Determinants of functional connectivity of holm oak woodlands: Fragment size and mouse foraging behavior

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ABSTRACT

Demographic and genetic connectivity of fragmented plant populations will depend on effective propagule flow across the landscape. We analyze functional connectivity in a holm oak (*Quercus ilex*) fragmented landscape by considering three important stages driving recruitment: effective pollination, acorn production and acorn dispersal. We used a network approach to (1) determine if pollen-mediated gene exchange across the landscape was spatially structured; (2) estimate the effects of limited acorn dispersal on functional connectivity; (3) identify which landscape traits could drive source–sink dynamics of gene flow.

Although long distance dispersal was relatively frequent, most effective pollen flow occurred over short distances (<100 m). This resulted in a significantly modular structure of the mating network, yielding higher gene flow among nearby fragments. Limited mouse acorn hoarding activity had a strong impact on landscape connectivity, decreasing male gametic immigration rates into forest patches by one order of magnitude Besides, our results show that big forest fragments (>10 ha) are the main pollen sources, while small ones (<1 ha) are important pollen sinks. Thus, big fragments are critical to maintain functional connectivity, while small forest fragments may provide acorn crops better representing regional genetic diversity. In addition to area effects, less isolated and more central fragments showed higher migration rates and exchanged effective pollen with more fragments. Hence, we expected that landscapes with uniform or clumped distribution of big forest fragments would show optimal connectivity traits. However, despite that simulated gene flow was more evenly distributed across the landscape, connectance and migration rates decreased. Our results call for caution before translating patch-level management guidelines to the landscape scale. They also show that the level of functional connectivity may change throughout the recruitment process, suggesting that large-scale conservation strategies may fail if local effective seed establishment is disregarded.

1. Introduction

Land use changes have led to intense fragmentation of many temperate woodland ecosystems, replacing large continuous forests by a mosaic of isolated fragments, embedded in agricultural or urban matrices (Riitters et al., 2000). The demographic and genetic connectivity of fragmented plant populations depend on the effective dispersal of pollen and seeds across the landscape (Sork et al., 1999; Sork and Smouse, 2006). The intensity and scale of both seed and pollen dispersal across forest fragments are thus central to the decade-long debate about the extent to which tree populations are resilient to fragmentation (Kramer et al., 2008). High tree genetic diversity and outcrossing rates, and extensive long distance pollen dispersal may buffer the negative effects of forest fragmentation (Hamrick, 2004), while localized seed dispersal tends to increase spatial genetic structure and relatedness. Seed dispersal determines plant recruitment patterns, the successful establishment of both male and female migrant gametes and the final distribution of genotypes (Sork and Smouse, 2006; Sork et al., 2015). Pollen movement sets the template of male gametic gene flow, which depends on and is subsequently shaped by effective seed dispersal (Fig. 1). Integrating the joint effects of pollen and seed dispersal is thus necessary to gain realism in functional connectivity studies (Sork and Smouse, 2006; Jordano, 2010; Bacles and Jump, 2011).
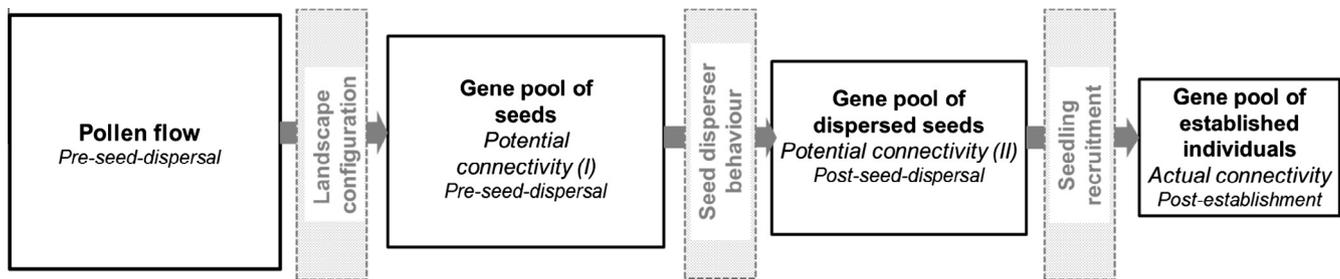
Fig. 1. Conceptual model of functional connectivity including changes along different recruitment stages (modified from Rico et al., 2012).

Analyzing fragmentation effects for trees using a metapopulation perspective is a daunting task, due to their typically long life span, delayed maturity, high recruit mortality, and long generation times (Bacles and Jump, 2011). Functional connectivity analyses provide a middle way between data-intensive (and costly to validate) metapopulation models and topological measures not underpinned by biological processes. Functional connectivity usually refers to demographic and genetic exchange among fragmented populations (Taylor et al., 1993; Rico et al., 2012). The application of graph theory methods to functional connectivity studies (Dyer and Nason, 2004, reviewed in Laita et al., 2011) has paved the way for evaluating which factors govern mating patterns and effective pollen- and seed-mediated gene flow at a landscape scale, and to detect genetic bottlenecks on fragmented populations (Dyer et al., 2010; Fortuna et al., 2008; Herrera-Arroyo et al., 2013). However, few landscape connectivity studies have integrated the effects of fragmentation on the functionally-related processes of pollen movement, effective mating, seed dispersal, and post-dispersal seed mortality, even if connectivity patterns may vary substantially throughout recruitment stages (Rico et al., 2012; Fig. 1).

Holm oak (*Quercus ilex*) forests are a suitable study system for addressing the effects of limited seed dispersal in functional connectivity. Like other wind-pollinated species, holm oaks typically exhibit leptokurtic distributions of pollen dispersal distances, combining a rapid decay at short distances with fat tails (Hampe et al., 2013; Ortego et al., 2014). As for acorns, forest management typically hampers their effective dispersal over long distances (Santos and Tellería, 1997; Pulido and Diaz, 2005; Morán-López et al., 2015, 2016). Eurasian jays (*Garrulus glandarius*), the main long-distance acorn disperser in Europe, are absent in small forest fragments (Andren, 1992; Brotons et al., 2004), where dispersal relies on wood mice (*Apodemus sylvaticus*), a moderately efficient local acorn disperser. Most acorns handled by mice remain within a close range of mother trees (<5 m), and many of them are finally predated (Gómez et al., 2008).

In fragmented holm oak woodlands, functional connectivity will thus depend on effective pollen flow among fragments, and subsequent mouse-mediated effective local acorn dispersal. The latter may in turn be strongly negatively affected by fragmentation (Santos and Tellería, 1997; Morán-López et al., 2015). The resulting recruitment failure within fragments may compromise the establishment of long-distance male gametic migrants, which may explain the low genetic diversity of holm oak saplings in small forest fragments (Ortego et al., 2010) in spite of extensive pollen flow (Ortego et al., 2014). If this were the case, any conservation strategy aimed at enhancing genetic cohesiveness at the landscape scale should explicitly include measures aimed at restoring local acorn dispersal.

The main goal of this study was to quantify functional connectivity of a holm oak fragmented landscape and to detect critical demographic factors potentially compromising gene exchange among fragments. For this purpose we built spatial networks of gene flow among forest patches considering effective pollination, acorn production and acorn dispersal. This allowed us to (1) gauge if pollen-mediated gene exchange within the landscape is spatially structured; (2) estimate the effect of mouse activity on functional connectivity; (3) determine which patch traits define source–sink dynamics within the network; and (4) evaluate if conclusions drawn from patch-level approaches can be directly scaled up to management guidelines at a landscape scale. We expected (a) a modular structure of the gene exchange network due to predominantly local pollen flow (Ortego et al., 2014), (b) a significant decrease of landscape connectivity with diminishing effective acorn dispersal (Rico et al., 2012), (c) an important effect of the spatial extent, centrality and isolation of fragments on pollen source–sink dynamics, and (d) a problematic extrapolation of these findings to the landscape scale, due to emergent properties in the connectivity network (Baguette et al., 2013).

2. Materials and methods

2.1. Study area

Field work was carried out in a Holm oak (*Q. ilex*) archipelago (14.4×10^3 ha; see Appendix B Fig. B1) located in the southern plateau of central Spain, near Quintanar de la Orden (39°35′N, 3°02′W, 870 m a.s.l.). Cereal cultivation has reduced forest cover to ∼8% in the study area, with 401 patches ranging from 0.02 to 793 ha. The dominant tree is holm oak and the understory is composed of shrubby kermes oak (*Quercus coccifera*) and xeric Mesomediterranean shrubs (e.g. *Rhamnus lycioides*, *R. alaternus*, *Cistus ladanifer*, and *Asparagus acutifolius*). Annual precipitation and mean temperature are 421 mm and 14 °C, respectively. We chose this study site because it is representative of holm oak woodlands in central Spain (Santos and Tellería, 1998).

2.2. Sampling design

In autumn 2012, we simultaneously measured acorn production, acorn dispersal patterns by wood mice (*Apodemus sylvativus*) (Morán-López et al., 2015) and pollen flow. Eurasian jays are supposed to be absence in these patches (Tellería et al., 1999; Palomino et al., 2012), but we surveyed corvid presence during autumn–winter anyways. Two observers walked along 2–5 km transects, uniformly distributed throughout the study area (over 150 km in total), stopping every 100 m. Monitoring was performed at dawn and dusk (periods of peak bird activity). The openness of the study area, both among and within forest fragments, ensured easy acoustic or visual detection 200 m around observers, as ascertained by frequent contacts with magpies *Pica pica*, carrion crows *Corvus corone* and similar-sized birds. We did not detect any Eurasian jay, either during transects or while performing field work with mice and oak crops during 2012–2014 (Morán-López et al., 2015). Jay absence was further corroborated by interviews to local farmers and hunters.

We selected three large (>100 ha) and 10 small (0.05 ha on average) forest fragments. Within large fragments, we defined interior and edge areas considering a 60 m threshold distance to the cultivated border (García et al., 1998). We thus considered three fragmentation categories: large fragment interior, large fragment edge, and small fragment, which allowed us to assess global patterns of acorn production and seed and pollen dispersal taking into account edge effects.

To assess local acorn production, we monitored acorn crops of 90 focal trees (30 per fragmentation category) and their four nearest mature trees in random directions, whenever possible. We visually estimated acorn crop size with a semi-quantitative measure ranging from 0 (no crop) to 4 (more than 90% of canopy producing acorns), referred to as acorn score hereafter (Koenig et al., 2013). Acorn dispersal patterns by mice were evaluated for 45 focal trees (15 trees per fragmentation category). In each focal tree we offered 27 acorns to rodents (1250 acorns in total). A metal wire (0.6 mm $\varnothing$) with a numbered plastic tag was attached to each acorn (Xiao et al., 2006). Marked acorns were placed beneath the canopies of focal trees and protected with 35 cm × 35 cm × 15 cm wire cages with a mesh size of 6 cm that only allowed the entrance of rodents. Offered acorns were tracked and categorized as predated or cached (buried and intact) (see Morán-López et al., 2015, for further details). Finally, to estimate pollen dispersal distances we selected and georeferenced 28 mother trees, collecting young leaves and 15–20 fully mature acorns from each of them (460 seeds in total; see next section).

2.3. Pollen dispersal kernel estimation

We used mother-embryo diploid genotypic data and the POLDISP software (Robledo-Arnuncio et al., 2007) to estimate the probability density function of pollen dispersal locations from a source tree (pollen dispersal kernel), which was then used to parameterize landscape connectivity models. POLDISP relies on the expected decay with distance of a normalized measure of paternal identity among maternal sibship pairs. To gauge such decay empirically, mother trees should sample as many pairwise-distance classes as possible, from neighboring to long-distance pairs. Guided by this requirement and by viable acorn availability, the 28 sampled mother trees were distributed as follows: 10 in the interior of large fragments, 6 in large fragment edges, and 12 in small fragments. Pairwise inter-mother distances ranged from 4 to 5477 m. We initially intended to estimate a separate kernel for each fragmentation category, since changes in canopy structure are expected to alter air flow (Dyer and Sork, 2001; Bacles et al., 2005). However, sample sizes were insufficient to achieve good fits (results not shown), so all 28 mothers were pooled to obtain an average dispersal kernel.

Acorns were soaked for 24 h and then placed in a germination chamber. Because some acorns did not contain viable embryos, the final number of offspring per mother was 12 ± 0.32 (mean ± S.E). We used Invisorb DNA Plant HTS 96 Kit/C to extract and purify genomic DNA from adults and progeny. A total of ten nuclear microsatellites transferred from *Quercus petraea*, *Q. robur* and *Q. macrocarpa* were used to genotype all mothers and embryos (see Appendix Table A1). Primers were dyed labeled (FAM, PET, VIC, NED) and DNA was amplified following Ortego et al. (2014). Amplification products were sent to an ABI Genetic Analyzer (ABI, St. Louis, MO) and genotypes were scored using Genemarker (Holland and Parson, 2011).

The rank correlation coefficient between the probability of paternal identity among maternal sibship pairs and inter-mother distance was negative and significant ($r_s = -0.13$; $p < 0.01$). We fitted a two-parameter, two-dimensional exponential-power pollen dispersal kernel (Clark, 1998; Austerlitz et al., 2004):

$$P(d|a, b) = \frac{b}{2\pi a^2 \Gamma(2/b)} \exp(-(d/a)^b) \tag{1}$$

where d is the pollen movement distance, Γ is the gamma function, b is the shape parameter, and a is the scale parameter. The effective number of pollen donors was estimated as the inverse of the probability of paternal identity within maternal sibships ($N_{ep} = 1/r_p$) (Ritland, 1990), calculated separately for small and large fragments.

2.4. Design of landscape connectivity network

We constructed the functional connectivity network of our landscape through three consecutive steps: (1) computation of expected pollen migration rates among fragments, based on the empirically estimated pollen dispersal kernel; (2) simulation of the number of acorns produced and the realized number of effective pollination (mating) events within fragments and among fragment pairs, given the expected pollen migration rates; and (3) simulation of acorn caching by rodents.

2.4.1. Expected pollen migration rates

A digital ortophotography of the study area (20 × 7.2 km) was used to create a gvSIG shape file (Steiniger and Hay, 2009) of all fragments. For each forest fragment, we calculated its centroid, area and perimeter. The shape file was then imported to Netlogo and rasterized (Wilensky, 1999). Final pixel size was 20 m × 20 m (1000 pixels × 360 pixels within the landscape) and each pixel contained information of its habitat (cropland *vs* forest) and the type of forest fragment.

For each pair of fragments i and j, we defined the expected pollen migration rate from j to i (M_{ij}) as the probability that an acorn produced by a mother in the i-th fragment has been fertilized by pollen dispersed from the j-th fragment, which was calculated as

$$M_{ij} = \frac{1}{n_{\chi_i}} \sum_{\chi_i=1}^{n_{\chi_i}} m_{\chi_i j} = \frac{1}{n_{\chi_i}} \sum_{\chi_i=1}^{n_{\chi_i}} \left(\frac{\sum_{\chi_j=1}^{n_{\chi_j}} P_{\chi_i \chi_j}}{\sum_{k=1}^{K} \sum_{\chi_k=1}^{n_{\chi_k}} P_{\chi_i \chi_k}} \right) \tag{2}$$

where $P_{\chi_i \chi_j}$ is the probability of pollen transport between the centres of recipient pixel χ_i and source pixel χ_j (from the i-th and j-th fragments, respectively), given by the assumed pollen dispersal kernel (Eq. (1)); and where n_{χ_i} is the total number of pixels in the i-th fragment and K the total number of fragments. The term $m_{\chi_{i,j}}$ is the probability that an acorn produced by a mother in the χ_i-th pixel of the i-th fragment has a paternal parent from (any of the pixels of) the j-th fragment, so M_{ij} is calculated as an average probability of pollen immigration from fragment j across all pixels of the recipient fragment i. Note that M_{ii} yields the probability of local mating within the i-th fragment. If M_{ii} is close to zero, then the i-th fragment can be considered a pollen sink. On the other hand, the j-th fragment can be considered an important pollen source if the average M_{ij} over all i recipient fragments is comparatively large. All the M_{ij} values are stored in the $K \times K$ matrix **M**.

2.4.2. Simulating acorn production and mating events

We estimated acorn production across the landscape in 2012. Each pixel was assigned to one of the three forest fragmentation categories. The number of acorns produced in the i-th fragment ($Prod_i$) was estimated as:

$$Prod_i = \sum_{\chi_i=1}^{n_{\chi_i}} Prod_{type,\chi_i} \times 28.75 \times (\pi 2.5^2) \times 5 \tag{3}$$

where $Prod_{type,\chi_i}$ is the acorn score (see above) for the fragmentation category of the χ_i-th pixel of fragment i (1.28 ± 0.03, 2.28 ± 0.07 average ± SD acorn score for forest interiors and edges, respectively; $N = 171$ and $N = 153$ trees respectively); 2.5 is the

average canopy radius in our study area (±0.01 m; $N = 96$; 32 per fragmentation category), 5 is the estimated number of stems per pixel (corresponding to an average stem density of 125 stems/ha). The constant 28.75 converts the semi-quantitative acorn score data into acorns per square meter of canopy projection, following the regression model developed by Díaz et al. (2011) from data taken in the National Park of Cabañeros during 2008–2010 (acorns/$m^2 = 28.75^* $ acorn score; $P < 0.01$, $R^2 = 0.87$).

The mating network was then constructed by simulating acorn production. For each (recipient) forest fragment, we generated the number of acorns sired by pollen donors from each of the potential K (source) fragments by randomly drawing from a multinomial distribution with K classes with probabilities given by vector $\mathbf{M}[i]$ (see Eq. (2)), and $Prod_i$ trials, using the multinomRob package (Mebane and Sekhon, 2013). This way, network nodes represented forest fragments and link weights realized pollen migrant proportions among the corresponding $Prod_i$ acorns. By replicating realized numbers of mating events using the multinomial distribution (rather than using the multinomial frequencies directly as link weights), we could assess the sensitivity of network structure to stochastic mating and acorn caching (see next section), conditional on differences in acorn production and caching rates between fragmentation categories.

2.4.3. Simulating acorn caching vs predation

We obtained average acorn caching rates per fragmentation category from our field acorn predation/removal experiments. We calculated the number of acorns cached per fragment using the equation:

$$Cached_i = \sum_{\chi_i=1}^{n_{\chi_i}} Prod_i \times Cached_{type,\chi_i} \qquad (4)$$

in which $Cached_i$ is the estimated number of acorns cached in a pixel of forest fragment i, $Cached_{type,\chi_i}$ is the caching rate corresponding to the fragmentation category of the χ_i-th pixel of the i-th fragment (decreasing from 0.12 ± 0.05 in large fragment interiors and 0.09 ± 0.03 in large fragment edges, down to 0.02 ± 0.01 in small fragments). $Prod_i$ is acorn production, as calculated using Eq. (3).

The post-acorn-dispersal (effective) mating network was built in the same way as the pre-acorn-dispersal mating network, but using $Cached_i$ (instead of $Prod_i$) as the number of trials of the corresponding multinomial for the i-th fragment. In this case, the weights of network links corresponded to realized migrant proportions among the corresponding $Cached_i$ acorns (Fig. 1), i.e., the (i, j) link weight was the proportion of the $Cached_i$ acorns within fragment i whose paternal parent was located at fragment j.

2.5. Quantifying network structure

We explored the structure of the two mating networks (before and after acorn dispersal) by constructing 100 replicates for each and performing modularity analyses. For each network, we calculated the following parameters of functional connectivity: (1) connectance, defined as the ratio between the number of realized links and the number of possible links; (2) number of isolated (unconnected) patches; (3) modularity of the network (raw and relative; see below); (4) number of modules; (5) mean number of nodes per module; (6–7) minimum and maximum module size; and (8) mean immigration rates.

The modularity function (M) determines if the network is organized in well-defined separate modules, and was calculated using Newman and Girvans' (2004) algorithm, as implemented in the igraph R package (Csardi and Nepusz, 2006):

$$M = \sum_{s=1}^{Nm} \left[\frac{ls}{L} + \left(\frac{ds}{2L} \right)^2 \right] \qquad (5)$$

where Nm is the number of modules, L is the number of links in the network, ls is number of links between nodes in module s, and ds is the sum of degrees (total number of links) of module s. A higher modularity implies a more uneven distribution of gene flow across the landscape. Modules represent subareas of the landscape within which among-fragment gene flow is more intense. To test the significance of modularity we compared the observed M value with values of 100 randomization of the network, keeping the same connectance, (Guimera and Amaral, 2005) using the package tnet (Opsahl, 2009). The modularity of the networks depends on their connectance and hence, to control for this difference when comparing modularity between networks, we calculated relative modularity following (Fortuna et al., 2008).

$$M^* = (M_{real} - \overline{M}_{random})/\overline{M}_{random} \qquad (6)$$

Wilcoxon rank sum tests were used for comparing topological parameters (connectance, relative modularity, number of isolated nodes, etc.) between pre- and post-acorn-dispersal mating networks. To compare immigration rates we used generalized binomial regression models (link logit). Besides, to evaluate the impact of seed dispersal by mice on functional connectivity (Fig. 1), we calculated the percentage of variation between pre- and post-acorn-dispersal mating networks for each parameter of functional connectivity, represented in Eq. (7) as P ($\Delta_{pre-post}$).

$$\Delta_{pre-post} = \left(\frac{P_{post} - P_{pre}}{P_{pre}} \right) \times 100 \qquad (7)$$

2.6. Factors determining effective pollen source–sink dynamics

We evaluated which factors determined the role of forest fragments as sources or sinks of effective pollen across the landscape (i.e. source *vs* sink nodes within the network). Our response variables were migration rates and the degree of the fragments within the network. Immigration rates are given by the proportion of non-local mating (equal to $1 - M_{ii}$ for each fragment i). Emigration rates for every fragment j were calculated as the average proportion of seeds sired by pollen donors from j over all recipient fragments i (average M_{ij} over all i). The degree of a node is defined as its number of outgoing and ingoing links, which in our case translates into the number of source or recipient fragments exchanging effective pollen with the target fragment. We divided out forest fragments into five size categories: $\geqslant 100$, $[10, 100)$, $[1, 10)$, $[0.1, 1)$ and $\leqslant 0.1$ ha. Our fixed effects were fragment area, distance to the center of the archipelago, mean distance to other forest fragments (isolation) and their interaction. Distance to the center of the archipelago and isolation were not correlated ($P = 0.22$, Pearson correlation = -0.06). We used generalized quasibinomial and quasipoisson models for the analysis of migration rates and fragment degrees, respectively.

2.7. Scaling up to regional-scale management from patch-level measures

To explore whether our fragment-level analyses can be extrapolated to landscape management guidelines, we generated six alternative landscape scenarios: (1) Control landscape, in which the centroid and the area of forest fragments are equal to those of the real landscape; (2) Area reduction landscape, in which the area of large forest fragments (>10 ha) is reduced by 10%; (3) Uniform landscape, in which centroids of large forest fragments are

uniformly distributed within the landscape, while keeping the same area; (4) Clumped landscape, in which centroids of large fragments are aggregated in the center of the archipelago following an exponential decay function; (5) Combined uniform and area reduction landscape; (6) Combined clumped and area reduction landscape. Each fragment was built by spreading the forest habitat from its allocated centroid to neighbor cells until the desired area was reached, producing a tumor-like shape. The control landscape was used as a benchmark (excluding shape effects) in our connectivity simulations. A 10% of area reduction corresponds to reported values of habitat loss (per decade) in evergreen woodlands of the Iberian Peninsula (Costa et al., 2014). For each theoretical landscape scenario, we built a mating network following the same protocol as for the real landscape, and compared the corresponding functional connectivity parameters against those of the control landscape ($\Delta control_{LANDS}$).

To assess the impact of enhanced caching rates on landscape connectivity, we built two post-acorn-dispersal mating networks in the control landscape: a control one, in which caching rates were the same as those observed in the study area, and an alternative increased-caching network in which caching rates were fixed to 0.17 for all pixels, corresponding to observed values in the interior of well-preserved forests (Morán-López et al., 2015). As before, we calculated landscape connectivity parameters and compared them between both caching scenarios (control and increased-caching; $\Delta control_{INCREAS}$).

3. Results

3.1. Structure of pre- and post- acorn dispersal networks

The estimated pollen dispersal kernel had an average of 1149 m, was fat-tailed ($b = 0.16$) and rather leptokurtic, with a rapid decay of pollen dispersal probability within 100 m of source trees (see Appendix Fig. A1). Modeled effective pollen movement across the landscape was significantly modular, both for the pre- and post-acorn-dispersal networks (Table 1). In the modularity analysis of the pre-acorn-dispersal mating network, Newmans' algorithm (see Methods) detected an average (over independent replicates) of seven subareas of more intense gene flow (modules). Modules were of highly variable size (ranging from 5.87 to 129.66 nodes per module, Fig. 2A). Almost no isolated fragments were detected in any of the replicates of the network, even if only 22% of potential links between fragments were realized on average (Table 1).

After simulating mouse foraging decisions (caching vs predation), the post-acorn-dispersal mating network was globally less connected (−36.62%), more spatially structured (61.62%), and with one order of magnitude lower immigration rates, relative to the mating network before seed dispersal (Table 1). Besides, the probability distribution of effective pollen immigration rates became substantially positively skewed after acorn dispersal, with one third of fragments showing very low (⩽2%) immigration rates (Fig. 2B and C). Overall, the considered post-seed-dispersal

demographic filter resulted in a more labile effective mating network, due to the disappearance of low probability links and a lower strength of the remaining ones.

3.2. Factors determining effective pollen source–sink dynamics

Considering first the pre-acorn-dispersal mating network, we found that large fragments (>10 ha) acted predominantly as sources of effective pollen, while small forest fragments (<0.1 ha) were mostly effective pollen sinks (Fig. 3A and B, gray bars). Fragment area positively affected the number of ingoing and outgoing links, though this effect was weak (Fig. 3C and D, gray bars), resulting in a lower link number to fragment area ratio in large forest fragments (mean ± S.E. links per pixel, 17.69 ± 1.14 and 0.01 ± 0.00 in fragments <0.1 ha and >10 ha, respectively). In agreement with these results, the effective number of pollen donors was much higher in trees located in small forest fragments than those from large ones (32.29 ± 9.74 vs 12.66 ± 1.95 in small and large forest fragments respectively). Pollen emigration rates and the degree of fragments also depended on their spatial configuration. More isolated stands donated less pollen and to a lower number of neighbor fragments. In addition to isolation, distance negatively affected the number of ingoing and outgoing links. In general, spatial configuration effects were stronger in smaller fragments (Table 2, pre-acorn-dispersal network).

Considering now the post- acorn-dispersal mating network (Fig. 3, black bars), results were similar in terms of emigration rates and fragment degree (in and out). However, immigration rates showed a bell-shape response (Fig. 3A, black bars, Table 2, post-acorn-dispersal network). Medium-sized fragments showed the highest immigration rates. In fact, as a consequence of strong post-dispersal seed mortality, small forest fragments showed rather low effective pollen immigration rates (<2%), relative to the high proportion of immigrant pollen at the seed-crop stage (>60%).

3.3. Scaling up to regional-scale management from patch-level measures

A 10% area reduction of large fragments (>10 ha) in the control landscape resulted in a lower number of links within the mating network (−9.34%), smaller modules (−6.99%) and weaker links (−3.13%), but the modular structure of the network did not change significantly, as indicated by the relative modularity index (Table 3, see area reduction landscape). Contrary to our expectations, both clumped and uniform spatial distributions of large forest fragments negatively impacted landscape connectance (−36.60% and −33.99% respectively) and average immigration rates (−24.6% and −16.7%, respectively). Nevertheless, links were more homogeneously distributed across the landscape (Fig. 4), resulting in a reduction of network modularity (−49.59% and −52.88% respectively). Besides, the size of the smallest modules was about three times larger (2.87 and 3.20 respectively, for clumped and uniform

Table 1

Summary of modularity analysis on pre-acorn and post-acorn dispersal networks. *Connect*, connectance; *Isol*, number of isolated patches; *Mod*, Newmans' modularity; *S*, significance of the network modularity; *M**, relative modularity; *Nmod*, number of modules of the network; $\overline{N}$, average number of nodes per module; *Min* and *Max*, minimum and maximum number of nodes per module, respectively; *Mig*, immigration rates (%, with respect to acorns produced). $\Delta_{pre-post}$, percentage of variation between pre- and post-acorn dispersal network parameters. Significant values of $\Delta_{pre-post}$ are in bold.

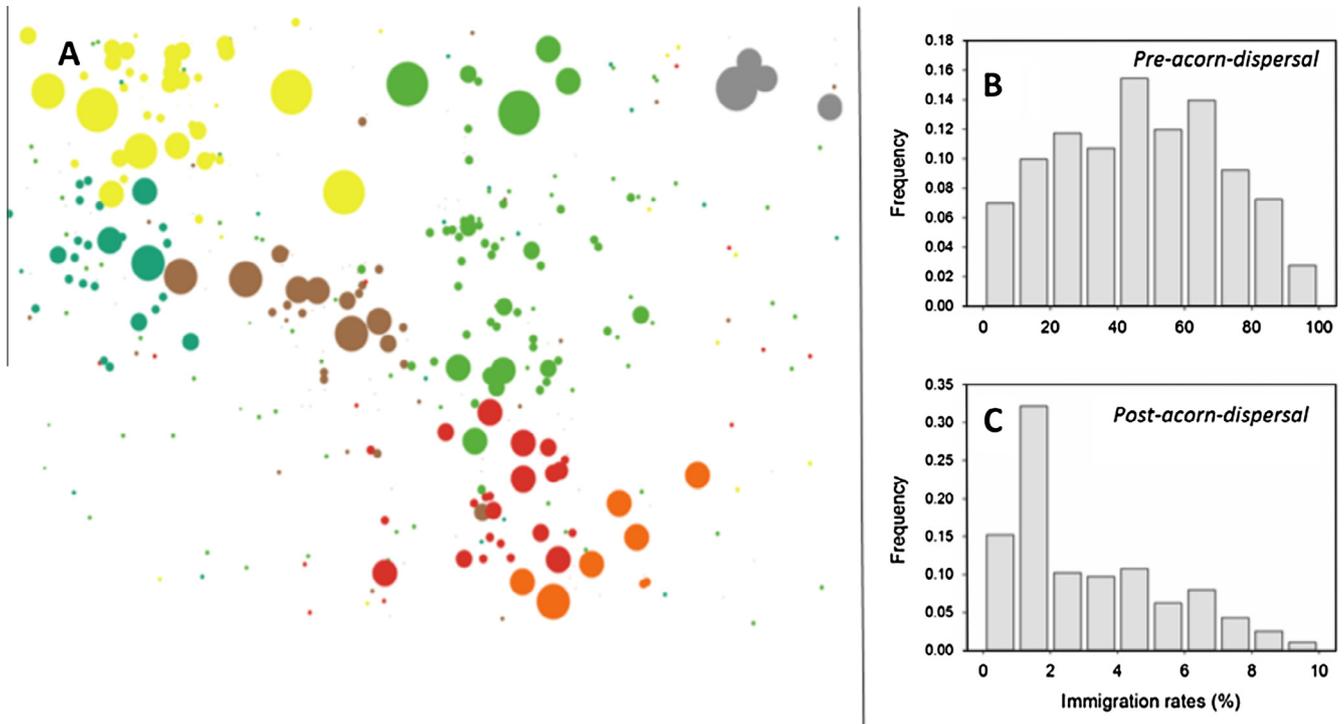| Network	Filter	Connect	Isol	Mod	S	M*	Nmod	$\overline{N}$	Min	Max	Mig
Pre-acorn dispersal	Landscape	0.22 ± 0.00	0 ± 0.00	0.47 ± 0.00	−**	0.91 ± 0.00	6.88 ± 0.22	62.63 ± 1.49	5.87 ± 0.18	129.66 ± 0.81	46.64
Post-acorn dispersal	Acorn predation	0.14 ± 0.00	0 ± 0.00	0.51 ± 0.00	−**	1.47 ± 0.01	6.97 ± 0.23	63.15 ± 1.81	5.82 ± 0.16	109.61 ± 3.30	3.11
$\Delta_{pre-post}$		**−36.36****	–	–	–	**61.54****	1.30	0.83	−0.85	**−15.46****	**−93.32****

** Denotes *P* < 0.01.

Fig. 2. (A) Modular organization of the mating network of _Quercus ilex_ population (pre-acorn dispersal). Nodes represent forest fragments. Each color depicts a module detected by the algorithm (areas of higher estimated gene flow). (B and C) Histogram of immigration rates of the pre and post-acorn-dispersal networks.
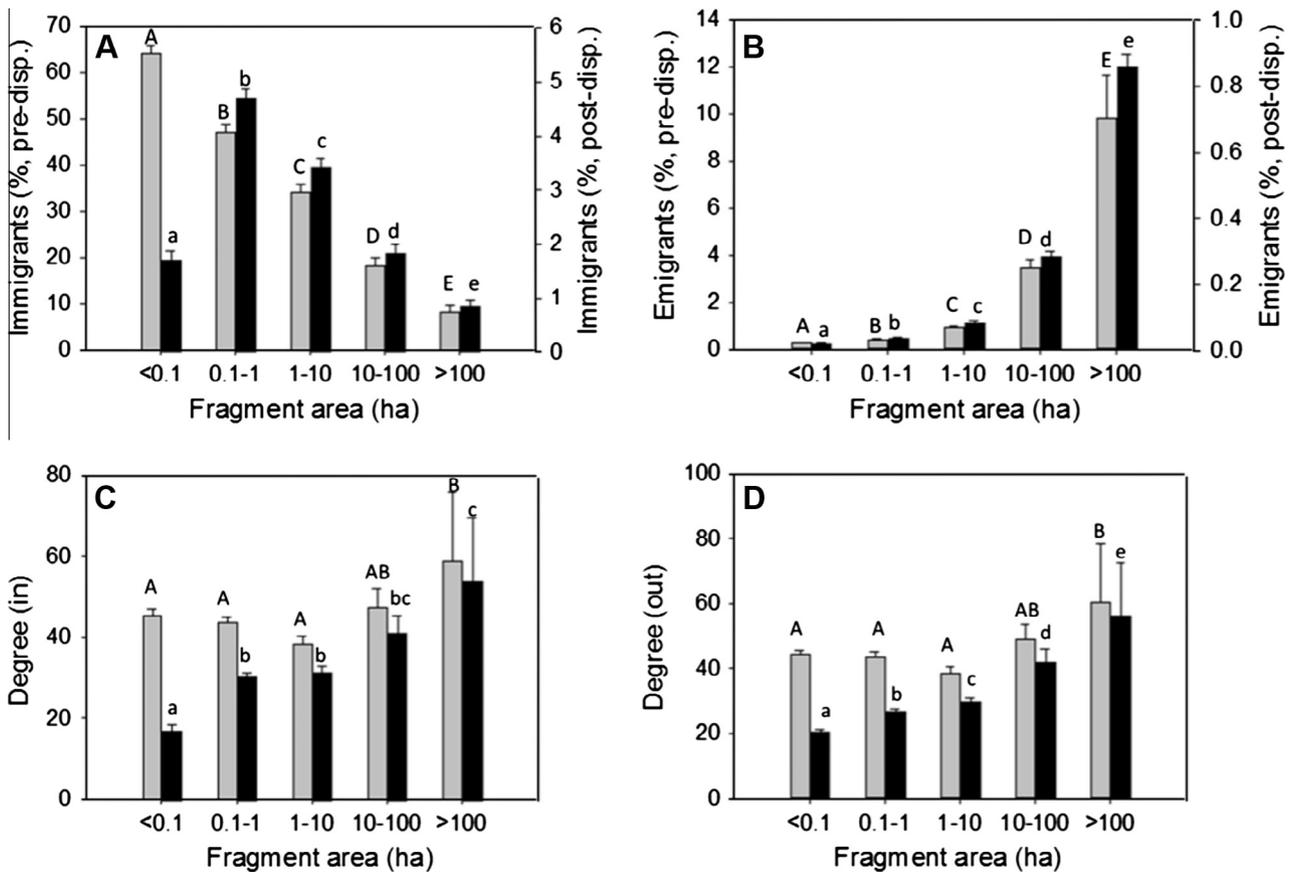

Fig. 3. Area effects on (A) immigration and (B) emigration rates, (C) links arriving to the fragment (degree in), and (D) links emerging from the fragment (degree out). Gray and black bars represent the pre- and post-acorn-dispersal networks, respectively. Letters represent homogeneous groups according to Tukey tests (capitals for pre-acorn-dispersal, lower case for post-acorn-dispersal). Note that the axes of immigration and emigration rates have different scale for each network.

Table 2
Effects of fragment area, distance to the center of the archipelago, and isolation on immigration and emigration rates, and number of ingoing and outgoing links. Only significant effects are shown. *LRT*, likelihood ratio test; *df*, degrees of freedom; *Sign*, sign of the effect.

Network	Response	Effect	LRT	df	Sign	pseudoR2
Pre-acorn dispersal	Immigration rates	Area	4152.20	4	–	0.91
	Emigration rates	Area	1018.06	4	+	0.77
		Isolation	16.05	1	–	
		Area * Isolation	10.88	4	–	
	Degree (in)	Area	20.45	4	+	0.30
		Distance to center	87.00	1	–	
		Isolation	41.24	1	–	
		Area * Distance	9.12	4	–	
		Area * Isolation	15.00	4	–	
		Distance * Isolation	12.05	1	+	
	Degree (out)	Area	29.51	4	+	0.18
		Distance to center	42.92	1	–	
		Isolation	96.8	1	–	
		Area * Distance	11.36	4	–	
		Area * Isolation	15.75	4	–	
		Distance * Isolation	12.11	1	+	
Post-acorn dispersal	Immigration rates	Area	5093	4	±	0.92
	Emigration rates	Area	1164.31	4	+	0.79
		Distance to center	3.69	1	–	
		Isolation	18.63	1	–	
		Area * Isolation	15.24	4	–	
	Degree (in)	Area	1141.54	4	+	0.29
		Distance to center	25.36	1	–	
		Isolation	12.00	1	–	
		Distance * Isolation	5.81	1	+	
	Degree (out)	Area	165.89	4	+	0.35
		Distance to center	34.95	1	–	
		Isolation	23.28	1	–	
		Area * Distance	33.25	4	–	
		Area * Isolation	14.17	4	–	

Table 3
Landscape configuration effects on connectance (*Con.*), number of isolated patches (*Isol*), raw modularity (*Mod*), relative modularity (*M**), number of modules (*Nmod*), mean number of nodes per module ($\overline{N}$), number of nodes of the smallest module (*Min*), number of nodes of the biggest module (*Max*) and migration rates (*Mig*). $\Delta control_{LANDS}$, percentage of variation of pre-acorn dispersal network with respect to the control landscape; $\Delta control_{INCREASE}$, percentage of variation between post-acorn dispersal and increased-caching networks in the control landscape. *S* indicates the significance of modular structure of the landscape in relation to null networks (see text). *Pre-disp* and *Post-disp*, pre- and post-acorn dispersal networks, respectively.

Effect	Landscape	Analysis	Con.	Isol	Mod	S	M*	Nmod	$\overline{N}$	Min	Max	Mig
No effect	Control	*Pre-disp*	0.14	0.00	0.62	–**	1.45	9.39	45.47	8.73	89.31	30.27
		Post-disp	0.10	7.23	0.63	–**	1.79	11.19	36.63	2.78	84.45	2.35
Area	Area reduction	*Pre-disp*	0.12	0.03	0.63	–**	1.45	9.88	42.29	10.10	91.41	29.33
		$\Delta control_{LANDS}$	**−9.34****	–	–	–	−0.04	5.16	**−6.99***	15.74	2.35	**−3.13****
Configuration	Clumped	*Pre-disp*	0.09	0.00	0.63	–**	0.73	8.59	48.86	25.95	72.49	22.83
		$\Delta control_{LANDS}$	**−36.30****	–	–	–	**−49.59****	**−8.56****	**7.43***	**197.34****	**−18.84****	**−24.6****
	Uniform	*Pre-disp*	0.09	0.00	0.66	–**	0.68	7.87	54.64	27.87	79.96	25.22
		$\Delta control_{LANDS}$	**−33.99****	–	–	–	**−52.88****	**−16.22****	**20.16****	**219.34****	**−10.47****	**−16.7****
Configuration & area	Clumped Area	*Pre-disp*	0.09	0.00	0.61	–**	0.65	8.64	49.18	24.38	72.30	21.12
		$\Delta control_{LANDS}$	**−34.54****	–	–	–	**−55.36****	**−8.03***	**8.14***	**179.35****	**−19.05****	**−30.23****
	Uniform Area	*Pre-disp*	0.09	0.00	0.66	–**	0.69	7.15	59.01	21.76	90.10	24.45
		$\Delta control_{LANDS}$	**−31.23****	–	–	–	**−52.11****	**−23.88****	**29.77****	**149.37****	0.88	**−19.43****
Increased caching	Control	*Post-disp*	0.12	0.84	0.63	–**	1.63	10.13	41.15	5.57	82.43	5.15
		$\Delta control_{INCREAS}$	**15.51****	**−88.38***	–	–	**−8.99****	**−9.47****	**12.33****	**100.36****	−2.39	**118.88****

Significant differences are in bold.
* *p < 0.05*.
** *p < 0.01*.

landscapes; Table 3). Superimposing a fragment area reduction on the clumped or uniform landscapes had low additional impact on observed parameter changes (Table 3, Uniform area and Clumped area landscapes).

The simulated enhancement of acorn caching rates increased all connectivity parameters. It increased network connectance (15.51%), decreased the number of isolated patches down to about 1 (*vs* 7 in the control network), and increased immigration rates by a factor of two (2.35% *vs* 5.15%). Besides, increased seed caching relaxed the modular structure of the network (−8.99%), which

showed bigger modules (12.33%) and a smallest module much larger (100.36%) (Table 3, Increased caching effect).

4. Discussion

We analyzed functional connectivity in a holm oak fragmented landscape taking into account three key stages of the recruitment processes: effective pollen dispersal, acorn production and local acorn dispersal.
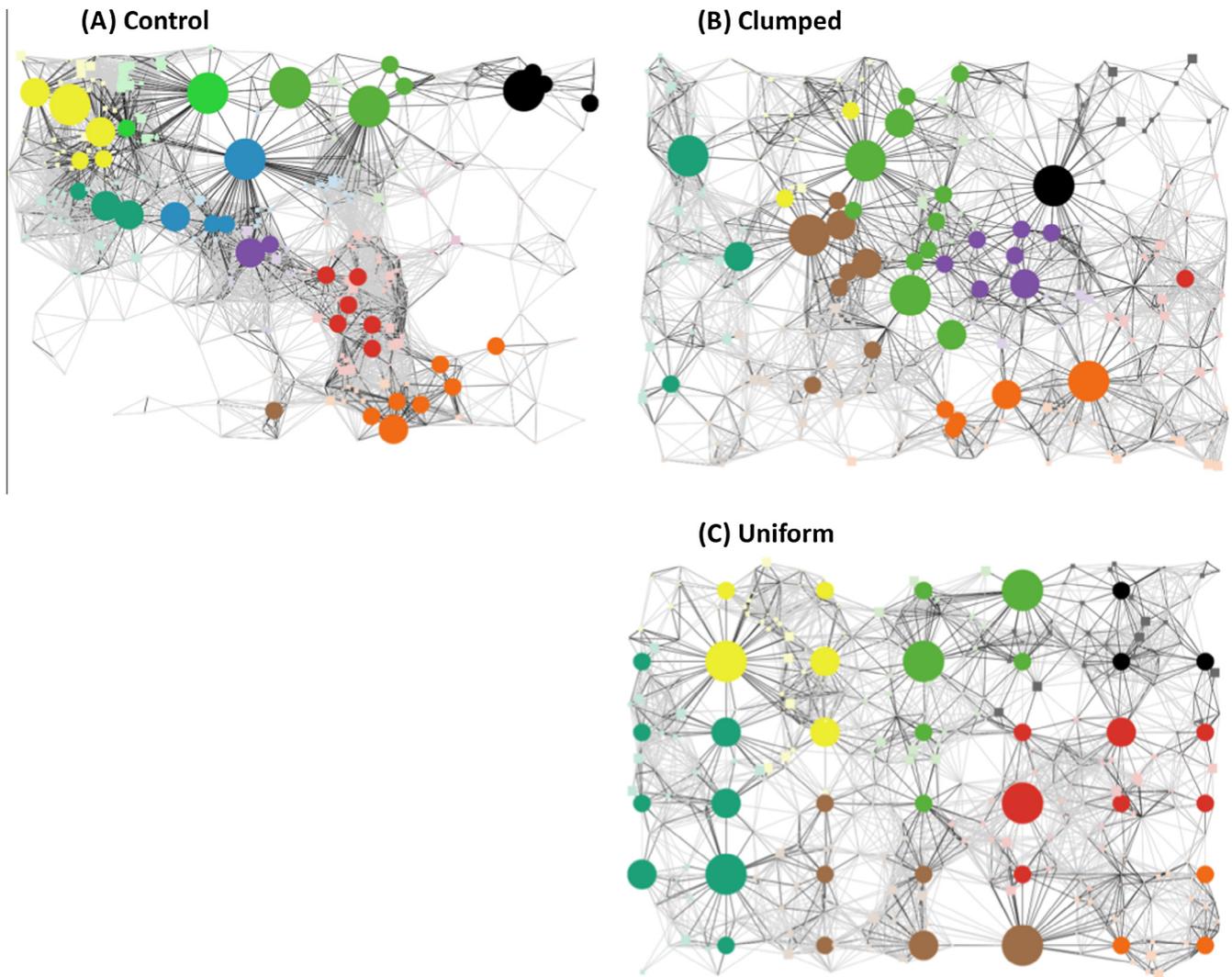
Fig. 4. Modular organization of connectivity in theoretical landscapes. Nodes represent forest fragments (*N* = 401; solid-color circles and light-color squares represent large (>10 ha) and small (<10 ha) fragments, respectively). Each color represents a module (group of patches highly connected, as detected by Newmans' algorithm). Lines indicate pollen-flow links between fragments (increasing gray darkness of links indicates larger pollen migration rates). (a) Control landscape, preserving the centroid and area of the fragments; (b) Clumped landscape, where centroids of large fragments are aggregated in the center of the archipelago, following an exponential decay function; (c) Uniform landscape, where centroids of large forest fragments are uniformly distributed within the landscape. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

As expected, effective pollen flow within the landscape was spatially structured. As with other *Quercus* species (Streiff et al., 1999; Pakkad et al., 2008; Pluess et al., 2009), most mating events occurred over short distances, while showing at the same time a fat-tailed distribution. The modular structure of the mating network implies that there are landscape subareas within which effective pollen exchange is substantial and that show comparatively less frequent mating with other subareas Notably, decreased effectiveness of local seed dispersal had a great impact on the landscape reproductive connectivity, diminishing the robustness of the mating network modules. Not only the number of connections in the mating network decreased after accounting for post-dispersal seed mortality, realized effective pollen immigration rates were an order of magnitude lower on average. In line with previous work, our results show that limited acorn dispersal can have pervasive effects on the maintenance of reproductive and genetic connectivity of fragmented oak populations (Fernández-M and Sork, 2005, 2007; Grivet et al., 2009; Luisa Herrera-Arroyo et al., 2013; Sork et al., 2015).

The role of forest fragments in the mating network reflected both their sizes and locations. Large fragments (>10 ha) were the main sources of effective pollen, enabling mating and thus pollen gene flow among fragments across the landscape. This result suggests that the system may become more genetically disjoint and fragile if a minimum number of large oak woodlands are not preserved. Conversely, small forest fragments (<1 ha) acted as major pollen sinks, showing the highest effective pollen immigration rates. Likewise other anemophilous species, our model predicts that as population size decreases the paucity of local mating neighbors results in higher immigration rates, as a consequence of the mass-action law (reviewed in Sork and Smouse, 2006). In addition to the amount of immigrant pollen, the diversity of sources is important in maintaining the genetic diversity of the acorn pool. Although larger fragments received migrant pollen from a higher number of external sources, the number of external pollen sources per area unit was larger for smaller fragments. Thus, individual trees within large fragments sampled less diverse pollen clouds (also reflected in N_{ep} estimates).

It has been advocated that small fragmented populations should be avoided in seed-collection management practices, due to impoverished genetic diversity of seed pools (Breed et al., 2013), but our results suggest that potential trade-offs between

fewer effective mothers and more effective fathers need not invariably result in reduced seed pool genetic diversity. Germplasm bank sampling strategies should consider including acorns not only from mothers in large forest fragments, but also from small ones, scattered across the landscape. The number of sampled mothers per fragment could in principle be proportional to fragment area (since the effective pollen pool diversity per unit area appears to be inversely related to fragment size), but more precise recommendations would require empirical genotypic and phenotypic information (e.g. common garden experiments). Given that fine-scale spatial genetic structure in adult trees is not uncommon in *Quercus* species (e.g. Aldrich et al., 2005; Soto et al., 2007), and also that correlated paternity among maternal families decreases with distance, usual recommendations about separation distance between sampled mother trees should also be considered.

In addition to area effects, the spatial distribution of forest fragments across the landscape modulated gene flow source–sink dynamics. As could be expected, forest patches located in central areas or less isolated played a more important role within the network. This suggests that a uniform or a clumped distribution of large forest fragments would enhance landscape connectivity. In the former case, gene flow would be more evenly distributed across the landscape. In the second one, a large central core area with a high number of patches and redundant connections would be created. However, functional connectivity may emerge from a complex interplay between habitat availability and landscape topology (Minor and Urban, 2008; Laita et al., 2011; Baguette et al., 2013), leaving aside potentially important selective effects influencing gene flow patterns across the landscape.

By simulating gene flow in theoretical landscape scenarios we were able to test whether fragment-level analyses can be scaled up to landscape management guidelines. In relation to area effects, the mating network was quite resilient to a ten percent area reduction of large forest fragments, even if the latter act as landscape connectivity hubs. Although reproductive connectivity may be minimally impacted by such a habitat loss, it is important to bear in mind that acorn predation may be exacerbated by increasing edge areas (Costa et al., 2014; Morán-López et al., 2015). As expected, the spatial arrangement of large forest fragments significantly impacted functional connectivity. However, not all the effects followed the expected connectivity enhancement. For instance, a uniform distribution of large forest fragments decreased the modularity of the network and increased the size of the smallest module, but diminished the number of connections among patches and average immigration rates. Similar results were obtained in the clumped landscape scenario. Overall, these results suggest that translating patch-centered approaches into conservation planning strategies may not be straightforward, due to the interaction between landscape configuration, habitat availability and edge effects. On the contrary, caching rate enhancement in edge areas improved all connectivity parameters. Process-based models, like our network approach, can help to evaluate the potential effectiveness of costly, large scale conservation measures before their implementation (Bolliger et al., 2014; Humphrey et al., 2015). Used carefully, they draw the attention to the potential of local management practices in the maintenance of functional connectivity at the landscape scale.

Our network approach allowed us to evaluate concomitantly pollen and seed dispersal considering the structure of the entire landscape. However, several simplifying assumptions should be acknowledged. Due to sampling constraints, we estimated a single global pollen dispersal kernel, without considering potential variation in pollen movement probabilities associated with local heterogeneity in vegetation structure or wind direction. Therefore, our model could be underrating pollen flow in open areas or along certain directions (Robledo-Arnuncio et al., 2014). However, estimated pollen dispersal distances and simulated immigration rates were comparable to those found in previous studies developed in a variety of landscape scenarios and *Quercus* species (Streiff et al., 1999; Nakanishi et al., 2004; Lorenzo Rodríguez, 2006; Soto et al., 2007; Pakkad et al., 2008; Pluess et al., 2009; Gerber et al., 2014; Ortego et al., 2014).

For the sake of simplicity, our model did not assume differences in male fecundity among individuals, which is substantial in many empirical studies (e.g. Kang et al., 2003). However, this assumption should not have biased our pollen dispersal kernel estimation, since the employed method has been shown to be accurate under non-uniform fecundity scenarios (Robledo-Arnuncio et al., 2006). The sensitivity of the mating network topology to potential variation in male fecundity among fragmentation categories across space, is more difficult to predict, and would be worth exploring theoretically and empirically. We additionally assumed that
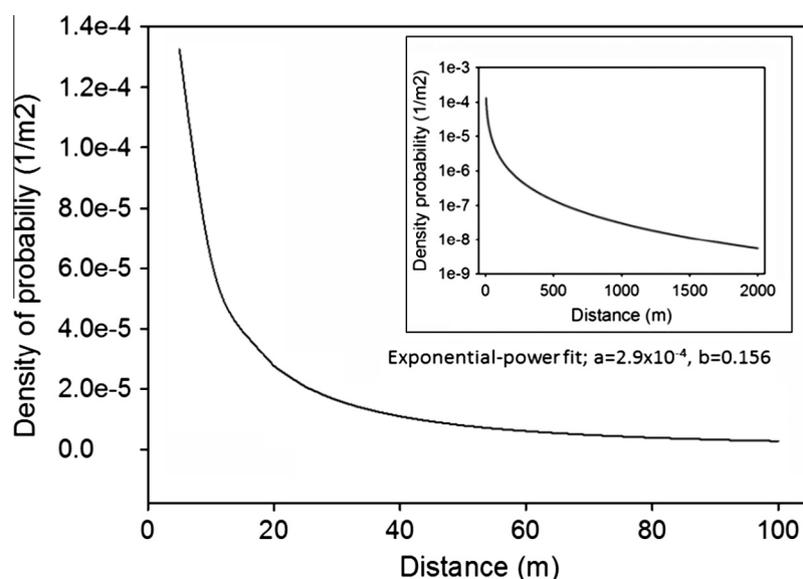
Fig. A1. Pollen dispersal kernel for *Quercus ilex* estimated with the exponential power function using the indirect method KINDIST. In the upper right corner is a log-plot version of the same curve providing a better representation of the tail.

fragmentation did not induce pollen limitation. It is not clear whether pollen availability is a limiting factor of acorn production as density decreases in holm oak stands (Garcia-Mozo et al., 2007; Ortego et al., 2014), but the positive edge effect on acorn production observed in our study area may suggest that this is not the case in our landscape. Our model did not include fragmentation effects on seedling establishment, even if cropland ploughing will

reduce recruitment success in edge areas and may act as a subsequent filter in functional connectivity (Fig. 1). However, we were interested in evaluating the endogenous potential of patchy woodlands to maintain genetic connectivity. The massive disappearance of emerging seedlings due to ploughing would make unfeasible to tease apart differences in recruitment driven by changes in disperser behavior from purely anthropogenic impacts. Acorn tracking experiments in abandoned croplands would help to incorporate edge effects on potential seedling recruitment in the model. Finally, in long lived species like holm oaks, the contribution of individuals to population demography and genetics occurs over decades. Albeit our network approach is based on extensive field data, it is temporally fine-scaled and hence it is difficult to evaluate the overall impact of fragmentation on the long term population structure and dynamics. For instance, although seedlings of managed holm oak woodlands show lower genetic diversity (Ortego et al., 2010), recent studies suggest that long distance dispersal may prevent genetic erosion in extremely isolated populations (Hampe et al., 2013). Models including multiple generations, based on mechanistic demographic and genetic approaches, could help to scale up our findings (Robledo-Arnuncio et al., 2014).

Table A1
Microsatellite loci used to genotype *Quercus ilex*. Ne = number of effective alleles, Ho: observed heterozygosity, He: unbiased expected heterozygosity, T_a = annealing temperature. Data corresponds to the analysis of the 28 mother trees.

Primer	Ne	Ho	He	T_a	Primer origin
MSQ13	5.92	1	0.85	50	Dow et al. (1995)
ZAG7	2.48	0.96	0.62	57	Kampfer et al. (1998)
ZAG9	4.98	0.64	0.76	55	Steinkellner et al. (1997)
ZAG11	1.55	0.43	0.36	50	Kampfer et al. (1998)
ZAG15	9.56	0.96	0.91	50	Steinkellner et al. (1997)
ZAG20	4.09	0.86	0.77	55	Kampfer et al. (1998)
ZAG112	2.88	0.64	0.66	55	Kampfer et al. (1998)
ZAG46	3.74	0.71	0.75	53	Steinkellner et al. (1997)
PIE020	2.27	0.57	0.57	50	Durand et al. (2010)
PIE258	6.37	0.68	0.86	55	Durand et al. (2010)
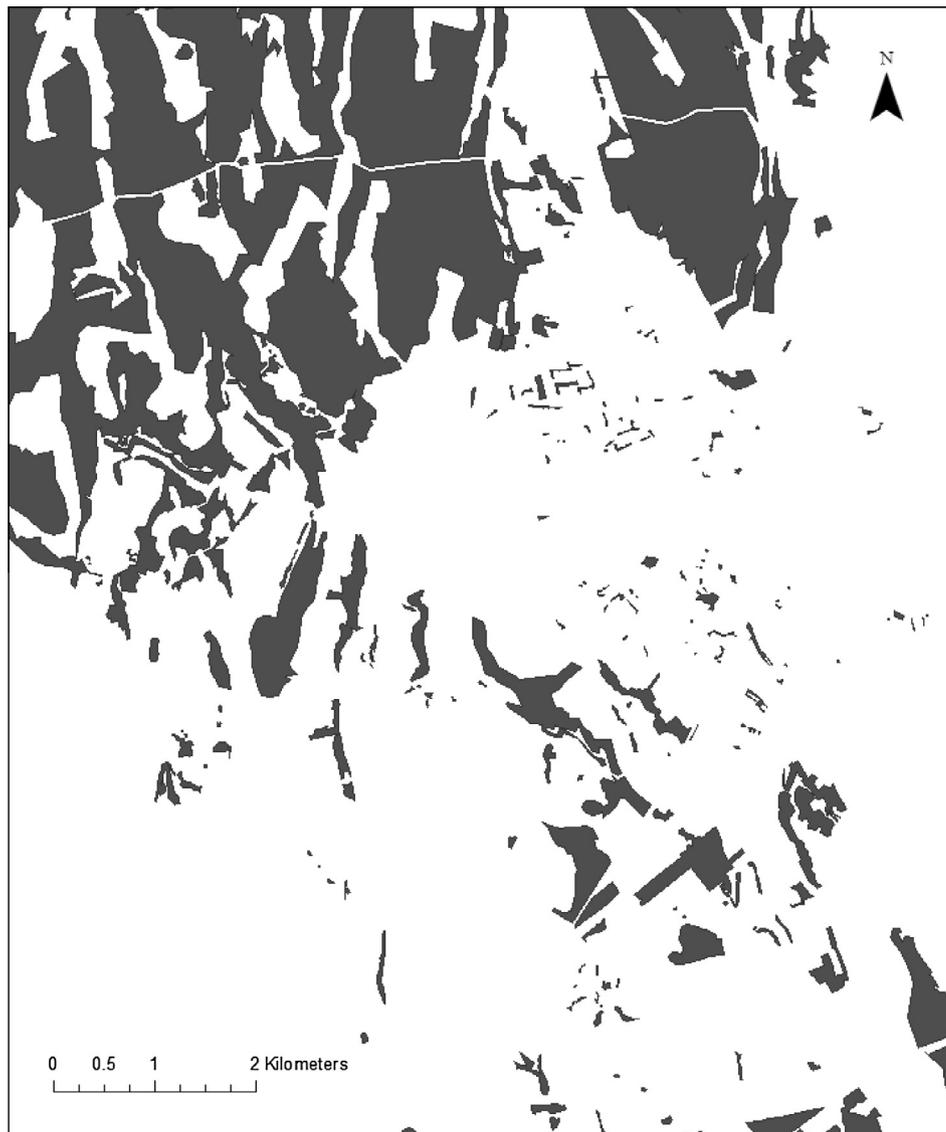
Fig. B1. Map of the study area. Fragments are depicted in gray. Not all the study area is represented in order to visualize small forest fragments.

5. Conclusions

Our results showed that limited seed dispersal can constrain genetic cohesiveness of fragmented populations despite extensive pollen flow. Besides, they suggest that the preservation of large forest fragment (>10 ha) is essential to maintain functional connectivity, as they act as predominant pollen sources. By contrast, acorn crops at small forest fragments may potentially harbor higher genetic diversity. Despite the clear source–sink dynamics found here, our results highlighted the need for caution when scaling up patch-centered approaches into landscape conservation guidelines. Overall, our study shows that costly large scale management practices envisioned to enhance landscape connectivity may not be effective if local recruitment processes are disregarded.

Acknowledgements

Appendix A

See Table A1 and Fig. A1.

Appendix B

See Fig. B1.

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