



Regional vs local effects of habitat loss and fragmentation on two plant–animal interactions

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The effects of habitat fragmentation on plant–animal interactions may emerge at different spatial scales, depending on the species-specific perception response of the interacting animals. Furthermore, changes in habitat cover and configuration commonly occur simultaneously, hampering efforts to understand and mitigate the impact of fragmentation on these biotic interactions. In order to account for the relative influence of habitat loss and fragmentation on plant–animal interactions, we quantified habitat structure in sixteen sectors (nested circular areas of 100 and 200 m radii) in four different localities (four sectors per locality) across the Cantabrian Range in NW Spain. In the center of each 100 m radius sector, we measured the magnitude of two ecologically opposite (mutualistic vs antagonistic) interactions in individual holly trees *Ilex aquifolium* which strongly determine the regeneration process in this plant species: frugivory by birds and seed predation by rodents. We found that habitat fragmentation, though not habitat loss, affected the magnitude of both plant–animal interactions. However, these effects were conditioned by the strong differences in spatial heterogeneity in habitat structure between localities. In fact, the effect of habitat fragmentation on both plant–animal interactions disappeared when the locality in which sectors were sited was taken into account. This study highlights that 1) habitat spatial configuration, far from being a negligible component of habitat structure, is in fact able to influence key ecological processes such as plant–animal interactions, and 2) the potential spatial and structural complexity of localities makes a regional approach (i.e. that involving many localities) indispensable in the quest for comprehensive understanding of the effects of habitat structure on biodiversity in real-world fragmented landscapes.

Habitat loss and fragmentation have been identified as major threats to the conservation of global biodiversity in terrestrial ecosystems (Solé and Bascompte 2007). The extent of remaining habitat patches (Bascompte and Solé 1996, Bodin et al. 2006) and their degree of isolation (Dunning et al. 1995, Davies et al. 2000) markedly affect species richness and abundance in human-impacted landscapes. However, it is increasingly evident that patch-based approaches alone might be insufficient since they do not take into account the context of patches, i.e. the habitat structure surrounding a patch, which also seems important in predicting the effects of habitat modification. This is not only true in relation to biodiversity (Mazerolle et al. 1999) but also for key ecosystem functions driven by interactions between species (Steffan-Dewenter et al. 2001, 2002, García and Chacoff 2007, González-Varo 2009).

Growing research indicates that habitat loss and fragmentation have pervasive effects on the networks of relationships between plants and animals (Tylianakis et al. 2008). Interactions such as pollination, seed dispersal, and herbivory are increasingly being proposed as primary conservation targets in human-impacted landscapes, given their pivotal role in plant regeneration processes (Wang and

Smith 2002), plant community sorting (Beckage and Clark 2005), and even biodiversity evolution and maintenance (Bascompte et al. 2006). It is also being suggested that the outcome of plant–animal interactions might be used as passive restoration tools and thus suitable for restoring not only plant populations but also community structure in human-impacted landscapes. For example, through their role in seed dispersal, frugivores ensure seed delivery into degraded patches (Wunderle 1997, Chapman and Chapman 1999), enhance functional connectivity across the landscape (Bodin et al. 2006, Herrera and García 2009) and even contribute to ecosystem resilience in real-world landscapes (Elmqvist et al. 2003, García et al. 2010). Nevertheless, mutualistic and antagonistic interactions are subsequently linked throughout the process of plant regeneration (Steffan-Dewenter et al. 2002, García and Chacoff 2007), hampering efforts to understand the net outcome of plant–animal interactions. Indeed, both habitat loss and fragmentation have nearly always been found to simultaneously alter mutualistic and antagonistic relationships (Santos and Tellería 1994, García and Chacoff 2007), thus obscuring the net effect of habitat structure on the outcome of plant–animal interactions.

Traditionally, ecological studies have tested the effects of habitat loss and fragmentation on plant–animal interactions over a limited spatial extent, that is, within a given locality, even when considering multiscaled approaches (Steffan-Dewenter et al. 2001, 2002, García and Chacoff 2007). However, human-promoted processes such as timber exploitation, agriculture and urban development, are spatially non-random mechanisms of environmental change and therefore patterns of habitat structure are commonly locally determined (Lindenmayer and Fischer 2006). Therefore, studies carried out exclusively within a given locality may neglect the potential spatial heterogeneity in habitat structure that could possibly exist at a regional scale, i.e. between localities. The human resource demands and time consuming nature of designs required to cover the regional scale are often the major reasons for the lack of process-oriented fragmentation studies at this spatial scale (Brennan et al. 2002).

In this study, we applied a regional-based approach to test whether and how habitat loss and fragmentation affect two plant–animal interactions across the highly anthropized Cantabrian Range in NW Spain. Specifically, we studied two opposite, key drivers of initial recruitment of holly *Ilex aquifolium* trees: frugivory by birds (i.e. a mutualistic interaction) and seed predation by rodents (i.e. an antagonistic interaction) (García et al. 2005a, b). We considered

both habitat loss and fragmentation as changes in habitat structure (set by habitat composition and configuration; Gustafson and Parker 1992) although their effects were analyzed separately by means of independent metrics. In particular we were interested to know: 1) whether plant–animal interactions determining plant recruitment of holly were similarly affected by habitat loss and fragmentation over a regional extent, 2) the relative influences of habitat loss and habitat fragmentation, and 3) the appropriate scale(s) (whether locality or regional) at which to observe trends in the response of both plant–animal interactions. With respect to this third aim, we believe that a solid understanding of the scale at which the effects of habitat structure emerge is a pre-requisite for the establishment of management guidelines to recover plant populations and vegetation in fragmented landscapes.

Methods

Study system and species

We carried out our study in mid-elevation forests of the Cantabrian Range (Asturias region, NW Spain; Fig. 1). Although presumably originally covered by Atlantic deciduous forests, a long history of deforestation for cattle grazing and selective logging has transformed the original

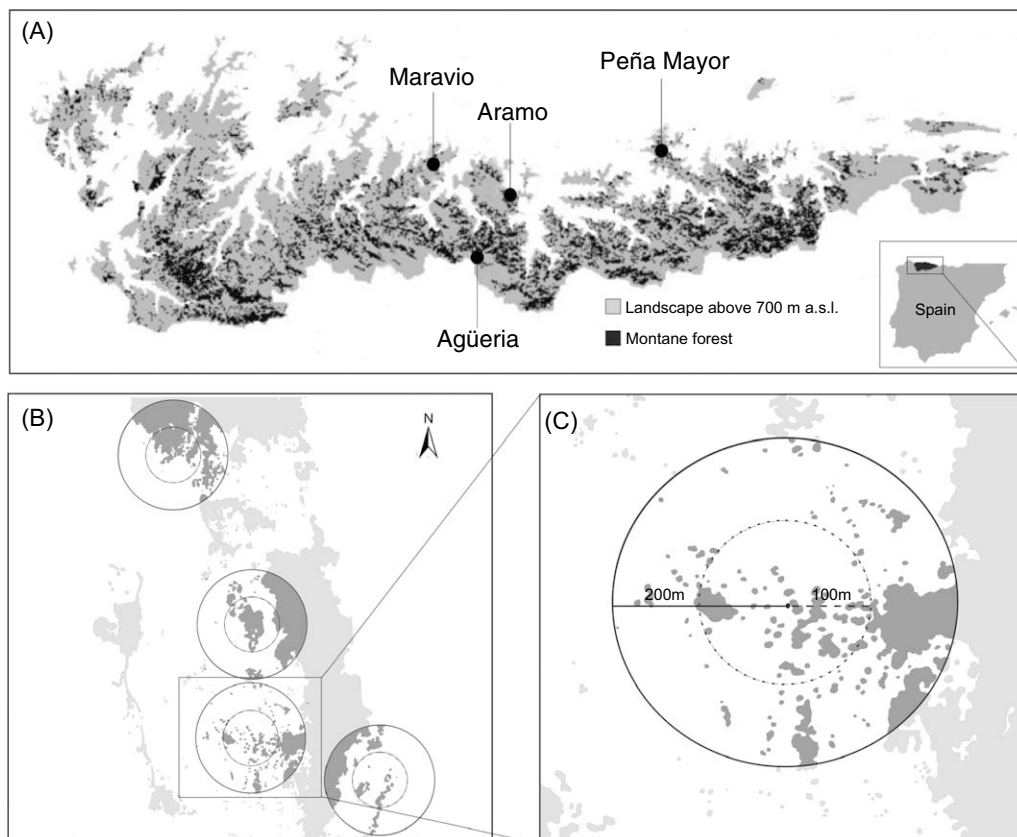


Figure 1. (A) Study region and localities across the Cantabrian range in the Asturias province (Spain). Landscape above 700 m a.s.l. is indicated in grey, black areas indicate forests. (B) Distribution of the four sampling landscape sectors in the locality of Peña Mayor (a similar approach was carried out at the remaining three localities). (C) Detailed information of a given landscape sector with the two nested radii at 100 and 200 m. In (B) and (C), light grey area corresponds to forest cover and the dark grey area to forest cover considered for landscape metrics measurement.

primary forests of beech *Fagus sylvatica*, birch *Betula alba* and oak *Quercus* spp. into pasture and heathland. After their abandonment for the last few decades, many pastures are being recolonized by secondary-growth forests rich in animal-dispersed trees such as holly *Ilex aquifolium*, hawthorn *Crataegus monogyna*, yew *Taxus baccata*, hazel *Corylus avellana* and rowans *Sorbus* spp. Thus, the current forest-landscape in the Cantabrian Range shows a highly variegated pattern (sensu McIntyre and Hobbs 1999), characterized by a heterogeneous mosaic of occasional large remnants of deciduous forest and numerous small and isolated fragments of primary and secondary-growth forest, all embedded in a dominant (ca 75% cover) matrix of pasture, heathland (*Erica* spp., *Ulex europaeus*) and rural settlements (García et al. 2005c).

We focused on secondary-growth forest as a target habitat, and on holly *Ilex aquifolium*, Aquifoliaceae; *Ilex* hereafter, as a case study species. *Ilex* is a major canopy component of Cantabrian secondary-growth forests (García et al. 2005c), and it shares a common network of interacting animals, such as pollinators, frugivorous seed dispersers and seed predators, with other tree species in these forests such as *C. monogyna*, *T. baccata* and *Sorbus* spp. (García et al. 2005a, b, Martínez et al. 2008). We thus considered that the potential *Ilex* regeneration constraints associated to habitat structure would be generalized to other tree species of secondary-growth forests. Moreover, *Ilex* is a pioneer species that quickly colonizes abandoned pastures and facilitates the establishment of other trees like *T. baccata* (García and Obeso 2003). Therefore, we assumed that animal-mediated *Ilex* regeneration processes in fragmented habitats represent a large portion of the forest recovery dynamics in a degraded matrix (see Kollmann 1995 for a similar approach).

Ilex is a dioecious evergreen tree that flowers mainly in the second half of May and early June. Ripe fruits are red berries with 2–4 pyrenes which are available from September to January. Holly fruits average ca 9 mm in diameter and are small enough to be consumed by all the principal fruit eating species in our study region. In the northern Iberian Peninsula berries are mainly consumed by frugivorous birds of the genus *Turdus*, especially by the overwintering migrant *T. iliacus* (Gutián and Bermejo 2006, Martínez et al. 2008). All *Turdus* species interact with holly as legitimate seed dispersers (sensu Jordano 1992). Mammals such as badgers *Meles meles*, and foxes *Vulpes vulpes* may occasionally consume *Ilex* fruits, but their relative contribution to seed dispersal services is negligible as demonstrated by fecal sampling analysis (Martínez et al. 2008). After deposition by birds, seeds suffer predation by rodents (*Apodemus* spp.) during late winter. Previous studies reveal intermediate seed predation rates relative to other fleshy-fruited species of the Cantabrian secondary-growth forests, such as *T. baccata* and *C. monogyna* (García et al. 2005a). Recruitment potential in this species is directly positively related to the number of dispersed seeds and negatively to seed predation rates (García et al. 2005a, b). Rodents do not act as seed dispersers (García et al. 2005a).

Habitat structure

In order to account for the effect of habitat loss and fragmentation on plant–animal interactions, we measured habitat structure using a “hybrid patch-landscape scale approach” (Brennan et al. 2002). In 2006, we selected four main localities (Aramo, Peña Mayor, Maravio and Agüeria; Fig. 1), with similar habitat composition (highly fragmented secondary-growth forests embedded in a pasture-heathland matrix). However, differential historical land use and forest management have resulted in considerable variability in the size and degree of isolation of fragments between localities (Results). All localities were on north-facing slopes, at altitudes of between 900 and 1300 m a.s.l. and 25–40 km apart (Fig. 1).

At each locality, we selected four non-overlapping circular sectors as study replicates ($n = 16$) in which to evaluate habitat structure (Fig. 1B–C); each consisting of two concentric circular sectors with radii of 100 and 200 m. We considered these observation extents to accurately represent the gradients of spatial heterogeneity caused by habitat fragmentation (Brennan et al. 2002). More importantly, these scales have previously been shown to be important to the study of habitat structure effects on plant–animal interactions in fragmented landscapes (García and Chacoff 2007). For each 100 m and each 200 m radii circular sector, we measured the proportion of remaining forest cover (COVER), and fragmentation metrics such as mean forest patch size (SIZE), length of forest edge (EDGE) and the number of forest patches (NUMBER) from a Geographic Information System derived from orthophoto 1:5000 maps. In the center of each 100 m radius circular sector, we selected 4 fruiting *Ilex* trees ($n = 64$) in which we measured the magnitude of frugivory by birds and seed predation by rodents (see below). We selected trees of similar size (diameter at breast height 10–15 cm) and individual fruit availability (i.e. crop size $\approx 10\,000$ fruits) in order to prevent the potential influence of these factors in the response of plant–animal interactions. Due to the degree of fine-grain fragmentation in some localities, the trees within a given circular sector were sometimes located in different forest patches, but they were never further than 15 m apart. In addition, we ensured there was a similar amount of protective canopy in the 10 m radius surrounding each focal tree, as this habitat feature has previously been shown to strongly influence the functional response of frugivorous birds (García and Chacoff 2007). To do this, we visually estimated the forest cover, irrespective of plant species, in a 10 m radius around each focal tree in the field and the cover of the total projection of overlapping canopies (see García and Chacoff 2007 for a similar procedure). We did not find differences in forest cover surrounding focal trees neither between trees nor between sectors or localities (ANOVA; all comparisons were $p > 0.05$).

Frugivory by birds

In September 2006 we established three permanent 50×50 cm sampling quadrats beneath each focal tree and, each

fortnight, collected all fallen *Ilex* fruits and counted the number of fruits pecked by thrushes. Fallen pecked fruits result from birds' handling failures when perching and pecking fruits on branches, and they are easily recognizable by the V-shaped beak marks on the fruit coat (Sallabanks 1993). We assumed pecked fruits to be directly related to frugivory activity and not to fruit rejection due to selective pressures on fruit traits given that: 1) in our study region holly fruits are mostly (ca 80%) consumed by a single frugivore species (*T. iliacus*; above) (Martínez et al. 2008) and 2) there are no significant differences between trees in fruit size within a locality (unpubl.). Following the procedure reported by García and Chacoff (2007), the magnitude of frugivory was estimated as the cumulative number of beak-marked fruits with respect to the total number of fallen fruits at the end of the dispersal season (February) (Sallabanks 1993). The consumption of *Ilex* fruits from quadrats by nocturnal mammals like foxes or badgers was considered negligible as no seed or fruit remains were observed in mammal faeces during the sampling period (Martínez et al. 2008). Furthermore, a previous work in one of the study sites (Peña Mayor) evidenced a negligible loss of seeds from open quadrats for the target species (García et al. 2005a). We are therefore confident that our method provides a reliable estimate of fruit abundance, free of fruit loss due to environmental conditions such as rainfall. For these reasons, although an indirect measurement, we consider our estimate of the magnitude of interaction of each individual tree with avian frugivores a good approximation of seed dispersal services (Sallabanks 1993, García and Chacoff 2007).

Seed predation by rodents

We quantified the magnitude of seed predation in three experimental seed depots beneath each focal tree. Each depot consisted of 10 seeds of *Ilex* selected from a pool of bird-dispersed seeds previously collected in the study sites. Seeds were glued with a low odour, rain-proof thermo-plastic glue, to a 10 × 6 cm plastic mesh nailed to the ground (Herrera and García 2009). We located seed depots below focal trees since this is the principal microhabitat receiving dispersed seeds in fragmented landscapes (Herrera and García 2010). Furthermore, seed removal rate is independent of the density of seeds and the distance to potential source trees (unpubl.). In this sense, we consider our distribution of seed deposits an accurate representation of the potential spatial variability in seed predation rate across the landscape. We revisited seed depots fortnightly assuming that rodents had preyed on a seed when it was missing from the plastic mesh or still on the mesh but gnawed and empty (see Alcántara et al. 2000 for a similar procedure). The number of seeds established in the seed depots (n = 10) was within the range of seed abundance found in the field in natural conditions (García et al. 2005a, b). We calculated seed predation rate as the proportion of preyed seeds per individual tree (i.e. the average between depots under each tree; García and Chacoff 2007, Herrera and García 2009).

Data analysis

We were interested in assessing the effects of habitat structure on the magnitude of plant–animal interactions, especially in distinguishing the relative influence of habitat loss and habitat fragmentation. To do this, given that the metrics we used to quantify fragmentation (i.e. SIZE, EDGE and NUMBER; see above) were correlated to forest cover (COVER), we regressed the values of these fragmentation metrics against the values of the amount of forest cover to obtain the residuals from these regressions. Following Fahrig (2003), we applied a quadratic polynomial regression to the length of forest edge and a linear regression applied to the other metrics. After extracting the residuals, we integrated them using principal component analysis (PCA; Jolliffe 2002) and subsequently used them as fragmentation metrics that were statistically independent to the amount of forest cover (McGarigal and Cushman 2002). We built separated PCAs for each set of fragmentation metrics, that is, one for those at 100 m radius and another for those at 200 m radius. The first two PCA eigenvectors at 100 m (hereinafter FRAG1_100 and FRAG2_100) and 200 m (FRAG1_200 and FRAG2_200) radii, accounted for >90% of the variation in these metrics. Thus, we considered three independent habitat metrics for each spatial extent, at 100 m (COVER_100, FRAG1_100 and FRAG2_100) and at 200 m radius (COVER_200, FRAG1_200 and FRAG2_200; see Results). To calculate the magnitudes of both frugivory and seed predation, we averaged the values of all four focal trees per sector (see Steffan-Dewenter et al. 2002 for a similar procedure). We assessed the effects of habitat structure on plant–animal interactions by means of linear and, where necessary, quadratic regressions using the amount of forest cover (COVER_100 and COVER_200) and the PCAs (FRAG1_100, FRAG2_100, FRAG1_200, FRAG2_200) as predictor variables and the associated magnitude of both plant–animal interactions as response variables.

We were also interested to know whether the influence of habitat structure on plant–animal interactions, if any, was due to spatially correlated factors derived from the intrinsic nature of localities. In other words, we sought the relative weight of locality identity on the relationship between habitat structure and response variables. To this end, we fitted general linear models (GLM) considering the locality identity as a main factor and the magnitude of frugivory by birds and that of seed predation by rodents as response variables, and obtained the residuals from these GLMs. We took these residuals to represent the variability in magnitude of plant–animal interactions independent of the locality, and they were thus used as response variables in linear and quadratic regression tests with the amount of forest cover and PCAs again as predictor variables. We considered that the disappearance of significant effects of habitat structure after the inclusion of locality identity would indicate that the effects of habitat loss and fragmentation on response variables were related to the inherent nature of localities in terms of the spatial heterogeneity in habitat structure. All statistical analyses were performed using JMP 7.0 (SAS Inst.). Means ± SE are reported throughout the text and tables.

Results

Habitat structure

Two eigenvectors for the set of fragmentation metrics at 100 m radius, and two for the set at 200 m radius were judged meaningful by the PCA (Table 2) and explained >90% of the total variance in the set of fragmentation metrics at both spatial scales (Table 1). The first eigenvector at 100 m radius (FRAG1_100) represented a negative gradient in the number of patches simultaneous to a positive gradient in the average patch size, indicating that circular sectors with higher positive eigenvalues for FRAG1_100 contained fewer, but relatively bigger forest patches. The second eigenvector at 100 m (FRAG2_100) was strongly positively related to length of edge (Table 1). The first eigenvector at 200 m radius (FRAG1_200) represented a positive gradient mainly related to the average number of patches whereas the second (FRAG2_200) collected the variation in length of edge and average patch size, with large eigenvalues representing sectors with a lot of edge and small average patch size.

Regarding the average amount of forest cover, we found no significant differences between sectors per locality either at 100 m (GLM, $F_{3, 12} = 0.88$, $R^2 = 0.18$, $p = 0.782$) or at 200 m radius ($F_{3, 12} = 0.34$, $R^2 = 0.07$, $p = 0.843$) (Table 2). However, there were strong differences between localities in the average values of fragmentation metrics. In all but one of the eigenvectors representing fragmentation metrics, significant differences among localities appeared at 100 m for FRAG1_100 ($F_{3, 12} = 4.23$, $R^2 = 0.39$, $p = 0.002$) and FRAG2_100 ($F_{3, 12} = 10.63$, $R^2 = 0.71$, $p < 0.0001$), and at 200 m for FRAG1_200 ($F_{3, 12} = 18.64$, $R^2 = 0.82$, $p < 0.0001$) but not for FRAG2_200 ($F_{3, 12} = 0.93$, $R^2 = 0.18$, $p = 0.452$) (Table 2).

Effects of habitat structure on plant–animal interactions

The proportion of beak-marked fruits (i.e. frugivory rate) averaged $0.31 \pm 0.13SE$ ($n = 64$) in the circular sectors. There were large differences in the average frugivory rate between localities (ANOVA; $F_{3,13} = 18.17$, $p < 0.001$). In descending order, the proportions of beak-marked fruits were $0.49 \pm 0.03SE$ (Maravio), $0.31 \pm 0.03SE$ (Peña Mayor), $0.30 \pm 0.03SE$ (Aramo) and $0.16 \pm 0.03SE$ (Agüeria). Amount of forest cover had no effect on frugivory

rate at 100 m (linear regression, $R^2 = 0.05$, $F_{1,14} = 0.81$, $p > 0.05$) or at 200 m radius ($R^2 = 0.03$, $F_{1,14} = 0.48$, $p > 0.05$, Table 3). However, there was a significant negative relationship between frugivory rate and FRAG2_100 at 100 m radius (linear regression, $R^2 = 0.32$, $F_{1,14} = 6.87$, $p < 0.05$) indicating that the longer the length of edge at 100 m radius, the lower the frugivory rate (Fig. 2A). At 200 m radius, frugivory rate was positively related to FRAG1_200 although better fit was provided by a quadratic relationship (quadratic regression, $R^2 = 0.67$, $F_{2,13} = 13.49$, $p < 0.001$, Table 3) indicating that frugivory rate increased in sectors with a moderate number of patches, but decreased in sectors with both very few or many patches (Fig. 2B).

Rodents preyed on holly seeds beneath 93.7% of target trees ($n = 64$), with an average predation rate of 0.57% ($\pm 0.34SE$). Overall, differences in seed predation rate between localities were smaller than for frugivory rate: Agüeria ($0.78 \pm 0.33SE$), Aramo ($0.55 \pm 0.35SE$), Maravio ($0.50 \pm 0.32SE$) and Peña Mayor ($0.47 \pm 0.29SE$). Seed predation rate was positively correlated to FRAG2_100 (linear regression, $R^2 = 0.27$, $F_{1,14} = 5.26$, $p < 0.05$, Fig. 3A) indicating that longer edge length led to higher seed predation rates. Seed predation rate was also positively correlated to FRAG2_200 ($R^2 = 0.25$, $F_{1,14} = 4.69$, $p < 0.05$, Fig. 3B) meaning that the larger the mean patch size, the lower the seed predation rate at 200 m radius.

We repeated the regression analyses above, but this time using the residuals of the magnitudes of frugivory and seed predation after the GLMs. We found that only the positive relationship between seed predation rate and FRAG2_200 remained significant ($R^2 = 0.26$, $F_{1,14} = 4.94$, $p < 0.05$, Fig. 2C–D, Fig. 3C–D, Table 3). This means that, irrespective of the locality identity, the longer the edge length, the higher the seed predation rate.

Discussion

We examined the relative effect of habitat loss and habitat fragmentation on two ecologically opposite interactions in individual holly trees which strongly determine the regeneration process in this plant species: frugivory by birds and seed predation by rodents. We found that habitat fragmentation, but not habitat loss, affected the functioning of both biotic interactions. Moreover, our approach over a regional scale (i.e. involving several localities) revealed that the

Table 1. Results of principal components analyses to obtain fragmentation metrics from the residuals of mean forest patch size (SIZE), length of forest edge (EDGE) and the number of forest patches (NUMBER) against the amount of forest cover (COVER) at both radii.

	100 m radius		200 m radius	
	FRAG1_100	FRAG2_100	FRAG1_200	FRAG2_200
Cumulative explained variation (%)	66.4	99.7	78.8	99.1
Principal component structure				
Res. number of patches	−0.70	−0.01	0.64	−0.01
Res. total edge length [‡]	0.00	0.99	0.53	0.72
Res. mean patch size [†]	0.70	−0.01	0.54	−0.69

[†]Logarithmically transformed before PCA.

[‡]Obtained by polynomial regression analysis using habitat proportion (AREA) as the explanatory variable. FRAG1 and FRAG2 represent the two first PCA eigenvectors at each radius (see text for details).

Table 2. Landscape metrics for sectors in different localities evaluated at two observation scales (100 and 200 m radii circular areas). Means \pm SE are given (n = 4). Numbers in brackets show minimum and maximum values.

Locality	100 m radius			
	Cover (%)	Mean patch size (m ²)	Number of patches	Perimeter (m)
Peña Mayor	27.7 \pm 21.9 (6.4, 56.1)	708.8 \pm 732.3 (112.2, 1732.7)	21.0 \pm 15.8 (6, 42)	1174.7 \pm 455.7 (577.3, 1549.5)
Maravio	25.0 \pm 19.5 (6.2, 42.8)	327.0 \pm 353.9 (32.6, 791.8)	38.2 \pm 28.4 (17, 60)	1229.5 \pm 307.1 (1002.1, 1683.0)
Agüeria	38.2 \pm 24.1 (13.0, 64.0)	799.6 \pm 620 (205.6, 1513.2)	17.2 \pm 4.2 (11, 20)	3685.7 \pm 431.8 (3196.0, 4248.0)
Aramo	39.5 \pm 29.4 (8.7, 68.9)	485.2 \pm 485.3 (57.2, 1083.2)	36.7 \pm 15.1 (20, 51)	1751.0 \pm 490.3 (1170.8, 2367.2)
200 m radius				
Peña Mayor	26.2 \pm 18.1 (8.8, 48.8)	457.4 \pm 3740.2 (9.2, 55964.2)	72.0 \pm 39.6 (21, 117)	3649.5 \pm 1180.7 (2483.0, 4946.1)
Maravio	19.8 \pm 15.4 (6.5, 40.2)	230.7 \pm 2492.8 (5.3, 48402.2)	108.2 \pm 46.3 (65, 163)	3453.5 \pm 719.8 (2433.0, 4126.0)
Agüeria	27.9 \pm 10.3 (13.1, 37.3)	703.0 \pm 3477.1 (6.0, 36612.0)	50.1 \pm 14.0 (39, 72)	1126.0 \pm 206.2 (842.0, 1328.1)
Aramo	44.8 \pm 28.9 (11.3, 70.9)	582.2 \pm 4347.3 (4.5, 63940.3)	96.7 \pm 40.1 (43, 128)	5576.5 \pm 1164.4 (4259.1, 7044.0)

detection of fragmentation effects was conditioned by the large differences in habitat spatial configuration between localities. In fact, the effect of habitat fragmentation on both plant–animal interactions disappeared after taking locality into account. This study reveals that habitat spatial configuration, far from being a negligible component of habitat structure, is in fact able to influence key ecological processes such as plant–animal interactions. Furthermore, our results highlight that the effects of habitat fragmentation on frugivory, and to a lesser extent on seed predation, mostly emerge when applying an observation window large enough to adequately represent the spatial heterogeneity in habitat structure that may influence both biotic interactions, that being, in our case, the regional scale.

Relative influence of habitat loss and fragmentation

Our results are consistent with other studies which show plant–animal interactions being influenced by habitat fragmentation (Donoso et al. 2003, García and Chacoff 2007, Cordeiro et al. 2009). However, we consider that our findings expand on previous works as we discern how

fragmentation effects might actually be related to changes in configuration, rather than to habitat loss (see also Farwig et al. 2009). Traditionally, fragmentation-related research has not discriminated between these two effects thereby making difficult the identification of the mechanisms by which habitat fragmentation actually affects biodiversity (Lindenmayer and Fischer 2007). Furthermore, among the few known empirical studies disentangling both effects (Villard et al. 2001, Farwig et al. 2009), very few (but see Farwig et al. 2009) have analysed their relative influences on ecosystem functions driven by biotic interactions. Our findings on plant–animal interactions will help to fill this gap, closely matching as they do growing research evidence showing changes in habitat spatial configuration to be a major factor behind species occurrence in fragmented habitats (Villard et al. 2001, Yamaura et al. 2006).

The effect of habitat fragmentation was additive at 100 m radius in terms of plant reproduction (sensu García and Chacoff 2007) as habitat fragmentation simultaneously decreased frugivory and increased seed predation rates. Higher seed predation rates in fragmented habitats are commonly related to increased population sizes of generalist

Table 3. Coefficients of determination of regressions between the amount of forest cover and fragmentation metrics obtained from previous PCA (see Table 1 caption) at 100 and 200 m radius (predictor variables), and frugivory and seed predation rate (response variables). *p < 0.05; ***p < 0.001.

Interaction	100 m radius			200 m radius		
	COVER_100	FRAG1_100	FRAG2_100	COVER_200	FRAG1_200	FRAG2_200
With locality effect						
Frugivory rate	0.03	0.05	0.32*	0.05	0.67***†	0.01
Seed predation rate	0.03	0.05	0.27*	0.06	0.07	0.25*
Without locality effect‡						
Frugivory rate	0.00	0.00	0.00	0.00	0.01†	0.00
Seed predation rate	0.16	0.02	0.01	0.08	0.00	0.26*

†A polynomial quadratic regression was adjusted.

‡The response variables were the residuals of frugivory rate and seed predation rate after GLMs considering locality as the main factor.

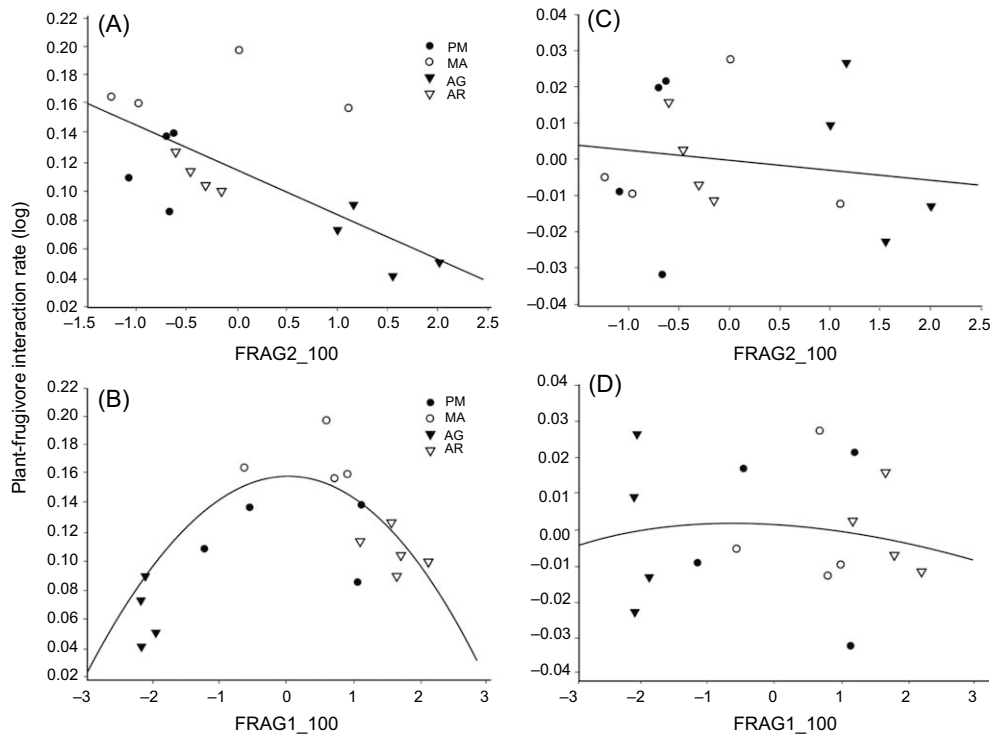


Figure 2. Relationship between the two PCA eigenvectors and frugivory rate (log-transformed) before (A–B) and after (C–D) the GLM considering locality as the main factor (see text for details). Dots correspond to different landscape sectors, different symbols indicate localities: Peña Mayor (PM), Maravio (MA), Agüeria (AG) and Aramo (AR). (A): $y = 0.115 - 0.024 \times \text{FRAG2}_{100}$; $R^2 = 0.32$; $F_{1,14} = 6.87$; $p < 0.05$. (B): $y = 0.155 + 0.005 \times \text{FRAG1}_{200} - 0.018 \times \text{FRAG1}_{200}^2$; $R^2 = 0.67$; $F_{2,13} = 0.13$; $p < 0.001$. (C): $y = -2.54e^{-18} - 0.001 \times \text{FRAG2}_{100}$; $R^2 = 0.06$; $F_{1,14} = 0.08$; $p > 0.05$. (D): $y = 0.003 - 0.001 \times \text{FRAG1}_{200} - 0.001 \times \text{FRAG1}_{200}^2$; $R^2 = 0.01$; $F_{2,13} = 0.12$; $p > 0.05$. Note the different scale of x- and y-axes.

small mammals (Tallmon et al. 2003), or stronger functional responses among seed predators in edge-increased areas (García and Chacoff 2007). At the same time, frugivory rates may decline if frugivores become scarce or less diverse (Farwig et al. 2006). In any case, our findings at 100 m radius reflect a common trend in fragmented habitats: habitat fragmentation disrupts mutualistic interactions and increases antagonistic ones (Santos and Tellería 1994, Steffan-Dewenter et al. 2001, García and Chacoff 2007, Cordeiro et al. 2009, González-Varo 2009).

We found that moderate fragmentation at 200 m radius (i.e. intermediate values in the number of forest patches, edge length and average patch size) favoured the interaction between *Ilex* and their seed dispersers, as judged by the quadratic relationship between frugivory rate and fragmentation (Fig. 2B). Previous studies evaluating animal occurrence and abundance in heterogeneous landscapes support the idea that those highly-mobile species able to use the non-forested matrix tend to be more abundant in moderately edge-increased, fragmented habitats (Farwig et al. 2006). As a consequence of the increased number of frugivorous animals, seed dispersal services have been reported to rise in fragmented or disturbed areas (Farwig et al. 2006). Frugivorous birds, in our case, thrushes, are able to move easily between forest patches, and even to frequently use isolated trees in the non-forested matrix (Herrera and García 2009). In this way, despite habitat fragmentation decreased frugivory rates in edge-increased areas at 100 m (above), intermediate values of habitat

fragmentation at 200 m favoured the activity of frugivorous thrushes. We suggest that increased availability of fruits in those sectors of the landscape with moderate fragmentation and forest openness attracted more birds, thus enhancing the likelihood of interaction with fruiting plants and the concomitant seed dispersal service in these sectors (García et al. 2010).

We also found that fragmentation increased seed predation rates. Accordingly, assuming that our interaction measures were indeed indicative of seed dispersal and post-dispersal seed survival (García and Chacoff 2007, Herrera and García 2010), our study suggests that moderate fragmentation could even favour tree regeneration, at least at 200 m radius, as a result of the balance between enhanced frugivory and moderate seed predation. However, data on seedling establishment would seem necessary to adequately test this hypothesis.

Regional vs local effects of habitat fragmentation

In most cases, the significant effects of fragmentation on the magnitude of both plant–animal interactions disappeared after controlling for the effects of locality identity. We acknowledge that this trend might be due to spatial autocorrelation in the response of biotic interactions to habitat structure since proximate circular sectors within a given locality will behave more similarly than distant ones, i.e. those sited in another locality. However, all original

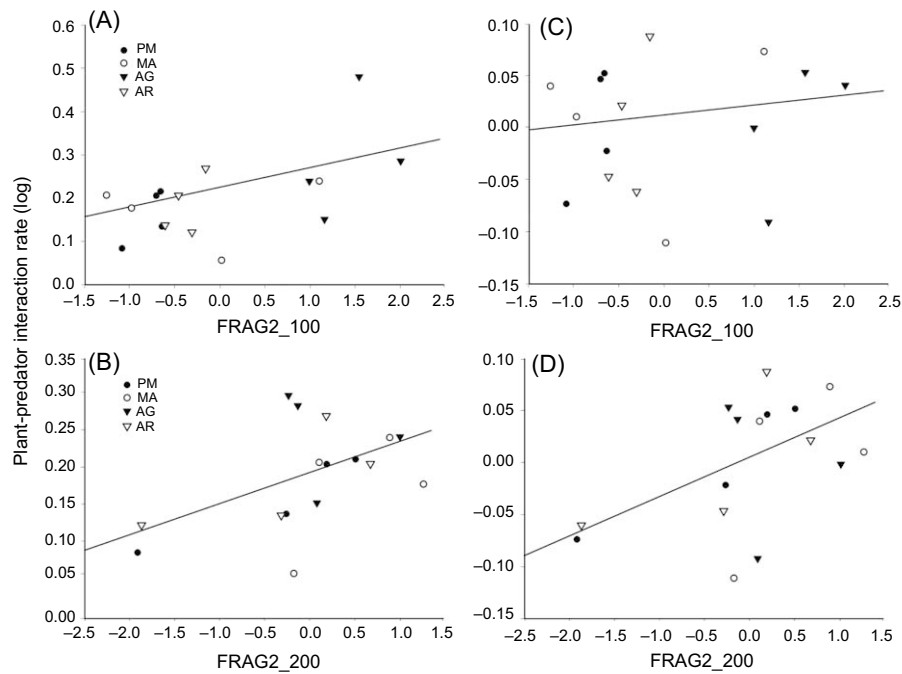


Figure 3. Relationship between the two PCA eigenvectors and frugivory rate (log-transformed) before (A–B) and after (C–D) the GLM considering locality as the main factor (see text for details). Dots correspond to different landscape sectors, different symbols indicate localities: Peña Mayor (PM), Maravio (MA), Agüeria (AG) and Aramo (AR). (A): $y = 0.187 + 0.035 \times \text{FRAG2}_{100}$; $R^2 = 0.27$; $F_{1,14} = 5.26$; $p < 0.05$. (B): $y = 0.187 + 0.040 \times \text{FRAG2}_{200}$; $R^2 = 0.25$; $F_{1,14} = 4.69$; $p < 0.05$. (C): $y = 0.001 + 0.008 \times \text{FRAG2}_{100}$; $R^2 = 0.01$; $F_{1,14} = 0.25$; $p > 0.05$. (D): $y = 0.001 + 0.035 \times \text{FRAG2}_{200}$; $R^2 = 0.26$; $F_{1,14} = 4.94$; $p < 0.05$. Note the different scale of x- and y-axes.

significant relationships between the magnitude of interactions and fragmentation were also significant after the correction for spatial autocorrelation constraints ($p < 0.1$ in all pair-wise correlations, data not shown). We therefore suggest that this disappearance of the effects of habitat structure is significant in understanding the functioning of biotic interactions in real-world fragmented landscapes. Patterns of habitat change, habitat loss and fragmentation, are rarely random processes at broad spatial scales (Lindenmayer and Fischer 2006). Indeed, patterns of human land-use are known to be locally determined, especially in areas where resource exploitation results from historical and extensive practices of agriculture and cattle grazing. Accordingly, if the spatial heterogeneity in habitat structure able to influence ecological processes is locally-aggregated, then taking into account spatial extents ranging from local to regional scales seems necessary (Schooley and Branch 2007). If not, the actual spatial scale at which the pattern emerges could be overlooked (Habeeb et al. 2005). This could be the picture emerging from our results given that habitat structure, as measured locally, affected plant–animal interactions only when considering spatial heterogeneity in habitat fragmentation covered by all localities (i.e. at the regional scale). In fact, the only relationship between habitat fragmentation and biotic interaction that remained significant after controlling for locality identity, was that caused by a fragmentation metric that presented large differences between sectors within a given locality, but weak differences between localities (i.e. edge length and patch size at 200 m radius on seed predation; Fig. 3B–D). To summarise, this

study suggests that regional approaches are crucial in determining the strength of habitat fragmentation effects on biodiversity, and that these effects can be quantified and used to predict patterns of ecological interactions at broad spatial scales.

Concluding remarks and management implications

Our results suggest that the effects of habitat structure on biotic interactions such as plant–animal interactions might be exclusively driven by fragmentation, even at levels of habitat cover above the theoretical thresholds suggested for an exponential increase of fragmentation effects (20% habitat cover, Andrén 1994, Fahrig 1997). Our findings indeed suggest there are no simple rules dictating thresholds in habitat quantity when related to ecological processes and therefore further research should be aimed at elucidating this issue. In any case, while the rules remain unclear, we support Villard et al.’s (2001) conclusions that conservation and management actions should simultaneously be aware of the problem of net habitat loss and that of configuration effects, irrespective of the quantity of habitat cover (Fahrig 1997).

Our findings on mutualistic and antagonistic interactions occurring sequentially throughout the reproductive cycle of *Ilex* suggest that an intermediate degree of habitat fragmentation might even favour the regeneration dynamic of this species. This finding closely matches results from Montoya et al. (2010) which showed the large-scale distribution of *Ilex* to be weakly sensitive to, and even

benefitted by, moderate-to-high habitat fragmentation in localities with forest cover over 20%. Considering that *Ilex* is not only a major component of secondary-growth forests in the Cantabrian Range, but also a pioneer tree in abandoned pastures and a nurse plant for other tree species (García and Obeso 2003), we assume that higher recruitment rates of *Ilex* might translate into enhanced forest regeneration within moderately fragmented areas, including regeneration within forest fragments as well as forest recovery in the non-forest matrix. In this sense, this study suggests that the outcome of plant–animal interactions might be used as passive restoration tools and thus suitable for restoring not only plant populations but also community structure in human-impacted landscapes.

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