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The role of landscape and species attributes in insect community assembly, population genetics and plant-insect interactions in expanding Quercus ilex forests

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PhD Thesis

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A mis padres.

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Y por fin llega el momento de decir: ¡TESIS ACABADA!

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Abstract

The mechanisms driving species colonization and coexistence in habitats, is one of the main puzzles in community ecology. Several theories, including niche, neutral and coexistence theories, have tried to explain the biodiversity observed in natural ecosystems as the result of deterministic, neutral, or a combination of both processes, respectively. This biodiversity will assemble in communities and ultimately establish biotic interactions, which will play an important role in the ecosystem dynamics and its resilience. Species interactions can only be fully approached by the double perspective of landscape ecology and metapopulation/metacommunity dynamics, and therefore both, landscape attributes and species' traits must be taken into account. Loss of communities and biotic interactions has been thoroughly studied in the context of shrinking and fragmented habitats, as it is one of the main threats to global biodiversity; however, the assembly of communities and biotic interactions in new expanding natural ecosystems is a much less explored topic. In the current context of global change, land transformation has resulted in loss and fragmentation of habitat in many different ecosystems, however, recovery of natural habitats has also been observed. Particularly in Europe, cropland abandonment has led to an intense process of forest expansion and nowadays we can find patchy afforested areas all over the continent in which an ongoing process of assembly is taking place. Especially in these patchy habitats, neutral processes modulated by spatiotemporal landscape attributes as well as dispersal ability of organisms could be playing a key role in the assembly of communities and biotic interactions. Nevertheless, deterministic processes might as well be shaping communities and interactions at other spatial scales

The aim of this thesis has been, therefore, to deepen in the knowledge of factors driving community and interaction assembly, taking as study system the plant-insect interaction established between species that have successfully colonized these expanding forest patches, specifically *Quercus ilex*, a key species of the Mediterranean forests, and its community of herbivore (folivore and granivore) insects. As a first objective, this thesis proves the impact of spatiotemporal attributes at the tree scale on the assembly of the Lepidoptera community. **Chapter 2** shows a complex interplay of age, canopy size and connectivity of trees interspersed in an expanding savannah-like *Q. ilex* landscape, leading to more abundant, richer and more diverse Lepidoptera communities in older, larger and more connected trees, while alpha and

beta diversity depending exclusively on the distance between canopies, therefore validating the importance of neutral and random processes on the assembly of the herbivore community. As a second objective, this thesis explores the impact of spatiotemporal and genetic attributes at the tree scale on herbivory. **Chapter 3** shows a direct effect of tree genetics on the leaf damage suffered by holm oaks, as well as an edge effect benefiting insects at the core of the patch, while an indirect effect of age and connectivity, resulting in associational resistance, is revealed among mature developmental stages of holm oak. Additional support of tree characteristic impact on herbivory is found in **Chapter 4**, where seed-predation is regulated by acorn size and tree connectivity, while it does not change between habitats or landscapes at the local and regional scales, due to compensatory dynamics of the different species within the seed-predator guild. This homogeneity in the interaction, contrasts with the heterogeneity shown by seed-predator assemblages at the three spatial scales, with colonization credits of the poorer dispersers in the most new and isolated patches. Therefore, results of **Chapter 4** evidence, indeed, the importance of studying community and interaction assembly at different spatial scales as well as revealing different seed-predator assemblages at the local scale but zero-sum landscape effects on seed-predation at the landscape scale. In addition to effects on the composition of the community, the colonization process mediated by landscape and species' attributes can also have genetic effects. Population genetic theory predicts a strong correlation between population size and genetic variation, which in turn, is expected to correlate with fitness and adaptation potential. In **Chapter 5** we explore the effects of age and connectivity of the habitat in combination with the dispersal vs. dormancy trade-off exhibited by some seed-predator species. Results reveal significant genetic structure and low genetic variability only for the poor disperser *Curculio elephas*, as well as restricted gene flow between old and new-isolated patches, in consonance with colonization credits found for this species in **Chapter 4**. In spite of the founder effects, consistent larger populations of *C. elephas* compared to the better disperser *C. glandium* suggest that other factors are favoring the fitness of the former species

Overall, results included in this thesis provide a detailed insight on the processes that, at different spatial scales, are mediating the *Quercus ilex* – herbivore assembly in expanding forests, revealing the participation of all, niche, neutral and random processes. Findings also suggest that the patchy arrangement of these expanding habitats are favoring the existence of different herbivore assemblages, but with a resultant zero-sum impact of herbivory on holm oaks. Differential colonization dynamics, mediated by differences in species dispersal ability, have already left a genetic signature, which may impact seed-predator fitness and potential of

adaptation. The information provided in this thesis may be of great help for the management of both, *Q. ilex* and herbivore populations in expanding forests, supporting decisions such as size/shape patch maintenance of enhancement/reduction of inter-patch connectivity.

Chapter 1: *General Introduction*

1.1 Biodiversity in a context of land-use change

In the present context of global change, land transformation is considered a primary threat to biological diversity because it has resulted in loss and fragmentation of habitat in many different ecosystem types (Vitousek *et al.* 1997). Estimates of complete habitat change vary by biome from 0.4% (tundra) to 48.5% (tropical/subtropical dry broadleaf forests), with a 21.8% of global land area converted to human-dominated uses (Hoekstra *et al.* 2005). Usually, the term “land-use change” brings up implicitly the idea of loss of natural habitats and associated species (Sala *et al.* 2000) due, obviously, to the massive extent of natural habitat loss compared to that of natural habitat recovery. However, a change in the use a land is given can also open the door to the emergence of new natural habitats and the assembly of new communities; indeed, recovery of natural habitats (grasslands, forests, shrublands, savannas, etc.) has been also observed.

According to the Global Forest Resources Assessment 2015 (Keenan *et al.* 2015), in spite of the global 3%-decline in forest area from 1990 to 2015, temperate forests have expanded. This forest expansion has profound ecological implications due to the key functions and ecosystem services forests globally provide, including maintenance of wildlife habitat (Foley *et al.* 2005; Loo 2009). It has been estimated that only in Europe, forest cover has increased by about 25% during the second half of the twentieth century as a consequence of cropland abandonment and natural transition to woodlands (Fuchs *et al.* 2013). Recent afforestation might be especially important in Mediterranean forests, considering their historical deforestation (Cervera *et al.* 2015). In consonance with the forestation trends across the continent, some regions of the Iberian Peninsula have also experienced intense forest expansion, with about 25% of the current forest cover emerged in the last decades (Baśnou *et al.* 2013).

Mediterranean forests, compared to temperate or boreal forests, are characterized by high spatiotemporal complexity and heterogeneity regarding functional dynamics at landscape and local levels, as well as species composition and richness (Lefèvre & Fady 2016). Identifying the most relevant drivers of community assembly, especially in these highly variable Mediterranean ecosystems, is not an easy task, but it is still crucial to guarantee the persistence of functional communities under the current context of global change (Fleishman *et al.* 2002; Opdam & Wascher 2004). Ecologists have added temporal, genetic and spatial structure to the concepts of population and community in order to address all the possible sources of variability. Although different species may respond differently to changes in the same landscape context, depending on their degree of habitat specialization, body size, resilience or dispersal abilities, very few studies have focused on the rearrangement of whole communities in these new habitats.

1.2 What drives the assembly of communities: The role of landscape attributes vs. species traits

To answer the question "what drives the assembly of communities?" we first need to state clearly what we understand by "community assembly". Following HilleRisLambers et al. 2012, we define community assembly, as "the process by which species from a regional pool colonize and interact to form local communities". That is, a subset of species belonging to a regional species pool, are available for the colonization of a particular site and form new local species pools. However, the myriad of processes influencing this community assembly is such, and the range of spatiotemporal scales involved so wide, that disentangling the identity and importance of community assembly drivers, seems an insurmountable task.

Historically, community assembly has been built on classical niche differentiation theory (Macarthur & Levins 1967; Chase & Leibold 2003; Tilman 2004; Leibold & McPeek 2006) that explains biodiversity as the result of deterministic processes. Despite its importance in ecology, the niche differentiation theory fails to explain many patterns in community assembly, and since the mid-20th century, it has been challenged by neutral theory, which emphasizes the importance of stochastic processes (Hubbell 2001; Rosindell *et al.* 2011). Neutral theory has three basic assumptions: the neutrality, the point mutation and the zero-sum ones. Despite the criticism on these restrictive assumptions, neutral theory fits very well some ecological patterns (abundance-occupancy relationships, species turnover, distance-decay relationships) in a variety of communities (Bell 2001; Chave and Leigh 2002; Ulrich and Zalewski 2007) and substantial improvements have been accomplished in order to make the original assumptions more realistic (Etienne et al. 2007; Rosindell et al. 2011; Rosindell and Cornell 2013; Matthews and Whittaker 2014). Hubbell's 'individual-based' neutral theory was explicitly formulated after the 'species- based' equilibrium theory of island biogeography (MacArthur & Wilson 1963, 1967), and one of its most important contributions to community theory is the emphasis on dispersal limitation as a dominant factor in determining species abundances. Niche differences, although they certainly exist, are effectively neutral and therefore do not drive local abundances and distributions of species.

In spite of these niche-based and neutral theories being the two main theoretical frameworks in ecology assembly, an emerging consensus about the complementarity and simultaneity of both, deterministic and stochastic processes in structuring communities, seems to be consolidating among researchers (Tilman 2004; Gravel *et al.* 2006; Vergnon *et al.* 2012). In coexistence theory, competition among species would not lead to exclusion but rather to a dynamic coexistence mediated by the shift in species' fitness according to the species abundance at a time. These so-called stabilizing differences, would be involved in key processes such as distance- and density-dependent recruitment and resource partitioning, driving population growth and dynamics (Chesson 2000; Chase & Leibold 2003).

Community assembly can be summarized as the outcome of both metapopulation and metacommunity dynamics determined by the interplay between landscape and species attributes (Hanski 1994; Ettema & Wardle 2002; Holyoak *et al.* 2005). Regarding landscape attributes, these metapopulation and metacommunity dynamics are primarily related to landscape structure (see McCoy & Bell 1991; Lomolino 1994; Hubbell 1997). Increasing habitat patch area and connectivity increases the probability of a species to reach and establish successfully in an unoccupied habitat and thus, may enhance local diversity at community level (Bailey *et al.* 2007; Brückmann *et al.* 2010).

Regarding species attributes, dispersal ability in space (including mobility across the landscape matrix) and time (i.e. dormancy/ diapause strategies) is a primary driver of metapopulation and metacommunity dynamics, as it determines the spatiotemporal scales at which species experience the landscape (Tscharntke *et al.* 2002). A great dispersal ability in space will reduce the risk of local extinction by reducing landscape matrix effects (Murphy & Lovett-Doust 2004; Tscharntke & Brandl 2004) and increasing the propagule pressure (i.e. propagule numbers; Simberloff 2009). Similarly, a variable dormancy will spread the local extinction risk by scaling emergence of adults, guarantying the survival of some individuals to years of unfavorable environmental conditions (Pélisson *et al.* 2013). However, both strategies are highly energy demanding, and so trade-offs between these two strategies are common in nature (Roff 1986; Zera *et al.* 1997; Zera & Harshman 2001). These trade-offs between both attributes might affect species composition in fragmented landscapes as well as rule the relative frequency of the species following one strategy or the other (Amarasekare 2013).

Still, community assembly will also depend on the inherent habitat succession dynamics (e.g. afforestation following crop abandonment) that will bring changes in the habitat quality and structure to which species will have to cope with and adjust. Thus, extinctions and colonizations of species in dynamic landscapes and habitats often occur with time lags (Jackson & Sax 2010), and both extinction debts (i.e. the number of species expected to go extinct as a new habitat reaches its equilibrium state; see Kuussaari *et al.* 2009) and colonization credits (i.e. the number of species committed to eventual immigration following a forcing event; see Cristofoli & Mahy 2010; Jackson & Sax 2010) have been identified following changes in habitat quality, amount or connectivity. Regarding colonization of new habitat patches, we would expect greater species richness as habitats get older and colonization credits are gradually paid off. In any case, while patch age *per se* may play a relevant role in structuring ecological communities (Borges & Brown 1999; Hubbell 2001), its effects have been less explored than that of habitat quality and structure (Drake *et al.* 2002).

1.3 Genetic dynamics and structure in expanding forests

Based on simple population genetic theory, a strong correlation can be expected between population size and genetic variation, which in turn, is predicted to also correlate with fitness (Leimu *et al.* 2006). Recently established secondary forests can exhibit founder effects, kinship structure and genetic drift (Petit *et al.* 2004) due to small population sizes and/or low genetic variability. At evolutionary scales, reduced genetic diversity decreases the potential of species to adapt to changing environments, and at the ecological scale, they may reduce fitness, especially in small populations (Ellstrand & Elam 1993). Nevertheless, this will only be true in natural populations if they are not regulated to a greater extent by other factors. In species exhibiting zoochory, recruitment in new patches can be assisted by long-distance dispersers carrying seeds from distant populations, thus enhancing genetic variability within the new forest and consequently, diluting the founder effect. Previous studies carried out in similar expanding forests have already documented the importance of regular assisted arrival of seeds from other patches in the expansion of the forest studied (Gerber *et al.* 2014; Gerzabek *et al.* 2017). This genetic variation will lead to individual phenotypic variation in resistance traits (i.e.

trichomes, leaf toughness, tanins, glycosides, etc.), which, in turn, has been proven to impact insect density and performance (Awmack & Leather 2002; Ruhnke *et al.* 2006; Hughes *et al.* 2008). However, literature on host-genotype effects on insect herbivory is scarce and results are highly context- and species- dependent (Mopper *et al.* 1991; Donaldson & Lindroth 2008; Maldonado-López *et al.* 2014) probably owing to not including in these studies other traits that may mediate in the relevance of genetics for herbivory damage: i.e. plant developmental stage and connectivity (Guyot *et al.* 2016; Moreira *et al.* 2017; Fernandez-Conradi *et al.* 2017).

Similarly to plants, a positive relationship between population size, genetic variation and fitness is expected for animal populations (Reed & Frankham 2003). Indeed, endangered species typically have lower levels of heterozygosity, smaller population sizes and/or lower genetic variability than related no endangered species (Frankham 2005; Haig & Avise 1996). In newly established populations, genetic structure is often determined both, by historical events (i.e. bottlenecks and founder effects) (Franklin *et al.* 2014) and species-specific characteristics (i.e. dispersal ability) (Duminil *et al.* 2007; Timm & Geertsema 2008). Species with good dispersal abilities will show a higher gene-flow across patches and a resultant weak genetic structure, compared to poorly dispersed ones; yet, local genetic diversity might be higher in the highly dispersed species due to the recurrent arrival of new immigrants (Hastings & Harrison 1994), even though a proportion of these immigrants might eventually die because of their limited resilience. In contrast, species that are more resilient could be less genetically diverse at local scale due to their lower dispersal abilities, especially in very isolated patches, yet this could be counterbalanced by a larger population size due to their lower likelihood to suffer local population bottlenecks (Frankham 2005). In the expanding forests of the Iberian Peninsula studied in these thesis, herbivores are known to differ greatly in their dispersal abilities (Venner *et al.* 2011; Pélisson *et al.* 2013) and therefore we expected that these differences had already impacted their population genetic structure and diversity.

1.4 Species interaction assembly and ecosystem functioning

Species interactions are often ignored in the context of global change even though they will be the first link disappearing from local communities as a precursor to local (and ultimately global) extinctions as habitat availability shrinks (Albrecht *et al.* 2007;Sabatino *et al.* 2010; Woodward *et al.* 2010). Understanding the processes underlying species interaction assembly is thus of vital importance to provide critical new insights into ecological responses to perturbations.

The metapopulation and metacommunity concepts have their roots in island biogeography and they have provided valuable insights in the regional persistence of interacting local populations/communities through spatial dispersal. However, species interactions can only be fully understood from a double-perspective approach: a population/community perspective, concerned with metapopulation/metacommunity dynamics, as well as a landscape perspective, concerned with ecological processes in explicitly structured ecosystems (Pickett & Cadenasso 1995; Turner *et al.* 2001). Therefore, species interactions should be addressed by explicitly considering communities into ecosystems, with abiotic constraints of the environment and feedbacks of the community influencing this environment. The meta-ecosystem concept is a natural extension of the metacommunity concept, which includes these abiotic constraints and feedbacks, providing a theoretical framework integrating population/community and landscape ecology in spatial ecosystem ecology (Loreau *et al.* 2003). Again, the dichotomy between niche and neutral models shape the proposed species-interaction assembly at meta-ecosystem level, involving several spatiotemporal scales and species' traits (Thies *et al.* 2003). While species sorting is often associated with niche differences among habitat patches and inter-specific competitive ability, both mass effects and neutral processes are frequently determined by the interaction of habitat spatial structure and animal dispersal abilities (Shmida & Wilson 1985; Pulliam 1988). Sorting, or environmental filtering, will modulate local species interactions through competitive exclusion, whereas movement among local communities would be the regional process that counterbalances sorting effects by homogenizing species distributions (Wang & Loreau 2016). It will be at intermediate movement levels, when reciprocal effects of local and regional processes will arise, with both regional and local diversity and relative species abundance being the result of a balance between local sorting and immigration from the regional metacommunity. As posited by the third assumption of neutral theory, this balanced dynamics would lead to competing species exhibiting zero-sum dynamics, i.e. density compensation or trade-off processes, in their abundance (Ernest *et al.* 2008), which could also extend to ecosystem processes like trophic cascades and nutrient cycles in a spatiotemporal context. Yet these meta-ecosystem effects (*sensu* Loreau et al. 2003) have been poorly investigated.

Effects of landscape structure and dynamics on metapopulation and metacommunity processes also drive species interactions and the resulting ecosystem functioning (Tscharntke *et al.* 2012). Indeed, habitat connectivity might help to promote mass effects on herbivore populations, thus increasing colonization success and damage on host-plant species, while specific patch dynamics might promote or arrest these landscape processes (Leibold *et al.* 2004). Landscape attributes may also determine zero-sum effects on plant-herbivore interactions if there is merely a substitution of species among patches. At a regional scale, more resilient species with poor dispersal abilities will be constrained to stable and highly connected habitat patches, while species with higher dispersal abilities will colonize new and isolated ones (Hendrickx *et al.* 2009; Hernández *et al.* 2014; Evans *et al.* 2015). Thus, dispersal ability (including mobility across the landscape matrix) is a primary driver for community assembly and governs the reorganization of species interactions in patch dynamics across the landscape (Thompson & Gonzalez 2017). Surprisingly, few studies have explored the effects of habitat recovery on the assembly of plant-animal interactions (de la Peña *et al.* 2016) despite a growing number of them have analysed the effect of landscape structure on plant-animal interactions in a context of habitat loss and fragmentation (Didham *et al.* 2012; Guardiola *et al.* 2017).

1.5 Plant-insect interaction assembly in new forest patches: The case of new Quercus ilex forests

Albeit largely ignored, specialist phytophagous insects are among the first organisms colonising new habitat patches, concurrently with plant communities (Lawton 1983). This process has a non-negligible, yet scarcely studied, spatiotemporal component (but see de la Peña *et al.* 2016). Indeed, a limited set of previous studies highlighted the importance of the interplay between landscape attributes (i.e. size, connectivity and age), and species' traits (mostly dormancy and dispersal ability) in the assembly of plant-insect interactions in new habitat patches (Bouget *et al.* 2015). For example, changes in habitat connectivity commonly affect frequency and abundance of specialist insects (Thomas *et al.* 2001; Kruess 2003). On the other hand, a number of studies have highlighted the combined effect of habitat structure and age on insect species richness and abundance (Inoue 2003; Tscharntke & Brandl 2004; Jeffries *et al.* 2006; Taki *et al.* 2010), with the poorest dispersers exhibiting colonization credits in recent habitats (Heiniger *et al.* 2014), yet results are highly idiosyncratic.

Specifically regarding plant-insect interactions, we can find some empirical studies on the role of habitat age and connectivity in the community assembly of herbivores and their consequences for the host plants (e.g. Tscharntke & Brandl 2004), while effects on predator insects have attracted much less attention (but see Orrock *et al.* 2003; Orrock & Damschen 2005). Plant expansion in younger or more isolated patches may be favoured by decreasing insect damage, in a sort of “enemy release” effect (Koivula *et al.* 2002; De la Vega *et al.* 2012; Fountain-Jones *et al.* 2015), or arrest it when hosts plants in new isolated patches are highly affected by herbivores, as these are released from their natural enemies (Roland 1993; Terborgh *et al.* 2001). Therefore, changes in the spatiotemporal pattern of habitat patches might lead to insect community shifts depending on species attributes like dispersal and resilience (e.g. Hendrickx *et al.* 2009; Hernández *et al.* 2014; Evans *et al.* 2015) yet the effects (i.e. positive, negative or neutral) on species-plant interactions are not clear.

Many studies exploring the effects of invertebrate herbivory on forest dynamics focused on tropical forests (Weissflog *et al.* 2018). The knowledge about herbivory impact on temperate forests is much scarcer (but see Piper et al. 2018, article in press), especially in forests that are in expansion (Lemoine *et al.* 2017). Interestingly, Europe has undergone a process of greening in the last decades, reversing centuries of deforestation (Meyfroidt & Lambin 2011), with transition to woodlands being the most extensive land-use change in recent years, followed far behind, by urban expansion (Meiner & Pedroli 2017). In particular, European Mediterranean forests, which historically showed strong reductions in their extent (Grove & Rackman 2001), have partially recovered in the last 50 years, especially in mountain and former agricultural areas (Lloret *et al.* 2002; Marull *et al.* 2010; Baśnou *et al.* 2013). As a result, nowadays these landscapes consist of habitat patches that have persisted through past centuries, but also younger dynamic patches with ongoing changes in their environmental conditions and species composition. Insect herbivores can influence the regeneration dynamics of these new forests through selective predation, altering the succession trajectories of the ecosystem particularly if herbivory affects recruitment and mortality of key species of the ecosystem (Lemoine *et al.* 2017).

Quercus ilex is one of the woody species that have successfully colonized new Mediterranean forest patches emerged in abandoned agriculture and pasture areas since the 1950s in many parts of the Iberian Peninsula (Guirado *et al.* 2008; Puerta-Piñero *et al.* 2012; Basnou *et al.* 2016). The expansion of these populations are expected to reflect the history of the landscape and specific life-history traits of this woody species, as well as have deeply determined the assembly of other animal species that interacts and find food and shelter in it. *Quercus ilex* can be considered a key species of Mediterranean forests due to the wide range of interactions it establishes as well as the services it provides (Plieninger *et al.* 2010; Iriarte-Goni 2013; López-Sánchez *et al.* 2016). Recent studies on plant community assembly in new established Mediterranean forests, including our study area, have revealed a delayed colonization of *Q. ilex*, compared to other species that have already exhausted their immigration credits (Basnou *et al.* 2016). In general, the genus *Quercus* supports species-rich assemblages of specialist phytophagous insects that are a major component of world biodiversity and form part of numerous trophic interactions (Novotny & Missa, 2000). Folivore specialists of *Q. ilex* in Europe belong to the Noctuidae, Geometridae, Tortricidae and Drepanidae families (Bellmann 2017) amongst others. Regarding seed-predation, *Curculio elephas*, *Curculio glandium* and *Cydia fagiglandana* are the most prevalent predator of *Q. ilex* acorns in the Iberian Peninsula (Espelta *et al.* 2009a). Herbivory and seed-predation can affect tree primary productivity and viable seed pool for regeneration and so they might be modulating *Q. ilex* expansion dynamics in these new forests. In turn, the maintenance of the abovementioned biological diversity and ecosystem services depends on the successful regeneration of holm oaks. There exist some recent literature on the assembly of plant community in these new Mediterranean forests (Guardiola et al. 2013; Bagaria et al. 2015; Basnou and Pino 2015), nevertheless these studies have not been extended to other organism communities (e.g. insects), neither considered the role of species and landscape attributes in the assembly of the insect-plant interactions and its consequences: i. e. herbivory and granivory.

With this thesis I aim to fulfil the current lack of knowledge about biotic interaction assembly in new Mediterranean forests taking as study system the holm oak and its community of insect herbivores.

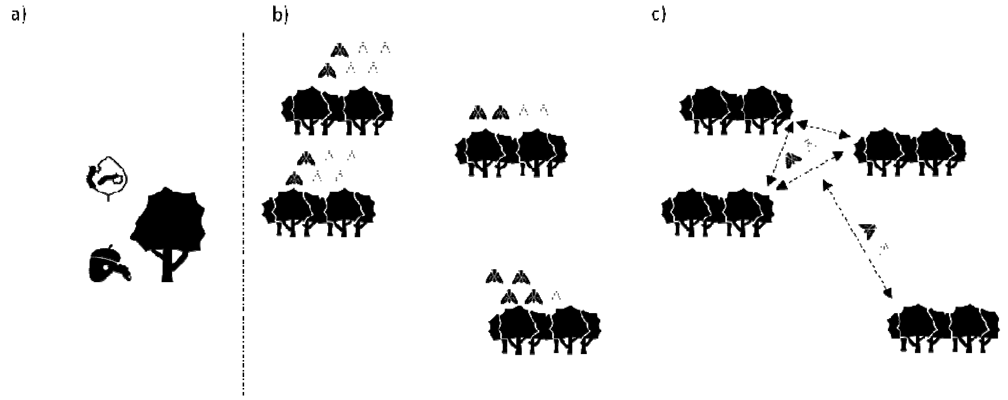
1.6 Main objectives

In this thesis I address the assembly of new insect communities and plant-insect interactions in expanding *Q. ilex* forests in order to shed some light on this complex process in the yet scarcely studied context of habitat recovery. We focus on the interaction of landscape attributes such as age and connectivity, and species' traits such as dispersal and dormancy ability of insects, as drivers of the assembly process. In addition, we include the importance of the spatial scale by covering landscape (regional), patch (local) and tree (individual) scales, at which the contribution of neutral- and niche-based processes to the community assembly and plant-insect interaction, might shift (**Figure 1**).

Summarizing, I investigate the interacting effect of habitat characteristics and species' traits on the herbivore community composition and genetics, the assembly of *Quercus ilex* and its insect community, and the functional consequences of these processes: folivory and acorn predation. The studies are conducted in emerging savannah-like landscapes in Toledo (center of Spain) and emerging forests of Catalonia (northeastern Spain), where abandonment of croplands during the second half of the 20th century have given rise to low-density wooded lands as well as young mixed forest patches with different degree of isolation. The main aims of this thesis are:

1. To quantify the effects of *Q. ilex* tree age, size and connectivity on the folivore community assembly in terms of abundance, richness, diversity and composition (Chapter 2).
2. To explore the effects of host individual traits such as genetic origin, age and spatial location on herbivory in expanding *Quercus ilex* forests (Chapter 3).
3. To study the effects of connectivity at the landscape, patch and tree level in combination with insect dispersal ability on the composition of the seed-predator community and seed-predation pressure (Chapter 4).
4. To identify the genetic signature left by dispersal vs. dormancy risk-spreading strategies in the metacommunity of seed-predators established among *Quercus ilex* patches of different age and connectivity (Chapter 5).

Figure 1. Different spatial scales addressed on the study, and ecological processes involved at each one. a) Tree (individual) level at which the herbivory (folivory and seed-predation) occurs according to characteristics of the host. b) Patch (local) level, at which local community assembles according to both, spatiotemporal patch attributes and species' traits. c) Landscape (regional) level, at which mass effects and metacommunity dynamics determine the regional pool of species and its distribution among patches.



Study system and study site

Regenerating wooded areas after land-use change

Field work of this thesis was carried out in two kind of regenerating habitats. Firstly, a savannah-like landscape located in Huecas (Toledo, Central Spain; **Figure 1**) with a very low density of holm oaks (from <1 to 100 oaks per ha; Bonal *et al.* 2012), with trees standing either as remnants of clutched holm oaks or as completely isolated individuals embedded in an agricultural matrix. Regeneration in the area started after the ease of agriculture, which allowed holm oaks kept, so far, at the boundaries and interspersed within the croplands, to grow. The trees chosen for the study differ in age, canopy size and connectivity and thus, Lepidoptera community feeding on this species is expected to colonize each tree according to these spatiotemporal characteristics. Secondly, forest patches spread throughout the Vallès-Penedés lowlands (northeastern Iberian Peninsula) with different degree of isolation and age (**Figure 2**). Landscape is a mosaic of forest patches of different ages (pre- and post-agricultural), *Quercus* species being mostly concentrated in pre-existing forests and *Pinus* species in recent ones (Guirado *et al.* 2008). These new forest patches in the Vallès-Penedés lowlands are embedded in highly anthropised landscapes (Terradas 1999), and their recruitment still depend on the activity of seed dispersers (e.g. jays *Garrulus glandarius* L. and wood mice *Apodemus sylvaticus* L.; see (Bossema 1979; Jensen & Nielsen 1986). In order to study the impact of landscape structure and age on community assembly, we established patch age using historic (1956) and present (2005) land cover maps available for all Barcelona province. In doing so, we were able to distinguish forests emerged after cropland abandonment or in a primary succession stage in 1956, from forests that were already present back that time (named “new forests” and “old forests” respectively).

Both ecosystems are the result of land-use changes but due to their marked differences in encroachment, they provide the study with a complementary view of the community assembly process.

Herbivore community

Holm oaks host a diverse community of insects (Müller & Goßner 2007), including specialist Lepidoptera species that feed on leaves and fresh shoots (Gómez de Aizpúrua 2003; Robinson,

G. S. *et al.* 2010) (see **Figure 3**). Crown structure can be of great importance for these organisms, especially for caterpillars, which are very sensitive to changes in microclimatic conditions and also suffer predation from birds. Regarding adults, they can easily select habitat patches thanks to their mobility, so they will show an active and quick response to habitat change through time. Therefore, this Lepidoptera community constitutes an ideal model for testing landscape structure and age impact on insect colonization dynamics.

Holm oaks are also the habitat for seed predators. In the Iberian Peninsula, the most important species predating upon holm oak acorns are moths of the family Tortricidae (e.g., *C. fagiglandana*) and weevils of the genus Curculio spp. (Coleoptera: Curculionidae) like *C. elephas* and *C. glandium* (Espelta *et al.* 2009b) (see **Figure 3**). Larvae of these species develop within acorns, commonly exhausting cotyledons and even killing the embryo, so they can lessen the potential recruitment of the nutritious plant. The extent of damage is partly dependent on life-history traits of the holm oaks related to predator satiation such as acorn size and acorn production, but also on life-history traits of predators related to extinction risk spreading spatially and temporally. These seed-predators differ markedly on these life-history traits such as dispersal ability and dormancy and so they constitute ideal guilds to study the effects of landscape structure and age on community assembly. Regarding flight abilities, the small- bodied *C. glandium* can disperse double the maximum distance estimated for the larger-bodied *C. elephas* (1200 m vs. 600 m, respectively), but it shows a fixed diapause of 2 years while C. elephas can spread the emergence of adults from the same cohort from 1 to 3 years (Pélisson *et al.* 2013). Compared to these two weevil species, Cydia exhibits good dispersal abilities but does not have any dormancy strategy (Schumacher *et al.* 1997). Moreover, there also exist differences in the way they predate upon acorns: while weevil larvae are constrained to develop inside a single acorn, single moth larva can shift acorns during its development, not always exhausting the cotyledon tissues (Soria 1999).

Figure 1. Left image: Aerial orthoimage of the savannah-like landscape in Huecas (Toledo), with the location of the holm oaks studied, marked with red dots. Right images: differential spatial connectivity within the study area, from highly isolated canopies to a low-density forest.

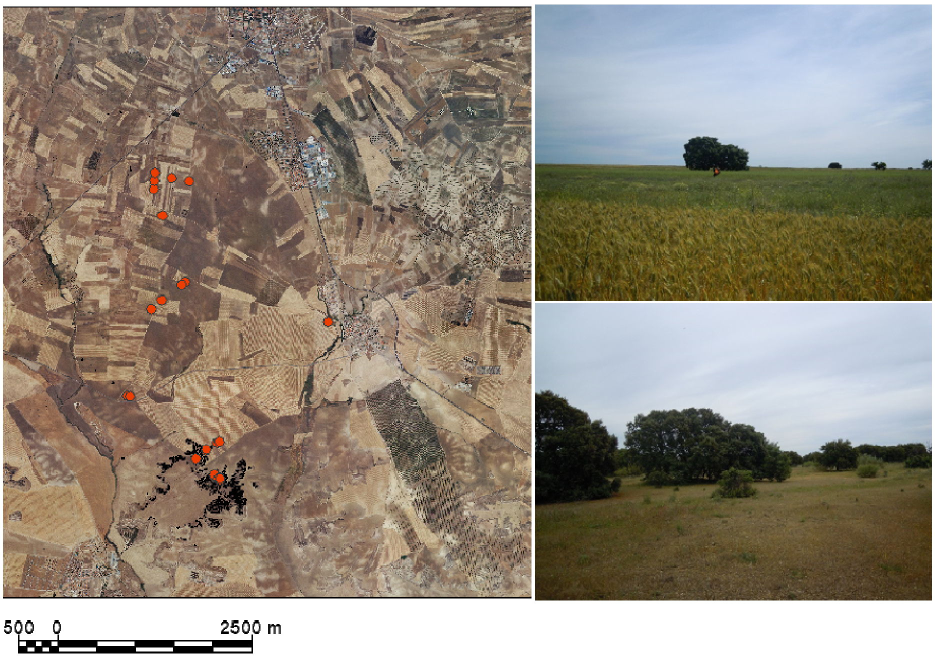


Figure 2. Upper image: Regional context of the study site, with little yellow dots indicating the location of new isolated patches studied in Chapter 2, and large red dots indicating the expanding forest patches studied in Chapters 3 and 4 within the Vallès-Penedès lowlands. Lower image: a) Orthoimages (historical and actual) of one of the isolated new forests studied in Chapter 3, with sampled individuals highlighted in yellow. B) Orthoimages (historical and actual) of one of the replicates studied in Chapter 4 and 5, with the OF (old forest), CNF (connected new forest) and INF (isolated new forest) patches highlighted in red.

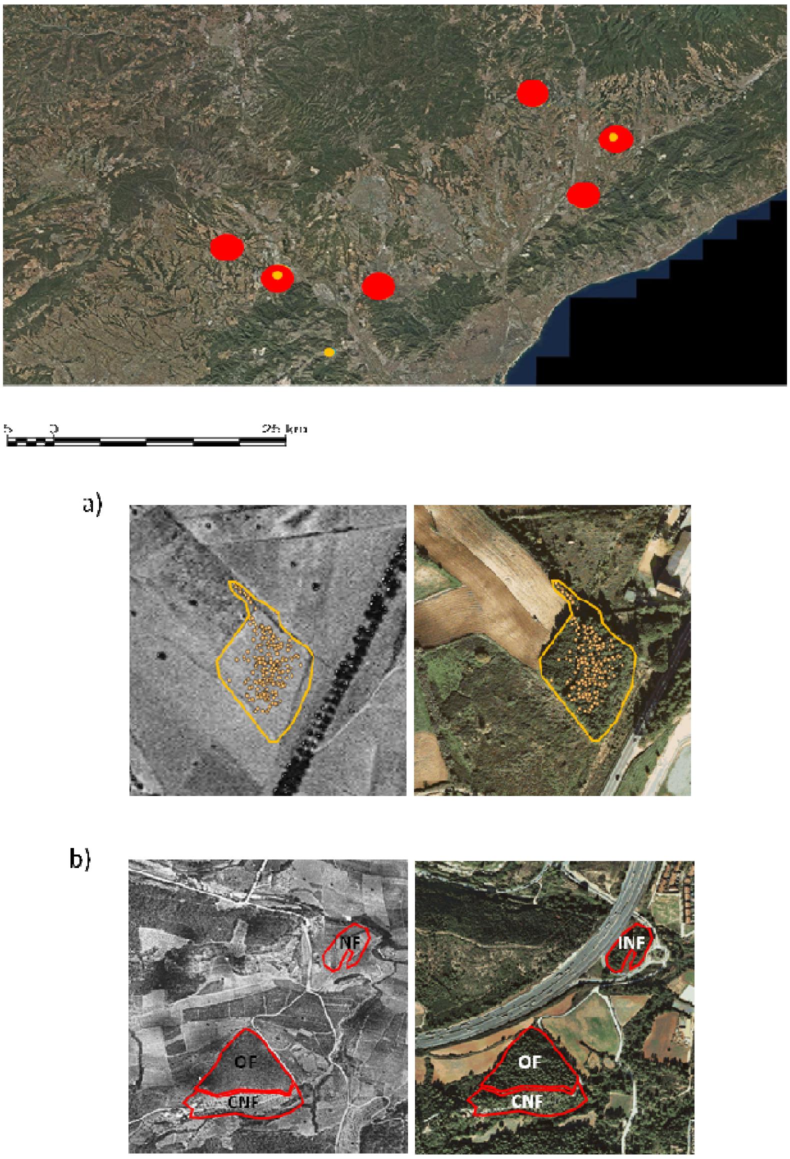
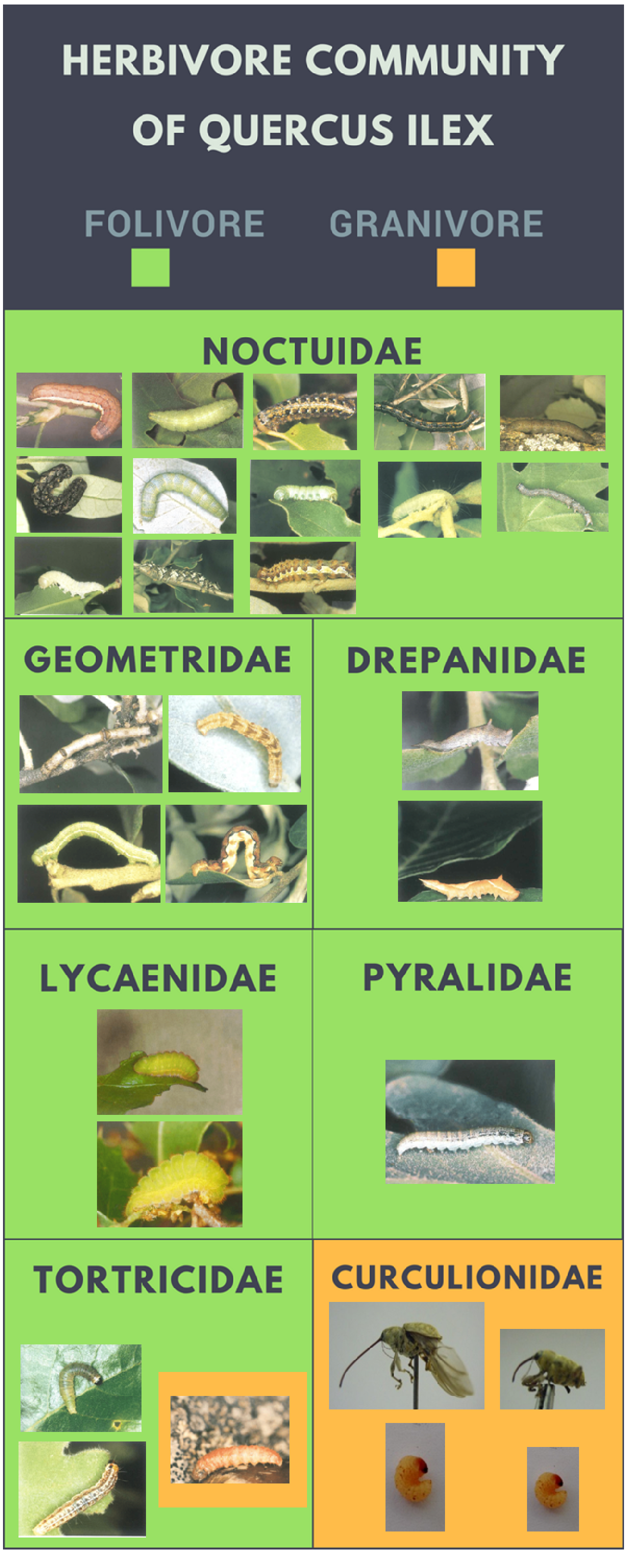


Figure 3. Families of the Lepidoptera folivore community and the seed-predator guild.



Chapter 2: Community assembly in time and space: the case of Lepidoptera in a *Quercus ilex* L. savannah-like landscape

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Abstract

1. The factors governing community assemblages in fragmented habitats remain a challenging topic in ecology, especially in the present context of global change. We studied the colonization of holm oak (*Quercus ilex* L.) woodland by specialist Lepidoptera in an afforested savannah-like landscape that has emerged as a result of the abandonment of croplands. We sampled herbivorous caterpillars for five years to assess the interplay between tree age and structure (canopy size and connectivity) on Lepidoptera species abundance, richness, diversity, and composition.

2. 1336 individuals belonging to 24 species of Lepidoptera were collected. Species abundance, richness and diversity increased in the best connected large trees and in the best connected young ones, probably because of the greater likelihood of colonization and the existence of colonization credits, respectively. Although species abundance, richness and diversity varied significantly between years in all trees, there were no effects for tree age, canopy size, or connectivity.

3. Although tree age and structure had little effect on overall species composition (alpha and beta diversity), thereby revealing a predominant random effect, the relative abundance of Noctuids and Pyralids was significantly affected by tree age, suggesting that differences in dispersal abilities exist between these families.

4. These findings suggest that habitat age and structural interactions play a significant role in the accumulation of Lepidoptera species but have little importance in determining their identities. From a conservation point of view, the results highlight the need to preserve old, large, and well-connected holm oak trees in agricultural landscapes as refuges for specialist insects.

Chapter 2: Community assembly of Lepidoptera

Keywords: Habitat connectivity, habitat change, insect colonization, niche effects

2. 1 **Introduction**

The debate on the mechanisms that drive species colonization and persistence in habitats has been alive in ecology since MacArthur & Wilson (1967) first broached the subject. During the past five decades, the classical theory of niche differentiation, widely advocated to explain community assembly (e.g. MacArthur & Levins, 1967; Chase & Leibold, 2003; Tilman, 2004; Leibold & McPeek, 2006), has been challenged by diverse approaches that emphasize the important function that species dispersal and propagule availability play in population dynamics (Levin, 1974; Hubbell, 2006). Currently, empirical data show that the assembly of ecological communities is governed by both niche- and dispersal-assembly rules (Cottenie, 2005), which thus implies that both environmental conditions and the spatial structure of habitats play major roles in community assembly (see Andrén, 1994; Ribas *et al.*, 2005; Sobrinho *et al.*, 2003; Spengler *et al.*, 2011). In terms of habitat structure, i.e. the composition and arrangement of objects in space (see McCoy & Bell, 1991), community dynamics are assumed to be primarily related to patch geometry (area and isolation). Most studies show that species richness increases with patch area and decreases as the degree of isolation grows (Lomolino 1994; Hubbell 1997 and references therein). This species-area positive relationship has been explained primarily in three different ways: (1) larger patches support larger populations that have lower extinction risks (Preston, 1962); (2) larger patches are more heterogeneous and have a higher number of vacant niches (Williams, 1964); and (3) larger patches are more 'apparent' and are more likely to be colonized (Gilpin & Diamond, 1976). The negative species-isolation relationship, on the other hand, depends on the differential dispersal ability of a species, the distance to the nearest colonized habitat, and the landscape matrix in between habitats (Murphy & Lovett-Doust, 2004; Tscharntke & Brandl, 2004). Consequently, (a) the poorer the dispersal ability of the species, (b) the greater the distance between patches, and (c) the less permeable the matrix, the lower the probability that the habitat patch will be colonized and the scarcer the colonizing species involved (MacArthur & Wilson, 1967; Simberloff & Wilson, 1969). Habitat structure in fragmented landscapes can vary as a result of natural succession and stochasticity and so the age of the habitat may also play *per se* an equal or even larger role in structuring ecological communities than environmental and spatial variables (Borges & Brown, 1999; Hubbell, 2001). Indeed, due to time-lags in colonization processes determining colonization credits (Jackson & Sax, 2010), we expect greater species richness as habitats get older. In any case, the role of habitat age as an explanatory variable of community attributes has been much less explored than the habitat structure and habitat conditions (Drake et al., 2002) in part owing to the lack of chronoseries of community data that actually capture this temporal component of the assembly process. In this regard, the use of certain short-lived animals such as insects as study models can be very illustrative as they respond particularly quickly to environmental changes (Lawton *et al.* 1998; McIntyre *et al*., 2001; Musolin, 2007). As an example, Badano *et al.*, (2005), Taki *et al*., (2010), Inoue (2003) and Jeffries *et al.*, (2006) have all shed some light on the way insect communities grow in different habitat scenarios taking into account the structure and age of the habitat; nevertheless, their results are not consistent and the response of the community depends greatly on the focus taxa. Such variability complicates the stating of general predictions and therefore the significance of spatiotemporal effects on insect community assembly remains largely unknown.

Besides the theoretical interest of this topic, spatiotemporal interactions in community assemblies have acquired greater relevance in recent years as part of the attempt to understand the consequences of changes in land use. For example, the abandonment of croplands in Europe from the second half of the twentieth century onwards has led to the appearance of new tracts of forest (Hermy & Verheyen, 2007; Bolliger *et al.*, 2007; Meyfroidt & Lambin, 2011; Basnou *et al*., 2013), which have been recolonized by many forest species. Specialist phytophagous insects are often among the first organisms to colonize young trees, where they find the shelter and reproduction sites that a hostile cropland matrix cannot provide (Lawton, 1983). This process is thus taking place on a massive spatial scale and should be studied in order to understand the factors and interactions driving the restoration of forest ecosystems.

In certain parts of the Iberian Peninsula, increases of over 20% in the forest surface area has been recorded in the last fifty years (Basnou *et al.*, 2013). Secondary forests are dominated by two main tree genera, *Pinus* and *Quercus* (Blanco *et al.*, 1997; Pons & Pausas, 2006; DeSoto *et al.*, 2010). The genus *Quercus* supports species-rich assemblages of specialist phytophagous insects that are a major component of world biodiversity and form part of numerous trophic interactions (Novotny & Missa, 2000). We studied an area of Central Spain where the holm oak (*Quercus ilex* L.) has progressively colonized former croplands and now appears in the form of small forest patches and isolated trees (Ortego *et al*., 2010), and where the natural reassembly of the Lepidoptera community associated with these oaks provides an interesting opportunity for exploring the processes underlying community assembly from an island-biogeographic perspective.

The aim of this study was to evaluate to what extent the community assembly of Lepidoptera in this new patchy habitat is affected firstly, by age, canopy size, and connectivity, and, secondly, by the spatial pattern of the host plant *Quercus ilex*. Specifically, we addressed the following questions: (1) Do Lepidoptera species abundance, richness, diversity, and species composition vary between holm oaks of different age, canopy size, and connectivity? (2) Do holm oak age, canopy size, and connectivity determine Lepidoptera species turnover (species beta diversity) and does spatial location of the trees also play an important role? We expect greater species abundance, richness and diversity of Lepidoptera caterpillars in older, larger, and more connected holm oak trees, as well as different patterns of colonization by Lepidoptera (different species assemblages) due to random dispersal and/or differences in species' dispersal abilities. To answer these questions we collected Lepidoptera caterpillars from 23 holm oaks for five years and assessed the effects of tree age, canopy size, connectivity, and their interactions on i) caterpillar abundance, ii) species richness, iii) species diversity, and iv) the species composition of the community.

2.2 Material and Methods

2.2.1 Study area

The study was performed in Huecas (40° 0´N, 04° 11´W, Central Spain; Fig.1). Mean temperatures range from 24–26 ºC in July to 4–6 ºC in January; mean annual rainfall is 350–450 mm, mainly concentrated in spring and autumn and with a severe drought in summer months. The landscape consists of both clustered and isolated holm oaks within an extensive cereal field matrix (see Ortego *et al.* 2010 for a more detailed description). Tree density ranges from <1 to 100 oaks per ha (Bonal *et al*., 2012). The comparison of historical and current photographs reveals the on-going holm oak regeneration process, concentrated at the boundaries of cropland areas and in marginal and abandoned land.

2.2.2 Study species

The evergreen holm oak (*Q. ilex*) is widespread in the Iberian Peninsula and forms primary and secondary mixed forests, as well as savannah-like landscapes. It hosts a diverse community of insects (Müller & Goßner, 2007), including several Lepidoptera species belonging to a number of families that feed on leaves and fresh shoots (Robinson *et al.*, 2010; see Appendix A, Table A.1). Many are highly specific to the genus *Quercus* (Gómez de Aizpúrua, 2003; Robinson *et al.,* 2010) and constitute ideal models for assessing insect colonization of new forest patches in fragmented landscapes. Moreover, given the mobility of adults, Lepidoptera can respond in a relatively short time to habitat succession processes and thus are also good models for testing the effect of habitat age on community assemblages. Specialist species are in general univoltine and their peak egg-hatching period is synchronized with the appearance of new shoots in their food plants (which begins in mid-April in the study site). These phenologic patterns give maximum species abundances in April and at the beginning of May (Fernández & Jordano, 2004).

2.2.3 Experimental design and tree measurements

We chose 23 *Q. ilex* trees in the study site with a broad range of tree ages and canopy sizes. Trees were distributed across approximately 680 ha, and the distances between them varied from 4 m to 2 km.

We geolocated the study trees in the field using a global positioning system (GARMIN GPSMAP 62st) and determined their age (20–74-years old) by tree-ring dating and counting after extracting a core from the trunk using a Pressler borer (see Gené *et al.*, 1993). We also took leaf area index (LAI) measurements from each tree crown along the four cardinal points using a ceptometer (LP-80, DECAGON). The mean LAI values for each tree crown thus obtained were used as a proxy of canopy density and biomass. In addition, we georeferenced all isolated holm oak trees and patches in the study area in 2014 and converted the resulting vector layer into a 1x1-m grid. Trees studied in the field were identified in the 2014 layer by their spatial position. We used orthoimages to determine tree crown perimeters and then the tree crown area inside the perimeter for selected oaks using Miramon GIS tools (Pons, 2004; www.creaf.uab.es/miramon). Canopy size values were in the range 12–104 m^2 (see Appendix A, Table A.2). Following the methodology used by Puerta-Piñero *et al.*, 2012, we estimated the connectivity for Lepidoptera of each study tree (C_i) using a modification of Hanski's connectivity index (Hanski, 1999):

$$C_i=\sum(I_j\cdot e^{-dij})$$

where I_j corresponds to each pixel of holm oak tree or patch different from the study tree i, and d_{ij} to its distance to the edge of the study tree. We constrained the calculation of C_i to 1 km around each tree in accordance with previous studies that suggest that for many moth and small butterfly species dispersal over 1 km is rare (Thomas *et al.*, 1998). Map photo-interpretation and calculation of the C_i was performed using Miramon (Pons, 2004) and Quantum GIS for Windows.

2.2.4 Lepidoptera sampling

We sampled the lower-canopy Lepidoptera community of holm oaks in five consecutive springs (2010–2014). Since caterpillar abundance may depend on holm oak phenology (Herrera, 2004), we always sampled trees well into the period of maximum activity (beginning-middle of May; own observations) to avoid any interannual bias. Micro-environmental changes within crowns can also affect Lepidoptera abundance (Summerville & Crist, 2008) and so we split tree crowns into four parts based on the four cardinal points to sample the overall conditions for the Lepidoptera community. In each position, we placed a beat sheet under the canopy and gently beat all the branches of the corresponding canopy quarter six times with a wooden stick. To control for sampling effort we always beat the trees the exact same number of times. The caterpillars collected from each canopy division were placed in plastic containers with ventilation and subsequently individually housed in plastic petri dishes. They were reared on fresh *Q. ilex* foliage, which was periodically renewed until they reached the adult stage. Caterpillars were checked for survival, pupation, and emergence every two days. We used taxonomic guidebooks (Gómez de Aizpúrua, 2003) for identification to species level based on external appearances. Many individuals were determined whilst still larvae, whereas others were identified on the basis of adult traits.

Species diversity was determined using the exponential form of Shannon's entropy (subsequently termed Shannon's Diversity to distinguish it from non-exponentiated Shannon's entropy): where *S* is the species richness and *pi* is the relative abundance of each species.

$$\exp\,(H') = \exp\left(-\sum_{i=1}^{S} pi \ln pi\right)$$

Due to its unique ability to weight elements precisely by their frequency without disproportionately favoring either rare or common species, Shannon's Diversity has proved to perform better than many other diversity measures (Beck and Schwanghart 2010).

2.2.5 Data analysis

Prior to modelling we checked our landscape predictor variables for colinearity using Pearson's correlation coefficients.

Due to incompleteness in the sampling (Appendix A, Table A.1), we examined whether differences in Lepidoptera species richness between holm oak trees were driven by differences in Lepidoptera abundance captured in trees of different size by constructing rarefaction curves for small (12–43 m^2), medium (43–79 m^2), and large (79–104 m^2) tree sizes, and then estimated cumulative species per subsample size (Appendix A. Figure A.1). In addition, a rank-abundance curve was plotted to determine whether the sampling effort was enough to capture the long ‘tail’ of rare species that is characteristic of communities with low evenness (Appendix A. Figure A.2). To determine how tree age, canopy size, and connectivity affect Lepidoptera species richness, Shannon’s Diversity, and species abundance (log-transformed), linear mixed effect models with different error structures were used to search for the best possible fit. In addition, we included a spatial correlation structure in the models to control for the presence of spatial autocorrelation in the data. We built saturated models with all fixed effects plus their second-degree interactions; model selection was performed based on the second-order Akaike Information Criterion (AICc) and adjusted R^2. Models were run with tree age, size, and connectivity. To better understand the resulting interactions, in the interaction graphics tree size and age were categorized as 3-level factors — tree size: small (12–43 m^2), medium (43–79 m^2), and large (79–104 m^2); tree age: young (20–43 years), middle-aged (43–56 years), old (56–74 years).

To assess the effect of tree age, canopy size, and connectivity on species beta diversity we constructed pair-wise distance matrices for (1) species abundances, (2) geographic location, and (3) habitat variables (age, canopy size, and connectivity) and then used them to run linear models with the distance matrix of species abundances as response variable and environmental distance matrices as factors (and correcting the sample size for the multiple pair-wise comparisons). The model was subjected to bootstrapping to obtain reliable relative weights of the explanatory factors.

Given that a detrended correspondence analysis (DCA) showed environmental range lengths >2 SD, we carried out a Redundancy Analysis (RDA) to test the relationship between species beta diversity and the environmental gradients. We constructed two data tables with information on (1) total species abundance in each tree and (2) environmental variables characterizing each tree (age, canopy size, and connectivity). RDA is based on Euclidean distances and so we used the Hellinger transformation to solve the inherent problems of this distance metric and to reduce the undue weight of species that may be abundant at a particular place but rare at study-site scale (Legendre & Gallagher, 2001).

The study area has a Mediterranean climate characterized by high interannual variability and so pair-wise differences in species abundance, richness and diversity between sampling years were tested using Kruskal-Wallis test. We then calculated (1) coefficients of variation (i.e. the ratio of the standard deviation to the mean) for species richness, Shannon's Diversity and species abundance in order to quantify the interannual variation in the Lepidoptera community, and (2) tested the habitat effects (tree age, canopy size, and connectivity) using general linear models. To check any potential effect for tree age, canopy size, and connectivity on the total and/or relative abundances of each Lepidoptera family, we carried out simple linear regressions. Finally, we also explored the relationship between LAI and tree age using linear regressions.

All statistical analyses were performed in R, version 3.0.3 (R Development Core Team, 2011).

2.3 Results

2.3.1 Description of the Lepidoptera community

In all, 1336 individuals belonging to 24 morphologically recognizable Lepidoptera species were collected from the 23 study trees during the five years in which they were sampled. The species belonged to the Noctuidae, Tortricidae, Pyralidae, Geometridae, Lycaenidae and Drepanidae families (see Appendix A, Table A.1), of which the Noctuidae were the most important in terms of the number of species (13). Seventeen species (80% of the total individuals gathered) were *Quercus* spp. specific leaf-feeders, while the other seven can potentially feed on other genera; however, at the study site all behave as specialists since no other woody species are present. During the five-year period, one third of the species was collected every year, another third was recorded in two, three, or four years, and the remaining third appeared only once. In terms of total abundance, the most abundant species was – by far – the Noctuid *Catocala nymphagoga*, followed by the Yortricid *Tortricodes alternella*, the Noctuid *Dryobotodes eremita,* and the Pyralid *Phycita torrenti* (Appendix A, Table A.1).

2.3.2 Effects of tree age, canopy size and connectivity, on species abundance, richness and diversity

The best models for community characterization were those considering tree age, canopy size, and connectivity plus their second-degree interactions (Appendix A, Table A.4). Results revealed complex interactions between tree age, canopy size, and connectivity that significantly affected Lepidoptera abundance, richness and Shannon's Diversity (Appendix A, Table A.4). For smaller canopies, Lepidoptera communities were more abundant, richer, and more diverse in older trees; nevertheless, the age effect was barely observable in the largest trees (Fig. 2: 1.a, 1.b, 1.c). In consonance with these results, although LAI values increased significantly with age for trees within the smallest size category (R^2=0.13; $F_{1,43}$=7.502; P=0.008), no significant increments were detected within the other two tree size categories (P>0.05). Yet, trees with larger canopies were most influenced by connectivity, i.e. in larger trees, species abundance, richness and diversity increased as the canopies became more connected (interaction tree canopy size and connectivity; see Table 1, Fig. 2: 2.a, 2.b, 2.c), suggesting thus that connectivity may only contribute to determining the Lepidoptera community assembly once the host plant reaches a certain size. Finally, the interaction between tree connectivity and tree age indicates that species richness and abundance – but not Shannon's Diversity – increased in younger trees as they became more connected (Fig. 2: 3.a, 3.b). Models explained 28%, 36%, and 36% of variance in species abundance, richness and Shannon's Diversity, respectively, with the random factor (i.e. year of sampling) accounting for 15%, 24%, and 26% of total variance, respectively. Although Lepidoptera communities were significantly more abundant, richer and more diverse in some years than in others (Fig. 3), tree age, canopy size, and connectivity had no significant effect on this interannual variability since coefficients of variation were unaffected by habitat characteristics (P>0.05).

2.3.3 Effects of tree age, canopy size, and connectivity on species composition

Beta diversity of Lepidoptera is mostly explained by the distance between trees (23%); tree connectivity (4%) explained very little of the composition, and tree age and canopy size had no significant effect on it (P>0.05) (Table 2). Consequently, the redundancy analysis decomposed the total variation in alpha diversity of Lepidoptera and revealed that only 5% of this variation was explained by environmental variables (Appendix A, Table A.5). Thus, no significant relationship between species composition within each tree and tree characteristics (age, canopy size, and connectivity) was detected ($F_{3,19}$=1.425; p=0.214. Appendix A, Figure A.3). When individually exploring the relationship between these variables and the relative abundance of each family (i.e. the individuals recorded in a given family vs. the total of recorded individuals), a significant linear regression is observed for Noctuidae and Pyralidae families vs. tree age (P<0.05). The ratio of Noctuidae is greatest in younger trees, while the opposite occurs in Pyralidae (Fig. 4a and 4b, respectively). Nonetheless, the regression between tree age and the total abundance of these two families was significant only for Pyralidae (Fig. 4c and 4d).

2.4 Discussion

Our study demonstrates that the interplay of tree age, canopy size, and connectivity shapes the species abundance, richness and diversity of the Lepidoptera community inhabiting holm oak (*Q. ilex*) trees in a savannah-like landscape. In addition, it reveals the absence of the same habitat effects (age, size, and connectivity) for species composition (alpha and beta diversity), thereby highlighting the spatial distance between trees as the main – and almost only – predictor of Lepidoptera species turnover. Overall, our models were able to explain a significant fraction of the variance in Lepidoptera species abundance, richness and diversity, as well as in species composition, and thus helps disentangle some of the habitat-related factors that underlie the assemblage of the studied Lepidoptera community.

The community that developed in this savannah-like landscape consists mostly of *Quercus* spp. specialist feeders and has a ‘tail’ of rare species that is characteristic of communities with low evenness (Colwell, 2009). It is clearly dominated by Noctuids, especially the 4-cm wingspan *Catocala nymphagoga* that appears very early in the succession process (i.e. it is very abundant in the youngest trees). This Lepidoptera community also had great interannual variability, with significant differences in species abundance, richness and diversity between consecutive sampling years; as well, a considerable amount of variance was explained by the random factor ‘year’. These two results reveal the high environmental stochasticity of the system – inherent to Mediterranean regions (Lionello *et al.*, 2006) – that our five-year data series detected.

In terms of the hypothesis that predicted differences in species richness, alpha diversity (Shannon’s diversity), and species abundance between holm oak trees of different age, canopy size, and connectivity, our results reveal a significant and complex combined effect of habitat age and structure on community attributes. The interaction between tree age and canopy size – which results in greater species abundance, richness and diversity only in the smallest trees as they age – can be explained by differential canopy encroachment in trees with different architectures. Due to human management activities such as pruning, mature holm oak trees of similar age can have very different canopy shapes and sizes. Pruning removes multiple leaders and encourages the production of new shoots (Attocchi, 2013). In our study area, pruned oak trees with better structured canopies and more new shoots will probably encroach more easily over time than trees with more spreading canopies. Lepidoptera will thus benefit from leaf clustering, either directly or indirectly, because it improves habitat conditions (more food and an adequate microclimate) and decreases caterpillars’ vulnerability to predators (Marquis *et al*., 2002). LAI measurements (our proxy for canopy density and biomass) reinforce this hypothesis since there was a significant positive slope of LAI with age for the group of smallest trees that did not appear for the other size groups. In addition, the time a tree has been standing in the fields may be of greater importance in smaller trees than in larger ones because they are less 'apparent' to Lepidoptera and initial colonization by Lepidoptera will thus place in a staggered fashion. Even though numerous studies have demonstrated that oak foliage quality is a major determinant of the distribution and abundance of oak herbivores (Feeny, 1970; Schultz & Baldwin, 1982; West, 1985; Faeth & Bultman, 1986), we consider it to be highly unlikely that foliage chemical composition can explain variability in Lepidoptera community parameters attributable to tree age given that significant differences in nutritional quality and palatability are only expected between individuals at different ontogenic stages (from saplings to mature and old trees) (Coley & Barone, 1996; Basset, 2001). Furthermore, Gripenberg & Roslin (2005) found that leaf quality variance within different parts of the same tree and between different trees varied little, which means that Lepidoptera see trees as habitat 'islands' of roughly equal average quality.

At our study site, tree canopy size also interacts with tree connectivity, thereby giving greater species abundance, richness and diversity in the largest trees as they become more connected, probably because Lepidoptera perceive a site such as this as a patchy habitat and so colonize connected trees more easily than isolated ones due to limitations in their dispersal abilities (e.g. Nieminen, 1996; Doak, 2000; Ricketts *et al.*, 2001). Even though both large and small tree crowns become more visible and reachable as they grow larger, this increase in the colonization probability, due to a better connectivity, only translates into greater species abundance, richness and diversity in large trees as in the smallest trees resource availability continues to be the main constraint to population dynamics (MacArthur & Wilson, 1967). These results agree with those obtained in similar studies such as those by Müller & Goßner (2007) and Gripenberg & Roslin (2005), which show that the proportion of herbivorous specialists in a given oak increased significantly as the number of oak trees increased in the surrounding area. Finally, the fact that Lepidoptera communities become more abundant and richer in the youngest trees as they become more connected may be due to the existence of colonization credits in the youngest isolated trees caused by random dispersal and differential dispersal abilities (i.e. the species accumulation curve in the youngest trees will not have yet reached the asymptote). These results suggest that in this archipelago of scattered trees the species-area-isolation paradigm of island biogeography (MacArthur & Wilson, 1967) and neutral models (Gyllenberg & Hanski, 1997; Hanski, 1999) is operating because we found greater species abundance, richness and diversity in older connected trees than in smaller isolated ones as the community is constrained mainly by habitat area and the time the tree has been standing (i.e. not long enough to have paid the colonization credit).

We found no ordination of the Lepidoptera species along either age, canopy size, or connectivity ranges that could throw light on whether holm oaks of different age, canopy size, and connectivity harbor different species assemblages. Similarly, the absence of tree age and canopy size effects (P>0.05) and the small effect of tree connectivity on beta diversity (only 4% variance explained) compared to the effect of the spatial distance between trees (23% variance explained) suggest that in our study system the spatial distance between trees overrides variation in their age, canopy size, and connectivity when determining Lepidoptera species composition and spatial distribution. As a result, the local Lepidoptera species assemblage will be strongly affected by random forces (Hubbell, 2001). However, for trees hosting either Noctuids or Pyralids, tree age did partially explain the variance in the relative abundance of these two families (24% and 16%, respectively). This relationship probably arises due to differences in dispersal ability linked to body size (Kuussaari *et al.*, 2014): i.e. Noctuidae, due to their larger body sizes (Sekar, 2012), are often the first to colonize trees, whereas the smaller Pyralids (e.g. 2-cm wingspan *Phycita torrenti*) take longer to reach the target patch and therefore are less well represented in young trees. This hypothesis is supported both by the lack of significance of tree age for the total abundance of Noctuidae (present at roughly equal abundances irrespective of tree age) and its significance for the total abundance of Pyralidae (that reach trees more gradually). Furthermore, this hypothesis is consistent with the colonization time hypothesis that relates species richness with the time a patch has been available for colonization (Borges & Brown, 1999).

This study is the first to address spatio-temporal patterns in the Lepidoptera community assembly in Mediterranean holm oaks (*Q. ilex*) in a study system with a savannah-like landscape. Overall, our results support the importance the interaction between habitat age and structure has in the assembly of a *Quercus*-specialist Lepidoptera community where connectivity effect is modulated by both tree age and size, two important factors that explain differences in species abundance, richness and diversity. The fact that habitat variables poorly explained alpha diversity and that beta diversity was mostly explained by the spatial distance between trees supports the importance of dispersal-based assemblies in this community. Likewise, given that a considerable proportion of variance remained unexplained by our models, niche-assembly processes may also be relevant. This paper provides guidelines for managing holm-oak savannah-like landscapes for diversity conservation and, for example, underlines the importance of maintaining old, large, and well-connected trees as important refuges for Lepidoptera. Moreover, these conservation efforts are likely to benefit other taxa that prey on Lepidoptera or share similar habitat requirements, thereby contributing to the restoration of forest ecosystem functioning.

Acknowledgements

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Fig. 1. Upper image: orthoimage of the study site (Huecas) in 1957. Lower image: orthoimage of the study site in 2014.

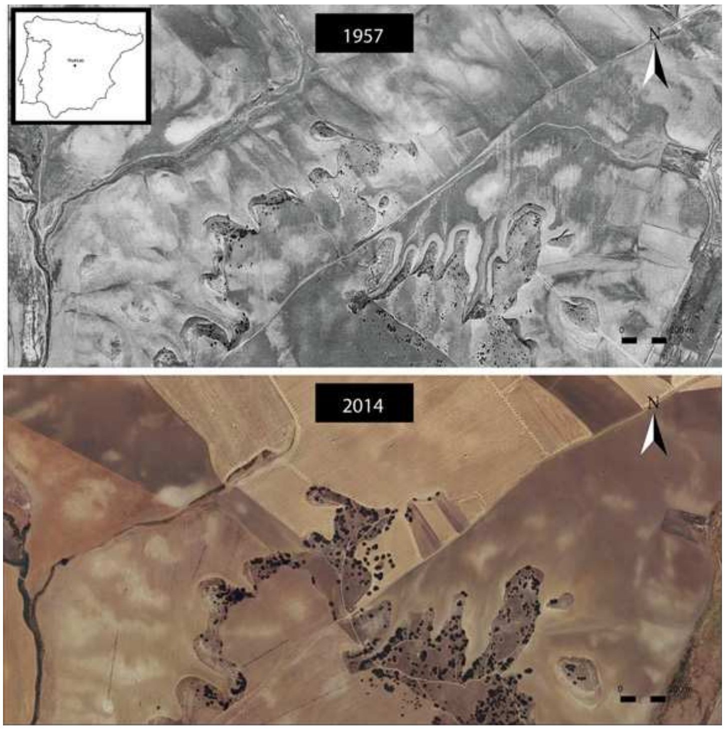


Fig. 2. Predicted effects (mean) of interactions between (1) tree age and tree canopy size; (2) tree connectivity and tree canopy size; and (3) tree connectivity and tree age on (a) species abundance (log scale), (b) species richness, and (c) Shannon's Diversity. Predicted values are based on the fixed effects of the best model (see Appendix A). Tree canopy size categories: Small (12–43 m2), Medium-size (43–79 m2), Large (79–104 m2). Tree age categories: Young (20–43 years), Middle-aged (43–56 years), Old (56–74 years). Units: years, square metres and square metres, for tree age, tree canopy size and tree connectivity respectively.

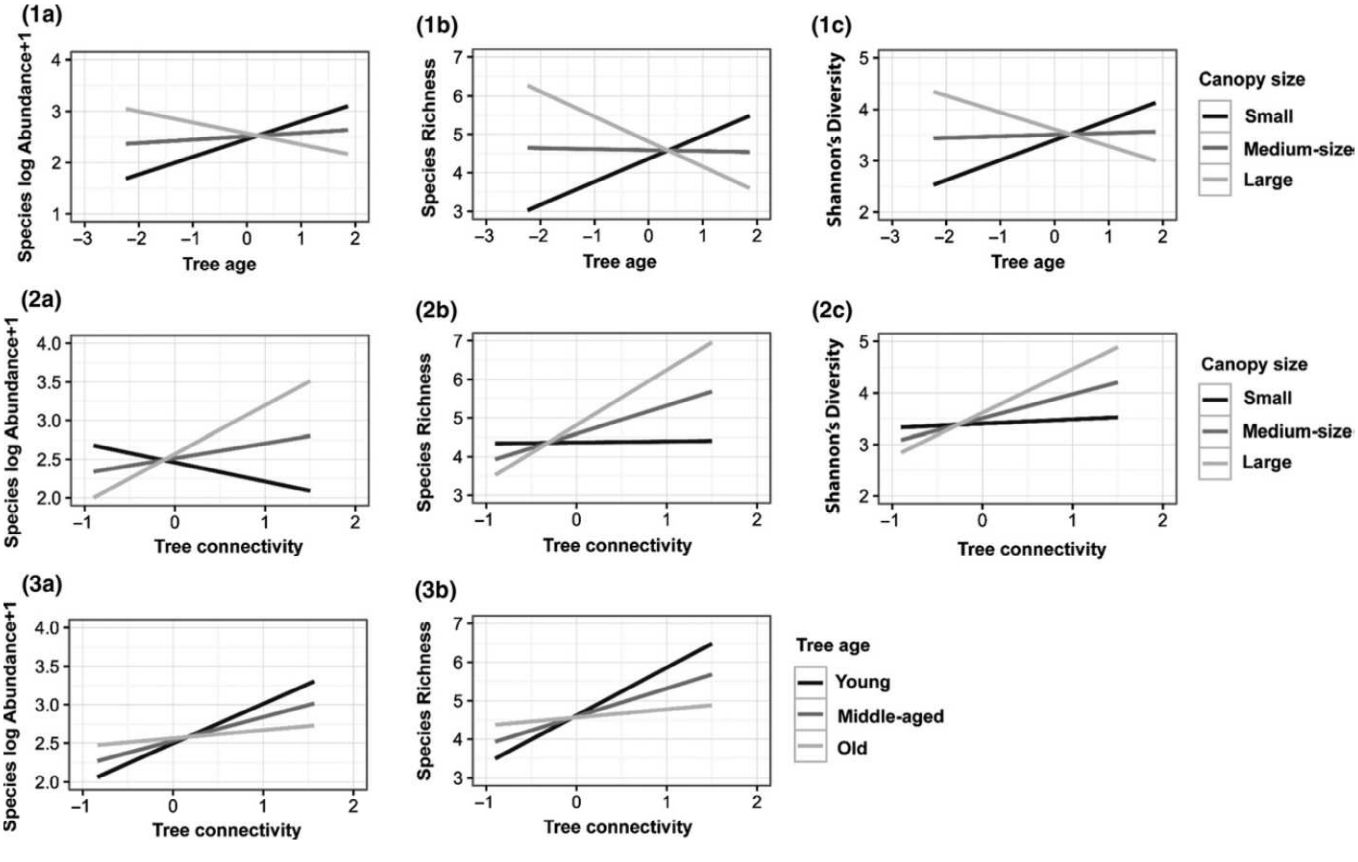


Table 1. Results of best mixed-effects linear models for species abundance, richness and diversity. Values in bold indicate significance at α of 0.05.

	Value	**SE**	**DF**	**t-value**	**P**
Species abundance					
(Intercept)	2.513	0.213	103	11.791	0.000
Size	0.055	0.103	103	0.532	0.596
Age	0.065	0.131	103	0.498	0.619
Connectivity	0.192	0.118	103	1.632	0.106
Size: Age	-0.280	0.106	103	-2.637	**0.010**
Size: Connectivity	0.438	0.119	103	3.665	**>0.001**
Age: Connectivity	-0.277	0.110	103	-2.528	**0.013**
Species richness					
(Intercept)	4.590	0.562	103	8.162	0.000
Size	0.223	0.244	103	0.913	0.363
Age	-0.026	0.310	103	-0.084	0.933
Connectivity	0.729	0.278	103	2.619	**0.010**
Size: Age	-0.625	0.251	103	-2.488	**0.014**
Size: Connectivity	0.700	0.282	103	2.484	**0.015**
Age: Connectivity	-0.517	0.259	103	-1.996	**0.049**
Shannon's Diversity					
(Intercept)	3.508	0.392	103	8.951	0.000
Size	0.105	0.167	103	0.629	0.531
Age	0.031	0.211	103	0.146	0.884
Connectivity	0.466	0.190	103	2.457	**0.016**
Size: Age	-0.359	0.171	103	-2.096	**0.039**
Size: Connectivity	0.386	0.193	103	2.004	**0.048**
Age: Connectivity	-0.307	0.177	103	-1.736	0.086

Fig.3. Boxplot illustrating species log-transformed abundance, richness and diversity (Shannon's Diversity) (1, 2 and 3 respectively) per tree in five sampling years. Different letters above and under bars indicate significant differences (multiple comparison test following Kruskal–Wallis) in the species abundance, richness and diversity of Lepidoptera per tree. Box plots show minimum, lower quartile, median, upper quartile and maximum values.

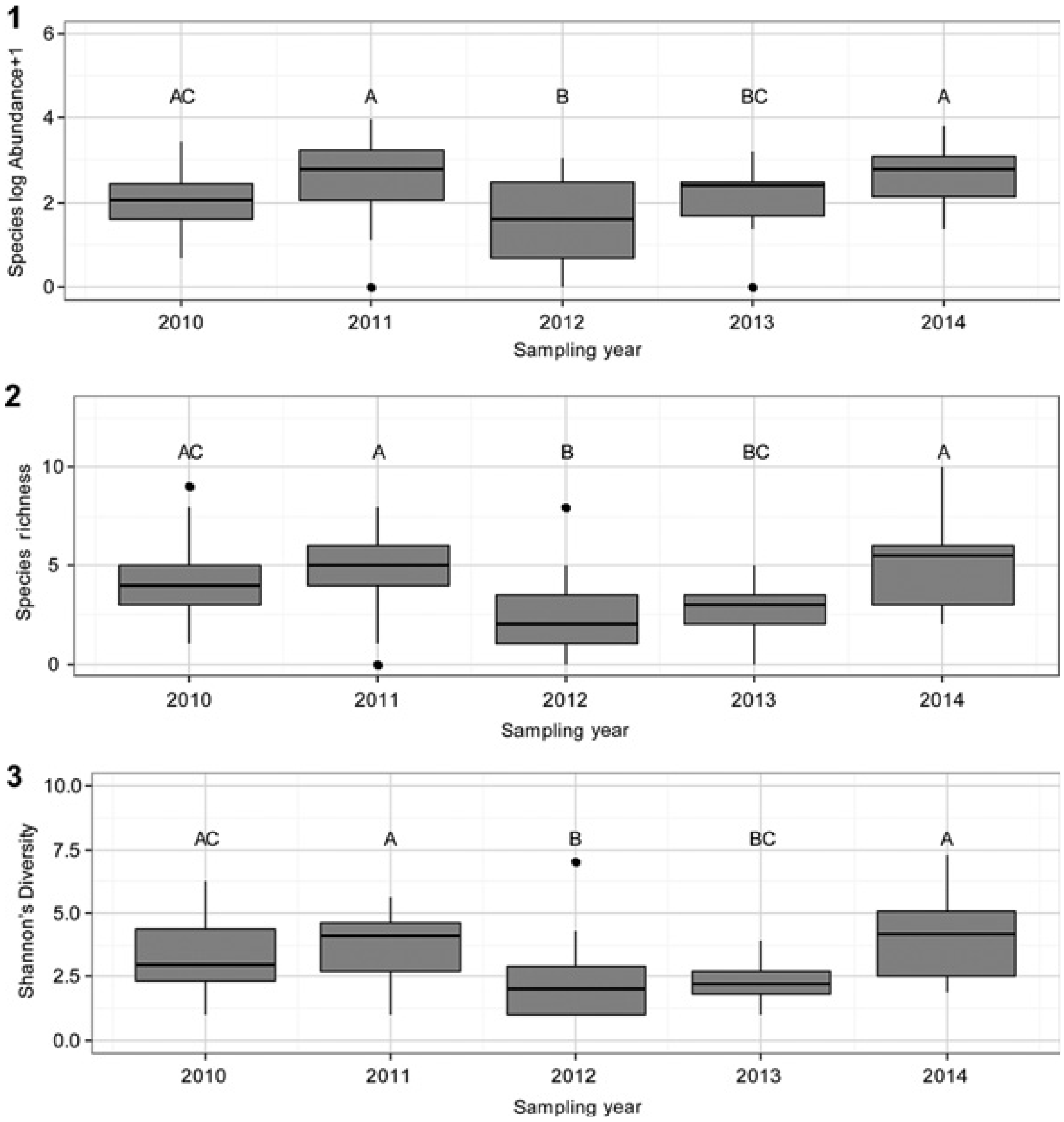
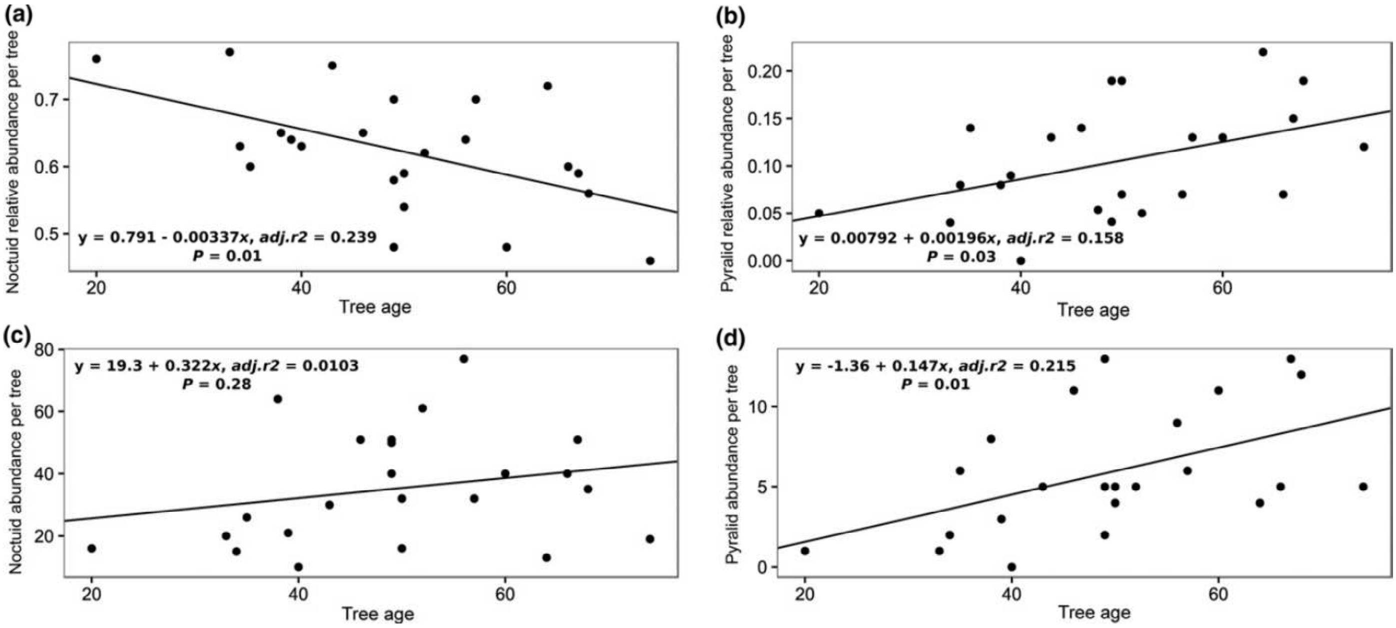


Table 2. Results of linear model for species composition in terms of abundance and environmental distance matrices, with the number of observations corrected as recommended by Qian & Ricklefs (2012). Values in bold indicate significance at a of 0.05.

	Estimate	SE	t-value	(Pr>|t|)
(Intercept)	4.365e-01	1.805e-02	24.177	**<0.001**
Spatial distance	6.988e-05	7.407e-06	9.433	**<0.001**
Tree age	-7.333e-03	7.134e-04	-1.028	0.318
Tree canopy size	-1.913e-04	3.168e-04	-0.604	0.553
Tree connectivity	-5.129e-06	1.068e-06	-4.805	**<0.001**

Fig. 4. Relative mean abundance of Noctuidae (a), relative mean abundance of Pyralidae (b), total mean abundance of Noctuidae (c) and total mean abundance of Pyralidae (d) per tree versus tree age. Regression equation, adjusted R2 (Adj.r2) and significance of tree age (P) are given in bold.



Chapter 3: *Disentangling the effects of host genetic identity, ontogeny and spatial distribution in insect herbivory in expanding oak forests*

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Abstract

Herbivory is expected to be influenced by host characteristics such as genetic characteristics, ontogeny and spatial location. However, the effects of these three factors have been seldom explored altogether neither their interaction. We investigated their impact on herbivory by insects on Holm oak *(Quercus ilex* L.) in spontaneously established forests after cropland abandonment. These *naïve* forests are an ideal system to study the assembly of plant-animal interactions as their genetic and age structure has not been yet significantly altered by forest management. We sampled *Q. ilex* individuals from seedlings to mature trees within forest patches and determined their location, age, genetic variability by means of SNP amplification and leaf herbivory damage. We identified two direct factors influencing herbivory: i.e. 5 genetic clusters with differences in susceptibility and a significant edge effect with damage being greater at the forest patch core compared to the edge. Conversely to the previous factors, oak ontogeny did not show any significant effect *per se* but in interaction with intra-specific connectivity: i.e. saplings and sub-dominant trees, but not seedlings, had lower leaf damage in the vicinity to mature trees. Our findings indicate that some host characteristics (i.e. genetics and tree location in the forest patch) may have direct and independent effects on herbivory while associational resistance, potentially mediated by plant-plant communication or differences in plant apparency, only occurs above a certain host size. In addition, these results shed light on how plant-animal interactions are assembled in new habitats and their potential consequences for forest expansion.

Chapter 3: *The role of host genetics, ontogeny and location on herbivory*

Keywords: edge effects, herbivory, holm oak, new forests, plant-insect interaction

3.1 Introduction

Plant-animal interactions are among the main processes shaping biodiversity (Valiente-Banuet et al. 2015). As interactions with herbivores have great impact on the survival, reproduction and recruitment of plants (Maron & Crone, 2006), they can largely affect ecosystem dynamics and services (Maguire et al. 2015). The intensity of herbivory in a host is modulated by intrinsic and extrinsic factors. Among intrinsic traits, genetic identity (Castagneyrol et al. 2012) and ontogeny (Moreira et al. 2017) have been observed to be relevant characteristics influencing host susceptibility. In addition, herbivory may be also mediated by extrinsic factors such as the spatial location (associational effects) of the host from conspecifics (Fernandez-Conradi et al. 2017), heterospecifics (Guyot et al. 2016), or in relation to the habitat characteristics (i.e. core area vs edge) (Régolini et al. 2014).

Susceptibility to herbivory can vary among host genotypes owing to differences in the expression of phenotypic traits affecting insect feeding preference such as architecture, trichome density, leaf toughness, nutrient or secondary chemical content (Barbour et al. 2015). Because herbivory can play a strong selective pressure, it has been suggested that natural selection may act on particular loci maintaining allele combinations that maximize fitness of individuals by providing these defense traits (Züst and Agrawal 2016). In this line, some studies have found a positive relationship between inter-individual resistance to herbivory and the number of heterozygous loci (Mitton and Jeffers 1989). However, the degree of herbivory damage suffered by a particular individual may not only depend on its particular genetic characteristics but be also influenced by the genetic diversity of neighbours (Barbosa et al. 2009; Barton et al. 2015). Yet, there is still a large disagreement among studies in the direction and strength of these associational genetic effects on herbivory. Genetic diversity of conspecific neighbours has been observed to increase susceptibility to herbivory by increasing host location and spill over of herbivores (Castagneyrol et al. 2012), to have neutral effects (Moreira et al. 2014) or increase resistance to damage owing to the negative impact in some groups of insects (e.g. chewing herbivores and gall makers in Barton et al. 2015). In addition to genetics, inter-individual differences in herbivory may be associated with shifts in defense traits during ontogeny (from seedlings to mature plants). Two hypotheses have been proposed to explain the potential variations that physical (e.g. leaf toughness) or chemical defenses (e.g. alkaloids) may experience throughout the lifespan of a plant: i.e. the plant-age and the growth-differentiation balance hypotheses. The plant-age hypothesis predicts a larger investment in defenses by young plants compared to older ones due to the higher risk and more negative effects they may suffer from herbivory owing to their reduced size (Kotanen and Rosenthal 2000). In contrast, based on the existence of resource constraints, the growth-differentiation balance hypothesis states that mature plants will be better defended owing to the existence of a growth vs. defense trade-off in the young ones (Herms and Mattson 1992). Interestingly, as for the role of genetic diversity on herbivory damage, these hypotheses have not been conclusively confirmed as it has been observed that plant defense traits may increase (e.g. Schaffner et al. 2003; Elger et al. 2009) or decrease (e.g. Goodger et al. 2006) during ontogeny. Consequently, some studies have reported a reduction in the susceptibility to herbivory with ageing (Moreira et al. 2017) while others have reported a larger damage in juveniles (Barton and Hanley 2013).

Contrasting results in studies analysing the role of genetic diversity and ontogeny in herbivory might arise from the fact that there have been little attempts to analyse both factors altogether in natural populations neither to evaluate also their interaction with plant location. Indeed, extrinsic factors such as the spatial distribution of hosts may also greatly affect herbivore’s activity, by altering the likelihood of a plant to be damaged according to the characteristics of neighbours. Associational resistance or susceptibility to herbivory are plant-plant interactions in which herbivory rates may respectively decrease or increase owing to the influence of defensive or appealing characteristics of the neighbouring plants (Barbosa et al. 2009). Plants in the nearby may either act as a deterrent, repelling herbivores from the area, or as a sink because of its greater palatability, leading in both cases to associational resistance for the focal plant. However, plants acting as sinks may also occasionally lead to associational susceptibility, as insect populations growing on suitable hosts often disperse to nearby trees. Interestingly associational effects have been observed to exist related to both characteristics: i.e. genetics and ontogeny. On the one hand, herbivory in a focal plant may depend on genetic characteristics of plants in the vicinity influencing palatability, volatile signals or differences in architecture (Marquis et al. 2002; Karban et al. 2006; Fernandez-Conradi et al. 2017). On the other hand, the developmental stage of a plant may influence its “apparency” compared to neighbours and, ultimately affect herbivory damage (Boege 2005; Castagneyrol et al. 2013; Moreira et al. 2017). Unfortunately, little research has been conducted on this sort of potential interactions between genetics, ontogeny and plant-plant location. In addition to these effects, host susceptibility may change according to the position in the habitat (e.g. core vs. edge), owing to changes in leaf palatability (Onoda et al. 2008) driven by differences in environmental characteristics (e.g. Guirado et al. 2006), the ability of herbivores to detect the host (Régolini et al. 2014) or herbivore abundance influenced either by environmental conditions (McGeoch and Gaston 2000) or by the presence of insect predators (Skoczylas et al. 2007) or parasitoids (Pare and Tumlinson 1999). Yet, there seems to be no consensus of forest edge effects on plant-herbivore interaction as some studies have reported a greater incidence of herbivory in forest edges because generalist herbivores benefit from favourable microenvironmental conditions, greater availability of high-quality food, and a weaker control from natural predators while others indicate less herbivory damages at the forest edge (e.g. see a review by Wirth et al. 2008). Once again, differences among studies might be partly arise from neglecting the other above-mentioned traits influencing herbivory: i.e. host genotype, ontogeny and their interaction (but see Barrett and Agrawal 2004).

The present study aims at disentangling the effects of intra-specific genetic variability, plant ontogeny, and host spatial distribution on leaf herbivory in Holm oak (*Quercus ilex* L.; Fagaceae) individuals within forest patches spontaneously established on abandoned croplands. These "new oak forests" offer an ideal study system to analyze the referred questions as their community composition, genetic characteristics and size structure has not been yet significantly altered by human influence (e.g. forest logging). Moreover, while there is abundant literature about the effects of forest fragmentation in plant-animal interactions, it has seldom been explored how they become assembled in new habitats as those originated by recent land use changes (e.g. Basnou et al. 2016) and how these interactions can influence the process of forest expansion itself (but see Ruiz-Carbayo et al. 2018 for granivorous insects). Previous studies have provided evidences that in these "new oaks forests" the community of insect herbivores can change from individual to individual depending on their age, canopy size and conspecifics connectivity (Ruiz-Carbayo et al. 2017) and other intrinsic host traits (e.g. for granivorous insects: seed size in Ruiz-Carbayo et al. 2018 and seed number in Bogdziewicz et al. 2018). In light of these results we predict that herbivory will be mediated by the interaction among genetics, ontogeny and location. We hypothesize that: (i) herbivory extent will be modulated by genetic variability, being higher in homozygous oaks (ii) younger trees will suffer from more intense damage compared to older trees because of their lower size and resource allocation to produce defenses; and (iii) both genetic and ontogeny effects will interact with the host location, with increasing herbivory in the vicinity to more mature trees and far from forest edges due to higher herbivore spillover and more suitable environmental conditions in the forest interior.

3.2 Material and Methods

3.2.1 Study area

This study was carried out in the Vallès lowland (Barcelona, Spain, 41° 33′N, 2° 2′E) (see Fig.1). Climate in the region is Mediterranean, annual rainfall averages 650 mm and mean temperatures range from 6°C in winter up to 23°C in summer. The Vallès lowland offers an ideal scenario to conduct spatiotemporal studies on plant-animal interactions because it includes a large number of new forest patches established in former croplands still embedded in a matrix of agricultural areas and pastures (Ruiz-Carbayo et al., 2018). Many of these forest patches have been successfully colonized by *Q. ilex* (Guirado et al. 2008) with the presence of different life-history stage classes: i.e. adult trees, saplings and seedlings. *Q. ilex* is a widespread oak species in the Mediterranean Basin (Terradas 1999). Previous studies have reported that this species host a large array of specialist phytophagous insects predating upon leaves including more than 25 Lepidoptera species (see Ruiz-Carbayo et al. 2017 for a comprehensive species list). Additionally, in this species a high allelic richness has been reported, and this genetic variability could influence leaf palatability among genotypes and, therefore, affect also host selection by herbivore insects (see Schädler et al., 2003).

3.2.2 Experimental design and sampling

By means of comparing 1956- and 2005-orthoimages available for all Barcelona province, we identified forest patches emerged after cropland abandonment in 1956 (hereafter “new forests”), and selected three of them with a mean distance of 27 Km among them (hereafter Cardedeu, Martorell, Cervelló). In order to carry out a demographic sampling as balanced as possible, we established five size categories that may also roughly correspond to different developmental classes (Class 1: Seedlings less than 20 cm in height. They are commonly seedlings recently established still bearing their cotyledons; Class 2: Seedlings 20-50 cm in height. Normally, two or more years old seedlings fully independent from the cotyledon reserves; Class 3: Saplings between 50-130 cm in height. Individuals moving from the seedling to the sapling stage often requiring environmental conditions suitable for establishment (e.g. increase in PAR) to continue their development; Class 4: Saplings more than 130 cm height but less than 5 cm dbh. Suppressed or sub-dominant saplings and, Class 5: individuals with dbh equal or greater than 5 cm. Adult trees with fully expanded canopies in the top layer of the forest (see Espelta et al., 1995, Zavala et al., 2011 for more information about the characteristics of these stage classes). We sampled a total of approximately 200 individuals in each site along parallel transects (see Appendix B, Table B.1), and a sub-sample of ca. 30 leaves were randomly gathered from each individual. Each individual was geolocated using a global positioning system (GARMIN GPSMAP 62st). For an accurate determination of the age, we harvested seedlings and saplings to get a trunk disc, or extracted a core from the trunk using a Pressler borer in adult oaks in order to apply the tree-ring dating technique later on the laboratory (see Gené et al., 1993).

To assess leaf herbivory damage, once in the laboratory we allocated each randomly selected leaf into a category of damage according to the percentage of area missing caused by leaf chewing by the herbivore caterpillars (category C0 = 0%, C1 = 1–5%, C2 = 6–25%, C3 = 26–50%, C4 = 51–75%, C5 = 76–100%). The estimation of the missing area was performed by the naked eye always by the same observer. Then, we quantified the herbivory pressure exerted by caterpillars upon each tree by calculating a herbivory index (H) after (Benitez-Malvido et al. 1999) as follows:

$$H = \sum ni(Ci)/N$$

Where i is the damage category, ni is the number of leaves in the ith damage category, Ci is the midpoint of each category (i.e. C0 = 0%, C1 = 3%, C2 = 15.5%, C3 = 38%, C4 = 63%, C5 = 88%), and N is the total number of leaves sampled per tree. In an attempt to standardize the value of herbivory across individuals with different size (from seedlings with only a few leaves to mature trees with voluminous crowns) we weighted the herbivory index by the diameter-at-breast height (dbh).

3.2.3 Data analysis

Connectivity to mature oaks of every individual in the sample (from seedlings to mature oaks) was estimated based on the Euclidean distance matrix calculated among individuals within the same forest patch using the *distance* function of the package *ecodist*. We chose the simplest Euclidean distance because of the short distance to be covered by insects from one tree to another (always <150 m) and the fact that dispersal is considered at the intra-patch level, with a homogeneous environment. For each individual, we averaged distances of the matrix to every mature tree within the patch, and used the inverse of this new variable as a proxy of connectivity. For the calculation of the minimum distance to the forest edge, we rasterized the vector layers containing the geolocation of the trees and the polygons of the sampling patches using packages *rgeos*, *rgdal* and *raster* in R, version 3.4.1. (R Core Team 2017). On the resulting raster layer we assigned null values to all forest pixels as well as a value of 1 to every non-forest pixel. We then calculated the minimum distance from the cell containing the oak coordinates, to the nearest non-forest cell using the *gridDistance* function within the *raster* package.

To determine the genetic characteristics of individuals (i.e. heterozygosity percentage, loci under selection, and genetic clusters) we used SNPs (Single Nucleotide Polymorphisms) information for all oaks. 66 SNP loci were identified, combined into a multiplex and sequenced on an iPLEX Gold genotyping kit (Sequenom, San Diego, CA, USA) at the Genomic and Sequencing Facility of Bordeaux (France) following the procedure described in (Chancerel et al. 2013). In order to obtain the heterozygosity percentage of each individual we divided the number of heterozygous loci by the total number of amplified loci and to identify candidate genes under selection that might be related to herbivory resistance/susceptibility, we used the software Lositan (Antao et al. 2008). The rationale behind the genetic model used by this program is the assumption of unusual low or high levels of heterozygosity in loci that have been influenced by natural selection. This differentiation can be quantified using the statistic F_{ST} and visualized in a plot of F_{ST} *vs*. heterozygosity using a null distribution generated by the genetic model (Beaumont and Nichols 1996). Genetic origin of each oak was determined by means of the *find.clusters* function of the *adegenet* package (Jombart et al. 2010). This function transforms data using a principal component analysis (PCA) and then, runs successive K-means with an increasing number of clusters (k). The goodness of fit measure selected was the Bayesian Information Criterion (BIC). The identified genetic clusters (5) were then included in the linear mixed models as a 5-level factor in order to test the effect of intra-specific genetic variability on herbivory patterns. Finally, to assess genetic structure partitioning among and within oak populations we performed analyses of molecular variance (AMOVAs) using ARLEQUIN software (Excoffier et al. 2005).

3.2.4 Statistical analysis

Prior to run the statistical analyses we checked our continuous predictors (distance to mature oaks, minimum distance to forest edge, oak age and percentage of heterozygosity) for collinearity using Pearson's correlation coefficients. As the highest r2 value was found for oak age and % heterozygosity (r2 = .60), we did not drop any explicative variable from the analysis.

Looking for the best goodness of fit, we built both, a generalized linear mixed model including the sampling site as a random term, as well as a simpler generalized linear model with no error structure. Since the AICc of the null model including random structure was lower in more than two units compared to the AICc of the generalized linear model (AICcglmm= 4148.633;

AICcglm= 4166.624) we performed generalized linear mixed models to test the effects of oak genetic variability, age and spatial distribution on the herbivory pressure including forest patch as a random factor. Once the presence/absence of random structure within the null model was stablished, we built a saturated model with the function *nb.glmer* as implemented in the R package *lme4* with the weighted herbivory index as a response and all fixed effects (genetic cluster, % heterozygosity, oak age, minimum distance to forest edge and connectivity to mature oaks) plus second-degree interactions. Model selection was performed based on the second-order Akaike Information Criterion (AICc) and adjusted R^2 (Appendix B; Table B.3).

The genetic structure of the oak populations was investigated by means of a Discriminant Analysis of Principal Components (DAPC) within the *adegenet* package. DAPC allows linear combination of alleles to perform as discriminant functions, therefore computing allele contributions and allowing inference of structure among genetic clusters. All data treatment and data statistical analyses were performed in R, version 3.4.1. (R Core Team 2017).

3.3 Results

The main characteristics of the forest patches surveyed and the level of herbivory per stage class are summarized in Table 1. The three forests showed a similar age structure (i.e. from 2 yr seedlings to 20-25 yrs adult trees) and a similar level of heterozygosis in the different stage classes (c.a. 20-25% except the highest values of the seedling class in Cardedeu). Concerning herbivory, in all forest patches the herbivory index never exceeded 15 and decreased in elder stage classes (Table 1). Following the maximum parsimony principle, the number of clusters that best explained the genetic structure present in our study sites were five clusters with a BIC value of 1551.524 (Appendix B, Table B.1). The high proportion of successful reassignment (based on the discriminant functions) of individuals to their original clusters, indicated clear-cut clusters. Concerning the three forest patches, Clusters 1, 2 and 3 were present in all three study sites (Cardedeu, Martorell and Cervelló), while cluster 4 was more abundant in Martorell and specially Cervellò. Finally, cluster 5 was exclusively present in the most distant study site, Cardedeu (except for a single individual in the other two sites). According to the AMOVA analysis, there were significant genetic differences attributable to age category (Appendix B, Table B.2). Nevertheless, in Cervellò and Martorell the percentage of variation in the number of different alleles among oaks of different age was less than 0.1%. Conversely, in Cardedeu, a significant amount of genetic structure was found among age categories within the patch (29.8%), as a consequence of the high values of pairwise F_{ST} (genetic differentiation) between the youngest seedlings (Stage class 1) and the rest of classes (pairwise $F_{ST} \geq 0.4$, $p<0.001$).

The best model for herbivory intensity was the model including genetic cluster, oak age, minimum distance to forest edge, connectivity to mature oaks, besides the interaction of age and connectivity (Supplementary material Appendix 2, Table 3). Best model revealed the importance of intra-specific differences in genetic lineages on herbivory resistance, with genetic cluster 3 (present in the three forest patches) being significantly less affected by herbivores than cluster 1 and 5 ($z = -3.101$; $P = 0.0136$ and $z = 2.928$; $P = 0.0235$, respectively; see Figure 2). Moreover, Lositan analysis, revealed the existence of four loci under positive selection (outlier loci that show lower F_{ST} compared to neutral expectations) and nine under balancing selection (excessively high F_{ST}), neutral and positive selection (Table 2), supporting the importance of the intra-specific genetic variability.

Concerning distance to forest edge, oaks located at the core of the forest patch exhibited more herbivory damage than oaks at the edge ($z = 2.185$; $p < 0.0289$). In addition, to this effect of location there was a significant interaction between oak age and connectivity to mature oaks ($z = -3.931$; $p < 0.001$), revealing lower levels of herbivory exclusively in elder stage classes as they were more connected to mature oaks (Fig. 3); however this interaction needs careful interpretation since most data are in the lower extreme of the connectivity gradient.

3.4 Discussion

By analyzing the combined effects of genetic variability, ontogeny, and spatial distribution of hosts in the herbivory susceptibility of *Q. ilex* individuals, we could detect that genetic differences had a direct effect on the damage by herbivore insects, irrespective of plant developmental stage and plant to plant distance, while the effect of these two other factors resulted in an associative resistance to herbivory in individuals closer to mature oaks but only above a certain developmental stage threshold (interaction age x distance). In addition, irrespective of genetic characteristics and size, herbivory intensity was directly influenced by the distance of the focal plant to the forest edge, suggesting that the shape and size of these recently established forest patches may be highly relevant in driving the outcome of plant-insect interactions (see for the composition of the Lepidopteran community Ruiz-Carbayo et al. 2017) and, ultimately, for the dynamics of *Q. ilex* establishment (see for acorn predation Ruiz-Carbayo et al. 2018).

The contrasting herbivory levels observed among the identified genetic clusters suggests that genetically-based chemical and/or structural traits likely affect palatability and susceptibility to herbivores within these oak populations (see Ito and Ozaki 2005; Castagneyrol et al. 2012 for other *Quercus spp.* and Silfver et al. 2009 for birch). Therefore, insect herbivory might be an important pressure favoring a particular genetic cluster (i.e. Number 3) in the assembly of these new oak populations and their future genetic composition. Population genetics theory predicts an increase in the fitness of individuals carrying a higher number of heterozygous loci, and many empirical studies have reported a positive effect of genetic variation on plant resistance to herbivory (Mopper et al. 1991; Bello-Bedoy and Núñez-Farfán 2011). However, contrary to this prediction, herbivory resistance in *Q. ilex* was not related to the percentage of heterozygosity, since this variable was not retained in the most adequate model in our study. This is corroborated by the low number of loci under balancing selection found, that is, loci with unusual high levels of heterozygosity. Since most of the loci considered for this study have been under neutral selection, there may be no advantages, either physiological or energetic associated to being either homozygous or heterozygous against herbivore's attack. Still, the genetic model identified a few candidate loci for herbivore resistance under selection (Appendix B, Fig. B.1), either balancing or positive, that would help explaining the differences among genetic clusters on plant susceptibility to herbivores.

We found significant changes in herbivory damage of *Q. ilex* leaves depending on plant developmental stage as previously described for other species (Del-Val and Dirzo 2003; Boege and Marquis 2005; Moreira et al. 2017). Yet developmental stage, only modified herbivory intensity in interaction with tree connectivity to mature oaks, indicating that the predictions made by the plant-age hypothesis or the growth-differentiation balance hypothesis may be strongly context-dependent and change. Previous studies suggest that in the case of associational susceptibility among neighbors due to herbivore spillover as part of their host-seeking behavior (Plath et al., 2012), whereas associational resistance hypothesis argues that emission of chemical compounds (e.g. VOCS) might promote attraction of predators and parasitoids and favor defense reactions in con-specific neighbor individuals (e.g. Barbosa et al., 2009). In the case of our new *Q. ilex* forests, the observation of oaks being less affected by herbivores nearby mature trees supports the associational resistance hypothesis, suggesting some sort of short-distance plant-plant communication mechanism from elder individuals, as previously reported by other authors (see Heil 2014 for a review on plant-plant communication). For example, Dolch & Tscharntke (2000) proved that defoliation of alder (*Alnus glutinosa* L.) by leaf beetles caused a local change of herbivore's attack on nearby plants, but especially on those located within a few meters of the damaged tree. In a similar study, sagebrush plants (*Artemisia tridentata* Nutt.), experienced less damage by herbivores only within 60 cm of plants subjected to leaf experimental clipping (Karban et al. 2006). Yet, the fact that the observed associational resistance of individuals when established close of mature oaks did mostly occur above a certain developmental stage (Connectivity to mature oaks * Oak age interaction in Tables 3), suggest that in smaller oaks the main characteristics driving their higher susceptibility to herbivory owing to more palatable leaves in a less dense canopy (see Ruiz-Carbayo et al. 2017 for similar results in terms of abundance of folivorous Lepidoptera in oaks related to age and connectivity, Fenner et al. 1999; Pearse 2011). However, the fact that we know neither the identity of the insect herbivores causing the damage nor their density does not discard that the observed differences in herbivory among developmental stage might be caused by differences in “apparency” or by hosting a distinct community of herbivores (see Castagneyrol et al. 2013; Haase et al. 2015).

A third relevant result is that herbivory damage in our new forest patches was higher in trees standing at the forest core compared to those located near the edges (see also McGeoch & Gaston, 2000, Skoczylas et al., 2007; Régolini et al. 2014). Several alterations in microclimatic conditions in forest edges such as reduction in air humidity, greater temperature variability, higher light exposure, and wind turbulences, may affect herbivore activity when compared with conditions at the forest interior (Laurance et al., 2002,). Moreover, differences in microclimatic conditions in forest edges, may result in higher leave toughness and lower leaf nutritional quality (Baraza et al., 2004; Barber and Marquis 2011) in comparison to more shaded leaves, especially in plants with carbon-based defenses (Dudt & Shure, 1994) In addition, edge effects are also expected to alter enemy abundance or behavior, by means of parasitoid signaling by VOC emission (Staudt and Lhoutellier 2007) that may be more intense in oaks benefiting from full-light regimes and consequently, at forest edges. Finally, in the case of larger predators, such as insectivorous birds, edges would provide maximum access to prey, making foraging for Lepidoptera larvae much easier compared to foraging at forest interior (Skoczylas et al. 2007).

Even though deforestation is still a major threat for biodiversity conservation, spontaneous establishment of forests in former cropland areas is a widespread phenomenon in some regions of the Northern Hemisphere (Meyfroidt and Lambin 2011). As plant-animal interactions are particularly affected by landscape changes there have been reported serious impacts on them owing to differential extinction of the concerned species mediated by habitat loss (Spiesman and Inouye 2013). Conversely, it is much more unknown how these interactions are assembled in new habitat patches, and how they can drive the dynamic of habitat recovery (see Basnou et al., 2016). Yet, recent studies have already demonstrated the importance of host traits at the individual level in modulating plant-insect interactions assembly in new expanding habitats. For example, Ruiz-Carbayo et al., 2017 reported complex interactions among oak age, size and connectivity in the Lepidoptera community present in oaks established in agricultural landscapes while Ruiz-Carbayo et al., 2018 revealed higher acorn predation driven by changes in acorn size in oaks highly connected in new established forest patches. In that sense, our results underscore the straightforward importance of *Q. ilex* genetics and location within the patch in modulating herbivory by insects in newly established patches. In addition, we provide evidences of the existence of associational resistance mediated by the interactive effects of developmental stage and connectivity and an influence of the forest shape/size with lower herbivory at the forest edge. Future research should be aimed to assess whether the differences in herbivory reported in this study may help to explain why some species, as *Q. ilex*, exhibit a somehow arrested recruitment in new established Mediterranean forests in comparison to other species that have already exhausted their colonization credits (Basnou et al., 2016, Stricker & Stiling, 2012).

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Figure 1. Location of the study site. Top left: situation of the region where the study was carried out in the Iberian Peninsula. Bottom left: landscape context for one of the sample locations. Note the heterogeneous mosaic of forest patches interspersed with agricultural and urban patches. Centre and right: historical and current view of one of the replicates as an example of the demographic sampling. Description of stage classes: Class 1: Seedlings less than 20 cm in height. They commonly represent recently established seedlings still bearing their cotyledons; Class 2: Seedlings 20-50 cm in height. Normally, two or more years aged seedlings fully independent from the cotyledon reserves; Class 3: Saplings between 50-130 cm in height. Individuals moving from the seedling to the sapling stage often requiring environmental conditions suitable for establishment to continue their development; Class 4: Saplings more than 130 cm height but less than 5 cm dbh. Supressed or sub-dominant saplings and, Class 5: individuals with dbh equal or greater than 5 cm.

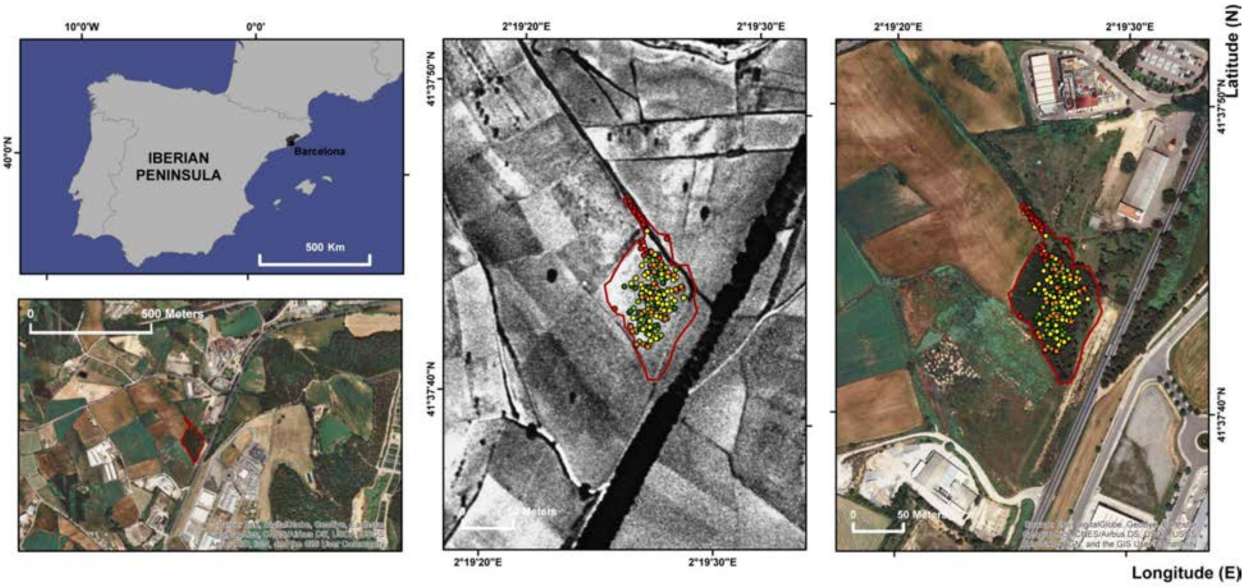

Figure 2. Leaf damage by lepidoptera on different *Q. ilex genetic* clusters identified by successive K-means. Boxplots represent median, 25th, and 75th percentiles, respectively. Different letters mean significant differences between genetic clusters. Means are represented by empty dots while outliers are depicted as black dots.

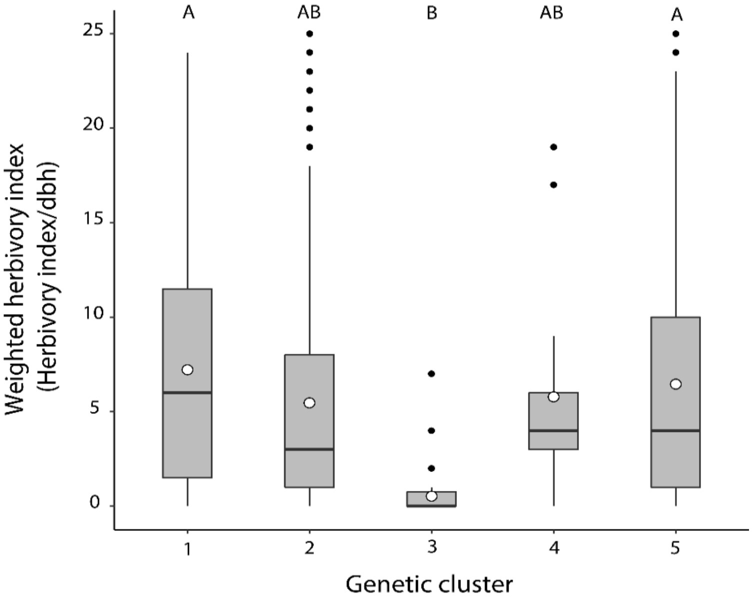

Figure 3. Interaction plot for the effects on herbivory damage of oak developmental stage (i.e. age) of the focal plant and the connectivity to mature oak trees. Connectivity (m^2), age (years) and herbivory index (dimensionless) are centered and scaled. Herbivory scale ranges from blue (low herbivory) to red (high herbivory) colors.

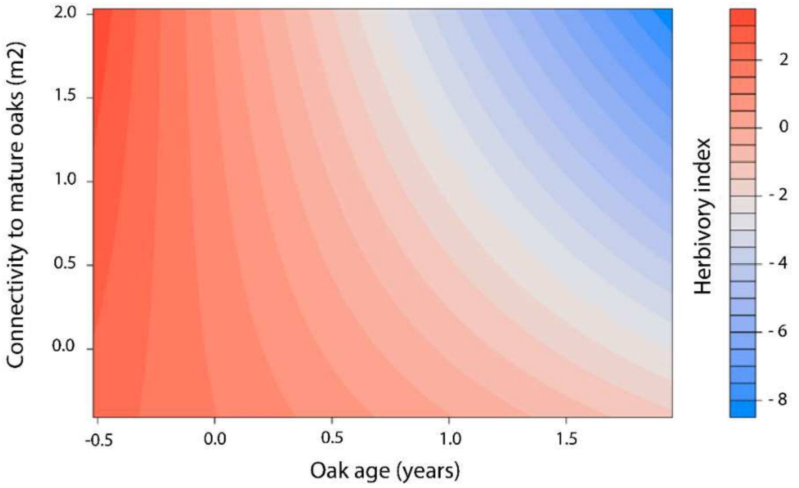

Table 1. Summary table of oak characteristics and herbivory damage per stage class at the three forest patches (Cardedeu, Martorell and Cervelló). Description of stage classes: Class 1: Seedlings less than 20 cm in height; Class 2: Seedlings 20-50 cm in height; Class 3: Saplings between 50-130 cm in height; Class 4: Saplings more than 130 cm height but less than 5 cm dbh; Class 5: individuals with dbh equal or greater than 5. Numeric variables are expressed as means ± standard deviation. Units of measure are indicated in brackets. % Heterozygosity refers to single nucleotide polymorphism (SNP) loci. Herbivory index is a dimensionless variable calculated after (Benitez-Malvido et al. 1999) as a proxy of herbivory pressure exerted by caterpillars upon each tree (See Material and Methods for further explanations).

Site	**UTM_X (long)**	**UTM_Y (lat)**	**Stage class**	**Age (years)**	**% Heterozygosity (% heterozygous loci/ total loci)**	**Min distance to edge (metres)**	**Connectivity to mature oaks (m^2)**	**Herbivory index**
Cardedeu	413672.565	4583290.7	1	2.9 ± 1.6	0.21 ± 0.06	11.33 ± 4.99	0.0116 ± 0.0053	15.04 ± 15.81
Cardedeu	413669.974	4583291.7	2	4.9 ± 1.7	0.22 ± 0.05	12.78 ± 6.29	0.0116 ± 0.0056	14.17 ± 15.30
Cardedeu	413672.714	4583289.6	3	7.9 ± 2.9	0.20 ± 0.07	12.39 ± 5.77	0.0128 ± 0.0068	7.16 ± 4.72
Cardedeu	413690.867	4583314.7	4	13.4 ± 5.3	0.22 ± 0.07	16.00 ± 5.01	0.0133 ± 0.0044	5.71 ± 7.17
Cardedeu	413691.194	4583316.7	5	23.0 ± 5.9	0.25 ± 0.05	17.04 ± 5.38	0.0386 ± 0.0097	0.12 ± 0.33
Martorell	443704.362	4608785.4	1	2.4 ± 1.2	0.29 ± 0.11	21.54 ± 8.40	0.0040 ± 0.0004	10.09 ± 8.96
Martorell	443706.723	4608779.7	2	4.7 ± 1.5	0.26 ± 0.10	21.16 ± 9.84	0.0041 ± 0.0004	7.45 ± 6.38
Martorell	443706.547	4608784.4	3	10.7 ± 3.0	0.24 ± 0.08	22.42 ± 9.11	0.0059 ± 0.0003	3.23 ± 3.47
Martorell	443709.066	4608782.7	4	17.6 ± 4.3	0.23 ± 0.06	17.35 ± 8.30	0.0065 ± 0.0010	1.58 ± 1.57
Martorell	443687.021	4608848.8	5	19.8 ± 5.2	0.24 ± 0.08	4.43 ± 4.09	0.0060 ± 0.0004	0.05 ± 0.22
Cervelló	408533.297	4593310.0	1	3.1 ± 1.4	0.19 ± 0.07	12.92 ± 7.69	0.0148 ± 0.0105	11.21 ± 9.45
Cervelló	408548.992	4593317.1	2	4.7 ± 1.6	0.18 ± 0.07	9.85 ± 6.20	0.0136 ± 0.0114	5.93 ± 5.65
Cervelló	408556.803	4593291.2	3	8.5 ± 2.9	0.20 ± 0.05	10.73 ± 6.58	0.0146 ± 0.0114	4.5 ± 5.65
Cervelló	408468.245	4593264.8	4	15.5 ± 4.7	0.18 ± 0.06	13.73 ± 5.81	0.0178 ± 0.0043	0.80 ± 0.86
Cervelló	408468.465	4593272.3	5	24.0 ± 4.4	0.22 ± 0.03	16.16 ± 3.29	0.0185 ± 0.0062	0.00 ± 0.00

Table 2. Lositan table summary describing SNP candidate loci under selection at each sampling site. The summarized information includes type of selection (neutral, balancing, positive), observed heterozygosity (i.e. number of individuals heterozygous for a particular locus), F_{ST} statistic (fixation index based on genetic distances for short divergence time) and associated P value (for a 95% CI).

Site	Locus	Selection type	Heterozygosity	F_{ST}	P (Simul F_{st} < sample F_{st})
Cardedeu	SNP 18	Balancing	0.483827	-0.010491	0.024998
	SNP 39	Positive	0.30288	0.099183	0.983846
	SNP 40	Balancing	0.500665	-0.010522	0.017272
	SNP 49	Balancing	0.410178	-0.010058	0.01277
	SNP 50	Balancing	0.247221	-0.009345	0.023255
	SNP 51	Balancing	0.258051	-0.011068	0.011921
Martorell	SNP 1	Balancing	0.267598	-0.01791	0.005353
	SNP 12	Positive	0.166667	0.133333	0.979187
	SNP 26	Balancing	0.500859	-0.014597	0.019767
	SNP 40	Positive	0.514859	0.139803	0.985193
	SNP 44	Balancing	0.1533	-0.013755	0.02303
	SNP 45	Balancing	0.303872	-0.014195	0.009302
	SNP 46	Balancing	0.10783	-0.020397	0.0
	SNP 47	Balancing	0.457467	-0.019525	0.00501
	SNP 53	Positive	0.487072	0.145515	0.992504
	SNP 55	Balancing	0.441523	-0.016314	0.002453
Cervelló	SNP 24	Balancing	0.25331	-0.007977	0.018051
	SNP 34	Balancing	0.152435	-0.008808	0.022873

Chapter 3: ***The role of host genetics, ontogeny and location on herbivory***

Chapter 4: *Zero-sum landscape effects on acorn predation associated to shifts in granivore insect community in new holm oak (Quercus ilex) forests*

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Abstract

Aim: Landscape attributes can determine plant-animal interactions via effects on the identity and abundance of the involved species. Since most studies have been conducted in a context of habitat loss and fragmentation, we know very little about interaction assembly in new habitats from a landscape approach. This study aimed to test the effect of forest age and connectivity on acorn predation by a guild of predator insects differing in dispersal ability and resilience mechanisms: two weevils (*Curculio elephas* and *C. glandium*) and one moth (*Cydia fagiglandana*) in expanding *Quercus ilex* forests.

Location: Barcelona, Spain.

Methods: We assessed the proportion of infested acorns and identified the predator at the species level in five patches of connected old forests, connected new forests and isolated new forests. Effects of habitat age and connectivity at three scales (tree, patch and landscape) were analyzed using generalized linear mixed effects models.

Results: Predation by weevils was positively associated with old connected forests, while moths, with better dispersal ability, were able to predate upon all patches equally. Moreover, *C. elephas*, the weevil with lower dispersal ability, exhibited colonization credits in the new isolated patches. In spite of these changes in the guild of seed predators, the proportion of infested acorns was non-significantly different among forests.

Main conclusions: The guild of seed predators may vary depending on forest age and connectivity. However, because those with higher dispersal ability may replace less mobile species, this resulted in zero-sum effects of landscape attributes on acorn predation (i.e. similar predation rates in well-connected old forests vs. isolated new forests).

Keywords: acorn predation, biotic interactions, forest regeneration, landscape structure, life-history traits, spatiotemporal effects, zero-sum effects

4.1 Introduction

Plant-animal interactions are among the most important processes shaping biodiversity (Rinker and Lowman 2004; Maron and Crone 2006; Valiente-Banuet et al. 2015). Even though interactions occur locally, they often depend on contrasting metacommunity processes operating at several spatiotemporal scales and involving different life-history traits of the organisms (Thies et al., 2003). While species sorting is often associated to niche differences among habitat patches and inter-specific competitive ability , both mass effects and neutral processes are frequently determined by the interaction of habitat spatial structure and animal dispersal abilities (Shmida and Wilson 1985; Pulliam 1988). Moreover, habitat change in the landscape frequently determines patch dynamics processes of species colonizations and extinctions. It is known for long that dispersal is a primary driver for community assembly and governs the reorganization of species interactions under environmental change (Thompson and Gonzalez 2017). In this context, landscape structure and dynamics exerts a myriad of effects on community processes from metapopulation to metacommunity dynamics , affecting species interactions and ecosystem functioning (Tscharntke et al. 2012).

Alternative (e.g. neutral, niche or trade-off) models of community assembly emphasize the importance of diverse mechanisms (e.g. demographic and niche differences among species, dispersal-limitation, and stochastic processes (Hubbell, 2001; Leibold et al., 2004; Tilman, 2004; Ernest et al., 2008). Despite these differences, however, most of these models incorporate in some extent three common processes: resource limitation, ecological niche overlap among species, and exchanges of species and individuals between local community and regional scales. Among these, resource limitation has recently concentrated much attention because it is a basic assumption of neutral theory (Hubbell, 2001). If some resource is limiting at the entire community level, a fixed limit on the total number of individuals can be set. Thus, species might exhibit density compensation or trade-off, thus manifesting as zero-sum dynamics or community-level carrying capacity (Ernest et al., 2008). Zero-sum effects from biodiversity trade-offs can also been extended to ecosystem processes like ecosystem metabolism (Ernest et al., 2009), despite these effects have been poorly investigated.

The role of landscape structure and dynamics on these zero-sum effects is also particularly unexplored, despite it is largely known that landscape structure determine species filtering and trade-offs across space and time. Many studies have highlighted the importance of landscape structure, and especially of habitat connectivity, for plant-animal interactions in the context of habitat loss and fragmentation (e.g. deforestation). Conversely, only few authors have explored the effects of habitat recovery on the assembly of plant-animal interactions (de la Peña et al., 2016). Previous studies highlighted the importance of landscape structure and its interaction with species attributes (e.g. dormancy and dispersal ability) in the assembly of plant-insect interactions in new habitat patches (Bouget et al. 2015; Ruiz-Carbayo et al., 2017). Recent formation and high isolation of habitat patches may result in time lags (namely, colonisation credits) for some species, especially those with poor dispersal ability (Heiniger et al. 2014; Bagaria et al. 2015). This may be especially relevant for interactions involving small specialists, like insects (Thomas et al. 2001; Kruess 2003). Beyond the interest in identifying the assemblage rules, the sign (positive or negative) of the interaction may ultimately influence the dynamics of the habitats where they occur, either arresting or speeding their expansion process.

Density-dependent effects, such as those considered in the Janzen-Connell hypothesis may provide a theoretical basis on which to analyse recruitment in new forest patches, considering the conspecific density of seeds, distance from parent trees (old forests) and foraging efficiency of seed predators (Hammond and Brown 1998; Wright 2002; Terborgh 2012). For example, it may take longer for herbivores or seed predators to find the target species in new and isolated patches, decreasing foraging efficiency either because they cannot disperse further (distance-response) or because the density is so low that searching is no longer profitable (density-response) (Beckman et al., 2012). Similarly, the shorter the time a new patch has been available, the lower the probability may be of plant enemies finding the host (Ruiz-Carbayo et al., 2017). In these cases, the young age or isolation of a patch may diminish insect damage and favour the expansion process of the new habitat, in a sort of “enemy release” effect (De la Vega et al. 2012). Conversely, expansion may take longer if plants established in new and isolated patches experience higher levels of herbivory, owing to the lack of natural enemies of herbivorous insects (Roland 1993; Terborgh et al., 2001) . From a metacommunity point of view (neutral-based models), habitat connectivity might help to promote mass effects on predator populations, thus increasing colonization success and damage on host plants across the landscape, while specific patch dynamics might help to promote or to arrest these landscape processes (Leibold et al., 2004). Still, landscape attributes may also have a zero-sum effect on the host-predator interactions if there is merely a substitution of species among patches with a fixed total amount of resources. At a regional scale, more resilient species with poor dispersal abilities will gather in the older forests while species with higher dispersal abilities will be able to reach the new and more isolated ones (Hendrickx et al. 2009; Hernández et al. 2014; Evans et al. 2015). At the local scale, niche characteristics will take over and also play an important role in the compensatory dynamics that maintain the zero-sum.

In the literature the effects of these landscape attributes on seed predator insects have attracted much less attention (but see Orrock et al. 2003 and Orrock and Damschen 2005) than effects on herbivores, in spite of the negative effects of seed predation on plant fitness by reducing the number of viable seeds (Siepielski and Benkman 2008; Espelta et al. 2009a). Since seeds can be viewed as sedentary preys (Janzen 1971; Hulme 1998), a vanishing of the "predator release" effect in new forest patches can be expected to occur as spatial connectivity increases and facilitates the movement of seed predators (Koivula et al. 2002; Fountain-Jones et al. 2015) or as the forest patch ages, thus allowing recurrent colonisation events. In this study, we have analysed the effects of age and connectivity of forest patches in the composition of the granivore guild of insects and the consequences (negative, positive, neutral) for acorn predation in expanding holm oak (*Quercus ilex* L.) forests in NE Spain. As in other regions in the world (Fuchs et al. 2013; Rudel et al. 2009) there is growing evidence of forest recovery in the Mediterranean Basin in recent decades, following economic development (Lambin and Geist 2006; Vilà-Cabrera et al. 2016). These new Mediterranean forests offer an ideal study system to assess these questions as *Q. ilex* can be considered a foundation species with large implications in ecosystem functioning (*sensu* Angelini et al. 2011) and its acorns are heavily depredated by a guild of predators. These include weevils (*Curculio glandium* Marsham and *C. elephas* Gyllenhal (Curculionidae)) and moths (*Cydia fagiglandana* Zeller (Tortricidae)) (Jiménez-Pino 2011; Espelta et al. 2009b), which differ in their dispersal ability (higher in *C. fagiglandana*, intermediate in *C. glandium* and lower in *C. elephas*) and resilience to unsuitable conditions (Schumacher et al. 1997; Soria 1999; Jiménez-Pino 2011; Pélisson et al. 2013). Moreover, besides potential differences in predation intensity, the identity of the guild predators can further affect recruitment as the amount of cotyledon consumed and embryo predation likelihood might differ among the abovementioned acorn predators, and this affects the survival of infested acorns (Siscart et al. 1999 ; Bonal et al. 2007). Specifically, we assessed whether: i) the composition of the guild of insect predators changed with the age and connectivity of forest patches; ii) the proportion of acorns predated differed according to dispersal abilities of predator species; iii) the overall acorn predation rates differed in new and more isolated patches; and iv) the composition of the insect guild prompted differences in embryo survival between oak patches. We hypothesise that, considering the abovementioned differences in terms of resilience and dispersal ability, *Cydia* will better colonise forests regardless of their age and spatial connectivity. On the contrary, *Curculio* will show a distance-decay pattern from old to new and isolated forests with further differences between the two weevil species (i.e. higher ability of *C. glandium* over *C. elephas* to colonise new distant sites). The lower presence of weevils in new forests would then result in lower acorn predation in these sites, and a higher presence of partially predated acorns (those attacked by *Cydia*) with higher germination potential.

4.2 Methods

4.2.1 Study area and study species

We conducted the study in the Vallès lowland (Barcelona, Spain, 41° 33′N, 2° 2′E) (see Fig.1) from August to October 2014. Climate in this region is Mediterranean, with annual average rainfall about 650 mm and mean temperatures ranging from 6°C in winter up to 23°C in summer. As in many parts of the Iberian Peninsula (Vilà-Cabrera et al., 2017), forest has noticeably increased in the Barcelona region in recent decades (Baśnou et al., 2013), thus determining a landscape mosaic of forest patches, both pre-existing and recent, mostly made up by mixed stands of *Pinus pinea*, *P. halepensis*, *Quercus ilex* and *Q. humilis.* Pine and oak cover in these forest stands mostly follow a well-known successional change associated to recent patch dynamics, with *Quercus* species being mostly concentrated in pre-existing forests and *Pinus* species in recent ones (Guirado et al. 2008). In any case, Holm oak (*Q. ilex*) has successfully colonised abandoned agriculture and pasture areas since the 1950s (Guirado et al. 2008; Puerta-Piñero et al., 2012; Basnou et al. 2016) in many parts of the Iberian Peninsula. The two major climatic factors limiting *Q. ilex* distribution are winter cold and summer drought (Terradas & Savé, 1992; Terradas, 1999), both related to hydric stress. Shortage in water availability has been seen to affect parameters related to reproduction (Sánchez-Humanes & Espelta, 2011; Espelta et al., 2008). Therefore, difficulties in the sexual regeneration of Mediterranean oak coppices have been extensively documented (Espelta et al., 1995; Gracia et al., 2001) and have been considered a potential constraint for their long-term persistence (Retana et al., 1999). However, the oak forests in the Vallès lowland are embedded in strongly transformed landscapes with large human populations and industrial areas (Terradas, 1999). Thus, the main constraint for the regeneration of *Q. ilex* in this county has been land-use changes associated to human activity (Basnou et al., 2016). Light-tolerant *Pinus* spp. pioneered colonization, followed by more shade-tolerant *Quercus* spp., whose upper-limit age has been estimated to be 35 years (H. Ruiz-Carbayo, unpublished results). Since the onset of reproduction in *Q. ilex* takes around 15-20 years (Sánchez-Humanes & Espelta, 2011), establishment and demographic dynamics of this woody species in these emerging forest patches can still be attributed to the activity of important seed dispersers (e.g. jays *Garrulus glandarius* L. and wood mice *Apodemus sylvaticus* L.; see (Bossema, 1979; Jensen & Nielsen, 1986). In fact, our own preliminary studies based on molecular techniques has shown that most oak seedlings and saplings in these new forest patches come from foreign seed arrival (unpublished results). The most important seed predators in Iberian *Q. ilex* forests are acorn moths of the family Tortricidae (e.g. *C. fagiglandana*) and weevils of the genus *Curculio* spp. (Coleoptera: Curculionidae), *C. elephas* and *C. glandium* being the most prevalent species foraging in these NE Spain forests (Espelta, Bonal and Sánchez-Humanes 2009). Adult weevils emerge from their underground overwintering earth cells in spring (*C. glandium*) or late summer (*C. elephas*) and climb to the oak canopies. Mating takes place in late summer-autumn, when acorns are already grown and available for oviposition. Shortly after mating, females oviposit into the developing acorns, which causes premature acorn abscission (Bonal & Muñoz, 2008). Larval development within the acorn lasts approximately 30 days (depending on the temperature) and when it is finished they leave the acorn to bury for diapause (Bonal and Muñoz 2009). *C. fagiglandana* (Lepidoptera: Tortricidae) has a univoltine cycle with five larval stages. Adults fly from June to September depending on the location, and egg-laying occurs from late June to late October (Jiménez et al., 2006). Eggs are individually laid inside new shoot leaves close to the acorns. Larval development lasts about 30-40 days and larvae can shift from one acorn to another, a trait that has been interpreted as a way to escape resource limitation (Soria, 1999). The mature larva exits the acorn to spend the diapause period underground, protected by a cocoon. In spring, they emerge to the surface and pupate.

Curculio and *Cydia* species differ markedly in terms of life-history traits of extinction-risk spreading such as dispersal ability and dormancy. *Curculio* species have moderate flight abilities and great diapause variability (with adults of the same cohort emerging over several years) (Pélisson et al., 2013), while *Cydia* exhibits good dispersal abilities but does not have any dormancy strategy (Schumacher et al., 1997). Within the *Curculio* genus inhabiting oak forests, there are also differences in dispersal abilities, the small-bodied *C. glandium* being a better disperser than the large *C. elephas* (1200 m vs. 600 m, respectively) (Pélisson et al., 2013). Moreover they also differ in the way they predate upon acorns: i.e. weevil larvae develop inside a single acorn, commonly exhausting the cotyledons prior to completing their development, while a single moth larva can predate upon several acorns, not always exhausting the cotyledon tissues (Soria, 1999).

4.2.2 Experimental design and sampling

Based on the comparison of 1956- and 2009- land cover maps and orthoimages available for all Barcelona province and complementary field visits, we established a dichotomy on forest patch age (old *vs*. new). Forest patches present in both 2009 and 1956 maps were considered "old patches", while those absent in 1956 (i.e. being croplands or scrublands) but present in 2009 were considered recent. We established five sampling sites in which habitat connectivity was studied at three scales: landscape, patch and tree. Landscape connectivity at each replicate site was estimated by setting 1-km radius buffers around each holm oak crown and merging all buffers to create a landscape buffer, in which we calculated forest cover percentage. Regarding connectivity at the patch level, since forests in the study area are arranged in patches rarely isolated from each other by more than 600 m, we set this distance as the threshold of the connected vs. isolated forest dichotomy and applied 600-m radius buffers of 20% of forest cover on the orthoimages. Then, within each site, we selected one old forest patch to act as control treatment, and two post-agricultural new forest patches, connected and isolated; hereafter called old forest (OF), connected new forest (CNF) and isolated new forest (INF). Finally, we estimated tree connectivity, setting 600-m radius buffers around each holm oak crown and calculating the total squared metres within that area covered by forest. In addition, previous literature has shown differences in pre-dispersal predation by *Curculio* species to be associated with differences in seed abundance (plant satiation) or the ability of seeds to survive infestation depending on their size (Espelta et al. 2009a). For this reason, we estimated tree acorn production and measured acorn volume to account for the possible interaction of these variables with the effects of age and connectivity.

Sampling started in late August 2014, when we randomly selected five seed-producing trees (i.e. 75 in total) within each forest patch and carried out a survey of seed crops at tree level (see Table 1 for summary information). We adapted the classical visual count method by Koenig et al. (1994) so two observers would simultaneously count the maximum number of acorns on the oak crown for one minute and would record the resulting average of both counts as the estimated yearly acorn production of the tree. In order to sample the whole time window available for oviposition by both *C. fagiglandana* and *Curculio* spp., we carried out three sampling events during the acorn season: beginning (mid-late September), peak (beginning of October) and end (late October), as the two groups show slightly different phenologies (earlier infestation in *Cydia*). In each oak, we gathered a random sample of 30 acorns (i.e. a total of 90 acorns) from several branches on its crown. We immediately transferred samples to the laboratory where we measured the length and width of all acorns. We separated them into sound and infested according to the presence of *Curculio* oviposition scars or external signs of *Cydia* infestation. We marked infested acorns following a unique numbering system, placed them in individual plastic vials opened above, and checked every two days for emerged larvae (Bonal and Muñoz 2009). We distinguished moth and weevil larvae with the naked eye but, since weevil larvae cannot be assigned to the species level by external morphological signs, we preserved them in 99% ethanol and stored them at low temperature (4ºC) for further genetic analysis. We recorded larval exit for ca. two months and, two weeks after the larvae stopped emerging, we opened all acorns in order to collect any remaining larvae inside and to record whether the embryo had been predated or not.

4.2.3 Genetic analysis

Curculio spp. and *C. fagiglandana* larvae can be easily distinguished according to morphology. However, there are no reliable morphological differences between *C. glandium* and *C. elephas* larvae to tell them apart. For this reason we made the identification by mitochondrial DNA sequencing and comparison with reference sequences from previously identified individuals (see Bonal et al. 2011). We randomly selected a total of 450 *Curculio* spp. individuals (30 from each forest stand population) to be analysed using species-specific mitochondrial primers. We carried out the extraction of total DNA from the posterior third of the larva’s body using NucleoSpin® Tissue (MACHEREY-NAGEL GmbH and Co. KG, Düren, Germany) according to the manufacturer’s instructions. We sequenced a fragment (826 bp) of the mitochondrial cytochrome oxidase subunit 1 (cox1) using the Pat and Jerry primers following methods described previously (Hughes & Vogler, 2004). We performed PCR amplification in a volume of 14 µL, consisting of 1.5 µL of template DNA sample, 11.2 µL of double-distilled water, 1.5 µL of PCR buffer, 0.6 µL of $MgCl_2$, 0.25 µL of deoxyribonucleotide triphosphate (100 mmol/L), 0.2 of each primer (10 µmol/L) (forward [F] and reverse [R]), and 0.06 µL of Taq polymerase (Linus). PCR cycles used the following conditions: a 9 min denaturation at 95ºC, 40 cycles of 30 s denaturation at 94ºC, a 45 s annealing at 50ºC, and 45 s min elongation at 72ºC, with a final extension step of 10 min at 72ºC. 4µL PCR product separated by electrophoresis on 3% (w/v) agarose gels with 1× Tris–borate–EDTA buffer at a voltage of 60 V during 25 min and 70 V for 15 min. The gel contained 0.005% Midori Green nucleic acid staining solution. We checked the stained gel under ultraviolet light looking for the presence of DNA bands. Sequencing allowed the identification of larvae identity and was performed using Big-Dye (Perkin-Elmer) technology and an ABI3700 sequencer. We edited forward and reverse strands using Sequencher 4.1 (Gene Codes, Ann Arbor, MI, USA). We edited sequences using SEQUENCHER 4.1 (Gene Codes Corp., Ann Arbor, MI, USA) and pooled and aligned with CLUSTALW supplied via http://align.genome.jp (gap open and gap extension penalties were those provided by default by the software, 15 and 6.66 respectively) including a set of reference sequences of Holarctic Curculio (Hughes & Vogler, 2004). According to their similarity to these references, we could assign all sequences to either *C. elephas* or *C. glandium*. The raw genetic divergence (ratio between the total number of differences and the total sequence length) of the sequences with respect to the reference with which each one was matched was always below 2%, much higher than the inter-specific divergence between *C. elephas* and *C. glandium*, which are distantly related (Bonal et al., 2011).

4.2.4 Statistical analysis

Prior to modelling we looked for collinearity among continuous predictors (landscape connectivity, tree connectivity, tree acorn production and acorn volume) using Pearson's correlation coefficients. The highest r^2 value was found for tree connectivity and tree acorn production ($r^2 = 0.45$), and so we did not drop any explicative variable from the analysis.

We analysed the effect of forest type (OF, CNF, INF), landscape connectivity and tree connectivity on acorn predation (overall infestation regardless of parasite identity, infestation by *C. fagiglandana*, infestation by *Curculio* spp, multi-infestation by several *Curculio* spp larvae, multi-infestation by both *C. fagiglandana* and *Curculio* spp, infestation by *C. elephas* and infestation by *C. glandium*) by means of generalised linear mixed-effects models (GLMM) for a variable with a binomial distribution (infested acorn vs. non-infested acorn) and a logit link function (see Table 2). In all models, we included forest type, landscape connectivity and tree connectivity as fixed factors (as well as tree acorn production and acorn volume to control for their effects); we used a nested error structure for the model (site: tree) to account for the inherent variability of the landscape. We also tested the effects of insect identity (*Curculio*, *Cydia* or both), forest type (OF, CNF, and INF), landscape connectivity and tree connectivity on the probability of survival of the acorn embryo by means of GLMMs. We built saturated models with all fixed effects plus their second-degree interactions; selection of the best models was made based on second-order Akaike Information Criterion (AICc) and adjusted R^2. We carried out the validation of the models by plotting observed data vs. residuals of the model and by performing overdispersion tests. We conducted all statistical analysis using R, version 3.0.3 (R Core Team 2017).

4.3 Results

The overall acorn predation percentage was 21%; *Curculio spp* were present in 58% of the infested acorns while *C. fagiglandana* had a lower occurrence, being present in 48%. Acorn predation was not significantly different among forest types (OF: old forest, CNF: connected new forest and INF: isolated new forest) when no distinction was made between predators (Figure 2.1) and the only significant effect observed was a higher predation in larger acorns and more connected trees (z-value = 6.19; P-value < 0.001; Table 2). When looking at predation by moths and weevils separately, differences in granivory frequencies between forest types arose. Infestation probability by *Cydia* was not affected by forest type ("forest type" predictor dropped from best model according to AICc; Figure 2.2). On the contrary, as shown in Figure 2.3, the probability of infestation by *Curculio* differed significantly between forest types, being higher in old forest than in either connected or isolated new ones (z-value = 0.56; P-value < 0.05 and z-value = 1.13; P-value < 0.001, respectively), in accordance with the differences in dispersal ability of moths (higher) and weevils (lower). At a tree level, models also revealed the existence of a positive acorn size effect on predation in trees with smaller acorn crops for *Cydia* and on more connected trees by *Curculio* (respectively, z-value = -3.390; P-value < 0.001 and z-value = 3.152; P-value = 0.002). Fixed factors in statistical models accounted for 11% of variance in *Cydia* and 17% in *Curculio* (Table 2). Multi-infestation of acorns either by *Curculio* and *Cydia*, or by the two *Curculio* species exclusively, showed very low occurrences (6.2% and 11.1% of infested acorns respectively) and was similar in the three forest types.

Almost two-thirds (64%) of the 450 *Curculio* spp. individuals identified (by the cytochrome oxidase I gene) belonged to *C. elephas*; the remaining third corresponded to *C. glandium*. When looking at acorn predation by either *C. elephas* or *C. glandium*, we observed differences between their infestation probabilities in all three spatial scales considered (landscape, forest and tree). Probability of infestation by *C. elephas vs. C. glandium* was significantly higher in more connected landscapes (z-value = 2.54; P-value = 0.01), in old forests than in isolated new ones (z-value = 2.11; P-value = 0.04; see also Figure 2.4) and in less connected trees (z-value = -3.10; P-value = 0.002).

Nearly 85% of the bitten acorns experienced depletion of the embryo. Acorn size showed strong, positive effects on the probability of acorn embryo survival (z-value = 11.11; P-value < 0.001). Acorns infested by *Cydia* experienced a significantly higher probability of the embryo being kept alive than acorns infested by *Curculio* (z-value = 3.75; P-value < 0.001; see also Figure 3), while no significant effects were found when comparing sibling weevil species (P-value > 0.05). However, embryo survival probability was not significantly different among forest types (P > 0.05).

4.4 Discussion

Our study shows that the community of acorn-predator insects in *Q. ilex* forests changes across space and time according to habitat attributes (age and connectivity), and that the effects of these attributes can be observed at tree, patch and landscape scales. Colonisation credit has been found in *Curculio* species in consonance with their limited dispersal ability, while *Cydia* populations do not show any significant association with habitat connectivity at any scale. The study also shows that these changes in predator community determines, however, zero-sum effects on acorn predation intensity, which is only modulated by habitat connectivity at local (tree) scale. In consequence, no predator release effects have been observed.

While some studies have previously reported the effects of connectivity on the assembly process of insect communities, our results highlight the importance that habitat patch age (i.e. the time that a habitat patch has been available for colonisation) may have in this process, as it determines the colonisation credit of poor-disperser acorn predators like *Curculio* species. Similar results have been found in Lepidoptera communities in Holm-oak savannas subjected to very low habitat connectivity (Ruiz-Carbayo et al., 2017). On the other hand, neutral effects of habitat connectivity on seed predation, owing to the compensation of some absent species by an increase in others with higher dispersal ability, have also been previously reported (Hendrickx et al. 2009; Brudvig et al. 2015). Indeed, the way in which habitat connectivity modify herbivory or seed predation seems to be highly dependent on how similar or different predator species are in key life-history traits (Beckman et al., 2012). In some cases, there is a shift in the abundances of the different members of the guild; in other cases, some groups maintain their predation levels while others increase it. For example, Orrock et al. (2003) reported the lack of significant effects of patch connectivity on pre-dispersal seed predation due to a shift in the relative importance of predator species: i.e. invertebrates removed more seeds in unconnected patches where rodents removed fewer seeds, and vice versa in more connected patches. In our study system, the potential "release" effect of the colonisation credits exhibited by *Curculio* in new forest patches is counterbalanced by the even distribution of the good disperser *Cydia*. In addition, the identity of the predators and the way they predate upon the seeds revealed a negative effect by weevils on the probability of embryo survival compared to moths. Nevertheless, the potential advantage of embryo survival in acorns in new isolated forests, due to lower probability of infestation by *C. elephas*, did not attain significant values.

The fact that moths showed a similar probability of infestation in all forest types is in line with their good dispersal ability (maximum dispersal distance of 11 km in Schumacher et al. 1997). So, in our study area this moth finds no barriers to colonisation, no matter how long the forest patch has been available for colonisation (old or new) or its spatial connectivity, as linear distances between forest patches were always under 11 km. In contrast, *Curculio* species were significantly more present in old forests than in new ones in consonance with the poorer dispersal abilities of this group, being less efficient at selecting its habitat by active flight and having a risk-spreading strategy based more on the resilience offered by its variable diapause (Pélisson et al. 2012; Govindan and Swihart 2015). This strategy allows emerging local populations at new oak patches to avoid extinction even after eventual catastrophic events (e.g. null acorn production in a certain year) as the emergence of the offspring of each female is spread over three to four years depending on the species (Soula and Menu 2003; Pélisson et al. 2012). Interestingly, within this genus the higher infestation probabilities in old forests compared to both new connected and new isolated ones, were due exclusively to the decreasing trend shown by *C. elephas* from old to new isolated patches. By contrast, *C. glandium*, which emerges in spring (Venner et al., 2011) and has a greater dispersal capacity, showed an evenly distributed infestation probability among forest types, just as *Cydia* did. This matches previous literature on dispersal ability within *Curculio* spp. showing that *C. glandium* is able to disperse double the maximum distance estimated for *C. elephas* (1200 m vs. 600 m respectively) (Pélisson et al., 2013). In our study area, almost all forest patches had forested areas in a radius of 600 m, so the patches we considered isolated in the experimental design may not have been much for *C. glandium*, which could be colonising them just as easily as *Cydia*, in spite of the greater dispersal capacity of the moth.

The study also reveals that effects of habitat age and connectivity might operate at diverse scales including tree, patch, and landscape. This last factor is only positively associated to acorn infestation by the least dispersive species, *Curculio elephas*. This suggests that metapopulation processes (Leibold et al. 2004) associated to landscape pattern might play a key role in the colonization of *Q. ilex* forests by this species. In addition, the decreasing trend in the infestation probability of *C. elephas*, from old to new isolated forests, points out that not only spatial connectivity but also habitat age may play a key role in determining the assemblage of plant-animal interactions. Indeed, previous studies have highlighted the importance of habitat age in insect community colonisation in new expanding habitats (see Ruiz-Carbayo et al., 2017) because species need a certain amount of time to fully colonise the new patch (Heiniger et al. 2014). In this context of old well-connected forests and new expanding forests with different degrees of connectivity, old forests may be acting as a source habitat of weevil dispersion towards younger forests patches, in a scaled colonisation process that has not yet fully occurred in the new isolated ones. It takes around 15-20 years for *Q. ilex* to reach the onset of reproduction (Sánchez-Humanes & Espelta, 2011), depending on environmental conditions. Therefore, a plausible explanation for the immigration lag shown by *C. elephas* in new isolated forests may be that oaks growing in this kind of patches may not have been in place, at least in their mature reproductive stage, long enough for *C. elephas* to fully colonise them up to old-forest levels. Moreover, the added effect of isolation of these oaks would make the *C. elephas* colonisation pace even slower, revealing significant differences between old and new isolated forests, which do not appear between the old and the new connected ones. In addition, while *C. glandium* adults start emerging in June so they have several months to look for acorns for egg-laying throughout the forest mosaic, this is not the case of *C. elephas*, whose adult emergence strongly depends on late summer rains, just before oviposition (Bonal et al., 2010). Since the emergence from the soil of *C. elephas* adults occurs at the end of the acorn season in comparison to *C. glandium*, and it exhibits a reduced dispersal ability, they maximize their fitness by climbing to the same tree under which they have pupate, avoiding the risk of death inherent to searching for a distant tree. As a result, the movement of *C. elephas* between trees is infrequent, especially if trees are scattered (Debouzie et al., 1996) and the probability of a female reaching distant trees would be very remote. Furthermore, the stochastic nature of summer storms may cause periodic bottlenecks or even local population extinctions of *C. elephas* (Frouz & Kindlmann, 2015).

The likelihood of embryo survival differed among acorns attacked by different insect species: i.e. acorns infested by *Curculio* experienced a higher probability of embryo predation than acorns infested by *Cydia.* This stresses the likelihood that, not only the number of acorns infested, but also the identity of the predator and the way it consumes the acorn, would be vital factors to take into account when estimating the crop size available for plant recruitment. This positive effect of *Cydia* on embryo survival could be related to the differential ethology (of both adults and larvae) of the species within the predator guild. *Cydia* caterpillars start feeding on the cotyledons from the base of the acorn far away from the embryo, increasing the their chances to fully develop *ad libitum* before killing the embryo, also, caterpillars may leave the acorn and get into a new one if there is a pre-existing hole or crack in the seed coat. This is not the case of *Curculio*, whose larvae are constrained to develop within a single acorn (Bonal & Muñoz, 2008) and whose larger size *ad libitum* compared to *Cydia* caterpillars could make them more ravenous, increasing the probability of embryo depletion. Therefore, the immigration credit shown by *C. elephas* in new isolated forests, could benefit regeneration potential with respect to old forests, since a higher number of acorns could maintain their potential to develop into seedlings (Mack, 1998). However, the full colonisation of new isolated forests by the other weevil species, *C. glandium*, prevented the appearance of that advantage in isolated new forest patches.

Land-use changes limited to the socioeconomic development of many countries in the last few decades, have led to high rates of habitat loss and fragmentation, but forest expansion has also occurred in some areas due to land abandonment (Meyfroidt & Lambin, 2011). The speed of forest regeneration and species composition will depend, among other factors, on the outcome of biotic interactions. Recent studies analysing plant community assembly in new established Mediterranean forests, including our study area, have shown that immigration credit is quickly exhausted, except in the case of some particular species, such as oaks (Basnou et al., 2016). In addition, the demographic and genetic analyses of new established patches during the process of oak expansion, reveals that, even after the growth of adult and reproductive trees occurs, most established oak seedlings continue to come from the surrounding areas (more than 80% in *Q. ilex* patches (according to R. Bonal's *pers. obs.*). This arrested establishment of local oak seedlings may be the consequence of different processes but we cannot rule out the possibility that acorn predation by weevils and moth may be responsible for reducing seed crop size. Indeed, we have shown that not only the number of predators that make it to the patch and infest the acorns, but also the identity of these predators, affect the size of the seed viable pool. Nonetheless, we have shown that isolation of new established forest patches does not prevent the arrival of the more mobile predators and that, even though the guild of predators is different from the one in older forests, it does not translate into a release of the pressure exerted on the size of the acorn crop.

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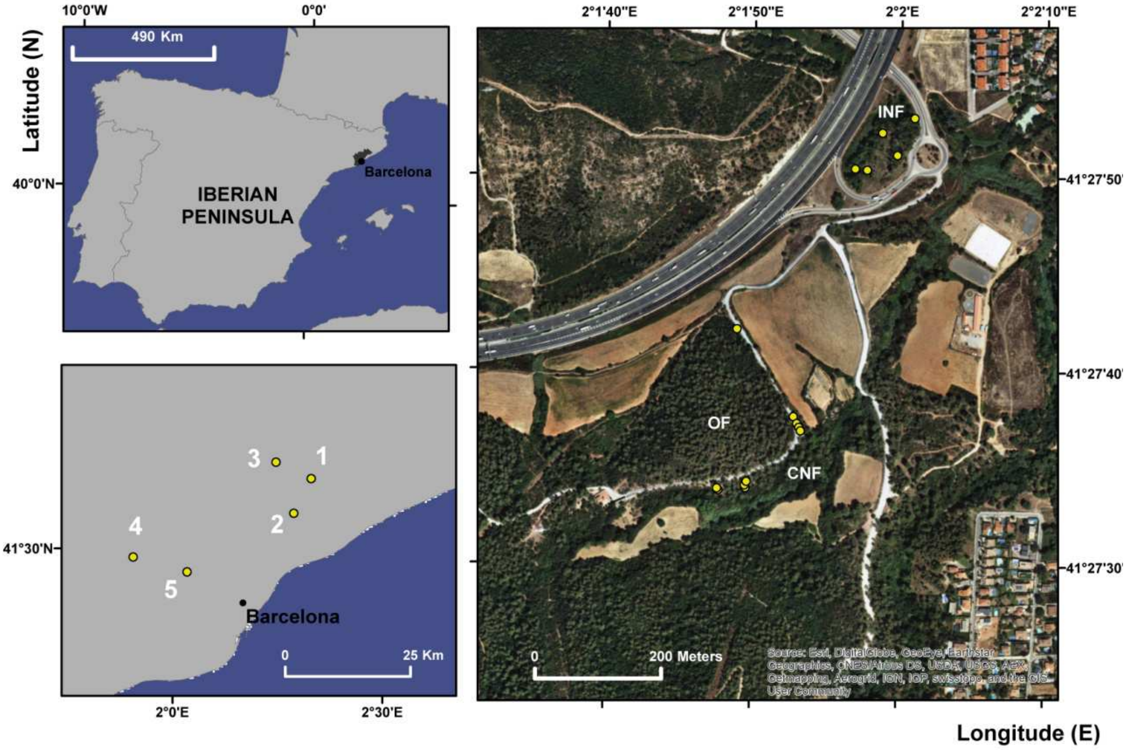
Figure 1. Location of the study site. Top left: context and study site. Bottom left: sample locations. Right: one of the replicates as an example of the experimental design



Source: Esri, DigitalGlobe, GeoEye, Earthstar Geographics, CNES/Airbus DS, USDA, USGS, AEX, Getmapping, Aerogrid, IGN, IGP, swisstopo, and the GIS User Community

Figure 2. Partial residual plots for the models of (a) overall, (b) Cydia, (c) Curculio, and (d) *Curculio elephas* infestation probabilities without interactions. Letters within each panel represent significant differences among forest types (OF: old forests, CNF: connected new forests, INF: isolated new forests) without interactions. Light grey shading indicates 95% confidence bands.

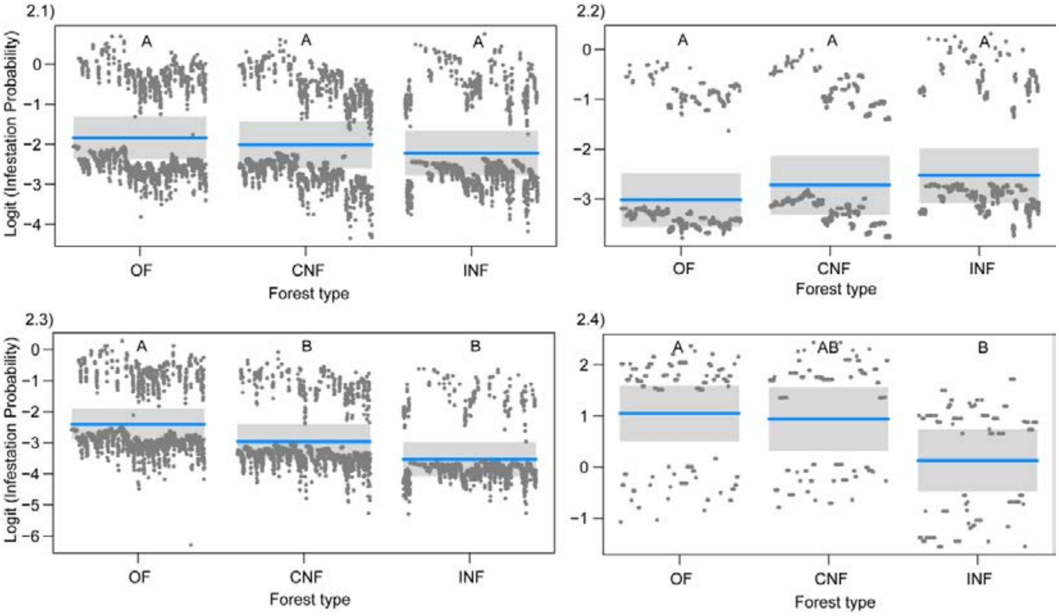


Figure 3. Partial residual plot for the embryo survival probability model without interactions. Letters within the panel represent significant differences among acorn predator. Light grey shading indicates 95% confidence bands.

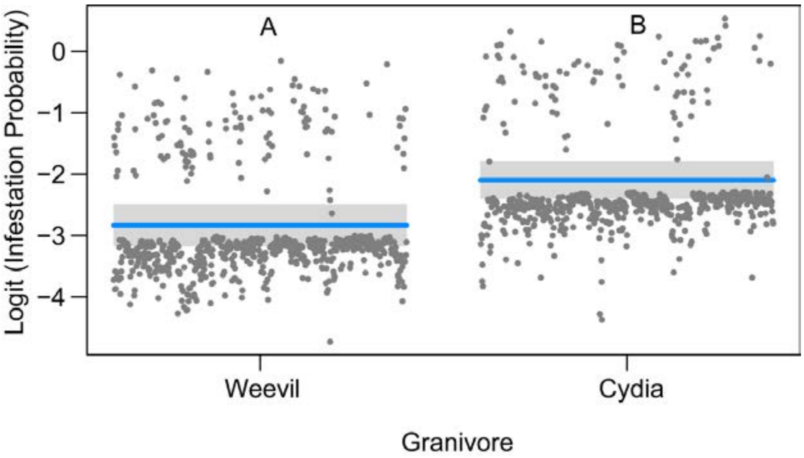


Table 1. List of study sites with their summary information according to tree ID, forest type (OF: old forest, CNF: connected new forest, INF: isolated new forest), latitude and longitude coordinates (given in decimal degrees), landscape connectivity (% forest cover), tree connectivity (m2 forest cover), tree acorn production (number of acorns) and mean acorn size (cm3).

Site	Tree ID	Forest	Latitude north	Longitude west	Landscape connectivity (% forest cover)	Tree connectivity (m^2)	Acorn production (number of acorns)	Mean acorn size ± SE (cm^3)
1	18	OF	41.630538	2.326706	37.2	587.0	229	0.11 ± 0.01
1	19	OF	41.630533	2.326769	37.2	631.6	340	0.44 ± 0.04
1	20	OF	41.630463	2.327110	37.2	865.1	330	0.79 ± 0.05
1	21	OF	41.630454	2.327153	37.2	902.8	264	0.32 ± 0.02
1	22	OF	41.630366	2.327333	37.2	1038.8	298	0.61 ± 0.04
1	108	CNF	41.626487	2.340318	37.2	1519.9	239	0.70 ± 0.04
1	109	CNF	41.625455	2.338392	37.2	785.0	217	0.84 ± 0.06
1	110	CNF	41.625445	2.338203	37.2	709.2	286	1.04 ± 0.05
1	111	CNF	41.625513	2.338085	37.2	598.8	177	1.17 ± 0.06
1	112	CNF	41.625767	2.338139	37.2	452.2	241	0.40 ± 0.03
1	1	INF	41.567878	2.285703	37.2	848.2	178	1.56 ± 0.06
1	28	INF	41.629461	2.323903	37.2	444.0	196	0.05 ± 0.01
1	29	INF	41.629503	2.323884	37.2	391.1	137	0.07 ± 0.01
1	30	INF	41.629533	2.323858	37.2	370.1	141	0.13 ± 0.02
1	31	INF	41.629590	2.323773	37.2	302.0	85	0.19 ± 0.02
2	7	OF	41.565440	2.284260	13.3	1205.9	147	0.69 ± 0.04
2	8	OF	41.565372	2.284274	13.3	1150.0	114	1.01 ± 0.06
2	9	OF	41.565194	2.284033	13.3	1287.3	312	0.72 ± 0.07
2	10	OF	41.565155	2.283989	13.3	1310.1	231	0.85 ± 0.08
2	11	OF	41.565070	2.283978	13.3	1223.1	252	0.77 ± 0.07
2	2	CNF	41.567870	2.285681	13.3	878.9	273	0.80 ± 0.06
2	3	CNF	41.567391	2.285135	13.3	1328.3	279	1.17 ± 0.08
2	4	CNF	41.566376	2.284032	13.3	1712.4	206	0.37 ± 0.04
2	5	CNF	41.566255	2.283811	13.3	1692.0	87	0.57 ± 0.05

2	6	CNF	41.566075	2.283663	13.3	1594.2	61	0.50 ± 0.05
2	13	INF	41.561718	2.289232	13.3	678.6	159	0.20 ± 0.02
2	14	INF	41.561718	2.289239	13.3	689.7	307	1.44 ± 0.08
2	15	INF	41.561730	2.289398	13.3	765.2	300	0.82 ± 0.05
2	16	INF	41.561707	2.289513	13.3	819.4	322	0.92 ± 0.06
2	17	INF	41.561716	2.289646	13.3	918.5	220	0.83 ± 0.06
3	97	OF	41.673756	2.218119	24.4	733.9	263	1.04 ± 0.06
3	98	OF	41.673809	2.218199	24.4	827.0	204	0.74 ± 0.06
3	99	OF	41.673818	2.218203	24.4	837.6	92	0.95 ± 0.08
3	101	OF	41.674192	2.218921	24.4	1762.5	213	0.74 ± 0.04
3	114	OF	41.675755	2.219445	24.4	1969.0	195	0.56 ± 0.04
3	89	CNF	41.675084	2.220417	24.4	1024.3	148	1.03 ± 0.07
3	91	CNF	41.676271	2.217079	24.4	1727.8	236	0.74 ± 0.06
3	93	CNF	41.676229	2.217949	24.4	1888.0	107	0.42 ± 0.05
3	94	CNF	41.675913	2.218063	24.4	1965.9	123	0.20 ± 0.02
3	113	CNF	41.675542	2.218387	24.4	2065.4	112	0.31 ± 0.04
3	102	INF	41.659679	2.241162	24.4	590.1	182	0.58 ± 0.06
3	103	INF	41.659912	2.241892	24.4	313.3	133	1.22 ± 0.05
3	104	INF	41.659634	2.241674	24.4	297.4	184	0.72 ± 0.05
3	106	INF	41.659651	2.241356	24.4	451.0	76	0.66 ± 0.05
3	107	INF	41.659951	2.241055	24.4	968.4	247	1.41 ± 0.06
4	48	OF	41.469973	1.889826	18.5	2001.7	113	0.79 ± 0.04
4	49	OF	41.469970	1.889804	18.5	2009.5	85	0.77 ± 0.04
4	50	OF	41.469835	1.889807	18.5	1960.9	125	0.58 ± 0.03
4	51	OF	41.469901	1.889743	18.5	2005.9	129	0.59 ± 0.04
4	52	OF	41.469476	1.889065	18.5	2326.2	177	1.07 ± 0.04
4	44	CNF	41.471689	1.890821	18.5	1396.1	41	0.84 ± 0.06
4	45	CNF	41.470951	1.889827	18.5	2171.1	49	0.36 ± 0.04
4	46	CNF	41.470368	1.890610	18.5	1791.5	58	0.39 ± 0.03
4	47	CNF	41.470288	1.890470	18.5	1833.8	84	0.35 ± 0.02
4	53	CNF	41.471507	1.890991	18.5	1439.7	39	0.13 ± 0.01
4	39	INF	41.485970	1.902348	18.5	1317.5	119	0.99 ± 0.08
4	40	INF	41.485477	1.903222	18.5	1286.8	214	1.31 ± 0.07
4	41	INF	41.485632	1.903116	18.5	1356.8	150	1.17 ± 0.07
4	42	INF	41.485618	1.903222	18.5	1383.6	75	0.67 ± 0.06
4	43	INF	41.485679	1.904579	18.5	1485.1	79	0.94 ± 0.07

5	59	OF	41.460452	2.031365	38.8	1835.8	88	0.55 ± 0.02
5	60	OF	41.460356	2.031431	38.8	1960.5	39	0.45 ± 0.04
5	61	OF	41.460301	2.031474	38.8	2014.0	41	0.50 ± 0.06
5	62	OF	41.460250	2.031502	38.8	2075.6	88	0.64 ± 0.04
5	63	OF	41.461701	2.030280	38.8	1090.3	156	1.26 ± 0.06
5	54	CNF	41.459399	2.029951	38.8	2603.8	93	0.74 ± 0.05
5	55	CNF	41.459423	2.029928	38.8	2608.0	96	0.57 ± 0.03
5	56	CNF	41.459432	2.030469	38.8	2627.8	57	0.44 ± 0.03
5	57	CNF	41.459471	2.030450	38.8	2635.9	50	0.49 ± 0.03
5	58	CNF	41.459520	2.030487	38.8	2634.5	80	0.79 ± 0.06
5	32	INF	41.464199	2.033289	38.8	784.0	173	0.30 ± 0.03
5	33	INF	41.463985	2.032720	38.8	837.3	252	0.60 ± 0.03
5	34	INF	41.464518	2.033005	38.8	820.3	119	1.64 ± 0.08
5	35	INF	41.464731	2.033612	38.8	545.7	169	0.91 ± 0.06
5	36	INF	41.464001	2.032494	38.8	855.3	81	0.93 ± 0.05

Table 2. Effects of landscape connectivity (% forest cover), forest type (OF: old forest, CNF: connected new forest, INF: isolated new forest), tree connectivity (m^2), acorn production (number acorns/tree) and acorn size (cm^3).

		Overall			**Cydia**			**Curculio**			**C. elephas/ C.glandium**			**Embryo survival**		
Random effects		**Variance**	**SD**		**Variance**	**SD**		**Variance**	**SD**		**Variance**	**SD**		**Variance**	**SD**	
Tree: Site		0.609	0.780		0.696	0.834		0.818	0.905		0.713	0.845		1.88e-01	4.33e-01	
Site		0.169	0.411		0.174	0.417		0.095	0.307		0.000	0.000		2.040e-14	1.43e-07	
Fixed effects		**Estimate**	**SE**	***z***	**Estimate**	**SE**	***z***	**Estimate**	**SE**	***z***	**Estimate**	**SD**	***z***	**Estimate**	**SD**	***z***
Intercept		-1.681	0.209	**-8.055*****	-2.712	0.231	**-11.726*****	-2.019	0.284	**-11.093*****	-1.028	0.270	**-3.811*****	-2.805	0.180	**-15.551*****
Landscape connectivity		0.340	0.210	1.620	---	---	---	0.332	0.183	1.816	-0.409	0.161	**-2.536***	---	---	---
Forest type	CNF	---	---	---	---	---	---	-0.564	0.287	**-1.961***	0.111	0.385	0.287	---	---	---
	INF	---	---	---				-1.134	0.332	**-3.419*****	0.919	0.436	**2.105***			
Tree connectivity		0.702	0.113	**6.192*****	0.230	0.152	1.515	0.500	0.170	**2.942****	0.584	0.189	**3.084****	-0.231	0.104	**-2.217*****
Acorn production		---	---	---	-0.314	0.145	**-2.162***	---	---	---	---	---	---	---	---	---
Acorn size		0.617	0.039	**15.943*****	0.030	0.052	0.578	0.951	0.048	**19.911*****	---	---	---	1.157	0.104	**11.112*****
Tree connectivity : Acorn size		---	---	---	-0.1241	0.054	-2.299*	0.161	0.051	**3.152****	---	---	---	---	---	---
Tree connectivity : Acorn production		---	---	---	-0.270	0.163	-1.658	---	---	---	---	---	---	---	---	---
Acorn size : Acorn production		---	---	---	-0.206	0.061	-3.390***	---	---	---	---	---	---	---	---	---
Parasite	Cydia	---	---	---	---	---	---	---	---	---	---	---	---	0.715	0.191	3.752***
	Both	---	---	---	---	---	---	---	---	---	---	---	---	-0.605	0.407	-1.487

Chapter 5: *Do dispersal vs. dormancy strategies of tracking unpredictable resources leave genetic signatures? A case of specialist insects inhabiting patchy habitats.*

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Abstract

Spatial dispersal vs. dormancy are contrasting strategies exhibited by short-lived organisms for coping with unpredictable pulsed resources in patchy and dynamic habitats. The trade-off affect species demography and population genetics, with good dispersers showing weaker genetic structure across patches while more resilient species being genetically poorer but showing larger local population sizes. While effects on community assembly regarding species distribution and abundance have been quite studied, genetic diversity and isolation of populations along patch dynamics are mostly unknown. Genetics will, however, condition population viability due to the relationship between genetic diversity and fitness. The present paper addresses this subject in an area in which former croplands are being colonized by holm oaks *Quercus ilex*, giving rise to a fragmented landscape with mature old forests, new oak plots adjacent to mature forests and new isolated oak plots. Holm oak acorns are predated by two specialist insects, *Curculio glandium* and *Curculio elephas* (Coleoptera: Curculionidae), which exhibit different strategies (dispersal and dormancy, respectively) to track food availability. Using mitochondrial and nuclear markers (single nucleotide polymorphisms) we assessed the genetic signature left by those strategies in their populations. As expected, the poor disperser *C. elephas* exhibited a significant population genetic structure among patches absent in *C. glandium*. In *C. elephas*, gene-flow was constrained in new isolated oak patches, as their isolation from mature forests and recent age hampers the arrival of immigrants. The populations of *C. elephas* (the species with bet-hedging dormancy) went through bottlenecks and their genetic diversity was lower than in *C. glandium* in all types of forest plots. The populations of the poor disperser species were not threatened in forest fragments though, as its populations were always more numerous probably thanks to a higher fecundity rate.

However, if more recurrent population bottlenecks this situation could change unless landscape management policies promote habitat connectivity.

Keywords: Curculio, dispersal/dormancy trade-offs, dispersal ability, environmental stochasticity, forest fragments, Quercus.

5.1 Introduction

Organisms that feed on pulsed resources have evolved different strategies to track unpredictable food availability in space and time. Spatial dispersal and dormancy (i. e. dispersal in time) are considered two of the main strategies exhibited by short-lived organisms like insects for coping with environmental stochasticity, as both of them reduce the risk of local extinction (Venable & Lawlor, 1980; Venable & Brown, 1988). Since both strategies are highly energy demanding, the emergence of trade-offs between these strategies is expected due to energetic constraints (Roff, 1986; Zera et al., 1997; Zera & Harshman, 2001). The existence of dispersal/dormancy trade-offs favours the co-existence of species exploiting the same pulsed resource, since they lower interspecific competition and this prevents any species from completely outcompeting the rest (Amarasekare 2003).

In patchy habitats, dispersal vs. dormancy trade-offs condition local community composition through their effects on species arrival likelihood and resilience. Species with great dispersal ability will be better able to move between suitable patches (Baguette et al., 2003; Doligez & Part, 2008, Ruiz Carbayo et al. 2017; 2018) than poor dispersers; however, increased dispersal will only lead to larger populations if the immigrants permanently establish and successfully reproduce in a patch. Conversely, poor disperser but more resilient species would be less likely to arrive but also to become locally extinct. Species resilience will strongly depend on the ability to circumvent temporal adverse conditions (i. e. dormancy). The balance between both selective forces will rule the relative frequency of the species following one strategy or the other (Amarasekare 2013; Ruiz-Carbayo et al. 2018)

Dispersal and dormancy will not only condition local species composition but also the population genetics of the species following each strategy. This could in turn also affect the viability of their populations, as genetic diversity is correlated with individual fitness (Vrijenhoek, 1994; Saccheri et al., 1996; Liersch & Schmid-Hempel, 1998; Gilligan, 2001; Reed & Bryant, 2001). Theoretically, species with higher dispersal ability would show a weak genetic structure and a higher gene-flow across patches compared to poor dispersers, but local genetic diversity would be higher due to the arrival of new immigrants (Hastings & Harrison 1994). In turn, more resilient species could be less genetically diverse due to their lower dispersal abilities, especially in very isolated patches, however, this could be counterbalanced by a larger population size due to their lower likelihood to suffer local population bottlenecks (Frankham 2005).

In the current context of Global Change, it is very important to assess how food-tracking strategies condition species composition and population viability in organisms colonizing new habitat patches. The appearance of new forest plots in former abandoned croplands is a widespread phenomenon (especially in Europe) and a good example of a new patchy habitat (Basnou *et al.* 2016)(Gerzabek *et al.* 2017). The species composition of new forest patches depends on their degree of isolation and age; poor dispersers will less likely reach new habitat patches far from old ones (sources of new immigrants). The effects of patch age and isolation on species composition have been studied in different taxa (Jeffries et al., 2006; Maldonado-López et al., 2015; Ruíz-Carbayo et al. 2017, 2018). However, we know much less about the genetic diversity and isolation of the new populations (but see Arias-Leclaire et al. 2017) and to which degree, depend on the species dispersal/resilience abilities. Yet, it may strongly affect their fitness and local population viability in the mid-long term.

We used two weevil species *Curculio glandium* and *Curculio elephas* (Coleoptera: Curculionidae), both specialist predators of oak *Quercus* spp. acorns, as study models to assess the effects of patch isolation and age on the genetic structure and diversity of their populations in new oak forests in Catalonia (north-eastern Spain). In Western Europe the appearance of new forests is an ongoing process that started some decades ago with the abandonment of agricultural lands; e.g. in our study area forest surface has increased in 20% in the last 50 years (Baśnou *et al.* 2013). The holm oak *Quercus ilex* is one of the most prevalent tree species in forest patches, which could be divided in: mature (i. e. old) forest plots, new forest patches adjacent to mature forests and new and isolated forest patches. The weevils *Curculio elephas* and *Curculio glandium* are the most important pre-dispersal acorn predators in Iberian Holm oak forests, being the most prevalent species in these NE Spain forest patches (Espelta *et al.* 2009a). They are trophic specialists, as their larvae can only develop within oak acorns (Desouhant et al., 2000; Bonal et al., 2007; Pélisson et al., 2011; Venner et al., 2011). The production of acorns vary greatly, both temporally and spatially (Espelta et al., 2008;Venner et al., 2011; Pélisson et al., 2013), what makes it an unpredictable food resource.

These insects have evolved both temporal and spatial risk-spreading strategies in order to cope with this resource stochasticity. *C. glandium* has greater dispersal abilities than *C. elephas;* the maximum dispersal distances have been estimated to be 1200 m in the former and 600 m in the latter. Also, *C. glandium* adults emerge from their overwintering underground refuges in spring and have several months to search and disperse to high-quality habitat patches for feeding and egg-laying before the acorns start growing in September. By contrast, *C. elephas* adults emerge in late summer and mate and oviposit soon after (Pélisson *et al.* 2013a), so that adult dispersal within the tree under which they emerge is the rule (Coutin, 1960; Debouzie et al., 1996), especially if trees are isolated (Menu & Debouzie, 1993;Bonal et al., 2012). In turn, *C. elephas* has a higher ability to cope with local stochasticity of food resources. The diapause of *C. glandium* has a fixed duration of two years, whereas in *C. elephas* diapause is variable and may last from one to three years (Pélisson *et al.* 2013b). This means that, despite the arrival of immigrants is less likely in *C. elephas*, this species will presumably better withstand temporal local scarcity of food resources as some individuals will emerge when the environmental conditions improve (Menu & Debouzie 1993). Besides, *C. elephas* is larger sized and larval survival is higher than in *C. glandium* (Bonal *et al.* 2011), what could also make it less likely to suffer local extinction.

Because *C. elephas* is more likely to escape poor conditions temporally, while *C. glandium* is more likely to do it spatially, landscape features can impact their colonization dynamics differently. Ruiz-Carbayo et al. 2018 revealed a distance-decay pattern in *C. elephas* populations from old mixed-forests to new recently established ones, with significant colonization credits in the most isolated new forests, while no significant effects of forest age and connectivity were detected for *C. glandium*. In this study we used two different types of genetic markers (mitochondrial DNA and nuclear single nucleotide polymorphisms) to assess the genetic signature that these contrasting dispersal strategies leave in the populations of both species. The effects on the population genetics of the species could condition the viability of the populations of these insects which, together with moth caterpillars (Ruíz-Carbayo et al. 2017), are at the base of the trophic chain as primary consumers feeding on oaks.

We specifically tested: i) whether population genetic structure differed between species ii) whether gene-flow and genetic diversity were lower in isolated new forest patches and whether it differed between species. We hypothesized that genetic structure would be less marked in *C. glandium* due to a stronger gene-flow among populations favoured by its higher dispersal abilities. Gene-flow between new isolated forests and the rest would be expected to be lower than in the case of new patches connected to old forests, especially in the case of the poor disperser *C. elephas*. Regarding genetic diversity, it could be higher in *C. glandium* although a larger population size in *C. elephas* resulting from its higher resilience could compensate for a lower arrival of immigrants.

5.2 Material and methods

5.2.1 Study area and species

The sampling was carried out in the Vallès lowland (Barcelona, Spain, 41° 33′N, 2° 2′E) (see Fig.1) in 2014. Climate is Mediterranean, with annual average rainfall about 650 mm and mean temperatures ranging from 6°C in winter up to 23°C in summer. The landscape is a mosaic of croplands, urban areas and forest patches dominated by pinus (*Pinus pinea* L., *Pinus halepensis* MiLL.) and oaks (*Quercus ilex* L., *Quercus pubescens* LMiLL.). In the last decades forest surface has increased at the expense of croplands in many parts of the Iberian Peninsula. In our study area the Holm oak *Q. ilex* has successfully colonized abandoned agriculture lands since 1950s, forming new oak patches more or less connected with mature forest stands (Basnou *et al.* 2016). The Holm oak *Quercus ilex* exhibits a marked inter-annual variability in seed-production synchronized at the population level (Espelta *et al.* 2008). Acorns mature in one year and are attacked by weevils of the genus *Curculio* spp. (Coleoptera: Curculionidae): *Curculio elephas* and *Curculio glandium* are the most prevalent pre-dispersal acorn predators in our study area (Espelta et al. 2009a; Bonal et al. 2011).

In early autumn acorns have grown to a size that allows oviposition by females of both species (Espelta et al. 2009a; Bonal et al. 2011). Weevil females perforate the seed coat with their specialized long rostrum, introduce the oviscapt through the puncture and oviposit into the acorn. Commonly a single egg is laid per acorn, but larger clutches have eventually been recorded (Bonal & Muñoz 2009). Weevil larvae have to complete their development (it lasts around 35 days) within a single acorn feeding on the cotyledons (Bonal *et al.* 2010). Oaks drop infested acorns prematurely and when larvae finish their development they drill an exit hole through the acorn coat and bury underground to overwinter within an earth chamber. Larvae undergo a diapause of variable duration depending on the species. In *C. glandium* larvae spend two years buried before pupating and emerging to the surface as adults, whereas in *C. elephas* diapause has a variable length and larvae of the same cohort may emerge as adults after one, two or three years (Soula and Menu 2003; Venner et al. 2011; Pélisson et al. 2013a). Adult emergence takes place in spring in *C. glandium* and in late-summer/early autumn in *C. elephas*. In *C. elephas* rainfall amount strongly conditions adult emergence in Mediterranean environments, as adults depend on the rain to soften the soil after the summer drought and be able to emerge to the surface (Espelta *et al.* 2017). Dispersal abilities also differ between species, being higher in *C. glandium* (1200 m *vs* 600 m in *C. elephas*). However, these maximum dispersal distances were estimated in captivity (wind tunnel experiments in Pélisson *et al.* 2013b) and might be lower in nature.

5.2.2 Experimental design

In order to sample forests of different age and connectivity, in autumn of 2014 we selected 18 stand forests grouped in 6 different sites (Fig. 1). Within each site, patch selection was made based on the comparison between 1956- and 2009- land cover maps and orthoimages, which allowed us to identify patches already present in 1956- (hereafter called “old forests”) and subsequent patches (hereafter called “new forests”). Since patches in the study are rarely isolated further than 600 m, we set this distance as a threshold to distinguish between connected vs. isolated new forests. We calculated patch connectivity applying 600-m radius buffers of 20% of forest cover on the orthoimages, which allowed us for the identification of connected new forests (CNF) and isolated new forests (INF). Old forests (OF) in the study area are present as large continuous patches, so no distinction in connectivity was made.

Sampling started in late August 2014, when we randomly selected five seed-producing trees within each forest patch. (i.e. 90 in total) We carried out three sampling events, covering the beginning, peak and end of the acorn season, as phenology differs between weevil species in some extent (Bonal *et al.* 2011). From each oak, we gathered a random sample of 30 acorns in each event (i.e. a total of 90 acorns) and once at the laboratory, we separated them into sound and infested acorns, according to the absence/presence of *Curculio* oviposition scars. Acorns were unequivocally identified following a unique numbering system, laid in individual trays and checked every two days for emerged larvae (Bonal & Muñoz 2009). Since larvae of *C. elephas* cannot be distinguished from larvae of *C. glandium* by morphological traits, we preserved them in 99% ethanol and stored them at low temperature (4ºC) for further genetic analysis.

5.2.3 Molecular laboratory methods

We selected a subsample of 540 larvae (10 per sampling event and forest patch) for genetic analysis. We extracted DNA from a small piece of larval tissue (approx. 2mm) using NucleoSpin® Tissue (MACHEREY-NAGEL GmbH and Co. KG, Düren, Germany) according to the manufacturer. As species identity cannot be determined in these species based on larvae's morphological traits, we carried out specific identification of individuals using molecular techniques (DNA barcoding). We sequenced a fragment (826-bp) of the mitochondrial gene cytochrome oxidase subunit 1 (cox1) and compared the obtained sequence with reference sequences from previously identified individuals (see Bonal et al. 2011 for a similar approach). Cytochrome oxidase subunit 1 (cox1) was amplified via PCR using the Pat and Jerry primers following methods described in Hughes & Vogler, 2004. 14 µL PCR reactions contained 1.5 µL of template DNA sample, 11.2 µL of double-distilled water, 1.5 µL of PCR buffer, 0.6 µL of $MgCl_2$, 0.25 µL of deoxyribonucleotide triphosphate (100 mmol/L), 0.2 of each primer (10 µmol/L) (forward [F] and reverse [R]), and 0.06 µL of Taq polymerase (Linus). PCRs were run in a PTC-100® Thermal Cycler (Bio-Rad Laboratories, Hercules, California) under the following conditions: a 9 min denaturation at 95ºC, 40 cycles of 30 s denaturation at 94ºC, a 45 s annealing at 50ºC, and 45 s min elongation at 72ºC, with a final extension step of 10 min at 72ºC. The presence of the 826-bp DNA fragment in the sample was determined by electrophoresis on 3% (w/v) agarose gels with 1× Tris–borate–EDTA buffer at a voltage of 60 V during 25 min and 70 V for 15 min. Next, we looked for the DNA band in the agarose gel stained with 0.005% Midori Green nucleic acid staining solution. Sequencing was performed using Big-Dye (Perkin-Elmer) technology and an ABI3700 sequencer. We accomplished forward and reverse strand editing with SEQUENCHER 4.1 (Gene Codes Corp., Ann Arbor, MI, USA), and aligned with CLUSTALW supplied via http://align.genome.jp. We collapsed alignment sets into unique haplotypes and compared them to the *Curculio* spp. reference sequences available for Holarctic Curculio (Hughes & Vogler 2004) at GenBank. The comparison showed that the sequences belonged either to *C. elephas* or *C. glandium*. The raw genetic divergence (total number of differences divided by the total sequence length) between our samples and the corresponding reference sequences was consistently below 2%. In turn, this intra-specific divergence was much lower than the divergence between the two species (Bonal *et al.* 2011).

We used mtDNA not only for species identification, but also in analyses of the genetic structure, gene-flow and genetic diversity. Even though mt-DNA is quite preserved, previous studies have shown that in *Curculio* spp. it exhibits enough variability at spatial scales of just a few kilometers (Toju & Sota, 2006; Toju et al., 2011; Arias-LeClaire *et al.* 2017).

Besides mtDNA we used nuclear markers, namely SNPs (Single Nucleotide Polymorphisms), which could potentially provide a finer resolution at smaller spatial scales. Genomic DNA was quantified using a Qubit Fluorometer (Invitrogen). We carried out sample dilutions to 20 ng/lL using Biomek® NXP Laboratory Automation Workstation (Beckman Coulter). DNA of four larvae from four different populations was isolated, tagged and pooled before next-generation sequencing on the Ion Torrent PROTON system. About 10Gb of data were generated and analyzed using the Ion Torrent Suite Software to detect SNPs distributed across the genome. Using stringent technical (depth of coverage >10x) and biological (high heterozygosity) criteria, we selected in silico a subset of 160 SNPs to genotype 96 individuals using the Mass Array Sequenom iPLEX Gold assay (Sequenom 2008) at the Genome Transcriptome Facility of Bordeaux (PGTB). The scatter plots of all genotyped SNPs were inspected using TYPER 4.0 and a subset of 80 di-allelic SNPs were chosen according to their amplification success and polymorphism across individuals. These 80 SNPs, arranged in two multiplexes of 40 SNPs each, were used to genotype all the samples.

5.2.4 Molecular analyses

We calculated for each *C. elephas* and *C. glandium* populations the following standard molecular indexes using mtDNA: haplotype richness, gene diversity (H) and nucleotide diversity (π). We also tested the signature of recent population bottlenecks or expansions by means of neutrality tests such as Tajima's D (Tajima 1989) and Fu's Fs (Fu 1997). In addition, for each study site and species we plotted haplotype networks with the *haploNet* function of the *pegas* package (Paradis 2010) in R to illustrate the effects of such demographic events on the population genetics. For each species we removed from the analyses all populations with less than 5 individuals (Papadopoulou *et al.* 2011). According to this criterion, all 18 populations of *C. elephas* where suitable for analysis whereas only 12 *C. glandium* populations remained (see Table C.1). In the case of SNPs we calculated observed heterozygosity (H_o) and expected heterozygosity (H_e) ant tested for deviation from Hardy-Weinberg equilibrium. All analyses were performed using ARLEQUIN software (Excoffier *et al.* 2005).

To quantify gene flow between populations, pairwise F_{ST} (Wright 1931) were estimated using ARLEQUIN software. F_{ST} significance was evaluated by means of a permutation test, with 1000 permutations. Associations between geographic and genetic distance (isolation by distance) were tested in both species using a Mantel's test. As genetic matrix we used the population pairwise F_{ST} yielded by ARLEQUIN, and a simple Euclidean distance matrix for geographic distances. The Mantel test was run for 1000 permutations to test for significance. Both distance matrices were calculated using the R package *ecodist*.

We tested the existence of significant population genetic structure with analyses of the molecular variance (AMOVAs) using ARLEQUIN software (Excoffier *et al.* 2005). To assess the existence of geographical patterns in the genetic structure we performed analyses using the software SAMOVA 1.0 (Dupanloup *et al.* 2002). We simulated different number of populations (K) ranging from K = 2 to 17 for *C. elephas* and K = 2 to 11 for *C. glandium*, looking for the optimal grouping option that maximizes the among-group component (F_{CT}) of the overall genetic variance.

In addition, we carried out a complementary analysis for the nuclear SNPs using STRUCTURE software (Pritchard *et al.* 2000) to also estimate the number of discrete genetic populations (K values) supported in the data, and check the congruence of both methods. The program STRUCTURE implements a Bayesian approach for inferring population structure from multilocus data by several models, including the no-admixture model, which assumes that each individual has inherited all its ancestry from a single population. We carried out analyses with most parameters set to their default values as advised in the user's manual of structure 2.0 (Pritchard & Wen 2003).

5.2.5 Statistical analyses

Linear Mixed Models (LMMs) were built to test for significant differences in population size (number of individuals) between forest types and species. Also by means of LMMs we tested the effects of population size, species and forest type on haplotype richness, gene diversity and nucleotide diversity. Saturated models included second-degree interactions of predictors. In addition, we evaluated whether the age and the degree of isolation of the patches had an effect on genetic flow. Within each study site we set three different types of pairwise comparisons: between old forests and connected new forests (OF-CNF), old forests and isolated new forests (OF-INF) and between connected and isolated new forests (CNF-INF). We assessed whether gene-flow differed depending on the type of pairwise comparison and whether it differed between the two species; the interaction between both factors was also tested. We used the inverse of pairwise population F_{ST} following a precise criterion (F_{ST} negative values converted to zero values) as a proxy of gene-flow. Models were performed with *lmer* function as implemented in the *lme4* package. Model selection consisted of a backward selection procedure in which less significant variables, based on the output of the *anova* function (package *lmerTest*), were progressively eliminated from the saturated model. Validation of the model included calculation of coefficient of determination (R^2) and normality of model residuals.

5.3 Results

5.3.1 Demography and parameters of genetic diversity

Curculio elephas was the most abundant species in our study area, the mean number of individuals per patch was significantly higher than that of *Curculio glandium* (t = 4.53; *P* <0.001; see Fig. 2). *C. elephas* was the most prevalent species irrespective of the age and the isolation of the patch, as the interaction between the factors *type of patch* and *species* was not significant (*C. glandium* * CNF: t = 0.77; *P* = 0.445 and *C. glandium* * INF: t = 1.08; *P* = 0.287; see Table C.2).

The type of forest patch had a significant effect on haplotype richness values, which were significantly higher in the old forests (OF) than in the isolated new ones (INF) (t = 5.97; *P* = 0.021; see Fig. 3); the mixed model explained 61% of variance of the response variable (Table C.2). Haplotype richness per patch increased with the number of individuals but the increase rate differed between species (Table C.2 and Fig. C.1). In *C. glandium* it increased more quickly than in *C. elephas*, hence, despite the larger population sizes of *C. elephas*, the mean number of haplotypes per patch did not differ between species (mean *C. elephas*: 4.00 ± 1.283; *C. glandium*: 3.94 ± 1.731).

Contrary to haplotype richness, genetic and nucleotide diversities differed between species; both were higher in *C. glandium* (Fig. 4 and Table C.3). In the best models only the factor *species* was significant in the case of nucleotide diversity (t = 8.113, *P* < 0.001) and only marginally significant for gene diversity (t = 1.915, *P* = 0.068; Table C.2) and. Nucleotide and genetic diversity were higher in *C. glandium* despite the lack of differences in mean haplotype richness because in *C. elephas* the genetic divergence among haplotypes is very low. The haplotype networks illustrate this low divergence very well (Fig. 5). In *C. elephas* most populations are dominated by one or few haplotypes and most of the less frequent ones differ from them in just one nucleotide base pair. The result is the “star-like” network typical of the populations that have gone through population bottlenecks. Accordingly, Fu´s FS parameter was negative and significant in 6 forest patches for *C. elephas* (5 of them old forests), what means that there is a departure from neutrality (excess of rare haplotypes close to the most prevalent one in terms of genetic divergence). Those weevil populations would be going through a recent population expansion after a bottleneck. In *C. glandium*, by contrast, neither of the two neutrality tests carried out (Tajima´s D and Fu´s FS) retrieved significant values at any of the populations (Table 1).

5.3.2 Dispersal and population genetic structure

The analysis of molecular variance (AMOVA) using mitochondrial DNA showed that there was a significant population structure in the poor disperser *C. elephas*. The differences among populations explained 10.5% of the total genetic variance taking all (18) forest patches and 12.3% when only the 12 patches that could also be used in the analysis for *C. glandium* were considered. By contrast, in *C. glandium* no significant population structure was found (Table 2) due to the higher gene-flow among patches. The SAMOVA analysis did not retrieve any significant geographic genetic structure above the population level ($P>0.05$).

Gene-flow for the mt-DNA marker was significantly greater in *C. glandium* than *C. elephas*, according to its higher dispersal ability (Fig. C.2). The analyses of gene-flow at a finer spatial scale among the three patches of each of the 6 study sites, showed that gene-flow is restricted in some of the newer and more isolated forest patches (INF) in *C. elephas*. Using mitochondrial DNA, the pairwise Fst values were significant in three of the study sites in the comparisons between old forests (OF) and isolated new forests (INF) in *C. elephas*, whereas in the rest of the contrasts for *C. elephas* and in all contrasts for *C. glandium* they did not differ significantly from zero. Using finer nuclear markers (SNPs) we found more significant pairwise F_{ST} values that confirmed the trend detected using mitDNA. Contrasts between OF and INF were significant in all (6) study sites, while CNF-INF and OF-CNF comparisons showed significance only in three and two sites respectively (Table C.4). This result was supported by the greater pairwise F_{ST} values found for OF-INF when compared to OF-CNF ($t = -2.480$, $P = 0.027$; see Fig. C.3). We could not carry out this analysis in *C. glandium* because the SNPs did not work in this species (reduced amplification success and very low levels of polymorphism).

The STRUCTURE analysis analyses based on SNPs revealed the existence of 2 or 3 genetic clusters depending on the study site (Fig. 6). However, despite the significant F_{ST} values that showed a significant gene-flow restriction in INFs, the genetic structuring between OF, CNF and INF was not clearly seen at all study sites. Only in some cases, like the study site 1, differences are evident, as half of the individuals of the INF belong to a genetic cluster (purple) were almost absent in the OF and CNF. The same was observed in Site 3, in which in the INF there was a mix of individuals of different genetic clusters whereas the OF was dominated by individuals of one of them (“the yellow cluster”). The genetic structuring would be the result of the restricted gene-flow between patches.

5.4 Discussion

The different strategies evolved by *C. glandium* and *C. elephas* to track food availability in a fragmented landscape have left a distinct genetic signature in their population genetics. *C. glandium* has better dispersal abilities (Pélisson *et al.* 2013b) and, accordingly, we did not find among populations the significant genetic structuring that we recorded in the poor disperser *C. elephas*. In this species gene-flow restriction was higher in new and isolated forest patches compared to new patches close to mature forest plots. Genetic diversity was higher in *C. glandium* compared to *C. elephas* but, contrary to our expectations, it was higher in all types of forest plots, not only in isolated new ones. Contrary to *C. glandium*, *C. elephas* can spread dormancy (i. e. dispersal in time) and persist in all types of patches, and so it was, in fact, the most abundant species practically in all plots. Yet, neutrality tests show that its populations went through marked population bottlenecks even in mature forest patches.

The lack of genetic structuring among populations in *C. glandium* agrees with its higher potential dispersal abilities (Pélisson *et al.* 2013b, Arias-Leclaire et al. 2017) and with the even and non-constrained dispersion of this species within the forest mosaic found for this species in previous works (Ruiz-Carbayo *et al.* 2018). Potential dispersal abilities in *C. glandium* double those of *C. elephas* (1200 *vs.* 600 m) what, given the distance among forest patches in our study area (Baśnou *et al.* 2013; Vilà-Cabrera *et al.* 2017), means that there are not dispersal barriers for *C. glandium*. According with a lower dispersal ability, gene-flow restrictions in *C. elephas* (measured either with mitochondrial DNA or SNPs), were higher in isolated new forests.

The maximum potential distance recorded in *C. elephas* (600 m) would, however, be high enough to avoid gene-flow constrains. The reason for these apparent incongruences could be the previous methods of measuring dispersal distances and the phenology of the species. The dispersal distances reported by Pélisson *et al.* 2013 correspond to wind-tunnel measurements what means that, if well weevils are physically able to fly over those distances, they may not actually do it in the field. Moreover, a later phenology may constrain dispersal in *C. elephas*; the adults of this species emerge in late summer from their underground earthen cells, whereas in *C. glandium* emergence occurs in spring (Pélisson *et al.* 2012; Espelta *et al.* 2017). *C. glandium* is a synovigenic species and adults spend several months in the trees before mating when acorns are available for oviposition in autumn. By contrast, *C. elephas* is proovigenic and females oviposit as soon as they mate just after emergence (Pélisson *et al.* 2012; Arias-LeClaire *et al.* 2017). *C. elephas* females may thus be time constrained to achieve their full potential fecundity (Desouhant *et al.* 2000b) what would prevent investing time in dispersal and thus, ultimately, reducing gene-flow among forest patches.

The higher genetic diversity in *C. glandium* suggests that the higher potential local resilience of *C. elephas* does not compensate for its lower dispersal abilities. The variable dormancy (i. e. variable length diapause) of *C. elephas* means that not all the larvae of the same cohort emerge after a fixed time period; rather, they may spend from one to three years in their underground refuges (Soula & Menu 2003). This strategy increases female fitness, as some of the offspring will be able to skip unfavorable years, and favors local population resilience against the dramatic inter-annual variability of acorn production (Crawley & Long 2008; Espelta *et al.* 2008). In fact, dormancy is common in many insect species that face stochastic seasonal environments and need to cope with unfavorable conditions (Kivelä *et al.* 2016). However, the low genetic diversity observed, the results of the neutrality tests and the shape of the haplotype networks, show that dormancy does not fully avoid population bottlenecks in *C. elephas*. These bottlenecks, which also occur in mature forest plots, could be provoked by the late phenology of this species. In the Iberian Peninsula, adult weevils depend on rainfall to soften the soil and be able to emerge from their underground refuges (Espelta *et al.* 2017), thus, the lack of late summer storms increases adult mortality and reduces *C. elephas* numbers (Bonal *et al.* 2010; Bonal *et al.* 2015). Besides, prolonged diapause reduces the risk of local extinction, but it might reduce population genetic diversity too (Suez 2012). Most individuals go through a one-year diapause (Pélisson *et al.* 2012), hence, if well an unfavorable year will not provoke total extinction, the effective population size will decrease. *C. glandium* will be less susceptible to the lack of rainfall because adults emerge in spring, before summer drought (Espelta *et al.* 2017). Also, despite their lower resilience (i. e. no variable length diapause), *C. glandium* higher dispersal abilities will facilitate recurrent colonization events from different patches, what will increase local genetic diversity (Chen *et al.* 2006; Roman 2006).

The larger but less genetically diverse populations of *C. elephas* suggest quick population growths. According to neutral theory, larger population size should support greater genetic diversity due to a decrease in the effects of genetic drift (Kimura 1983). However, *C. elephas* populations were less diverse than those of *C. glandium* and have gone through bottlenecks that could not be compensated by the arrival of immigrants due to its poor dispersal abilities (Pélisson *et al.* 2013a). The higher numbers of *C. elephas* in almost all forest patches could be explained by a higher fitness that might promote a stronger population growth. *Curculio elephas* is larger-sized than *C. glandium*, and body size is a key life-history trait in insects strongly related with individual fitness (Partridge & Farquhar 1983; Desouhant *et al.* 2000; Berger *et al.* 2008; Bonal *et al.* 2011). Larval survival is higher in *C. elephas* in oak forests where both species co-exist and larval size is correlated with adult size (Bonal *et al.* 2011). In turn, adult body size is correlated with potential fecundity; larger females lay more eggs in *C. elephas* (Desouhant *et al.* 2000a), and such relationship between body size and potential fecundity might hold at the inter-specific level between *C. elephas* and *C. glandium*. In fact, inter-specific comparisons among closely related species of butterflies have shown that larger sized species have a higher potential fecundity (number of eggs) than small sized ones (García-Barros 2000). These fitness advantages associated with a larger body size have been argued to underlie the inter-specific morphological diversification in acorn *Curculio spp.* (Hughes & Vogler 2004).

One of the limitations of our study is that we could not use nuclear markers (SNPs) in the case of *C. glandium*. Inter-specific comparisons based on mitochondrial DNA could be biased, as these genes are maternally inherited and strictly reflect female movements, what could be misleading in case of dispersal differences between sexes (Scribner *et al.* 2001)(Petit & Excoffier 2009). However, the concordance between mitochondrial and nuclear markers in *C. elephas* (higher gene-flow restriction in isolated new forests) suggest that such discordances do not exist in this species and have not likely affected the results of the comparisons between species.

C. elephas and *C. glandium* follow different strategies to track a pulsed food resource (i. e. acorns) what, in a fragmented landscape, conditions the relative frequencies of one or the other species according to the age and degree of isolation of the oak forest patch (Ruiz-Carbayo *et al.* 2018). Our results show that *C. glandium*, better able to track food in space, will easily colonize new and isolated forest plots and maintain a high genetic diversity. In *C. elephas*, dormancy (i. e. dispersal in time) does not make this species less vulnerable to eventual local food shortage, as it will go through bottlenecks further aggravated by the scarce arrival of immigrants (restricted gene-flow). The lower genetic diversity would be expected to lessen the resilience of populations to environmental perturbations (Frankham *et al.* 2002; Reed & Frankham 2003). Yet, *C. elephas* maintained large, but genetically poorer populations, probably due to a higher population growth favored by a higher fitness associated to its larger body size compared to *C. glandium*. In mature forest plots, a higher fecundity allows a quick recovery after a sharp population decline; in new isolated oak patches it would permit a quick population growth starting from a low number of new immigrants. The populations of the poor disperser species are thus not threatened in forest fragments, however, the situation could change if forecasted scenarios of more frequent drought episodes occur (Lionello *et al.* 2006). The vulnerability of *C. elephas* to drought could provoke more recurrent population bottlenecks from which its populations could not easily recovered unless connectivity is favored by the presence of isolated oaks that could act as stepping stones among forest patches (Manning *et al.* 2006).

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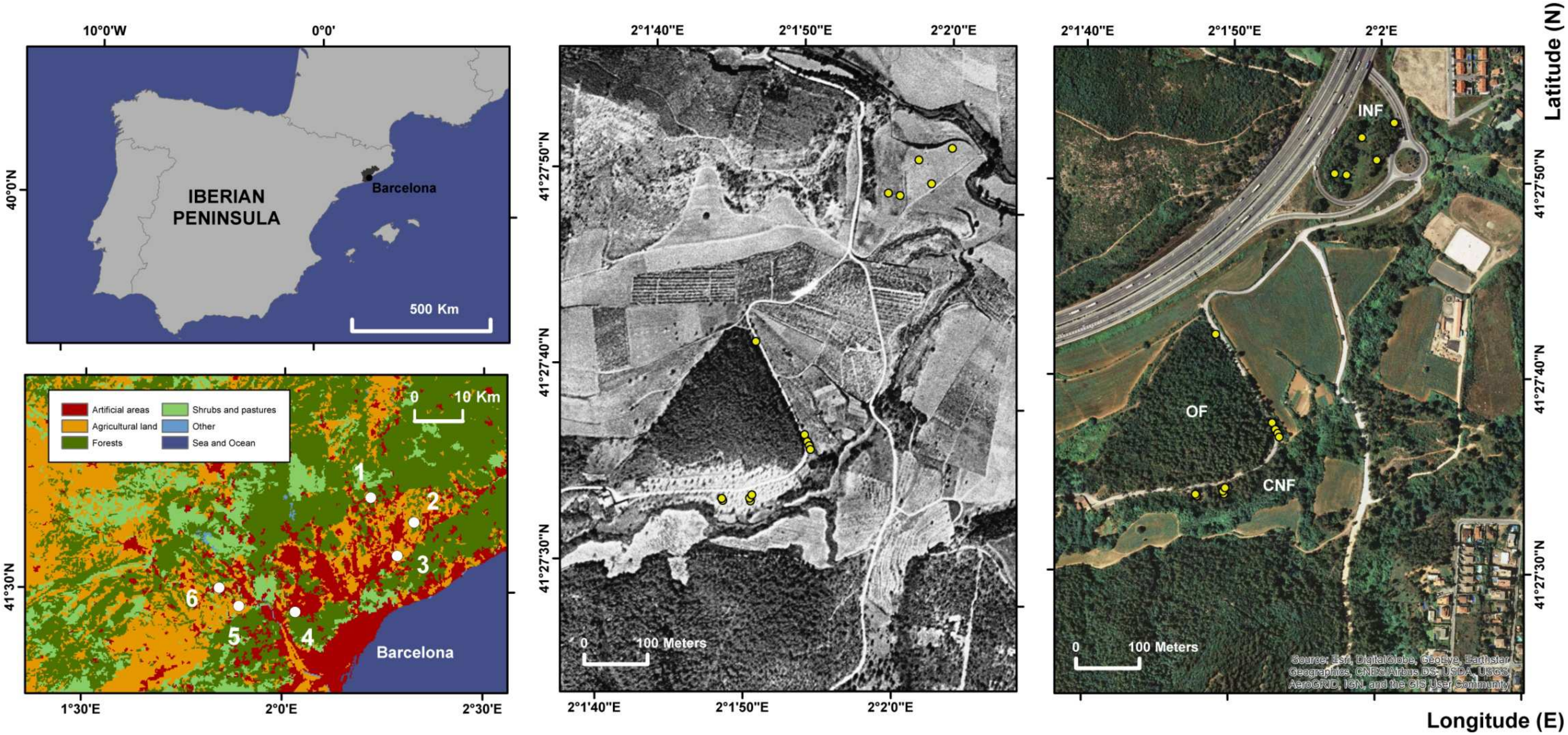
Figure 1. Location of the study site. Top left: Context and study site. Bottom left: Sample locations. Centre and right: One of the replicates as an example of the experimental design, in a 1956- American flight image (centre) and a 2005- orthoimage (right).



Source: Esri, DigitalGlobe, GeoEye, Earthstar Geographics, CNES/Airbus DS, USDA, USGS, AeroGRID, IGN, and the GIS User Community

Figure 2. Population size per patch for two weevil species: *C. elephas* and *C. glandium*. Boxplots represent median, 25th, and 75th percentiles, respectively. White circles represent mean population size. Black circles represent outliers.

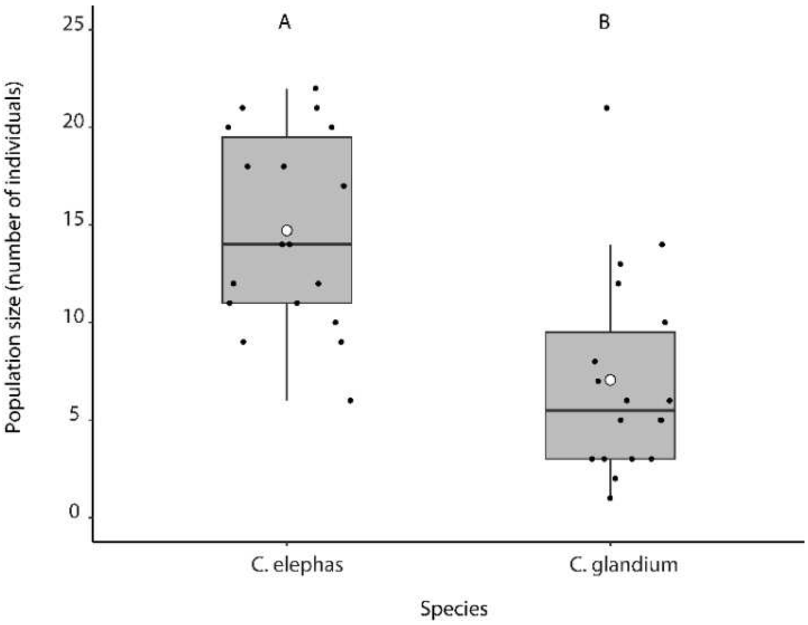


Figure 3. Boxplot illustrating haplotype richness by forest type (OF: old forest, CNF: new connected forest and INF: isolated new forest). Different letters indicate significant differences. Box plots show minimum, lower quartile, median, upper quartile and maximum values. Means are depicted as white dots.

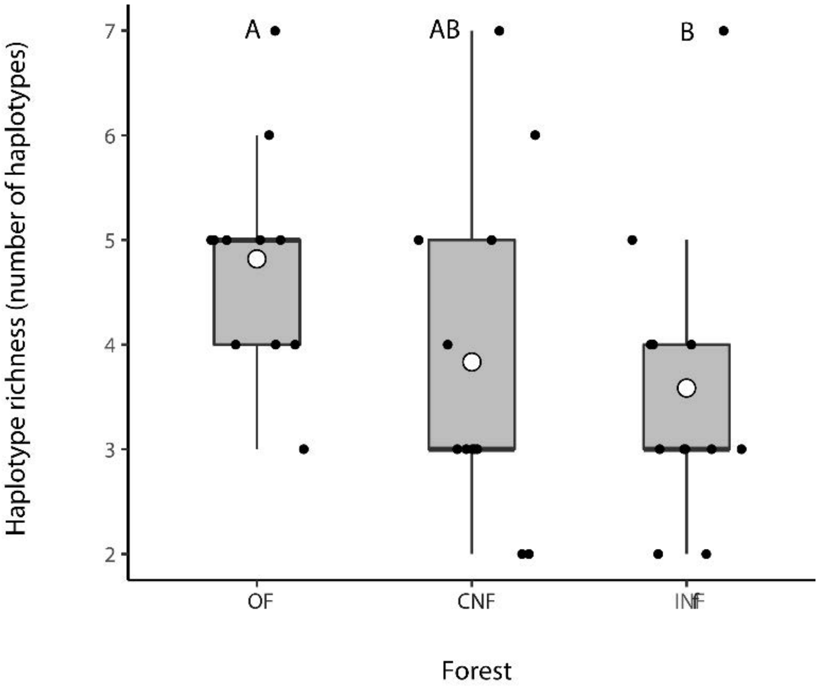


Figure 4. Boxplots illustrating differences in gene (a) and nucleotide (b) diversities between *C. elephas* and *C. glandium*. Box plots show minimum, lower quartile, median, upper quartile and maximum values. Means are depicted as white dots.

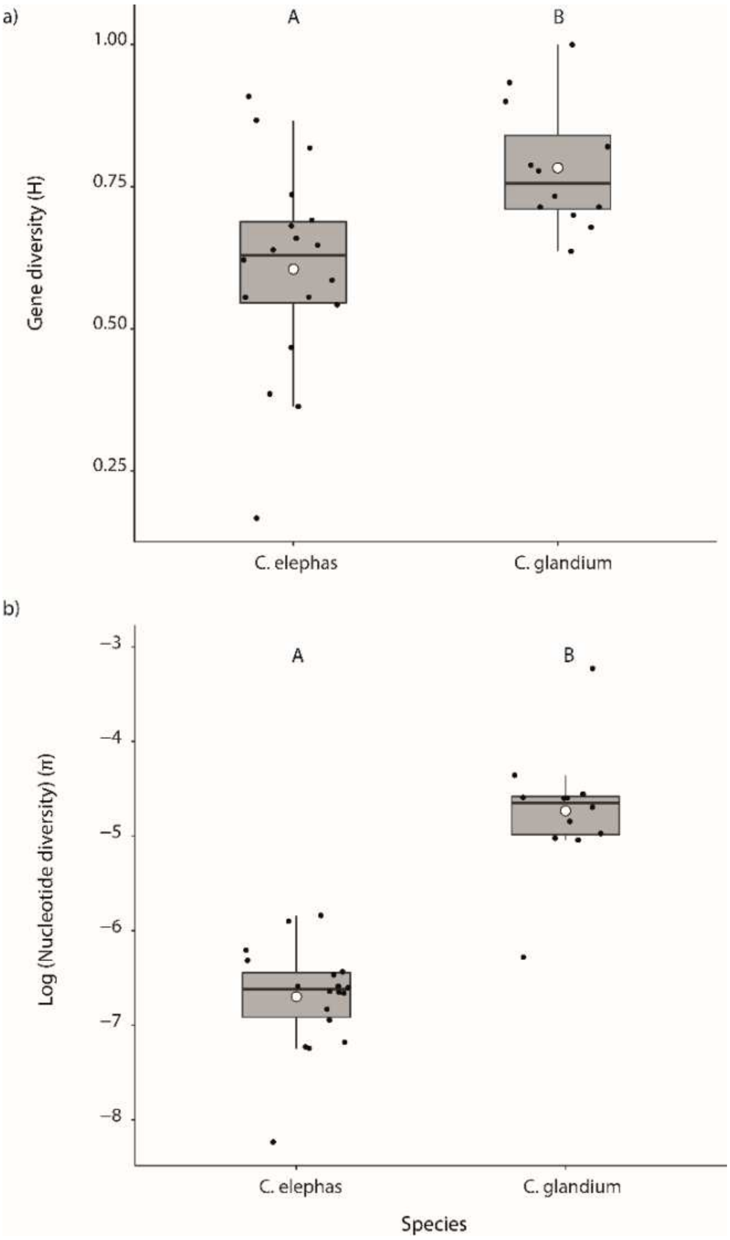


Figure 5. Parsimony network for mtDNA haplotypes of *C. elephas* and *C. glandium* in each study site. The size of the circles represents haplotype frequencies in each site. Bars represent unique mutation steps.

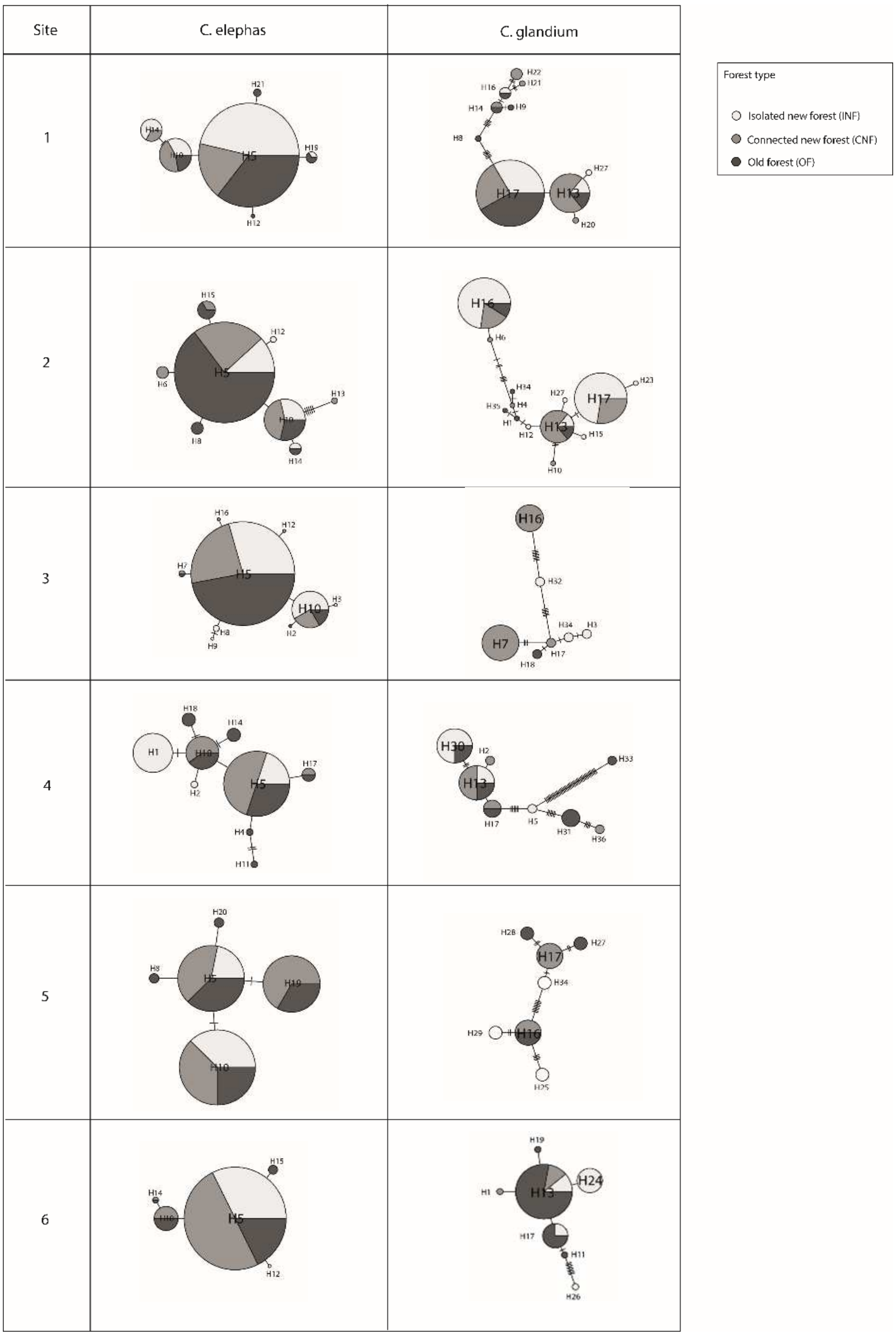


Figure 6. STRUCTURE graphs. Colors refer to different genetic clusters. Bars from top to bottom of the graphs represent % cluster membership of each *C. elephas* individual. Forest code: OF (old forest), CNF (connected new forest) and INF (isolated new forest).

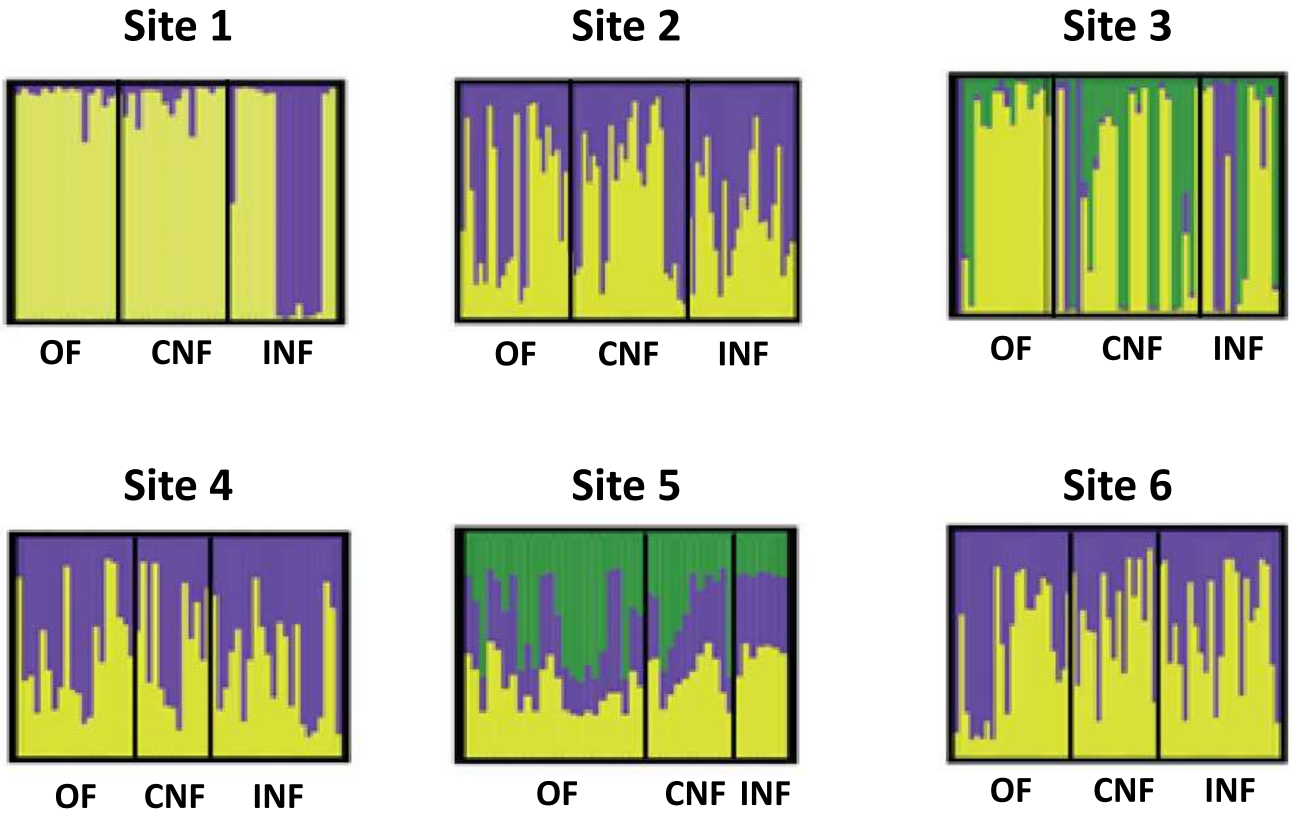


Table 1. Tajima's and Fu's neutrality tests for 825-bp COI sequences of *C. elephas* and *C. glandium* in six study sites. Significant values are highlighted in yellow.

	Statistics	B_INF	B_CNF	B_OF	C_INF	C_CNF	C_OF	H_INF	H_CNF	H_OF	I_INF	I_CNF	I_OF	M_INF	M_CNF	M_OF	V_INF	V_CNF	V_OF	Mean	s.d.
C. elephas																					
Tajima's D test																					
	Sample size	9	9	12	10	20	18	12	22	14	21	11	17	6	11	18	21	14	20	14.722	4.84
	S	3	2	3	1	2	4	1	2	3	3	2	4	3	7	4	3	4	3	3	1.33
	Pi	1.11	0.72	1	0.47	0.61	0.64	0.17	0.46	1	0.89	0.87	0.78	1.27	1.75	0.82	0.87	0.791	0.468	0.82	0.34
	Tajisma's D	0.03	-0.06	0.02	0.82	0.17	-1.35	-1.14	-0.37	0.18	0.16	0.85	-1.04	-0.19	-1.09	-0.88	0.10	-1.222	-1.191	-0.34	0.69
	Tajima's D p-value	0.56	0.37	0.58	0.87	0.65	0.08	0.16	0.36	0.64	0.60	0.83	0.21	0.4	0.16	0.24	0.59	0.128	0.087	0.42	0.25
Fu's FS test																					
	No. Alleles (unchecked)	3	3	7	2	3	5	2	3	4	4	3	5	4	5	5	5	5	4	4	1.25
	Theta_pi	1.11	0.72	1	0.47	0.61	0.64	0.17	0.46	1	0.89	0.87	0.78	1.27	1.75	0.82	0.87	0.79	0.47	0.82	0.34
	Exp. No. Of alleles	2.97	2.44	3.10	2.08	2.74	2.75	1.47	2.42	3.25	3.41	2.83	3.01	2.69	3.95	3.15	3.37	2.88	2.40	2.83	0.54
	FS	0.55	-0.24	**-5.06**	0.82	0.15	**-2.44**	-0.48	-0.31	-0.42	-0.24	0.32	**-1.99**	-1.35	-0.71	**-1.76**	-	**-2.24**	**-1.71**	-1.03	1.37
	FS p-value	0.62	0.35	**0**	0.54	0.45	**0.01**	0.12	0.31	0.36	0.39	0.48	**0.02**	0.06	0.26	**0.05**	0.11	**0.01**	**0.04**	0.232	0.20
C. glandium		B_INF	B_CNF	B_OF	C_INF	C_CNF	C_OF	H_INF	H_CNF	H_OF	I_INF	I_CNF	I_OF	M_INF	M_CNF	M_OF	V_INF	V_CNF	V_OF	Mean	s.d.
Tajima's D test																					
	Sample size	5	5	6				7		12	7	12	10	21	13	5		8		9.25	4.51
	S	9	8	53				12		4	11	13	13	13	13	11		13		14.42	11.93
	Pi	4	3.4	19.27				3.81		0.99	3.52	5.46	5.33	5.51	4.74	4.8		7.07		5.66	4.35
	Tajisma's D	-0.53	-0.81	-1.09				-1.21		-0.90	-1.16	1.13	0.73	1.88	0.55	-0.65		2.08		0.00	1.16
	Tajima's D p-value	0.42	0.30	0.15				0.12		0.21	0.15	0.91	0.80	0.98	0.77	0.34		1.00		0.51	0.34
Fu's FS test																					
	No. Alleles (unchecked	3	4	5				4		4	4	5	6	7	6	5		3		4.67	1.18

	)																				
	Theta_pi	4	3.4	19.27				3.81		0.99	3.52	5.46	5.33	5.51	4.74	4.8		7.07		5.66	4.35
	Exp. No. Of alleles	3.54	3.39	5.34				4.32		3.08	4.21	6.70	5.97	9.06	6.64	3.69		5.63		5.13	1.70
	FS	1.87	-0.13	1.91				1.15		-0.65	0.98	2.46	0.54	2.17	1.13	-1.41		5.22		1.27	1.64
	FS p-value	0.84	0.35	0.74				0.72		0.21	0.69	0.88	0.58	0.86	0.71	0.10		0.98		0.64	0.27

Table 2. Detailed variance analyses (AMOVA) for the two weevil species for all (18) populations in the case of *C. elephas* and for 12 populations in *C. elephas* and *C. glandium*.

	C. elephas	*C. glandium*
Variance among populations (18)	0.0469	-
F_{ST}	10.5%	-
P	< 0.001	-
Variance among individuals within populations	0.3987	-
F_{IS}	89.5%	-
P	-	-
Variance among populations (12)	0.0662	0.1533
F_{ST}	12.3%	5.6%
P	< 0.001	0.063
Variance among individuals within populations	0.4735	2.5960
F_{IS}	87.7%	94.4%
P	-	-

Chapter 6: *General discussion*

In this thesis I have addressed diverse aspects of the assembly of the herbivore insect community in *Quercus ilex* forests and the resulting effects of this plant-animal interaction for the host trees. Using a spatially explicit approach at contrasting scales, we have explored both, genetics and specific composition of some species (i.e. *Curculio spp.*) and communities (i.e. lepidoptera), as well as the intensity of folivory and granivory, in relation with species' life history traits, landscape context and their interaction. We have found that, in the studied context of land-use change and forest patch recovery in former agricultural areas, neutral processes are playing a particularly important role in assembling the community of insects and their interaction with *Quercus ilex* at landscape scale, as partially suggested by previous works on plant species assemblages (e.g. Basnou & Pino 2015; Basnou *et al.* 2016). As mentioned above, the spatial component -quite unexplored by now- has been thoroughly studied in the present thesis, with dispersal ability and habitat connectivity becoming key traits for the abundance and composition patterns observed, as well as the genetic structure and variability of herbivore populations.

Furthermore, the effect of time has been commonly overlooked in the literature on community ecology owing, partly, to the restriction of time-limited spatial data sets. Thus, we have successfully added the effect of time, simply comparing pre-existing and recent forests detected from land cover change maps, to the spatial effects of landscape (regional), patch (local) and tree (individual) scales. Results show that habitat age is as important as spatial attributes at structuring communities and biotic interactions in these new habitats; the herbivore community of *Q. ilex* in new forests is becoming assembled, at least to some extent, according to tree characteristics such as canopy size, connectivity and age. The interaction between these inherently spatiotemporal characteristics make well-structured, well-connected and mature holm oaks, into refugees of more abundant, richer and more diverse Lepidoptera communities (Chapter 2). Other characteristics such as tree genotype determines leaf chemical and physical defenses, and therefore, the quality and palatability of the host plant can also be a modulator of plant resistance/susceptibility to herbivory (Solla et al., 2016). As a result, uneven herbivory patterns can be observed within the same habitat patch, with some host individuals being more heavily damaged by insect herbivores than others (Chapters 3 and 4). Thanks to our integrative approach (**Figure 1**) we could prove how important interactions between size, age, connectivity, genetics and location of the host, are in the assembly of the herbivore community and the assembly of the resultant plant-animal interaction (herbivory). At the patch scale, this heterogeneous pattern in herbivore assemblages can still be observed among patches of different age and connectivity, with poor disperser insects gathering in old and well-connected patches and exhibiting colonization credits in the new isolated ones, while insects with better dispersal abilities colonizing old and new, connected and isolated patches, equally (Chapter 4). Nevertheless, habitat patches with different herbivore assemblages exhibit similar acorn predation damage, in what we have identified as a zero-sum effect of landscape attributes on herbivory assembly as a result of compensatory dynamics among species (Chapter 4). And at the landscape scale, this pattern of heterogeneous herbivore assemblages and homogeneous herbivory damage, persist. This assembly process has already left a genetic signature in the community, with a noticeable genetic structure among populations and lessened local genetic diversity of the poor dispersers due to bottlenecks and founder effects (Chapter 5).

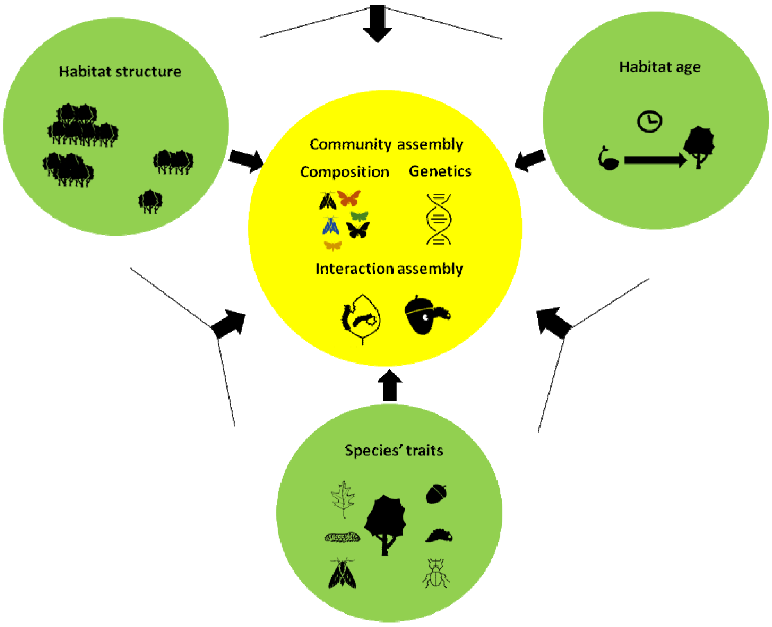


Figure 1. Integrative approach followed in the thesis, considering the interaction of spatiotemporal attributes of the landscape with species' traits, in order to study the assembly of a herbivore community and herbivory, in expanding habitats.

6.1 Islands in the stream: Assembly of a Lepidoptera community in an expanding savannah-like *Quercus ilex* landscape

In Chapter 2 we've tried to shed some light on this information gap on the interactive effects of species' traits and landscape spatiotemporal attributes on the assembly of a Lepidoptera community inhabiting holm oak (*Q. ilex*) trees in a savannah-like landscape emerged after cropland abandonment. The importance of spatial connectivity on the distribution of herbivore insects in patchy habitats, has been already highlighted in previous literature, where better connected trees host higher abundances and herbivore richness in both, highly isolated trees and trees embedded in closed mixed canopies (Gripenberg & Roslin 2005; Müller & Goßner 2007). This pattern has also been identified for other host species (e.g. grasses) and ecosystems (e.g. tropical forests; Doak 2000; Ricketts *et al.* 2001), but the temporal dimension of these habitats were overlooked in spite of the relationship that the colonization time hypothesis establishes between species richness and the time a patch has been available for colonization (Borges & Brown, 1999).

The most striking results in the second chapter of this thesis are that, indeed, there's a complex interplay between habitat structure and age (holm oaks are very distant so each tree act as a discrete habitat patch), with greater species abundance, richness and diversity of folivore Lepidoptera in old, larger and more connected trees than in young, small, isolated ones. Thus, we detect colonization credits (i.e. time-lags between the emergence of suitable habitat and the arrival of the organisms; Jackson & Sax 2010) in the youngest and most isolated trees, probably due to dispersal limitations of the smallest species within the Lepidoptera community. Heiniger *et al.* (2014), in one of the few studies available that tackles the spatiotemporal structure of habitats altogether, demonstrated the key role of habitat temporal structure for a Collembola community, revealing colonization credits in recent habitats, especially forests, in line with the results of our findings. On the contrary, insect species composition (alpha and beta diversity) found in each tree of the study site was independent of the age and/or structure of the tree, but merely a function of the spatial distance among trees, revealing a predominant random effect. Again, this result agrees with Ricketts *et al.* (2001) who, addressing the assembly of a Lepidoptera community in tropical forests, found that species composition changed exclusively as a function of distance between habitat patches and not due to differences in habitat quality. In general, results showed that in this archipelago of scattered trees the paradigm of island biogeography (MacArthur & Wilson, 1967) and neutral models (Gyllenberg & Hanski 1997; Hanski 1999) operate, as the community is constrained to some extent by habitat spatiotemporal attributes and spatial dispersal ability of Lepidoptera species. Similarly, in afforested areas with higher densities where *Q. ilex* trees are colonizing new forests patches (Chapter 4), the interplay of herbivore dispersal ability and landscape age and structure shapes the seed-predator guild formed by the tortrycid *Cydia fagiglandana* and the two sibling species *Curculio glandium* and *C. elephas*. Moreover, *C. elephas* exhibits colonization credits (*sensu* Jackson & Sax 2010) in the new and most isolated forest patches probably due to the low dispersal ability of this species. Thus, local herbivore community (both folivore and seed-predator) changes as a function of species' dispersal ability and habitat spatiotemporal attributes, and so different assemblages coexist at the regional level in patchy and heterogeneous landscapes, increasing community heterogeneity.

6.2 Fatal attraction: **The role of host genetic identity, ontogeny and spatial distribution in insect herbivory in expanding oak forests**

Simultaneously to the assembly of new herbivore communities in these new Mediterranean forests, the arriving insects will start interacting with the plants that form their habitat template and source of food and shelter (Bonal & Muñoz 2008; Valencia-Cuevas & Tovar-Sánchez 2015). In Chapter 4, again, we find that individual characteristics of *Q. ilex* trees such as identity, age, conspecific connectivity and location, create uneven patterns of herbivory within the same patch. Even though these tree characteristics have been largely suggested to have an impact on herbivory (McCrea & Abrahamson 1987; Urbas *et al.* 2007; Elger *et al.* 2009), little is known about the nature of the interactions among these factors. Within the new patches of mixed Mediterranean forests studied in this chapter, we proved that genetic identity and location of the tree, have direct effects on herbivory, with some tree clones being more heavily attacked by herbivores than others as well as holm oaks at the patch core compared to those located at the edge. These results agree with previous studies pointing out genetic-based differences in chemical defenses as a main driver of herbivory exerted by Lepidoptera (Hemming & Lindroth 1995; Osier *et al.* 2000). Even though a recent work of Solla *et al.* 2016 have reported no effect of foliar tannin content of *Q.ilex* on larvae performance, differences in other secondary metabolites could be behind leaf damage differences among holm oak clones in our study site. The lower herbivory rates at the edge of the patch, supports the hypothesis of leaf quality variations associated to differences in environmental conditions between the edge and the core of the habitat, regulating herbivory by insects (Angulo-Sandoval & Aide 2000; Rinker & Lowman 2004; Guirado *et al.* 2006). There are both, supporting and contradictory literature for this better regulation of herbivores on forest edges (McGeoch & Gaston 2000; Skoczylas *et al.* 2007; Wirth *et al.* 2008) and so further research should be carried out on this topic.

In spite of the potential differences in apparency (Castagneyrol et al., 2013; Régolini et al., 2014) and leaf quality (Bryant *et al.* 1992; Herms & Mattson 1992; Boege 2005) among developmental stages (from seedlings to mature trees), no significant changes in leaf damage were evidenced, challenging both, the plant-age hypothesis and the growth-differentiation balance hypothesis (Barton & Koricheva, 2010). There is a lack of information on ontogenic variation of chemical defenses on *Q. ilex* leaves and therefore, only a future study on the metabolomics of foliar tissue could generate conclusive results on this regard. Herbivory in new Mediterranean forests also seemed regulated by the interaction between tree age and intra-patch connectivity with mature holm oaks, resulting in associational resistance (Hambäck et al., 2000) against herbivores for trees but not for younger stages (seedling and saplings). This pattern could be the result of short-distance plant-plant communication among individuals that have already overcome the growth *vs.* defense trade-off (Herms & Mattson, 1992) mediated by the release of volatile signals, as already proved for *Q. ilex* in the face of herbivory damage (Staudt & Lhoutellier, 2007). Unfortunately, little research has been conducted in genetic-based traits and spatial location, and there is also a gap knowledge on the effects of associational resistance on the behavior and survival of herbivores and their natural enemies.

6.3 Different actors, same film: Shifts in the composition of the granivore guild has zero-sum landscape effects on acorn predation in expanding *Quercus ilex* forests

Even though the herbivore-plant interaction takes place at the tree (individual) scale, its dynamics will depend on processes operating at larger spatial scales, like the interplay between species' traits and spatiotemporal landscape attributes modulating community and metacommunity dynamics at the local and regional scales, as observed in the Vallès forests (Chapter 4). At a tree scale, we found higher infestation rates by *C. fagiglandana* in oaks producing small crops made up by large seeds, as well as higher infestation rates by weevils in connected oaks with large seeds, and so granivore and granivory assembly showed an heterogeneous pattern in host with different characteristics (Chapter 4) just as the Lepidoptera herbivore community and herbivory damage did (Chapters 2 and 3). Interestingly, at the patch scale, the colonization credits shown by the poorest disperser of the seed-predator guild, *C. elephas*, did not translate into a 'predator release' effect of holm oak crops in new isolated forests, that is, the interaction intensity (acorn infestation rate) did not decrease as a consequence of *C. elephas* lower abundance. We found an explanation for this lack of 'predator release' effect in the even distributions of the good disperser *C. fagiglandana* and *C. glandium*, which counterbalanced total abundances of species within the guild. Previous work pointed out that this kind of compensatory dynamics are rare in natural ecological communities (Houlahan et al., 2007) and that species tend to covary positively rather than negatively. In spite of that, the homogeneity found in the granivore-oak interaction agrees with the zero-sum assumption of Hubbell's Neutral Theory of Biodiversity, in which the changes in the abundance of one species are accompanied by compensatory changes in the abundances of others due to resource constraints (Hubbell, 2001). If this negative covariance is driven by interspecific competition among seed-predators, then the community would be at or near its carrying capacity. Nonetheless, almost 80% of acorns remained non-attacked by neither the acorn moth nor weevils and so most probably, resource limitation is not the key or the unique driver of the zero-sum dynamics observed in this guild, but differences in dispersal ability between species, as has been demonstrated for other study systems (Hagen et al., 2012).

Similarly, even though a predator identity effect was found on the survival likelihood of acorn embryo, with *Curculio* species destroying the embryo more often than *C. fagiglandana*, this potential advantage in new isolated patches would vanish due to the even distribution of *C. glandium*. Muñoz *et al.* (2014) already demonstrated that due to differences in larval size among weevil species (Espelta *et al.* 2009; Pélisson *et al.* 2011), they had different nutritional requirements and so the larval development of the largest bodied *C. elephas* was usually constrained by cotyledon depletion. This difference in the amount of cotyledons consumed linked to larval body size could also extend between weevils and the acorn moth. Supporting this idea, *Curculio* females avoid ovopositing in acorns already occupied by moths (Debouzie et al., 1996), while the opposite avoidance behavior has not been described for *C. fagiglandana*, whose smaller larvae could have lower nutritional requirements. In addition, differences in ethology (of both adults and larvae) could be important since weevil larvae are constrained to develop within a single acorn, while acorn moth larvae are able to switch from one acorn to another (Soria, 1999). Even though at the patch scale this "seed-predator identity" effect vanishes due to the zero-sum dynamics in the forests studied in this thesis, this finding underscores the importance that for the estimation of crop size available for plant recruitment, might have to consider the identity of the predator species and not only the number of acorns infested, since differences in seed survival have been revealed for other study systems when predators of different sizes are removed (e.g. see Hautier *et al.* 2010 for vertebrate herbivores). In agreement with the dynamics arisen at patch level, connected landscape hosted higher abundances of *C. elephas*, while herbivory was equally spread.

6.4 What lies beneath: Have dispersal vs. dormancy strategies of tracking unpredictable resources already left a genetic signature on *Curculio* populations inhabiting expanding *Quercus ilex* forests?

To sum up, Chapters 2, 3 and 4, support the importance of tackling the community and interaction assembly from interactive approaches and several spatial scales, since complex interplays can be missed and their relative importance on herbivore community and herbivore assembly, largely depends on the scale chosen. While there are some studies on spatiotemporal landscape attributes and species' traits impact on community assembly (Borges & Brown 1999; Tscharntke & Brandl 2004; Badano *et al.* 2005; Jackson & Sax 2010), much less is known about the genetic diversity and isolation of these emerging communities. Weevils have evolved dispersal strategies in time and space to cope with unpredictable acorn availability (Venner *et al.* 2011; Pélisson *et al.* 2012; Pélisson *et al.* 2013). Chapter 4 revealed that in new forests of the Iberian Peninsula, these differences in extinction risk spreading strategies have impacted community composition. As seen in Chapter 5, nevertheless, it seems to be only partially responsible for the genetic structure and variability observed in the same populations. In accordance with the colonization credits exhibited by C. elephas in new isolated forests in Chapter 4, this poor disperser showed a significant genetic structure among populations as well as restricted gene flow between old forests and new isolated ones in Chapter 5; on the contrary, *C. glandium* populations showed no genetic structure and no restrictions to gene flow. This results agree with the maximum 600-m dispersal ability estimated for *C. elephas* (Pélisson et al., 2013), which would experience this forest mosaic as patchy, while *C. glandium*, with an estimated 1200-m dispersal ability, would experience the same mosaic as a continuous habitat. Thus, dispersal limitation lowers genetic diversity of *C. elephas* populations in new isolated forests, while *C. glandium* recurrently colonizes new forests, even the most isolated ones, counterbalancing the potential loss of genetic variability due to bottlenecks associated to local environmental stochasticity.

This high genetic diversity in colonizing populations has been studied with special attention in the case of aquatic invaders, whose populations have increased their genetic diversity as a function of the size and frequency of human-assisted inoculations (Dupont *et al.* 2003; Voisin *et al.* 2005; Simon-Bouhet *et al.* 2006). In a similar way, recurrent colonization events would have increased the probability of establishment of *C. glandium* populations, vanishing the founder effect. On the contrary, large populations of *C. elephas* are poor in genetic variability, suggesting that this species experience quick population growths after both, bottlenecks and founder effects, in all forest types. We find strong support in the literature of fecundity benefits in large-bodied insects being behind this pattern of large populations with unexpected poor genetic variability. Body size is a key life-history trait in insects that is strongly related to individual fitness, and particularly in *C. elephas* larval size is correlated with adult size (Bonal et al., 2011) and larger females lay more eggs (Desouhant et al., 2000). Since previous work in related species of butterflies have shown that larger sized species have a higher potential fecundity (García-Barros, 2000), this could be also true between the small-bodied *C. glandium* and the larger-bodied *C. elephas*. The limitation in dispersal ability of *C. elephas* is paying its toll on population genetic diversity and even though large numbers guarantee the persistence of these populations in the short-term, the future resilience of this species in Mediterranean forests in expansion could be threaten due to the inherent environmental stochasticity of this habitats (Lionello et al., 2006) and the forecast scenario of drier summers in Mediterranean areas (Bonal et al., 2015).

6.5 Perspectives

Undoubtedly, this thesis leaves still some uncertainties about the underlying mechanisms and drivers of herbivore community assembly and herbivory assembly in new forests. The complexity of this topic, as well as the idiosyncrasy of study systems represent a challenge for attempts to identify key determinants and extract generalizable principles. From a practical point of view, this thesis provides valuable information for the management of herbivore impact on the expanding populations of *Q. ilex*, a key species of Mediterranean forests (Blanco 1998; Faivre-Rampant *et al.* 2011). Studies to date show no consistent response of herbivore insects to habitat fragmentation (McGeoch & Gaston 2000; Skoczylas *et al.* 2007; De Carvalho Guimarães *et al.* 2014; Maguire *et al.* 2016), however, evidences from Huecas and Vallès lowlands suggest that the patchy arrangement of these expanding habitats are favoring the existence of different herbivore assemblages, but with a resultant zero-sum impact of herbivory on holm oaks. Therefore, the maintenance of this patchy arrangement with moderate isolation among patches, could be a strategy to keep higher beta diversity within the regional herbivore community at the landscape level, without disrupting metacommunity functioning (Jamoneau et al., 2012). On the other hand, favoring connectivity by the maintenance of isolated holm oaks that could act as stepping stones for *C. elephas* dispersers would lessen the extinction risk of its populations in isolated patches (Manning et al., 2006). Regarding holm oak recruitment, small patches with large edge/core ratio can facilitate holm oak expansion due to the better regulation of herbivory at edges, as well as mixed patches with numerous holm oak clones and different woody species that could prevent the quick herbivore spillover among trees of the same origin (Dulaurent et al., 2012).

Worth studying in the future would be the management of isolated canopies in savannah-like landscapes, since better structured and encroached canopies host more abundant, richer and diverse Lepidoptera communities, could we enhance the establishment of these insect species by encouraging the production of new shoots and encroachment of holm oak canopies by means of pruning? Also, future research in areas with a more contrasting landscape connectivity, in which the effects of habitat age and habitat connectivity could be clearly tease apart, would be the next step on the study of community and interaction assembly in these expanding Mediterranean forests. Furthermore, we could study whether there exist a connectivity threshold below which, fitness advantage shown by *C. elephas* compared to *C. glandium*, could no longer counteract the negative effects of bottlenecks. Should exist such a threshold, new and highly isolated patches would experience a 'predator release' and holm oaks could pay their colonization credits quicker. Management strategies will vary depending on which ecological process we want to enhance, whether is the expansion of the key species *Quercus ilex*, or the promotion of biotic interactions that would reinforce the resilience of the ecosystem.

6.6 *CONCLUSIONS*

From the results obtained in this thesis, we can deduce the following general conclusions:

1. There exist complex interactions between holm oak canopy size, connectivity and age which, in combination with species' dispersal ability, have an impact on the abundance, richness and diversity of the Lepidoptera herbivore community colonizing holm oak trees in a recently created savannah-like landscape (Chapter 2).
2. Random effects mediated by the spatial distance between these holm oaks, are also shaping alpha and beta diversity patterns in the Lepidoptera community (Chapter 2).

3. At the local scale, individual host-characteristics such as genetic origin and location within the patch, have a direct impact on herbivory pressure, causing uneven leaf damage among different clones and an edge effect (Chapter 3).
4. On the contrary, age of the holm oak and connectivity with conspecifics, interact to affect herbivory pressure by associational resistance of trees in the vicinity of the oldest holm oaks (Chapter 3).
5. At the regional scale, dispersal ability plays a very important role in the community assembly of granivore insects, causing colonization credits in the poor disperser *C. elephas* in new and isolated forest patches, as well as unrestricted distributions of the good disperser *C. glandium* and *C. fagiglandana* (Chapter 4).
6. This compensatory dynamics in abundances vanishes the potential 'predator release' derived from *C. elephas* colonization credit. Consequently, the presence of patches with different age and connectivity promotes heterogeneity in seed-predator assemblages but homogenization of granivory at the regional scale, leading to zero-sum effects on acorn-predation intensity (Chapter 4).
7. The interplay of insect dispersal ability with habitat age and connectivity within the mosaic forest affect the herbivore community in terms of relative abundances but also at the finer genetic level (Chapter 5).
8. Limited dispersal causes restrictions in gene flow and a decrease in genetic diversity in *C. elephas* populations inhabiting new isolated patches, which exhibits bottleneck and founder effects, while no effects were observed in *C. glandium* (Chapter 5).
9. In spite of bottlenecks and founder effects in *C. elephas*, its populations are larger than *C. glandium* ones in all forest types, so other factors are certainly favoring the fitness of the former one (Chapter 5).

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APPENDIX A

Table A.1. Total number of individuals of Lepidoptera by family. Families are ordered according to decreasing species richness.

Family	Species	Number of individuals
Drepanidae	*Drepana binaria*	2
Drepanidae	*Drepana uncinula*	1
Geometridae	*Eupithecia abbreviata*	86
Geometridae	*Alsophila aescularia*	54
Geometridae	*Ennomos quercaria*	8
Geometridae	*Erannis defoliaria*	1
Lycaenidae	*Satyrium ilicis*	26
Lycaenidae	*Satyrium esculi*	3
Noctuidae	*Catocala nymphagoga*	455
Noctuidae	*Dryobotes eremite*	161
Noctuidae	*Dryobotodes monochroma*	64
Noctuidae	*Dryobota labecula*	48
Noctuidae	*Nycteola revayana*	31
Noctuidae	*Spudaea ruticilla*	27
Noctuidae	*Catocala nymphaea*	8
Noctuidae	*Dryobotodes tenebrosa*	5
Noctuidae	*Dryobotodes cerris*	3
Noctuidae	*Orthosia cerasi*	3
Noctuidae	*Bena bicolorana*	3
Noctuidae	*Catocala promissa*	1
Noctuidae	*Agrochola lychnidis*	1
Pyralidae	*Phycita torrenti*	136
Tortricidae	*Tortricodes alternella*	171
Tortricidae	*Tortrix viridana*	38

Table A.2. Habitat drivers and community variables included in the study. First column represent tree descriptors. Community variables are expressed as annual means ± standard deviation. Units of measure are indicated in brackets.

Habitat drivers					Community variables		
Tree ID	**Age (years)**	**Crown size (m^2)**	**Connectivity (m^2)**	**LAI**	**Species abundance (number of individuals)**	**Species richness (number of species)**	**Species Diversity (Shannon's Diversity)**
1	34	43	451	1,98	5.0 ± 4.5	2.6 ± 2.3	2.2 ± 2.0
2	64	104	307	2,48	3.6 ± 3.3	2.4 ± 1.9	2.4 ± 2.0
3	40	81	214	2,77	3.2 ± 2.2	2.6 ± 1.8	2.7 ± 2.0
4	20	29	109	0,79	4.2 ± 3.4	2.0 ± 1.0	1.8 ± 0.7
5	38	72	163	2,32	19.6 ± 16.8	4.4 ± 1.9	3.1 ± 1.2
6	49	91	156	2,69	15.0 ± 5.1	5.0 ± 1.0	3.5 ± 0.7
7	49	73	93	3,35	20.8 ± 15.4	4.8 ± 1.3	3.6 ± 1.1
8	52	26	83	1,51	19.8 ± 16.6	4.2 ± 1.9	3.4 ± 1.8
9	50	104	545	3,40	5.8 ± 6.0	3.0 ± 2.0	2.6 ± 2.2
10	43	89	565	2,54	8.4 ± 5.8	3.0 ± 1.9	2.4 ± 1.3
11	60	94	15704	3,15	17.4 ± 9.3	5.4 ± 1.1	4.0 ± 1.7
12	68	99	15052	3,25	12.4 ± 7.6	3.6 ± 1.7	2.8 ± 1.2
13	56	62	9838	3,54	24.8 ± 20.9	5.0 ± 3.9	3.2 ± 6.9
14	50	72	8963	3,61	12.0 ± 11.0	4.8 ± 2.2	4.0 ± 2.6
15	66	79	23169	2,81	13.8 ± 10.0	5.0 ± 3.3	4.0 ± 5.3
16	67	75	23362	2,39	17.4 ± 3.4	5.8 ± 2.8	4.4 ± 5.4
17	39	26	22667	2,56	6.6 ± 5.6	3.4 ± 2.6	3.2 ± 4.2
18	57	67	22842	1,49	9.2 ± 5.6	4.0 ± 2.0	3.1 ± 3.2
19	74	32	21256	1,87	8.2 ± 5.3	3.8 ± 2.5	3.3 ± 3.5
20	49	32	21161	2,15	14.0 ± 9.9	5.6 ± 3.8	4.0 ± 4.8
21	46	27	18116	2,55	15.8 ± 12.1	4.2 ± 1.9	3.2 ± 1.9
22	35	12	436	1,66	8.6 ± 6.8	3.2 ± 1.1	2.7 ± 0.5
23	33	43	318	1,57	5.2 ± 3.3	2.8 ± 1.1	2.4 ± 0.7

Table A.3. Identity of Lepidoptera species and total captures per tree and sampling year. Species: D.l: *Dryobota labecula* E., D.e: *Dryobotodes eremita* F., D.m: *Dryobotodes monochroma* E., D.t: *Dryobotodes tenebrosa* E., D.c:, C.noga: *Catocala nymphagoga* E., C.nea: *Catocala nymphaea* E., C.p: *Catocala promissa* D. & S., N.r: *Nycteola revayana* S., S.r: *Spudaea ruticilla* E., O.c: *Orthosia cerasi* F., B.b: *Bena bicolorana* F., A.l: *Agrochola lychnidis* D. & S., E.q: *Ennomos quercaria* H., E.d: *Erannis defoliaria* C., E.a: *Eupithecia abbreviata* S., A.a: *Alsophila aescularia* D. & S., T.v: *Tortrix viridana* L., T.a: *Tortricodes alternella* D. & S., S.i: *Satyrium ilicis* E., S.e: *Satyrium esculi* H., P.t: *Phycita torrenti* A., D.u: *Drepana uncinula* B., D.b: *Drepana binaria* H.

Tree	Year	D.l	D.e	D.m	D.t	D.c	C.noga	C.nea	C.p	N.r	S.r	O.c	B.b	A.l	E.q	E.d	E.a	A.a	T.v	T.a	S.i	S.e	P.t	D.u	D.b
1	2010	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
2	2010	1	1	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
3	2010	0	0	0	0	0	2	0	0	0	0	1	0	0	0	0	0	0	0	1	0	0	0	0	0
4	2010	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
5	2010	0	0	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
6	2010	0	0	0	0	0	5	0	0	0	0	0	0	0	1	0	1	0	1	0	0	0	0	0	0
7	2010	0	3	0	0	0	4	0	0	0	0	0	0	0	0	0	0	0	2	8	0	0	0	0	0
8	2010	0	6	0	1	0	11	3	0	0	0	0	0	0	1	0	0	0	2	6	0	0	0	0	0
9	2010	0	0	0	0	0	3	0	0	0	0	0	0	0	0	0	1	0	1	1	0	0	0	0	0
10	2010	0	0	0	0	0	5	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	1	0	0
11	2010	0	0	0	0	0	2	0	0	0	1	0	0	0	0	0	5	0	0	0	0	0	0	0	0
12	2010	0	0	0	0	0	4	0	0	2	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0
13	2010	0	0	1	0	0	6	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0
14	2010	0	0	0	0	0	1	0	0	1	0	0	0	0	0	0	1	0	0	1	0	0	0	0	0
15	2010	0	1	3	0	0	9	1	0	2	0	0	0	0	0	0	2	0	0	3	0	0	0	0	0
16	2010	0	2	2	0	0	6	0	0	1	1	0	0	0	1	0	3	0	1	0	0	0	0	0	0
17	2010	1	0	0	0	0	1	1	0	0	1	0	0	0	0	0	1	0	0	2	0	0	0	0	0
18	2010	0	0	0	0	0	2	1	0	1	1	0	0	0	0	0	0	0	1	0	0	0	0	0	0
19	2010	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	4	0	0	0	1	0
20	2010	1	2	1	0	0	7	1	0	0	0	0	0	0	1	0	0	0	0	3	0	0	1	0	0
21	2010	0	0	2	0	0	6	0	0	0	0	0	0	0	0	0	0	0	1	2	0	0	0	0	0
22	2010	0	1	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0
23	2010	0	0	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
1	2011	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
2	2011	0	0	0	0	0	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
3	2011	1	1	2	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0
4	2011	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0
5	2011	8	14	2	0	0	2	0	0	0	0	0	0	0	0	0	0	0	1	17	3	0	0	0	0
6	2011	2	7	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	6	2	0	0	0	0
7	2011	4	15	1	0	0	0	0	0	0	0	0	0	0	0	0	3	0	1	24	0	0	0	0	0
8	2011	1	12	0	0	0	11	0	0	0	0	0	0	0	0	0	0	0	0	18	0	0	0	0	0
9	2011	0	0	0	0	0	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

10	2011	0	5	0	0	0	5	0	0	0	0	0	1	0	0	0	1	0	0	3	0	0	0	0	0
11	2011	3	2	4	0	0	3	0	0	0	3	0	0	0	0	0	0	0	0	10	1	0	0	0	0
12	2011	0	0	1	0	0	7	0	0	0	0	0	0	0	0	0	2	0	0	4	1	0	0	0	0
13	2011	0	4	5	0	0	28	0	0	1	0	0	1	0	0	0	3	0	0	8	1	0	0	0	0
14	2011	1	2	1	0	0	6	0	0	0	0	0	0	0	0	0	0	0	0	1	4	0	0	0	0
15	2011	2	4	4	1	0	5	0	0	0	0	0	0	0	0	0	0	0	0	6	0	0	0	0	0
16	2011	0	3	7	0	0	0	0	0	0	2	0	0	0	0	0	1	0	1	9	0	0	0	0	0
17	2011	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0
18	2011	2	1	4	0	0	3	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
19	2011	1	1	2	0	0	3	0	0	0	0	0	0	0	0	0	0	0	0	3	1	0	0	0	0
20	2011	0	1	3	0	0	2	0	0	0	0	0	0	1	0	0	0	0	0	1	0	0	0	0	0
21	2011	0	3	5	0	0	18	0	0	1	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0
22	2011	2	7	0	1	0	2	0	0	0	0	0	0	0	0	0	0	0	0	8	0	0	0	0	0
23	2011	0	1	1	0	0	7	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0
1	2012	0	4	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
2	2012	1	1	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
3	2012	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
4	2012	2	7	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
5	2012	0	8	1	0	0	6	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0
6	2012	0	8	0	0	0	8	0	0	0	0	0	0	0	0	0	0	0	3	0	0	0	0	0	0
7	2012	1	4	0	0	0	7	0	0	0	0	0	0	0	0	0	0	0	2	1	0	0	0	0	0
8	2012	0	5	0	1	0	5	0	0	0	0	0	0	0	0	0	0	0	0	6	0	0	2	0	0
9	2012	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
10	2012	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
11	2012	1	0	0	0	0	5	0	0	0	1	0	0	0	0	0	3	0	2	0	0	0	0	0	0
12	2012	0	0	0	0	0	4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
13	2012	0	0	0	0	0	8	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
14	2012	0	1	0	0	0	3	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0
15	2012	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0
16	2012	2	2	1	0	0	1	0	0	0	1	0	0	0	0	0	4	0	2	0	0	0	1	0	0
17	2012	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
18	2012	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0
19	2012	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
20	2012	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
21	2012	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0
22	2012	0	5	1	0	0	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
23	2012	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
1	2013	1	2	0	0	0	5	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	1	0	0
2	2013	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
3	2013	1	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0
4	2013	0	0	0	0	0	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
5	2013	0	1	0	0	0	8	0	0	0	0	0	0	0	0	0	1	0	1	0	0	0	1	0	0
6	2013	2	0	0	0	0	6	0	0	0	1	0	0	0	0	0	0	0	0	1	0	0	0	0	0
7	2013	0	0	1	0	0	7	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0
8	2013	0	0	0	0	0	3	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0

9	2013	0	1	0	0	0	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
10	2013	0	3	0	0	0	8	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0
11	2013	0	0	0	0	0	4	0	0	3	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0
12	2013	0	0	0	0	0	7	0	0	2	0	0	0	0	0	0	1	0	0	0	0	0	1	0	0
13	2013	0	0	0	0	0	10	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0
14	2013	0	0	0	0	0	4	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	1	0	0
15	2013	0	0	0	0	0	4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
16	2013	0	0	0	0	0	12	0	0	2	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0
17	2013	0	0	0	0	0	8	0	0	0	0	0	0	0	0	0	1	0	0	0	0	2	0	0	0
18	2013	0	0	0	0	0	10	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	3	0	0
19	2013	0	1	0	0	0	7	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0
20	2013	0	0	0	0	0	17	0	0	1	0	0	0	0	1	0	1	0	0	0	0	0	4	0	0
21	2013	0	0	0	0	0	6	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	1	0	0
22	2013	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0
23	2013	0	0	0	0	0	5	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
1	2014	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	3	3	0	0	0	0	1	0	0
2	2014	1	0	1	0	0	2	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	4	0	0
3	2014	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	1	0	0	0	0	0	0	0
4	2014	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	1	0	0
5	2014	0	0	0	0	0	12	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	7	0	0
6	2014	1	0	0	0	0	9	0	0	0	0	0	0	0	0	0	2	1	2	0	0	0	2	0	0
7	2014	0	0	0	0	1	2	0	0	0	0	0	0	0	0	0	2	0	3	0	0	0	5	0	1
8	2014	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0
9	2014	0	0	0	0	0	2	0	0	2	3	0	0	0	0	0	0	3	0	0	0	0	5	0	0
10	2014	0	1	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	4	0	0
11	2014	2	0	0	0	0	5	0	0	1	0	0	0	0	0	0	9	3	0	0	0	0	9	0	0
12	2014	0	0	0	0	0	8	0	0	0	0	0	0	0	0	0	0	1	0	1	3	0	11	0	0
13	2014	0	1	0	0	0	6	0	0	4	2	0	0	0	0	0	10	6	5	1	0	0	8	0	0
14	2014	0	1	2	0	0	2	0	0	0	6	0	0	0	0	0	1	7	0	0	7	0	3	0	0
15	2014	0	0	0	0	0	3	0	0	0	0	0	0	0	2	1	3	4	0	1	0	0	4	0	0
16	2014	0	0	0	0	0	3	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	12	0	0
17	2014	0	0	0	0	0	5	0	1	2	0	0	0	0	0	0	1	1	0	0	0	0	3	0	0
18	2014	1	1	0	0	1	3	0	0	0	0	0	0	0	0	0	0	5	0	0	0	0	3	0	0
19	2014	0	0	1	0	0	1	0	0	0	0	0	0	0	0	0	4	3	1	0	0	0	4	0	0
20	2014	0	0	1	0	1	0	0	0	0	0	1	0	0	1	0	2	4	1	1	0	0	8	0	0
21	2014	0	2	0	0	0	4	0	0	2	0	1	0	0	0	0	3	7	0	0	0	0	10	0	0
22	2014	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	5	0	1
23	2014	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	2	2	0	0	0	0	1	0	0

Figure A.1. Sample-based species accumulation curves for Lepidoptera in small, medium-size and large Holm oak trees.

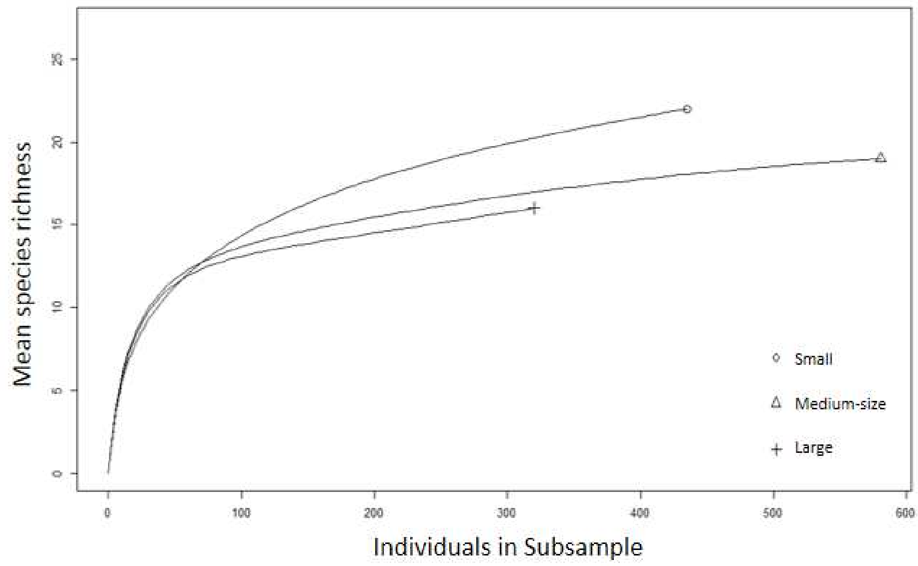


Figure A.2. Rank-abundance curve for the Lepidoptera community, from the most abundant to the rarest species.

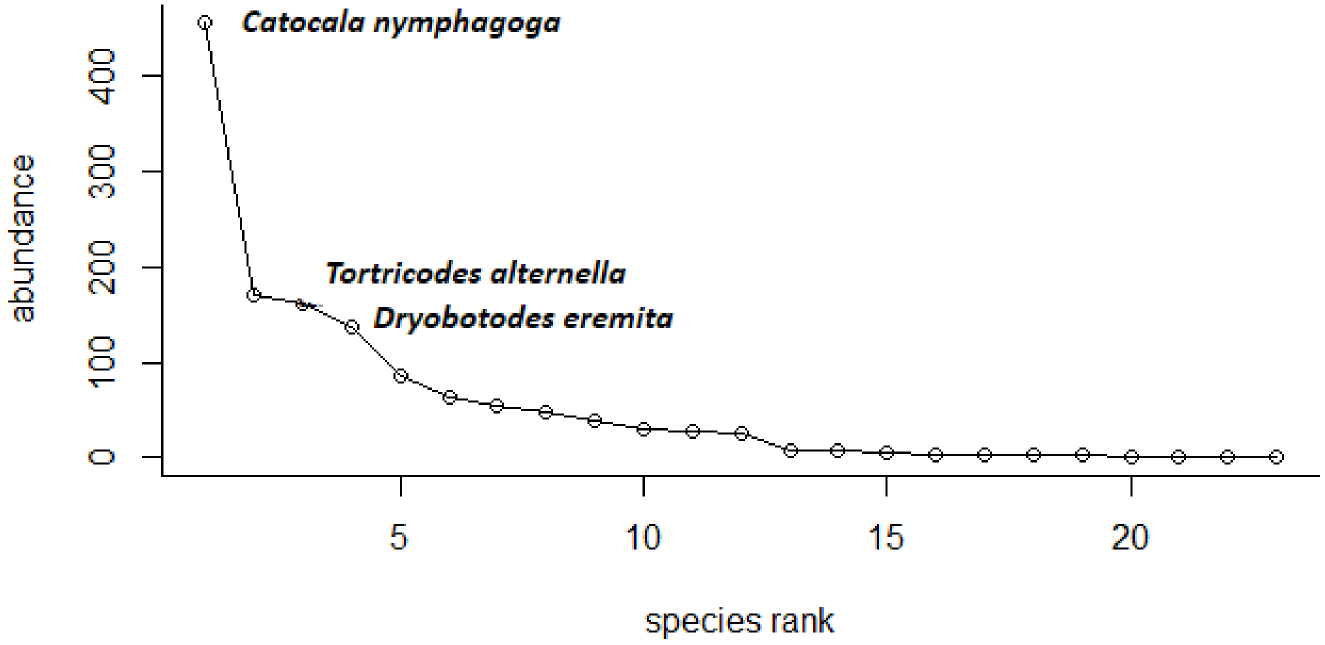


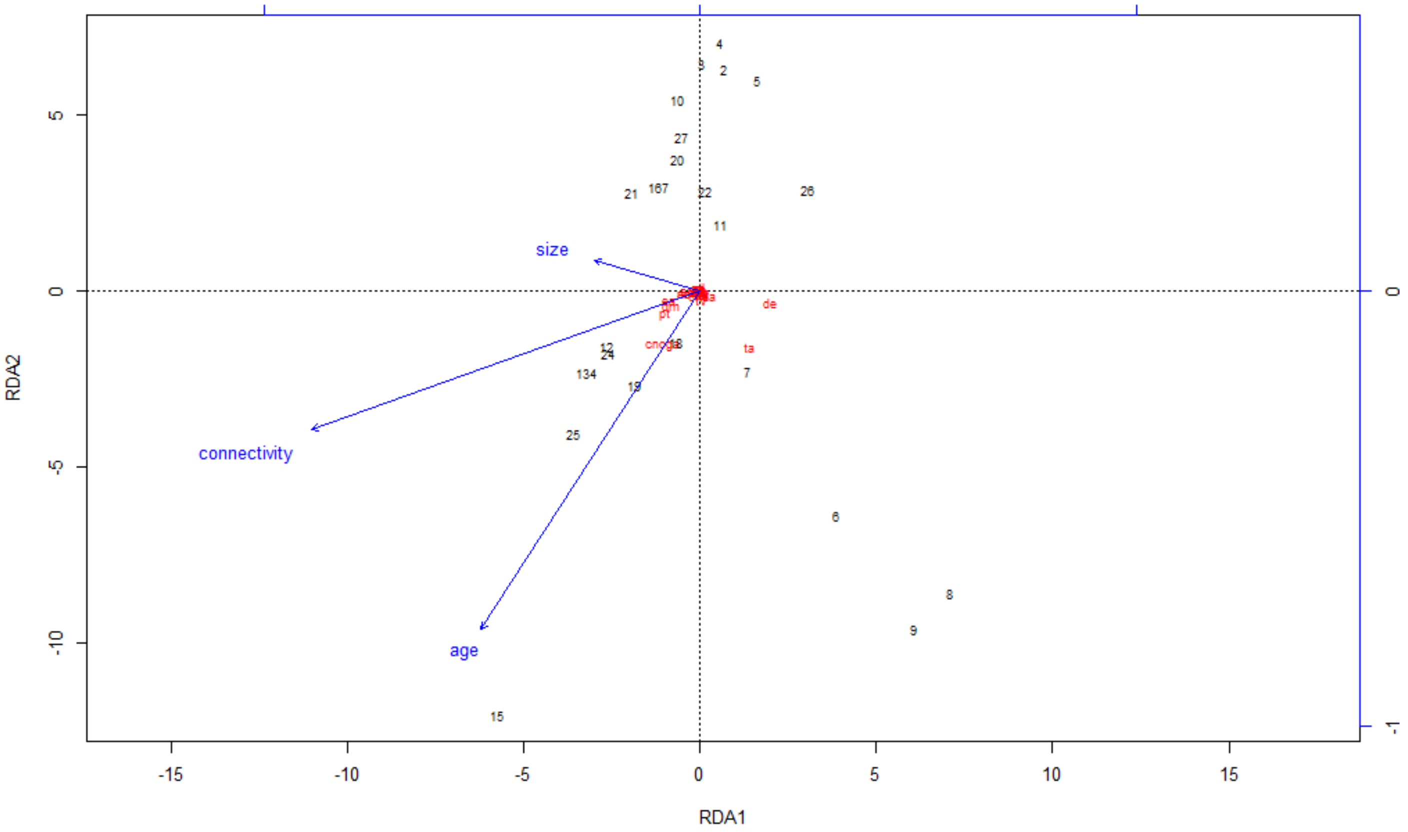
Table A.4 Selection of mixed effects linear models based on AIC.

Response	Model	Predictors		Method	AIC
		Fixed	**Random**		
Species richness	Saturated	·Tree age ·Tree canopy size ·Tree connectivity ·Tree age * Tree canopy size · Tree age * Tree connectivity ·Tree connectivity * Tree canopy size	·Year	ML	496.0154
	Best	**·Tree age** **·Tree canopy size** **·Tree connectivity** **·Tree age * Tree canopy size** **· Tree age * Tree connectivity** **·Tree connectivity * Tree canopy size**	**·Year**	**ML**	**496.0154**
	Parsimonious	·Tree connectivity	·Year	ML	496.7367
Shannon's Diversity	Saturated	·Tree age ·Tree canopy size ·Tree connectivity ·Tree age * Tree canopy size · Tree age * Tree connectivity ·Tree connectivity * Tree canopy size	·Year	ML	405.9241
	Best	**·Tree age** **·Tree canopy size** **·Tree connectivity** **·Tree age * Tree canopy size** **· Tree age * Tree connectivity** **·Tree connectivity * Tree canopy size**	**·Year**	**ML**	**405.9241**
	Parsimonious	·Tree connectivity ·Tree age · Tree connectivity * Tree age	·Year	ML	**405.4339**
Log (abundance+1)	Saturated	·Tree age ·Tree canopy size ·Tree connectivity ·Tree age * Tree canopy size · Tree age * Tree connectivity ·Tree connectivity * Tree canopy size	·Year	ML	287.1725
	Best	**·Tree age** **·Tree canopy size** **·Tree connectivity** **·Tree age * Tree canopy size** **· Tree age * Tree connectivity** **·Tree connectivity * Tree canopy size**	**·Year**	**ML**	**287.1725**
	Parsimonious	--------------------	-----	----	**----**

Table A.5 Total, unconstrained and constrained variance, main eigenvalues, adjusted R^2 and significance of the RDA performed between Lepidoptera species and tree characteristics (predictors).

	Inertia	Proportion			
Total	351.7	1.0000			
Constrained	64.6	0.1837			
Unconstrained	287.1	0.8163			
Eigenvalues for constrained axes	RDA1	RDA 2	RDA 3		
	40.2853	22.51940	1.79968		
Eigenvalues for unconstrained axes	PC1	PC2	PC3	PC4	PC5
	170.7675	69.5162	14.59797	8.65634	7.14901
Adjusted R^2	0.054				
$F_{3,19}$	1.425				
P value	0.214				

Figure A.3 Redundancy Analysis biplot showing the explanatory variables, in relation to selected variables along the two first significant axes. Pressures are shown in bold and physical correlates in italics



APPENDIX A

APPENDIX B

Table B.1. BIC values associated to the number of genetic clusters from cluster identification using successive K-means.

K	BIC
1	1613.621
2	1585.133
3	1572.550
4	1561.717
5	1551.524
6	1545.707
7	1541.079
8	1539.653
9	1538.226
10	1536.907
11	1536.046
12	1537.796
13	1538.045
14	1539.480
15	1541.496

Table B.2. Results of the intra-patch AMOVA analyses describing the partition of the total genetic variance of *Q. ilex* populations at different hierarchical levels.

1- Cardedeu

Source of variation	df	SS	Variance	Percent	*p*-value
Among stage classes	4	1043.198	2.87548	29.76	<0.001
Among individuals within stage classes	221	2006.758	2.29239	23.72	<0.001
Within individuals	226	1016.000	4.49558	46.52	<0.001
Total	451	4065.956	9.66344		

2- Martorell

Source of variation	df	SS	Variance	Percent	*p*-value
Among stage classes	4	31.268	0.02301	0.40	<0.001
Among individuals within stage classes	153	992.887	0.74473	12.91	0.986
Within individuals	158	790.000	5.00000	86.69	<0.001
Total	315	1814.155	5.76774		

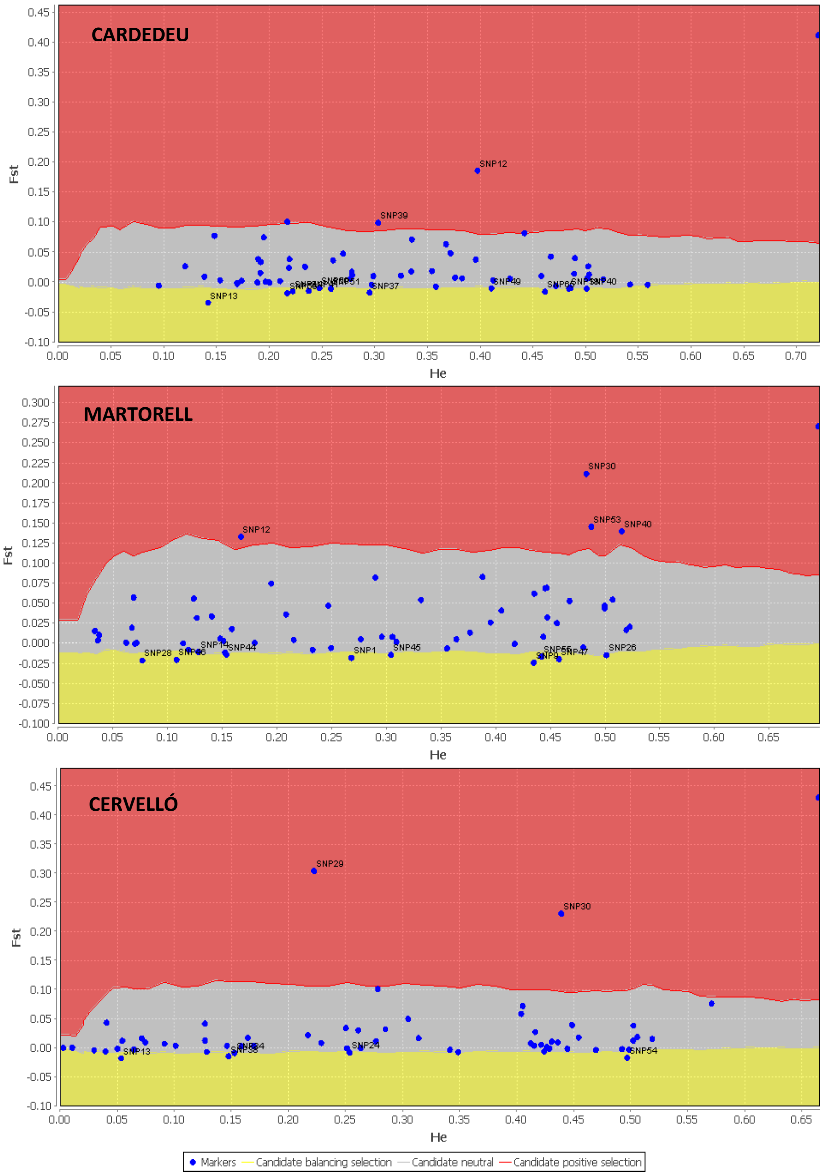
3- Cervelló

Source of variation	df	SS	Variance	Percent	*p*-value
Among stage classes	4	48.736	0.06999	1.14	0.006
Among individuals within stage classes	215	1351.453	0.23837	3.90	0.004
Within individuals	220	1278.000	5.80909	94.96	<0.001
Total	439	2678.189	6.11745		

Table B.3. Selection of mixed effects linear models based on AIC. Saturated models include all descriptors plus their second-degree interactions (represented by an asterisk).

Response	Model	Predictors		Method	AIC
		Fixed	**Random**		
Herbivory index	Saturated	· Heterozygosity percentage · Connectivity to mature oaks · Minimum distance to edge · Oak age · Heterozygosity percentage * Connectivity to mature oaks · Heterozygosity percentage * Minimum distance to edge · Heterozygosity percentage * Oak age · Connectivity to mature oaks * Minimum distance to edge · Connectivity to mature oaks * Oak age · Minimum distance to edge * Oak age · Genetic cluster	· Site	ML	3085.3
	Best	· Genetic cluster ·Connectivity to mature oaks · Minimum distance to edge · Oak age · Connectivity to mature oaks * Oak age	· Site	ML	3079.9

Figure B.1. Output from F_{ST} outlier analyses performed in LOSITAN testing for SNP loci under selection. Individual loci are represented by dots. Loci are expected to be under positive selection if they are embedded within the red area (higher differentiation than expected by chance), under neutral selection if they are in the grey area and under balancing selection in case they are in the yellow area. Limits of the grey area correspond to the 95% confidence interval thresholds of neutral expectations. Note that some markers have the same F_{ST} and He, and thus appear overlapped in the graphs



APPENDIX B

APPENDIX C

Table C.1. Population details of 18 *C. elephas* and *C. glandium* populations.

Site	Forest Type	ID code	Latitude (˚N)	Longitude (˚E)
B_INF	INF	1	41.5043	1.8410
B_CNF	CNF	2	41.5070	1.8403
B_OF	OF	3	41.5032	1.8386
C_INF	INF	4	41.6296	2.3238
C_CNF	CNF	5	41.6264	2.3403
C_OF	OF	6	41.6305	2.3267
H_INF	INF	7	41.5617	2.2892
H_CNF	CNF	8	41.5678	2.2856
H_OF	OF	9	41.5654	2.2842
I_INF	INF	10	41.6596	2.2411
I_CNF	CNF	11	41.6755	2.2183
I_OF	OF	12	41.6739	2.2184
M_INF	INF	13	41.4857	1.9017
M_CNF	CNF	14	41.4716	1.8908
M_OF	OF	15	41.4699	1.8898
V_INF	INF	16	41.4639	2.0327
V_CNF	CNF	17	41.4593	2.0299
V_OF	OF	18	41.4604	2.0313

Table C.2. Linear mixed model selection. Saturated models included fixed factors plus their second-degree interactions (depicted by asterisks). Factor reference level were set to: *C.elephas* for *species* and old forest (OF) for *forest*. Significant t-values at α = 0.05 are shown in bold. R^2 marginal (variability explained by fixed factors) and R^2 conditional (variability explained by both, fixed and random factors) are provided for each model.

Response		Predictor			Method	Coefficient	t	*P*-value	R^2 marginal	R^2 conditional
		Fixed		Random						
Population size	Full model			· Site	REML				0.369	0.369
		Species	***C. glandium***			**-10.333**	**-3.391**	**0.002 ****		
		Forest	CNF			-2.000	-0.656	0.517		
			INF			-3.333	-1.094	0.283		
		Species * Forest	*C. glandium* * CNF			3.333	0.774	0.445		
			C. glandium * INF			4.667	1.083	0.287		
	Selected model			· Site	REML				0.360	0.360
		Species	***C. glandium***			**-7.667**	**-4.410**	**< 0.001 *****		
		Forest	CNF			-0.333	-0.157	0.877		
			INF			-1.000	-0.470	0.642		
Haplotype richness	Full model			· Site	REML				0.494	0.622
		Population size				**-0.03124**	**-1.212**	**0.2379**		
		Species	***C. glandium***			**-0.71650**	**-2.121**	**0.0449 ***		
		Forest	**CNF**			**-1.09219**	**-2.426**	**0.0243 ***		
			INF			**-1.08605**	**-2.523**	**0.0196 ***		
		Population size * Species				**0.04738**	**2.716**	**0.0120 ***		
		Population size * Forest	CNF			0.04856	1.812	0.0844 .		
		Population size *	INF			0.04220	1.657	0.1124		

		Forest								
		Species * Forest	*C. glandium* * CNF			0.52492	1.748	0.0954 .		
		Species * Forest	*C. glandium* * INF			0.55282	1.886	0.0733 .		
	Selected model			· Site	REML				0.462	0.610
		Population size				0.012	1.044	0.30565		
		Species	*C. glandium*			-0.190	-0.895	0.37858		
		Forest	**CNF**			**-0.260**	**-2.759**	**0.01085 ***		
			INF			**-0.308**	**-3.223**	**0.00357 ****		
		Population size * Species				**0.042**	**2.442**	**0.02109 ***		
Gene diversity	Full model			· Site	REML				0.514	0.590
		Population size				**-0.055105**	**-3.589**	**0.00192 ****		
		Species	*C. glandium*			-0.297053	-1.576	0.13208		
		Forest	**CNF**			**-0.679509**	**-2.440**	**0.02563 ***		
			INF			**-0.949012**	**-3.636**	**0.00191 ****		
		Population size * Species				0.004528	0.437	0.66730		
		Population size * Forest	**CNF**			**0.038141**	**2.270**	**0.03619 ***		
		Population size * Forest	**INF**			**0.051673**	**3.305**	**0.00402 ****		
		Species * Forest	*C. glandium* * CNF			0.334200	2.014	0.06052 .		
		Species * Forest	***C. glandium* * INF**			**0.399665**	**2.435**	**0.02600 ***		
	Selected model			· Site	REML				0.253	0.426
		Population size				-0.008696	-1.553	0.1326		
		Species	***C. glandium***			**0.113286**	**1.915**	**0.0677 .**		
Nucleotide diversity	Full model			· Site	REML				0.761	0.826
		Population size				**-0.19646**	**-3.111**	**0.00598 ****		
		Species	*C. glandium*			0.17899	0.233	0.81881		
		Forest	**CNF**			**-3.00407**	**-2.659**	**0.01687 ***		

				INF			**-3.40570**	**-3.208**	**0.00522** **		
			Population size * Species				0.01886	0.445	0.66207		
			Population size * Forest	CNF			0.17986	2.640	**0.01758** *		
			Population size * Forest	INF			0.19030	2.997	**0.00826** **		
			Species * Forest	*C. glandium* * CNF			1.38791	2.074	0.05500 .		
			Species * Forest	*C. glandium* * INF			1.62787	2.447	**0.02608** *		
		Selected model			**· Site**	**REML**				0.701	0.793
			Population size				-0.01337	-0.623	0.539		
			Species	*C. glandium*			1.82555	8.113	**3.08e-08** ***		
Gene flux (population pairwise F_{ST})											
	COI	Full model			· Site	REML				0.338	0.338
			Forest pair	OF - CNF			-0.09283	-1.253	0.2239		
				CNF - INF			-0.04682	-0.632	0.5342		
			Species	*C. glandium*			-0.17246	-1.901	0.0711 .		
			Forest pair * Species	OF - CNF			0.16221	1.264	0.2200		
				CNF - INF			-0.08601	-0.670	0.5099		
		Selected model			**· Site**	**REML**				0.251	0.251
			Forest pair	OF - CNF			-0.03876	-0.616	0.5437		
				CNF - INF			-0.07549	-1.201	0.2422		
			Species	*C. glandium*			-0.14706	-2.701	**0.0128** *		
	SNP	Full/Selected model			· Site	REML				0.189	0.478
			Forest pair	OF - CNF				-1.312	0.2107		
				CNF - INF			-0.00721	-2.480	0.0266		

Table C.3. Population statistics for *C. elephas* and *C. glandium* for both mitochondrial and SNPs markers.

Mitochondrial marker					
Population ID	Species	N	r	Gene diversity (H)	Nucleotide diversity (π_n)
1	*C. elephas*	9	3	0.556 ± 0.165	0.0018 ± 0.0015
	C. glandium	5	3	0.700 ± 0.218	0.0079 ± 0.0055
2	*C. elephas*	9	3	0.639 ± 0.126	0.0013 ± 0.0012
	C. glandium	5	4	0.900 ± 0.161	0.0066 ± 0.0047
3	*C. elephas*	12	7	0.909 ± 0.056	0.0027 ± 0.0022
	C. glandium	6	5	0.933 ± 0.122	0.0396 ± 0.0237
4	*C. elephas*	10	2	0.467 ± 0.132	0.0007 ± 0.0008
	C. glandium	3	3	NA	NA
5	*C. elephas*	20	3	0.542 ± 0.105	0.0010 ± 0.0009
	C. glandium	3	2	NA	NA
6	*C. elephas*	18	4	0.556 ± 0.130	0.0011 ± 0.0010
	C. glandium	3	4	NA	NA
7	*C. elephas*	12	2	0.167 ± 0.134	0.0003 ± 0.0004
	C. glandium	7	4	0.714 ± 0.181	0.0069 ± 0.0045
8	*C. elephas*	22	3	0.385 ± 0.113	0.0007 ± 0.0007
	C. glandium	2	2	NA	NA
9	*C. elephas*	14	4	0.736 ± 0.075	0.0016 ± 0.0012
	C. glandium	12	4	0.636 ± 0.128	0.0019 ± 0.0015
10	*C. elephas*	21	4	0.586 ± 0.103	0.0014 ± 0.0011
	C. glandium	6	3	0.714 ± 0.181	0.0065 ± 0.0043
11	*C. elephas*	11	3	0.691 ± 0.086	0.0014 ± 0.0012
	C. glandium	14	7	0.788 ± 0.090	0.0105 ± 0.0061
12	*C. elephas*	17	5	0.647 ± 0.116	0.0013 ± 0.0011
	C. glandium	10	6	0.778 ± 0.137	0.010044 ± 0.005988
13	*C. elephas*	6	4	0.867 ± 0.129	0.0020 ± 0.002
	C. glandium	21	7	0.733 ± 0.068	0.0101 ± 0.0056
14	*C. elephas*	11	5	0.818 ± 0.083	0.0029 ± 0.0021
	C. glandium	13	6	0.821 ± 0.082	0.0091 ± 0.0054
15	*C. elephas*	18	5	0.621 ± 0.120	0.0013± 0.0011
	C. glandium	5	5	1.000 ± 0.127	0.0101 ± 0.0069
16	*C. elephas*	21	5	0.681 ± 0.072	0.0014 ± 0.0011
	C. glandium	3	3	NA	NA
17	*C. elephas*	14	5	0.659 ± 0.123	0.0016 ± 0.0014
	C. glandium	8	3	0.679 ± 0.122	0.0128 ± 0.0077
18	*C. elephas*	20	4	0.363 ± 0.131	0.0008 ± 0.0008
	C. glandium	1	1	NA	NA

Table C.4. Pairwise population F_{ST} matrix for SNPs for SNP nuclear markers in *C. elephas*. Numbers refer to site code. INF: isolated new forest, CNF: connected new forest, OF: old forest. Significant values are in bold.

	1_INF	1_CNF	1_OF	2_INF	2_CNF	2_OF	3_INF	3_CNF	3_OF	4_INF	4_CNF	4_OF	5_INF	5_CNF	5_OF	6_INF	6_CNF	6_OF
1_INF	0.00000																	
1_CNF	0.00901	0.00000																
1_OF	**0.04467**	**0.04201**	0.00000															
2_INF	0.01047	**0.01890**	**0.03991**	0.00000														
2_CNF	-0.00041	0.00689	**0.03605**	0.00607	0.00000													
2_OF	0.00478	**0.01995**	**0.02790**	**0.01200**	0.00429	0.00000												
3_INF	**0.01558**	**0.02399**	**0.02626**	**0.01344**	**0.01613**	**0.01061**	0.00000											
3_CNF	**0.01176**	**0.02831**	**0.04339**	**0.01413**	0.00692	**0.01539**	**0.02304**	0.00000										
3_OF	0.00608	**0.01573**	**0.03900**	**0.01951**	0.00278	**0.01470**	**0.02120**	**0.01053**	0.00000									
4_INF	0.00452	**0.01731**	**0.03525**	0.00555	0.00107	0.00271	**0.01736**	**0.02013**	0.00942	0.00000								
4_CNF	**0.01834**	**0.02461**	**0.03729**	**0.01260**	0.00312	0.00710	**0.01754**	**0.01389**	0.01093	-0.01121	0.00000							
4_OF	0.00239	**0.02116**	**0.05005**	**0.01316**	0.00331	**0.01166**	**0.02286**	**0.02360**	**0.02370**	**0.01886**	**0.01904**	0.00000						
5_INF	**0.00943**	**0.02244**	**0.03015**	0.00616	0.00742	0.00339	**0.01587**	**0.01756**	**0.01686**	0.00186	**0.01078**	**0.01221**	0.00000					
5_CNF	**0.01823**	**0.03061**	**0.05977**	**0.01604**	0.00723	0.01160	**0.03023**	**0.01187**	0.01099	0.00378	0.00810	0.01911	0.01188	0.00000				
5_OF	**0.02215**	**0.03056**	**0.07755**	**0.03438**	0.01167	**0.03253**	**0.05138**	**0.02618**	**0.02578**	**0.02767**	**0.02539**	**0.02599**	**0.03481**	0.02062	0.00000			
6_INF	0.00668	**0.02482**	**0.03479**	**0.00851**	0.00669	**0.01596**	**0.03082**	**0.01012**	**0.01623**	0.00786	0.01329	**0.02044**	**0.01582**	**0.01672**	**0.02542**	0.00000		
6_CNF	0.00557	**0.02298**	**0.01977**	0.00869	0.00471	0.00577	0.01026	**0.01270**	**0.01615**	0.00824	**0.01442**	**0.01983**	0.00832	**0.01665**	**0.02440**	**0.01336**	0.00000	
6_OF	0.00811	**0.01479**	**0.01561**	**0.00882**	0.00535	0.00725	**0.01557**	**0.02004**	0.00138	0.00170	0.00813	**0.01800**	0.00341	**0.02013**	**0.03854**	**0.01360**	0.00538	0.00000

Figure C.1. Scatterplot illustrating haplotype richness in relation to population size for each species.

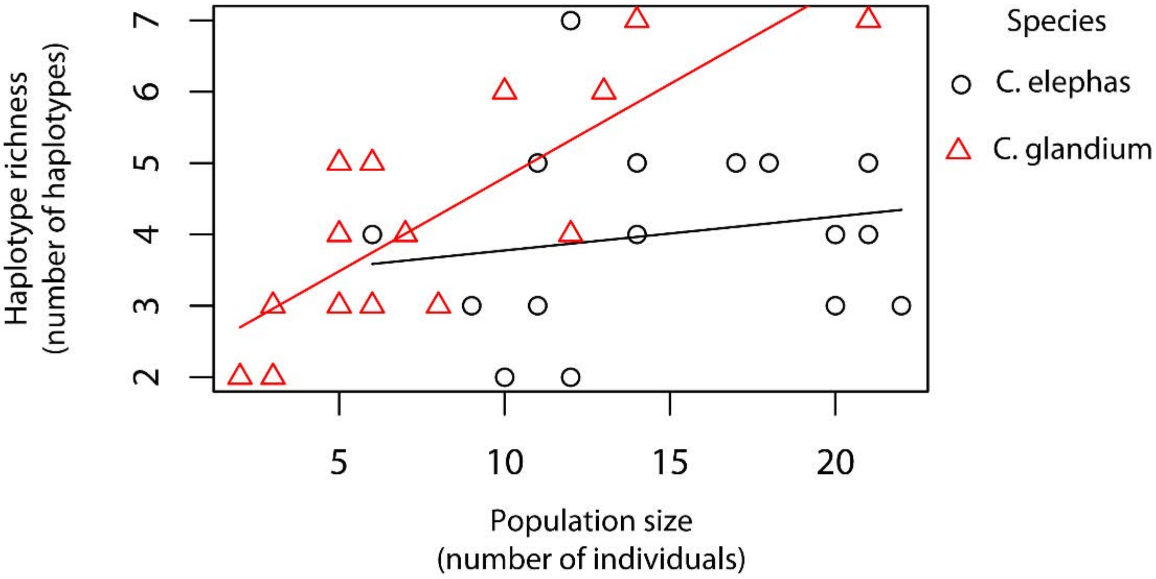


Figure C.2. Boxplot illustrating differences in population pairwise F_{ST} between species for the COI sequence. Box plots show minimum, lower quartile, median, upper quartile and maximum values. Means are depicted as white dots.

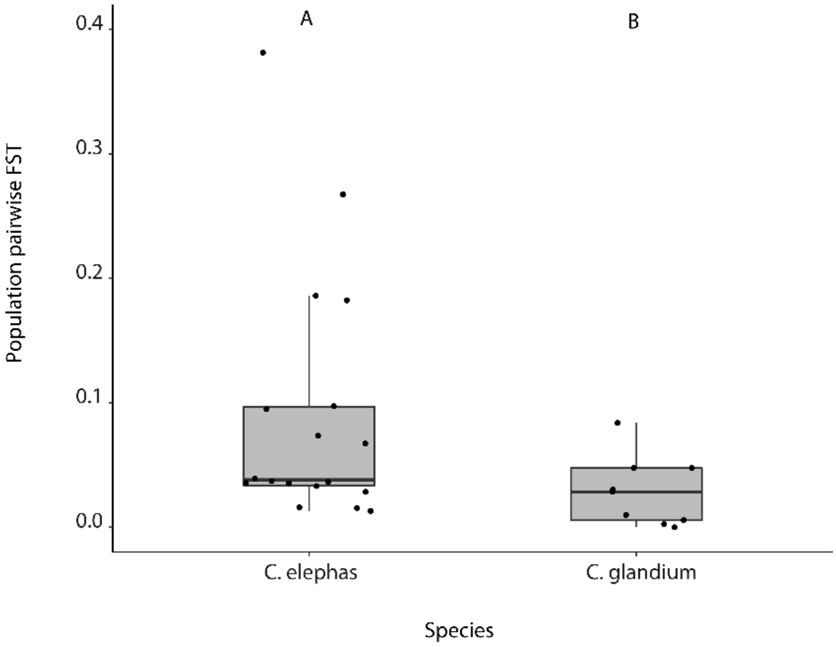


Figure C.3. Boxplot illustrating differences in population pairwise F_{ST} between forest type for SNPs. Box plots show minimum, lower quartile, median, upper quartile and maximum values. Means are depicted as white dots.