs(F13-F12)

Tr2<-rbind(Count,Abs,F13,F12,Absf)  # calculate Mean turnover
tr3<-t(Tr2)
#Mean if we Consider the first option (NA values ==4 in Change of state)
MeanAbs<-mean(Abs);MeanAbs
#Mean if we Consider the second option (amount of subsquares that changed state)
MeanAbsf<-mean(Absf);MeanAbsf
```