Scale-Dependent Effects of Habitat Fragmentation on Hawthorn Pollination, Frugivory, and Seed Predation

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Abstract: *Habitat fragmentation is a major cause of functional disruption in plant-animal interactions. The net effect on plant regeneration is, however, controversial because a given landscape change can simultaneously hamper mutualism and attenuate antagonism. Furthermore, fragmentation effects may emerge at different spatial scales, depending on the size of the foraging range of the different interacting animals. We studied pollination by insects, frugivory by birds acting as seed dispersers, and postdispersal seed predation by rodents in 60 individual hawthorn (*Crataegus monogyna *Jacq.) trees in relation to structural fragmentation in the surrounding habitat. We evaluated fragmentation at three spatial scales by measuring the percentage of forest cover in three concentric areas around each tree of, respectively, 10-m, 20- to 50-m, and 50- to 100-m radius. The number of developing pollen tubes per flower style and fruit set decreased in proportion to the decrease of forest cover. Similarly, the magnitude of frugivory in focal trees was negatively affected by habitat loss. In contrast, seed predation was higher under plants in highly fragmented contexts. The effect of fragmentation was additive in terms of reducing the potential of plant regeneration. Moreover, the functional scale of response to habitat loss differed among interactions. Fragmentation effects on pollination emerged at the largest scale, whereas seed predation was mostly affected at the intermediate scale. In contrast to expectations from the larger foraging range of birds, fragmentation effects on frugivory mainly operated at the finest scale, favored by the ability of birds to cope hierarchically with spatial heterogeneity at different scales. Given that two opposing demographic forces (frugivory and seed predation) would be potentially affected by fine-scale features, we propose structural scale as the primary spatial dimension of fragmentation effects on the process of plant regeneration.*

Keywords: Cantabrian Range, *Crataegus monogyna*, foraging range, forest availability, plant-animal interactions, spatial scale, structural fragmentation

Efectos de la Fragmentación Dependientes de la Escala sobre la Polinización, Frugivoría y Depredación de Semillas de *Crataegus monogyna*

Resumen: *La fragmentación del hábitat es una de las principales causas de interrupción funcional en las interacciones planta-animal. Sin embargo, el efecto neto sobre la regeneración de las plantas es controvertido porque un cambio dado de paisaje puede, simultáneamente, obstaculizar el mutualismo y atenuar el antagonismo. Más aún, los efectos de la fragmentación pueden emerger a escalas espaciales diferentes, dependiendo del tamaño del área de aprovisionamiento de los diferentes animales interactuantes. Estudiamos la polinización por insectos, la frugivoría por aves que actúan como dispersantes de semillas y la depredación postdispersiva de semillas por roedores en 60 árboles individuales de* Crataegus monogyna *Jacq. en relación con la fragmentación estructural del hábitat circundante. Evaluamos la fragmentación en tres escalas espaciales midiendo el porcentaje de cobertura forestal en tres áreas concéntricas con radios de, respectivamente, 10-m, 20 a 50-m, y 50 a 100-m, alrededor de cada árbol. El número de tubos polínicos en desarrollo por estilo floral y el cuajado de frutos decreció en proporción a la disminución de la cobertura forestal. De forma similar, la magnitud de frugivoría en los árboles focales fue afectada negativamente por la pérdida de hábitat. En contraste, la depredación de semillas fue mayor bajo plantas en contextos muy fragmentados. El efecto de la*

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fragmentación fue aditivo en términos de reducción del potencial de regeneración de las plantas. Además, la escala funcional de respuesta a la pérdida de hábitat difirió entre interacciones. Los efectos de la fragmentación sobre la polinización emergieron a la escala mayor, mientras que la depredación de semillas fue más afectada a escala intermedia. En contraste con las expectativas por la mayor área de aprovisionamiento de las aves, los efectos de la fragmentación sobre la frugivoría operaron principalmente en la escala más fina, favorecidos por la capacidad de las aves para enfrentarse jerárquicamente a la heterogeneidad espacial a distintas escalas. Dado que dos fuerzas demográficas opuestas (frugivoría y depredación de semillas) se verian potencialmente afectadas por factores a escala fina, proponemos la escala estructural como la dimensión espacial primaria de los efectos de la fragmentación sobre los procesos de regeneración de las plantas.

Palabras Clave: área de aprovisionamiento, cordillera Cantábrica, *Crataegus monogyna*, disponibilidad de bosque, escala espacial, fragmentación estructural, interacciones planta-animal

Introduction

Plant-animal interactions such as pollination, seed dispersal, and herbivory are primary conservation targets because of their pivotal role in plant regeneration processes (Jules & Rathcke 1999; Cordeiro & Howe 2001), plant community structure (Wright 2002), ecosystem functioning (Kremen 2005; Tscharntke et al. 2005), and biodiversity evolution (Bascompte et al. 2006). There is increasing empirical evidence that relates the disruption of these ecological interactions with habitat fragmentation. For example, net habitat loss and the concomitant changes in the habitat spatial configuration cause severe reductions in pollinator populations and richness, decreasing reproductive output and increasing inbreeding depression in plants in remnant habitats (e.g., Aizen & Feinsinger 1994; Young et al. 1996; Lennartsson 2002). Similarly, decreases in numbers of frugivorous seed dispersers leads to recruitment losses among plants in fragmented landscapes (e.g., Cordeiro & Howe 2001, 2003; McConkey & Drake 2006).

The net effect of fragmentation on the outcome of plant and animal interactions remains, however, controversial, given that different interactions are subsequently linked throughout the process of plant regeneration and the same scenario that disrupts mutualistic relationships may benefit plants by decreasing antagonistic relationships. In fact, the results of the few known integrated studies of fragmentation (mostly involving pollination and predispersal seed predation by insects) show similar landscape responses for both pollinating and seed-predating insects, which thus counterbalance the effects in terms of plant reproduction (Cunningham 2000*a*; Steffan-Dewenter et al. 2001; Duncan et al. 2004; but see Santos & Tellería 1994 for a system involving seed dispersal and predation by vertebrates). This counterbalancing effect may be attributed to the relative similarity between the different interacting animals in terms of life history and mobility.

Consideration of the spatial scale of fragmentation effects is fundamental to understanding the response of plant and animal interactions to habitat fragmentation (Kattan & Murcia 2002). Some response patterns are only evident along large-scale, landscape gradients of decreasing habitat availability (Steffan-Dewenter et al. 2002) or fragment size (Cordeiro & Howe 2001) or increasing patch isolation (Steffan-Dewenter & Tscharntke 1999). Nevertheless, fragmentation may operate at finer spatial scales (e.g., the contact between the original habitat and the surrounding, highly contrasting matrix; i.e., edge effects; Murcia 1995) and lead to disruption of pollination and stronger interaction with propagule predators, which decreases plant recruitment within the original habitat (Jules & Rathcke 1999; Donoso et al. 2003; Lienert & Fischer 2003). On the other hand stronger isolation of individual plants may involve decreased pollination (Duncan et al. 2004), frugivory (Guevara & Laborde 1993), and seed predation (Holl & Lulow 1997). The effects of modifying small-scale and internal features of a habitat (i.e., structural fragmentation; Lord & Norton 1990) remain less understood than landscape-level patterns.

Although the effects of fragmentation on plant-animal interactions appear to be strongly scale dependent, few attempts have been made to evaluate this spatial idiosyncrasy. Recent studies suggest particular scales of fragmentation effects for different interacting groups by correlating habitat availability at different spatial extents with the abundance of pollinators, herbivores, or predispersal seed predators (Steffan-Dewenter et al. 2001; Steffan-Dewenter 2003). These scale differences may even occur among different taxonomic groups participating in the same interaction (e.g., solitary bees, honeybees, and bumblebees pollinating the same plant; Steffan-Dewenter et al. 2002). The functional scale of fragmentation effects depends on the spatial grain of heterogeneity imposed by the particular fragmentation process (Lord & Norton 1990), and on the ability of the interacting animal to cope with the changed heterogeneity in the plant resource (Keitt et al. 1997; Steffan-Dewenter et al. 2002). In this sense, larger animals with wider foraging ranges are expected to be affected by fragmentation only when it occurs at larger scales (Tscharntke & Brandl 2004; Holland et al. 2005).

To examine the functioning of plant-animal interactions in relation to habitat structural fragmentation, we analyzed the network of the hawthorn tree (*Crataegus monogyna* Jacq., Rosaceae) in the Cantabrian Range (northern Spain). The system we studied included three animal groups that differ greatly in life-history traits and mobility: insect pollinators (flies and honeybees), frugivorous birds acting as seed dispersers (wintering thrushes), and rodent seed predators (woodmice). We investigated whether the effects of fragmentation on the different plant-animal interactions (mutualistic vs. antagonistic) would be additive or counterbalanced in terms of potential plant regeneration. We evaluated the functional scale of each interaction by relating its magnitude to habitat availability at progressively larger spatial scales around each individual plant. We predicted that fragmentation effects on seed predation would mainly emerge at fine scales, whereas pollination and particularly frugivory would be affected at large scales.

Study Site and System

We conducted our study from 2004 through 2005 in the Sierra de Peña Mayor (43°17′N, 5°30′W, 900-m asl, Asturias, Spain), which is a secondary mountain of the Cantabrian Range. The physiognomy of the area exhibits karstic features, with limestone outcrops alternating with areas of shallow soil. The climate of the region is Atlantic, with a mean annual temperature of 13° C and rainfall (approximately 1300 mm) distributed throughout the year.

The landscape is dominated (approximately 75% cover) by stony pastures and heathland (*Erica* spp., *Ulex europaeus* L.) resulting from historical deforestation for cattle grazing and, to a lesser extent, from natural fragmentation (rocky outcrops). The matrix surrounds a few large (approximately 20 ha; Fig. 1) fragments of hardwood forest (*Fagus sylvatica* L. and *Fraxinus excelsior* L.) adjacent to fringe patches of secondary forests (dominated by fleshy-fruited trees such as holly [*Ilex aquifolium* L.], hawthorn, yew [*Taxus baccata* L.], and hazel [*Corylus avellana* L.]) and numerous smaller (0.5- to 2-ha) fragments of similar secondary forest. Isolated trees or small groups of hawthorn and holly also occur scattered through the matrix. Thus, our study area represents a highly variegated and edge-dominated forest landscape in which habitat loss modifies heterogeneity even at fine scales (McIntyre & Barrett 1992).

We used hawthorn as the target plant species because it offered a wide gradient of configurations of individual context in the study site, from highly isolated, matrix-embedded trees to trees within forest patches (Fig. 1). Its population dynamic depends on plant-animal interactions; it relies on pollinators to set fruit, and recruitment is mostly limited by seed availability (García et al. 2005*a*, N.P.C. et al., unpublished data). We assumed that the response of hawthorn to fragmentation would be generalizable to other common trees in the same forests, such as holly, that share pollinators, frugivores, and seed predators and suffer similar regeneration constraints (García et al. 2005*a*, 2005*b*).

Hawthorn is a small tree that commonly grows up to 10 m in height. Flowers are hermaphrodite, white, have one style, with 5–25 stamens, and grow in small clusters of 9–18. In northern Spain hawthorn flowers between April and June, and its main pollinators are honeybees (*Apis mellifera*) and flies (Diptera: Calliphoridae, Syrphidae; Guitián & Fuentes 1992). This species is partially self-compatible, but relies on insect visits to set fruit, and fruit set improves with cross-pollination (N.P.C. et al., unpublished data). Fruits are single-seeded drupes, 7- to 12-mm in diameter, red when ripe (September-October), and remain attached to the trees during fall and winter. Most fruits are consumed by wintering frugivorous birds (thrushes, mostly *Turdus merula* L., *T. philomelos* L., *T. viscivorus* L., and *T. iliacus* L.; Guitián & Fuentes 1992). These birds defecate intact seeds, acting as dispersers. After dispersal seeds are frequently preyed upon by woodmice (*Apodemus sylvaticus*) and yellow-necked mice (*A. flavicollis*; García et al. 2005*a*). Consequently, different interacting animals (i.e., pollinators, frugivores, and seed predators) represented a wide gradient of foraging ranges (birds > insects > rodents) (Wolton & Flowerdew 1985; Steffan-Dewenter et al. 2002; García & Ortiz-Pulido 2004).

Methods

Fragmentation Context at Different Spatial Scales

To evaluate the variation in plant-animal interactions along an increasing gradient of structural fragmentation, we considered a variety of individual context configurations, from isolated trees in the nonforest matrix to trees embedded in forest fragments. We used percent tree cover as an inverse, synthetic measure of forest fragmentation (Steffan-Dewenter et al. 2002). We estimated tree cover in three nonoverlapping circular plots that surrounded each focal tree at, respectively, a 10-m (hereafter R10), 20- to 50-m (R20-50), and 50- to 100-m (R50-100) radius (Fig. 1). Each plot represented an independent fragmentation context at a different, progressively larger spatial scale (Tischendorf & Fahrig 2000). Measurements of cover in R10 were made in the field by visually estimating the cover of each tree species (individuals of height ≥1.5 m and dbh ≥4 cm) and the cover of the total projection of overlapping canopies. For R20-50 we estimated cover as above for each of 3, 10 × 10 m, nonoverlapping, alternate plots in each of four transects, following the cardinal points extending from 20 to 50 m from a

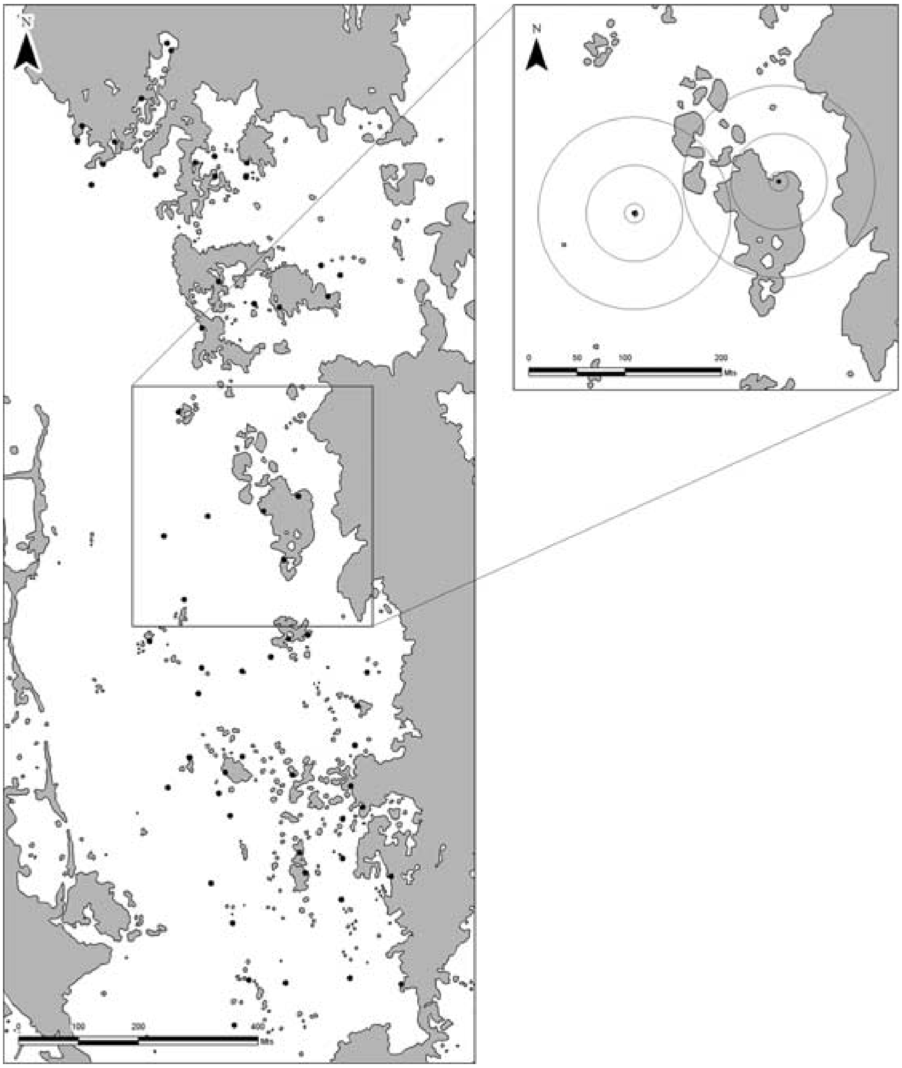

Figure 1. Position of sampled focal trees (black dots) and the configuration of forest cover (grey patches). The enlarged area shows the concentric circles of 10-, 50-, and 100-m radii used to establish the tree-cover sampling plots at different spatial scales.

focal tree. Average cover from the 12, 10 × 10 m plots was then extrapolated to the 20- to 50-m circular area. Cover in R50-100 was measured from a geographical information system derived from orthophoto quadrat maps (scale 1:5000) in which all focal trees had been georeferenced and tree cover digitized. Field and digital measures of tree cover were strongly correlated among them (Pearson correlation for measures at R20-50: $r = 0.948$, $p \leq 0.0001$, $n = 60$).

In September 2004 we chose 60 focal trees distributed over 75 ha (Fig. 1) for frugivory and seed predation sampling. We measured tree cover in October 2004, before leaf fall. In spring 2005 focal trees were resampled for the pollination study. Only 37 trees from the initial pool were included in pollination study because many trees did not bear enough flowers for analyses. We increased the sample size to 58 by sampling the flowering tree nearest to the original focal tree. Tree cover values were similar in both sets of trees for all scales (paired t test: $t < 1.7$, $p > 0.10$, $n = 21$).

Pollination

We measured the natural levels of pollination and reproductive success in terms of the frequency of visits to flowers by insects, the number of pollen tubes at the base of the flower style, and the proportion of flowers bearing ripe fruits (Table 1). Prior to sampling we visually estimated flower production in all sampled plants with a logarithmic scale (0, 1: ≤10 flowers; 2: ≤100; 3: ≤1,000; 4: ≤10,000; and 5: >10,000).

We observed insect visits to flowers between 10:00 and 18:30 on sunny and slightly cloudy days with low wind velocity and conducted censuses on 10 days distributed throughout the 2005 flowering season (27 May to 8 June). Each census consisted of observing a flowering branch for 10 minutes. The mean number of observed flowers ranged between 42 and 56 (95% CI) per census. We counted the number of open flowers, then recorded the number of visits the flowers received, and identified visitors to the lowest taxonomic level possible.

Table 1. Variables used to examine the magnitude of forest fragmentation at different spatial scales and the magnitude of different plant-animal interactions.

Variable	*Sampling unit*[a]	*Measurement unit*	*Spatial structure*[b]
Fragmentation context			
R10 tree cover (%)	10-m radius plot (1, 60)	percent tree canopy cover (field estimation)	no
R20-50 tree cover (%)	10 × 10 m plots in 20- to 50-m radius plot (12, 60)	percent tree canopy cover (field estimation)	yes, gradient
R50-100 tree cover (%)	50- to 100-m radius plot (1, 60)	percent tree canopy cover (GIS-based estimation)	yes, gradient
Pollination			
number of visits	10-minute census (2–4, 54)	number of visitor insects per flower	yes, gradient
number of pollen tubes	flower style (25–30, 56)	number of pollen tubes per style	no
fruit set	flowering branch (5, 58)	proportion of flowers setting fruit	yes, gradient
Frugivory			
proportion of beak-marked fruits	50 × 50 cm quadrat beneath the focal tree (3, 60)	proportion of fruits dropped by thrushes relative to the total number of fallen fruits	no
Postdispersal seed predation			
proportion of seed predation	seed depot (3, 60)[c]	proportion of seeds preyed by rodents	no

[a] *In parentheses, respectively, the number of sampling units per tree and the number of sampled focal trees.*

[b] *Presence and the shape, respectively, of a significant spatial structure detected by autocorrelation analysis.*

[c] *The same procedure was set up separately for holly and hawthorn seeds (10 seeds/depot).*

We performed a total of 94 censuses of 54 focal plants (on average two censuses per plant).

We collected 25–30 styles in each of 56 focal plants, just before the flowers started falling naturally, to estimate pollen-tube density. We fixed and stored the styles in individual microcentrifuge tubes containing FAA (formalin: acetic acid: ethyl alcohol, 5:5:90). In the laboratory we cleared styles in a 10 mL/L NaOH solution for 3 hours and stained them with 0.1% aniline blue in 0.1 mol/L K_3PO_4 (Aizen & Feinsinger 1994). We examined flattened preparations with epifluorescence microscopy (100×), counted the number of pollen tubes at the base of each style, and calculated the average number of pollen tubes per style per plant. To assess natural levels of reproductive success, we marked five flowering branches containing 25–30 open flowers per branch in each of 58 focal trees and then examined the branches on 27 June, counting the number of developed and ripe fruits. We determined fruit set as the quotient of ripe fruits per number of flowers.

Frugivory

In September 2004 we established 3, 50 × 50 cm quadrats beneath the canopy of 60 focal trees and collected all fallen hawthorn fruits in the quadrats in successive fortnightly surveys during the dispersal season (September to December). We considered the removal of fallen fruits from quadrats negligible because we never observed any thrushes eating fruits on the ground and did not find any mammal feces containing hawthorn seeds in the study site. We searched among fallen fruits for those picked by thrushes from the tree but dropped as a consequence of handling failure when perching, which are easily distinguishable by beak marks on fruit surface (Sallabanks 1992). We counted the cumulative number of beak-marked fruits and the total number of fallen fruits per quadrat and calculated interaction with frugivores as the average proportion of beak-marked fruits relative to the total number of fallen fruits per quadrat (Table 1). Although an indirect estimate we considered this parameter representative of the number of and the time spent by frugivores perching and feeding on an individual tree throughout the dispersal season (Sallabanks 1992). Prior to sampling we estimated individual fruit crop size by multiplying the average number of fruits on 10 randomly selected fruiting branches by the total number of fruiting branches per tree. The abundance of unmarked fallen fruits was independent of a tree's exposure to wind and was affected primarily by the fruit crop size (multiple regression; crop size: $F_{1,57} = 256.7, p \leq 0.0001$; R50 tree cover (%): $F_{1,57} = 0.1, p = 0.79$; $r^2 = 0.825$). Thus, confounding effects in the proportion of beak-marked fruits due to differences in the number of fallen fruits driven by tree isolation were considered negligible.

Postdispersal Seed Predation

From December 2004 to January 2005 we evaluated the removal of seeds beneath the canopy of each focal tree ($n = 60$) by predatory rodents. Previous studies reveal that the predation rate on hawthorn seeds is much lower than on other coexisting fleshy-fruited species such as holly (García et al. 2005*a*, 2005*b*). Thus, for accurate representation of the spatial variability in seed predation, and given that seed deposition of holly seeds is frequent under hawthorn trees (see below), we used both seed species. In each of the three frugivory quadrats we placed two seed depots, each consisting of a 3 × 6 cm plastic mesh (1.5-mm pore) containing 10 seeds of each species, glued firmly to the mesh by a low odor (rainproof glue that prevented seeds being dislodged by wind or rain) (García et al. 2005*a*). We nailed the depots to the ground 50 cm from each other. We monitored the seeds after 2 and 4 weeks. We considered that a seed had been consumed by a rodent if it was missing from the plastic mesh or was still on the mesh but was gnawed and empty. No seed removal was attributable to birds or ants (García et al. 2005*a*). After the first survey we only detected 31.3% of hawthorn and 39.9% of holly total seed losses; thus, the rate of seed predation was calculated as the average proportion of seeds preyed on per individual tree after 4 weeks (Table 1).

Prior to predation sampling, and simultaneously to the collection of hawthorn-fallen fruits for frugivory sampling, we collected all seeds from thrush droppings found on quadrats. These dispersed seeds belonged to holly (87%), hawthorn (12%), and yew (1%). We calculated the cumulative number of seeds per quadrat (the mean number of seeds per quadrat was 116.4 ± 20.1 SE) as an estimate of seed resource abundance for postdispersal seed predators beneath the canopy of focal trees. The high frequency of sampling, the relatively low predation rate at the beginning of the dispersal season, and the unusual secondary dispersal guaranteed a negligible seed removal effect on quadrats (as validated by the use of seed traps; García et al. 2005*a*, 2005*b*).

Statistical Analysis

We evaluated the relationship between fragmentation and plant-animal interaction outcomes at each spatial scale with simple regression models. We considered pollination, frugivory, and seed-predation, dependent variables and the percent tree cover in R10, R20-50, and R50-100 independent variables. This method enabled us to compare the effect of fragmentation across plant-animal interactions and scales, avoid collinearity in the independent variables, and cope with spatial constraints with additional straightforward tests. We tested the normality of distribution of errors for dependent variables and, when necessary, transformed data to the log- or arc-sine square root (all variables fitted to normal distributions with the exception of the frequency of flower visits by pollinators and the proportion of seed predation, Shapiro-Wilk test: $W \geq 0.902$, $0.05 > p > 0.002$). Transformation of the independent variables did not improve model fitting substantially, so we used original values (Zar 1996). Significant regression slopes obtained at different scales for a given dependent variable were compared by Student's t tests.

To assess whether flower production, size of the fruit crop of focal trees, and the abundance of seeds beneath focal trees affected the response of, respectively, pollinators, frugivores, and seed predators to fragmentation gradients, we checked for correlations between resource abundance and magnitude of the plant-animal interaction. If the correlations were significant we reevaluated the relationships between interactions and tree cover by considering flower, fruit, or seed abundances as covariables.

Given the implicit spatial configuration of the sampling design, the statistical significance of the regression tests could be affected by the presence of spatial structure in the studied variables (Legendre et al. 2002). We checked the spatial structure in all variables by means of Moran's I correlograms with 35 distance classes (50-m interval; Legendre & Fortin 1989). We considered that the probability of Type I error in the regression analyses increased significantly only when both the independent and the dependent variables were spatially structured (Legendre et al. 2002). In these cases we reevaluated the significance of the relationships between fragmentation and plant-animal interactions by means of Dutilleul's t test, which corrects the variance and the degrees of freedom of the correlation statistic in the presence of spatial autocorrelation (Legendre et al. 2002). We applied this test for coefficients of correlation between the residuals of both fragmentation and interaction variables after fitting for a polynomial trend-surface equation.

Another cause of increased probability of Type I error potentially affecting the results of regressions is pseudoreplication due to spatial overlap in the habitat sampling among closely located focal trees (Holland et al. 2004). We reevaluated the strength of the relationship between tree cover and the plant-animal interactions with a resampling technique. We used the Focus program (Holland et al. 2004) to conduct each regression model 100 times, with different sets of randomly selected, spatially independent focal trees. We considered trees independent if they were separated by a distance that is at least equal to the radius of the sampling area (10, 50, and 100 m). This distance resulted in ≤25% overlap at the largest scale and allowed a sample size of 24 trees for each iteration. We obtained the average (±SE) values of the coefficient of determination r^2 for each scale, establishing a scale hierarchy (the most significant scale of response to habitat characteristics) that was free of overlapping constraints.

Results

Plant-Animal Interactions and Fragmentation at Different Scales

We observed 310 insects visiting *C. monogyna* flowers during the flowering season. Flies were the most abundant group (88.8% of visits; Muscoidea, 40.1%; Empididae,

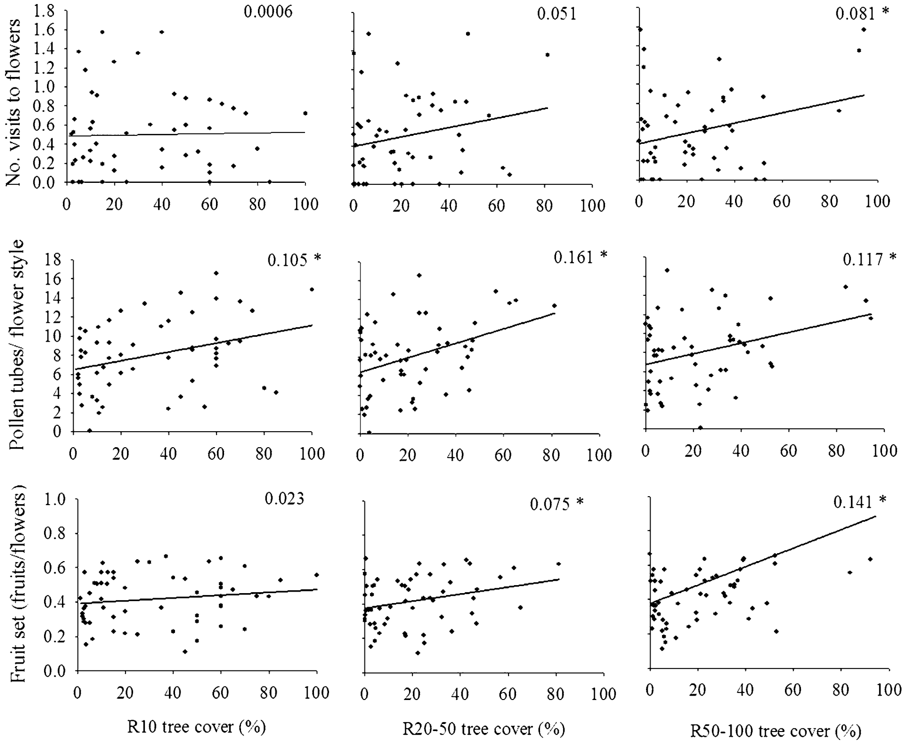

Figure 2. The number of pollinator visits to flowers, number of pollen tubes per flower style, and proportion of fruits per flower (fruit set) in hawthorn trees as a function of percent tree cover at different spatial scales (R10, within 10-m radius around focal tree; R20-50, 20- to 50-m radius; R50-100, 50- to 100-m radius). Each point represents a focal tree. Regression lines and r^2 *values (those marked with an asterisk, regression fit* $p < 0.05$*) are also shown.*

29.9%; Syrphidae, 10.2%; Calliphoridae, 7.6%), whereas honeybees accounted only for 10.1% of visits. Each flower received a mean number of 0.28 ($\pm$0.04 SE, $n = 54$) visits per 10-minute observation. The mean number of pollen tubes per style was 8.0 ($\pm$0.5 SE, $n = 56$). Mean fruit set was 17.7% ($\pm$1.3 SE, $n = 58$). All three measures of pollination were positively and significantly related to the percent tree cover, but the relationship was scale dependent. The frequency of visits to flowers was significantly higher for trees in areas of dense tree cover, but this effect was only evident at the largest scale (R50-100: $F_{1,52} = 4.59$, $p = 0.037$; Fig. 2). The number of pollen tubes per style was positively and significantly related to the percent tree cover at all scales. This relationship was stronger at the intermediate (R20-50) and largest (R50-100) scales ($F_{1,54} \geq 6.67$, $p \leq 0.01$; all comparisons among slopes were $t \leq 1.0$, $p \geq 0.319$; Fig. 2). Fruit set was also significantly higher for those trees in areas of low fragmentation, and this effect was mainly expressed at larger scales (R20-50: $F_{1,56} = 4.56, p = 0.037$; R50-100: $F_{1,56} = 9.22, p = 0.004$; comparison between slopes: $t = 0.38$, $p = 0.669$; Fig. 2). The frequency of pollinator visits to flowers was positively and significantly correlated with flower production ($r = 0.280$, $p = 0.040$, $n = 54$). The effect of tree cover at R50-100 on pollinator visitation rate was positive and significant even after considering flower abundance as a covariable ($F_{1,51} = 5.18, p = 0.030$).

Fruits dropped by frugivorous birds occurred beneath all except two sampled trees (average proportion of beak-marked fruits 0.08 $\pm$ 0.01 SE, $n = 60$). The proportion of beak-marked fruits was strongly and positively related to tree cover at R10 ($F_{1,58} = 16.17, p = 0.0002$; Fig. 3) and, to a lesser extent, at R20-50 ($F_{1,58} = 6.57, p = 0.013$; comparison of slopes between scales: $t = 0.27, p = 0.785$; Fig. 3). Nevertheless, frugivory was unrelated to forest availability at the largest scale ($F_{1,58} = 0.39$, $p = 0.533$; Fig. 3).

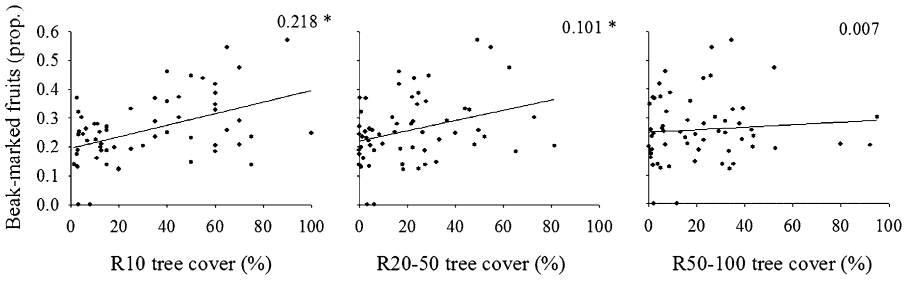

Figure 3. Proportion of beak-marked fruits beneath the canopy of focal trees as a function of percent tree cover at different spatial scales (R10, R20-50, R50-100; as explained in Fig. 2 legend). Each point represents a focal tree. Regression lines and r^2 *values (asterisk, regression fit* $p < 0.05$*) are also shown.*

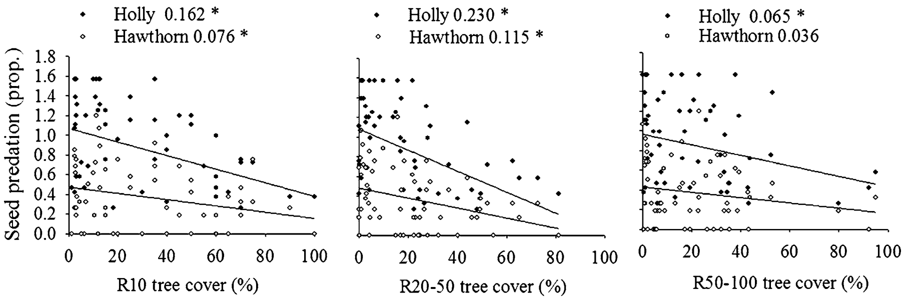

Figure 4. Representation of the proportion (prop.) of postdispersal predation in seeds of holly (filled circles) and hawthorn (empty circles) as a function of percent tree cover at different spatial scales (R10, R20-50, R50-100; as explained in Fig. 2 legend). Each point represents a focal tree. Regression lines and r^2 *values (asterisk, regression fit* $p < 0.05$*) are also shown.*

The proportion of beak-marked fruits was unrelated to the fruit crop size of focal trees ($r = 0.043$, $p = 0.741$, $n = 60$).

Rodents preyed on holly seeds in 96.6% of focal trees ($n = 60$), with an average predation rate of 0.54 (±0.04 SE), whereas predation frequency and rate were lower in hawthorn (respectively, 78.3% and 0.18 ± 0.03 SE). Predation rate was positively correlated between seed species (Pearson's correlation: $r = 0.728$, $p < 0.0001$, $n = 60$), suggesting a strong spatial concordance in predation patterns between seed species. The rate of predation was negatively and significantly related to the percent tree cover at all scales for holly seeds, indicating reduced survival for those seeds dispersed beneath trees in highly fragmented contexts (Fig. 4). Nevertheless, the strength of the relationship also varied with the observation scale and was stronger at the intermediate scale (R10: $F_{1,58} = 11.28$, $p = 0.0014$; R20-50: $F_{1,58} = 17.35$, $p = 0.0001$; R50-100: $F_{1,58} = 4.01$, $p = 0.049$, all comparisons among slopes were $t \leq 1.45$, $p \geq 0.155$; Fig. 4). Similar trends were found for hawthorn seeds (R10: $F_{1,58} = 4.78$, $p = 0.033$; R20-50: $F_{1,58} = 7.53$, $p = 0.008$; R50-100: $F_{1,58} = 2.18$, $p = 0.145$; Fig. 4). Predation rate was negatively and significantly correlated to the total abundance of seeds beneath focal trees (holly: $r = -0.542$, $p \leq 0.0001$; hawthorn: $r = -0.384$, $p = 0.003$). Percent tree cover related negatively to seed predation rate when including seed abundance as a covariable, but only when considered at R20-50 (holly: $F_{1,57} = 5.51$, $p = 0.022$; hawthorn: $F_{1,57} = 5.77$, $p = 0.019$).

Spatial Constraints

Percent tree cover at R20-50 and at R50-100 was spatially structured (Table 1; Fig. 5). Positive, significant Moran's I values at shorter distances and negative significant values at longer distances corroborated a gradient-type structure (Fig. 1). A similar gradient trend appeared in both the frequency of pollinator visits to flowers and fruit set (Fig. 5). Conversely, correlograms suggested random spatial distributions for frugivory and seed predation rates (Fig. 5). The correlations between habitat availability and fruit set were significant, or marginally significant, after spatial detrending (polynomial trend-surface equation with x, y and x, y^2 significant terms) and correction of degrees of freedom by Dutilleul's test (residuals of tree cover R20-50 and residuals of fruit set: $r = 0.237$, df $= 54$, $p = 0.079$; residuals of tree cover R50-100 and residuals of fruit set: $r = 0.330$, df $= 54$, $p = 0.013$). The relationship between tree cover at R50-100 and the frequency of pollinator visits to flowers was not significant (correlation between the spatial residuals of both variables: $r = 0.172$, df $= 50$, $p = 0.223$), indicating a potential effect of spatial position on the decrease in pollinator visitation in highly fragmented contexts. Focus-calculated average coefficients of determination (Fig. 6) suggested that the most important scale of response to fragmentation was R10 for the proportion of beak-marked fruits, R20-50 for the number of pollen tubes per style and the proportion of seed predation, and R50-100 for number of pollinator visits to flowers and fruit set.

Discussion

We examined the importance of forest availability in the functioning of several plant-animal interactions occurring sequentially throughout the reproductive cycle of hawthorn. Habitat loss altered all interactions, and trees in highly fragmented contexts had decreased pollination and frugivory but increased seed predation. The effect of fragmentation across mutualistic and antagonistic interactions was therefore not counterbalanced, but was additive in terms of reduction of plant reproductive potential. Nevertheless, the effects of fragmentation strongly depended on the spatial scale, the functional scale being characteristic of the interaction. The particular relationships between habitat loss and interaction outcome and the scale hierarchy of fragmentation effects were evident even when we considered the quantity of resource for interacting animals in the focal tree (flowers, fruits, and dispersed seeds for, respectively, insects, birds, and rodents) and the spatial and overlap constraints of sampling design.

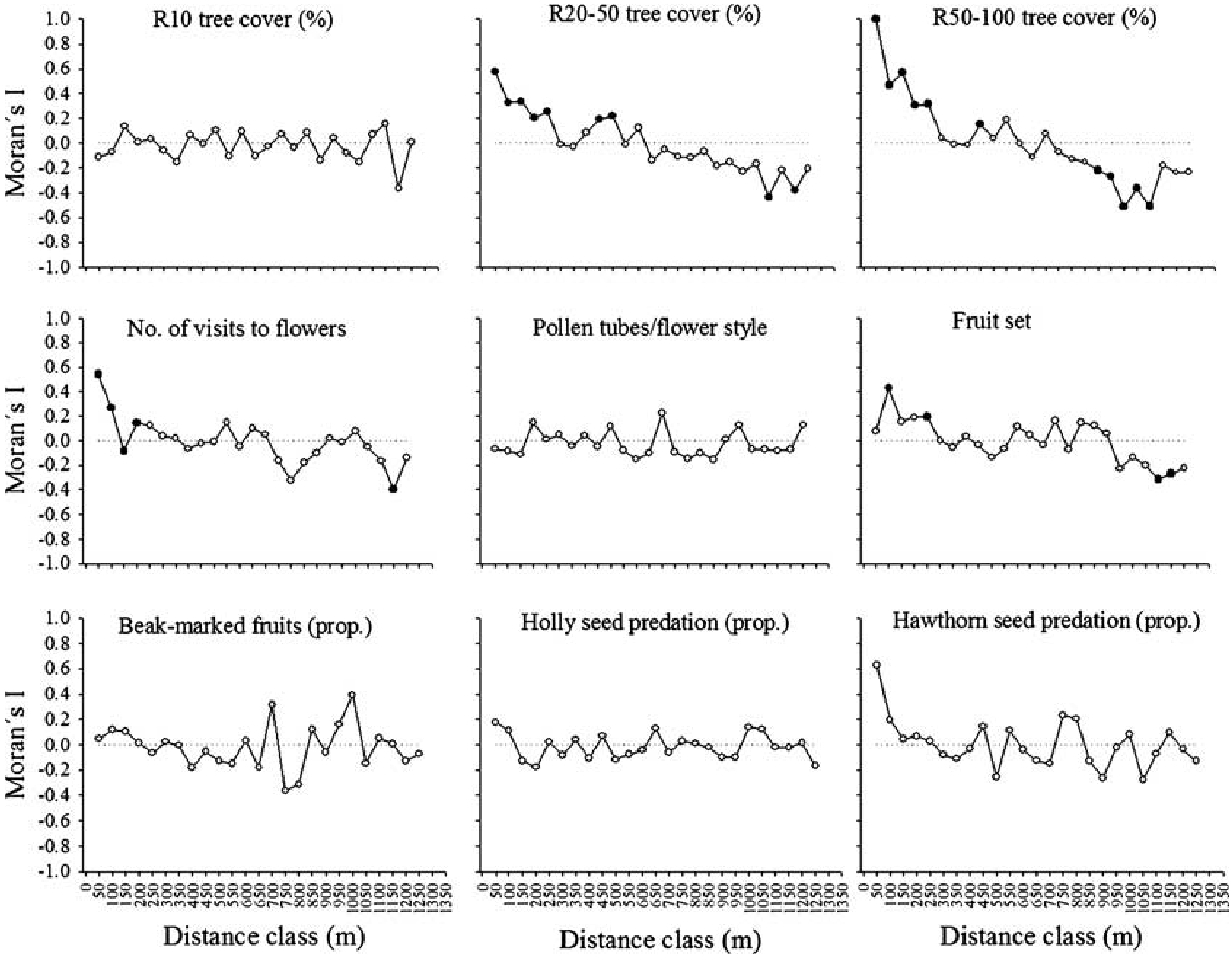

Figure 5. Spatial correlograms, with filled circles indicating significant Moran's I *values* (p $\leq$ *0.05, for globally significant correlograms after sequential Bonferroni adjustment) of fragmentation at different spatial scales (percent tree cover at R10, R20-50, and R50-100, as explained in Fig. 2 legend) and the interaction variables (number of pollinator visits to flowers, number of pollen tubes per flower style, fruit set, proportion [prop.] of beak-marked fruits, proportion of postdispersal predation in seeds of holly and hawthorn). Only distance classes from 50 to 1250 m are shown.*

Pollination

We observed a decrease in pollination levels and fruit set associated with habitat loss. This pattern may be driven by quantitative (reduced pollen load by decreased abundance of pollinators; Aizen & Feinsinger 1994; Cunningham 2000*b*) and qualitative (decreased deposition of compatible, outcross pollen on the stigma; Duncan et al. 2004) mechanisms. Quantitative effects appeared to be weak in our system, as judged by the low proportion of variance in the decay of insect visitation rate associated with habitat loss and the spatial constraints of this relationship. The fact that the hawthorn pollinator guild was dominated by generalist flies, evenly abundant in forest patches and matrix, probably precluded any strong effect of fragmentation on pollinator abundance (Ashworth et al. 2004). By contrast, the number of pollen tubes developed per style and fruit set clearly responded to changes in forest cover around focal trees. In the study site this species is partially self-compatible, but hand-outcrossing pollination increases the number of pollen tubes per style and fruit set (N.P.C. et al., unpublished data). Therefore, as shown in other small-scale fragmentation studies (Duncan et al. 2004), our results suggest that the mechanism determining pollination decay is mostly qualitative because of the low number of available, high-quality pollen donors in the surroundings of focal trees (hawthorn density was positively and significantly related to total tree cover, data not shown).

As judged by the fruit set (a parameter that combines the numerical effect of insect visitation and the qualitative effect of outcrossing), the disrupting effects of

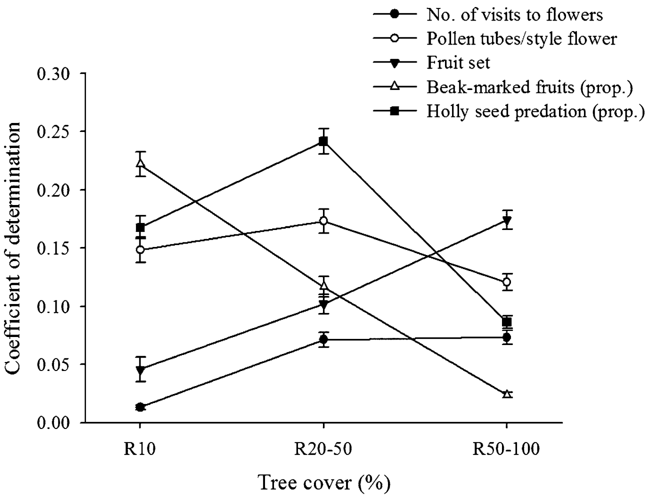

*Figure 6. Average (±SE) values of the coefficients of determination (r^2) of the regressions between percent tree cover at, respectively, R10, R20-50, and R50-100 (as explained in Fig. 2 legend) and the interaction variables (number of pollinator visits to flowers, number of pollen tubes per flower style, fruit set, proportion of beak-marked fruits, and proportion of postdispersal predation in seeds of holly) obtained by Focus resampling procedure. Each regression (*n = *100 iterations per scale) included 24 independent, low-overlap sampling points (hawthorn focal trees).*

fragmentation were most evident when evaluated at the largest spatial scale. The functional scale of fragmentation may be related to the wide foraging range and mobility of flying insects acting as pollinators (Steffan-Dewenter et al. 2002), fitting our earlier prediction. Nevertheless, given that our data failed to support any strong effect of fragmentation on pollinator abundance or activity, this pattern may also be explained by a threshold distance for the emergence of outcrossing effects (i.e., pollen quality drops sharply when plants are isolated far from forest stands; Duncan et al. 2004).

Frugivory

Hawthorn trees growing in highly fragmented contexts suffered decreased frugivory by birds, as found in other fleshy-fruited trees (Santos & Tellería 1994; Cordeiro & Howe 2001, 2003). Surprisingly, this fragmentation effect did not emerge at the spatial scale expected from the large foraging range and wide mobility of thrushes and was mostly significant at the finest scale. This pattern may be related to a hierarchy of levels of foraging behavior in these frugivores, rather than to reductions in their population size. Thrushes can move easily among fruit-rich patches performing high-height, broad exploratory flights in large flocks (García & Ortiz-Pulido 2004), reducing their susceptibility to coarse-grain fragmentation. Once within patches, however, birds choose in which tree to feed according to neighborhood fine-scale features, such as the presence of protective canopy and cofruiting individuals of the same and other species (García et al. 2001; Saracco et al. 2005). As a result focal plants that offer fruits and additional resources and protection in nearby areas (both the abundance of fruits and the cover by the perennial species holly and yew were positively correlated to forest cover in R10, data not shown) probably showed enhanced frugivory, independent of their isolation degree at a larger scale.

Assuming that our frugivory measure represented bird-feeding activity, we would expect more seeds to be dispersed beyond the canopy of mother plants in low-fragmented contexts. Assessing consequences in terms of recruitment would have required a better evaluation of seed fate and seedling establishment exceeding the scope of this work. Nevertheless, we believe that seed dispersal out from under the maternal canopy is beneficial for hawthorn because its recruitment is mostly limited by seed availability, and wider dispersal would lead to colonization of microsites free from conspecifics (García et al. 2005*a*, 2005*b*). In fact, dispersal to multispecific, high-seed-density patches under heterospecific canopy protects hawthorn seeds from predators (D.G. et al., unpublished data; see also Clark et al. 2004).

Postdispersal Seed Predation

Our findings are consistent with the results of other studies in temperate habitats that show increased postdispersal seed predation due to fragmentation (Jules & Ratcke 1999; Donoso et al. 2003). These studies related increased predation to higher densities of generalist rodents in small fragments and edge-increased areas. By contrast, woodmice abundance was much lower in open matrix than in mature and fringe forests in our site (I. Martínez & D.G., unpublished data). The stronger predation under more isolated trees may therefore be related to compensatory behavior of the scarcer seed predators, which, as suggested by the effect of seed availability, would respond negatively to seed density and richness. In fact, mice inhabiting nearby isolated trees must cope with more heterogeneous (concentrated under the focal tree) and less abundant seed resources than mice foraging in forest areas, where patches rich in more profitable seeds are common under focal trees and under cofruiting surrounding trees (García et al. 2005*b*).

The decrease in seed survival along the fragmentation gradient was significant at the finest and intermediate scales for both sampled seed species, but when considering seed abundance as a covariable, the pattern was only significant at the intermediate scale. This suggests that fragmentation effects are mostly governed by the indirect effect of tree cover loss on seed availability at the finest scale but mostly depend on landscape factors when considering a larger scale. This functional scale of fragmentation is consistent with our expectations, the stronger predictive power of the intermediate scale fitting well with the home range sizes described for woodmice (50-m radius plot covered 7850 m^2 and *Apodemus* sp. range extended between 1000 and 6000 m^2; Wolton & Flowerdew 1985). As for avian frugivory, a larger area was less suitable for evaluating the response of seed predation to habitat loss because of the uncoupling between the scale of observation and the actual scale at which the process mainly occurs.

Conservation Implications

Our results demonstrate a scale-dependent effect of fragmentation on different plant-animal interactions and emphasize the need for integrative and multiscale approaches for explaining the disruption of ecological functions in degraded habitats. This kind of approach identifies the scale(s) at which different species perceive the habitat depending on their dispersal abilities and foraging ranges and improves the understanding of the role of landscape structure in the changes of biotic interactions as factors shaping local populations and community dynamics (Steffan-Dewenter et al. 2001; Tewksbury et al. 2006). Our prediction on the funcional scale of fragmentation based on animals' foraging range sizes was only partially fulfilled. Thus detailed consideration of animal spatial behavior is required in future fragmentation studies.

The spatial idiosyncrasy of each interaction adds a source of complexity that hampers the establishment of management guidelines for these fragmented landscapes, given that a particular spatial grain of habitat loss may be pernicious for a type of interaction but harmless for another. Thus, effective conservation requires the identification of the spatial scale of fragmentation that determines the strongest disruption in the entire process of plant regeneration. The methodological constraints and the differences in the type of measurement of each interaction type in our study make it difficult to evaluate directly the combined interaction effects across scales (in terms of cumulative transition probabilities of a propagule through different regeneration phases; García et al. 2005*b*). Nevertheless, given that two opposing demographic forces (seed dispersal resulting from avian frugivory and seed predation) would be affected mainly by fine-scale features, we propose structural scale as the primary spatial dimension of fragmentation effects on the plant regeneration process. Further research should aim to elucidate the degree of dependence of large-scale patterns of landscape fragmentation on fine-scale, structurally operating mechanisms.

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