

Effects of habitat loss, habitat fragmentation, and isolation on the density, species richness, and distribution of ladybeetles in manipulated alfalfa landscapes

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Abstract. 1. Habitat loss and fragmentation are the main causes of changes in the distribution and abundance of organisms, and are usually considered to negatively affect the abundance and species richness of organisms in a landscape. Nevertheless, habitat loss and fragmentation have often been confused, and the reported negative effects may only be the result of habitat loss alone, with habitat fragmentation having nil or even positive effects on abundance and species richness.

2. Manipulated alfalfa micro-landscapes and coccinellids (Coleoptera: Coccinellidae) are used to test the effects habitat loss (0% or 84%), fragmentation (4 or 16 fragments), and isolation (2 or 6 m between fragments) on the density, species richness, and distribution of native and exotic species of coccinellids.

3. Generally, when considering only the individuals in the remaining fragments, habitat loss had variable effects while habitat fragmentation had a positive effect on the density of two species of coccinellids and on species richness, but did not affect two other species. Isolation usually had no effect. When individuals in the whole landscape were considered, negative effects of habitat loss became apparent for most species, but the positive effects of fragmentation remained only for one species.

4. Native and exotic species of coccinellids did not segregate in the different landscapes, and strong positive associations were found most often in landscapes with higher fragmentation and isolation.

5. The opposing effects of habitat loss and fragmentation may result in a nil global effect; therefore it is important to separate their effects when studying populations in fragmented landscapes.

Key words. Coccinellidae, fragmentation, habitat loss, isolation, ladybeetles, manipulative experiments.

Introduction

Habitat loss and fragmentation are the main causes of changes in the distribution and abundance of organisms, and have been considered major threats to compositional, structural, and functional biodiversity (Noss, 1991; Didham *et al.*, 1996). Initially,

the term fragmentation was used to refer simultaneously to habitat loss and the configuration of the remaining habitat after the event, but fragmentation *per se* (i.e. the breaking apart of a continuous habitat into a larger number of smaller patches) can vary at equal degrees of habitat loss (Fahrig, 1997). In the literature, habitat loss and fragmentation are often confused and few empirical studies have attempted to separate their effects (Wolff *et al.*, 1997; McGarigal & Cushman, 2002; Villard, 2002; Fahrig, 2003; Grez *et al.*, 2004a,b). It is thought that habitat

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fragmentation negatively affects the abundance and diversity of organisms (Wilcove *et al.*, 1986; Quinn & Harrison, 1988; Baur & Erhardt, 1995; Didham, 1997; Gilbert *et al.*, 1998), but these effects may be due only to the negative effect of habitat loss. Moreover, when fragmentation has been used to refer strictly to the change in the configuration of a particular habitat type, studies have found that fragmentation has a nil or even a positive effect on population abundance and community diversity (Wolff *et al.*, 1997; Caley *et al.*, 2001; Fahrig, 2003; Grez *et al.*, 2004a,b). Usually these positive effects have been attributed to a 'crowding effect' (Collinge & Forman, 1998; Debinski & Holt, 2000), immigration from outside the landscape (Bowman *et al.*, 2002), or social interactions (Collins & Barret, 1997; Wolff *et al.*, 1997; Caley *et al.*, 2001).

The distance, or isolation, between the remaining habitat fragments is a component of the configuration of a fragmented landscape that could affect the abundance and diversity of organisms. One should expect higher abundance of organisms in landscapes with closer fragments because when individuals leave a fragment, they have a high probability of reaching a neighbouring one. In landscapes with fragments that are far apart, individuals that leave a fragment may get lost and leave the landscape before finding a new fragment, or die because of adverse conditions (Roslin, 2000; Fahrig, 2001). On the other hand, species richness might be higher in landscapes with more distant fragments, if each fragment experiences independent colonisation events by different species compared with a group of fragments that are closer. Greater isolation of fragments may also make the redistribution of these species across the landscape more difficult (Gilbert *et al.*, 1998; Tschardtke *et al.*, 2002; Grez *et al.*, 2004b, 2005). Finally, more fragmented landscapes, with more isolated fragments, may enhance at the landscape scale the coexistence of potentially competitive species in comparison to landscapes with closer fragments or unfragmented landscapes, because in the former they can segregate by inhabiting different fragments (Nee & May, 1992; Dytham, 1994; Tschardtke *et al.*, 2002). Traditionally all these predictions were drawn from island biogeography theory (MacArthur & Wilson, 1967), but nowadays there is recognition that some important differences exist between the properties of the 'matrix' in terrestrial and oceanic ecosystems, and that they should be taken into account (Ricketts, 2001; Cook *et al.*, 2002; Driscoll, 2005).

Although habitat loss and fragmentation are generally considered a matter of conservation interest (Collinge, 2001), they are also closely related to biological pest control in agricultural landscapes (Tschardtke & Kruess, 1999; With *et al.*, 2002). From both theoretical and empirical studies, there is a general consensus that natural enemies are more vulnerable to fragmentation than their prey (Kruess & Tschardtke, 1994; Zabel & Tschardtke, 1998; Elliot *et al.*, 1999; Hunter, 2002). Additionally, when natural enemies are imported into a new geographical region, species at the same trophic level already present in the area may be displaced (Elliot *et al.*, 1996; Turnock *et al.*, 2003; Evans, 2004). To date, the ways in which fragmentation affects the coexistence of native and exotic species in agro-ecosystems is unknown (Hunter, 2002).

Coccinellids are among the most important natural enemies of aphids in numerous crops, especially alfalfa (*Medicago sativa*

L.), in which they play an important role in keeping aphid densities low (Obrycki & Kring, 1998; Dixon, 2000). In fragmented agricultural landscapes, aphids may reach high densities because of the delay in the arrival of coccinellids to fragments and prey patches (Kareiva, 1987; With *et al.*, 2002). Previously, it was reported that two coccinellid species were more abundant in more fragmented landscapes with closer fragments (Grez *et al.*, 2004a), in spite of a similar abundance of aphids in all landscapes (Grez *et al.*, 2005). Nevertheless, the sampling protocol used did not allow the evaluation of how habitat fragmentation affected the spatial distribution of the different species of coccinellids in the landscapes. In this paper, by sampling the whole surface of the same landscapes of Grez *et al.* (2004a), the short-term effects of habitat loss, fragmentation, and isolation on population density, species richness, and spatial distribution of coccinellids are studied. When previous theoretical and empirical evidence are considered, the predictions of this study are: (i) coccinellid density in the habitat should be negatively affected by habitat loss and isolation, and positively affected by fragmentation, (ii) coccinellid species richness in the habitat should also be negatively affected by habitat loss but positively affected by fragmentation and isolation, and (iii) the degree of spatial segregation of the different species of coccinellids should be higher in more fragmented landscapes with more isolated fragments; (iv) nevertheless, at the whole landscape scale (i.e. habitat and matrix) the negative effects of habitat loss on density and species richness should be maintained or increased, but the positive effects of fragmentation may disappear, because of the low abundance of individuals expected in the matrix.

Materials and methods

Experimental landscapes

The field research was conducted at Antumapu Experimental Research Station, University of Chile, Santiago, Chile (33°34'S, 70°37'W), during the 2002–2003 southern growing season. For this experiment ten 30 × 30 m alfalfa landscapes that were sown in August 2002, separated by at least 20 m and distributed in two blocks, were used. These landscapes were all originally continuous, but in December 2002 four randomly selected landscapes in each block were fragmented, removing 84% of the alfalfa by ploughing. In this way five types of landscapes were created: unfragmented control landscapes (0% habitat loss, one fragment), 4fr–2m (84% habitat loss, four 6 × 6 m fragments separated by 2 m), 4fr–6m (84% habitat loss, four 6 × 6 m fragments separated by 6 m), 16fr–2m (84% habitat loss, sixteen 3 × 3 m fragments separated by 2 m), 16fr–6m (84% habitat loss, sixteen 3 × 3 m fragments separated by 6 m). The matrix surrounding the alfalfa fragments was bare ground (see Fig. 1 for a scheme of the experimental landscapes). A detailed explanation of the crop management can be found in Grez *et al.* (2004a).

The degree of habitat loss used in the experiment was based on theoretical and empirical studies, which suggest that fragmentation effects on population abundance or dispersal behaviour should be apparent only at high degrees of habitat loss (i.e. over 70–80%, Andr n, 1994; Fahrig, 1997; With & King, 1999;

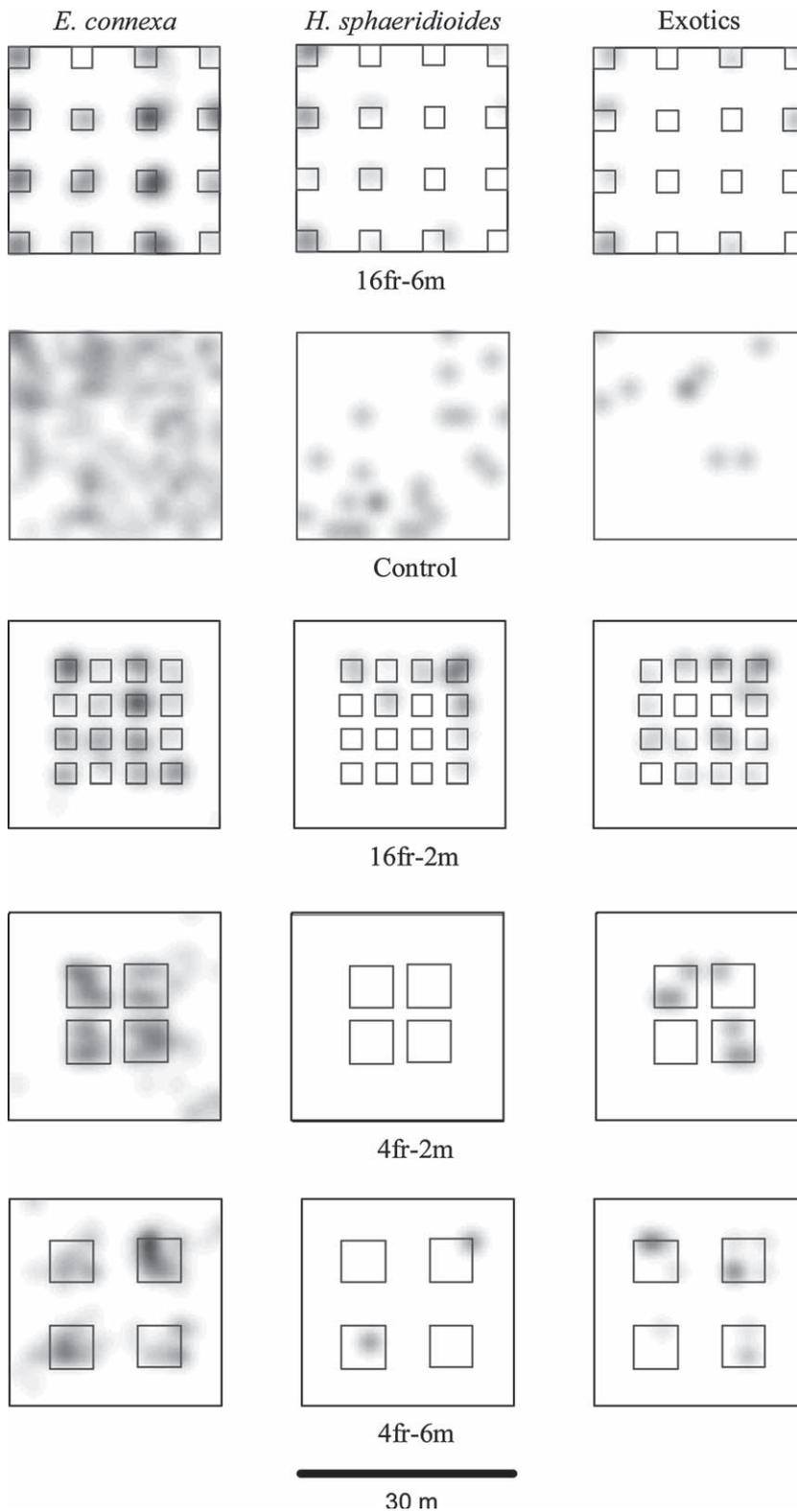


Fig. 1. Example (week 7, block 2) of the distribution and density of coccinellids in the five experimental landscapes (16fr-6m, control, 16fr-2m, 4fr-2m, 4fr-6m). The distribution and density of each species in the same landscape are shown in the rows. Darker areas represent higher densities. Exotics = *Hippodamia convergens* plus *Hippodamia variegata*.

Thies & Tschardtke, 1999; With *et al.*, 2002). Furthermore, the two isolation scales used (2 and 6 m) have been shown to significantly affect the inter-fragment movement of coccinellids

(Grez *et al.*, 2005). Most other studies dealing with similar questions in natural habitats (Braschler & Baur, 2003) as well as in agro-ecosystems (Kareiva, 1987; Ives *et al.*, 1993; Banks,

1999; With *et al.*, 2002) have used areas of considerably much smaller dimensions.

Insect sampling

One and seven weeks after fragmentation (December 20 and January 29 respectively) adult coccinellids in the whole landscape area (30 × 30 m), in all 10 landscapes, were sampled with sweep nets. To do this, each landscape was divided into a grid of 2 × 2 m cells (4 m²), which was the best scale or 'window size' to detect coccinellid distribution patterns in clover (With *et al.*, 2002), resulting in a total of 225 cells per landscape. For landscapes with 16 fragments of 3 × 3 m, some smaller cells (2 or 1 m²) were also used, to avoid having cells that included alfalfa and matrix. Each cell was sampled by passing the sweep net the number of times needed to cover its area (i.e. eight, four, and two times per cell of 4, 2 and 1 m² respectively). It has been demonstrated that sweep-netting provides accurate estimates of coccinellid abundance (Elliot & Michels, 1997). In order to standardise sampling conditions, all landscapes were sampled in the morning of one day, in warm sunny conditions, with a team of 8–10 persons sampling synchronously. Adult coccinellids were identified, counted immediately and released back into the cell previous to the one they were collected. Mark-recapture experiments, run parallel to this study, demonstrated that this protocol effectively prevented sampling the same insect twice (A. A. Grez & T. Zaviezo, unpublished data). The mean density (number of individuals per m²) of the different species in the fragments and matrix of each landscape and the number of coccinellid species found per unit area (i.e. species per m²) were calculated.

Data analyses

In order to have equal spatial grain for all data, the density and species richness of larger cells were converted into a 1-m² basis, and the weighted means were used for the analyses. In the case of the control, analyses were run with means calculated from only 32 randomly chosen cells, in order to consider an area equivalent to that of the fragmented landscapes (144 m²), although using the full data set gave the same results. Data were $\log(x + 1)$ transformed and analysed using GLM in SAS (SAS Institute, 2001). The mean density of each of the four most abundant species of coccinellids and the species richness in alfalfa and in the whole landscape was analysed by repeated measures ANOVA, with time as the repeated measure (1 and 7 weeks after fragmentation) and kind of landscape (unfragmented control, 4fr–2m, 4fr–6m, 16fr–2m, and 16fr–6m) as the independent variable, in a randomised block design. Then a series of planned comparisons were performed to test for the effects of habitat loss and fragmentation (unfragmented control vs. 84% loss), fragmentation *per se* (16 vs. 4 fragments) and isolation (2 vs. 6 m between fragments) on coccinellids density and richness. Planned comparisons were carried out for 4fr–6m and 16fr–2m landscapes, because these landscapes differ in fragmentation and isolation, but had similar spatial extent. Also

repeated measures ANOVA similar to the above, but excluding the control, were run to test for the effects of fragmentation, isolation, and their interaction on species density and richness, which was only partially tested in the previous analyses.

In order to study the spatial association between the most abundant native and exotic species of coccinellids, abundance data were analysed using spatial regression models, based on a second-order neighbourhood criterion (i.e. all eight 2 × 2 m cells around the target cell are considered in the model). This procedure takes into account the possibility that the variance-covariance matrix of the model is non-diagonal, and that some of the errors may be spatially correlated. As mentioned before, in this particular study, such correlations were assessed at the 2-m scale. Results are interpreted in the same manner as in the case of the normal multiple regression. Therefore, a positive association between the abundance of two species (one is the dependent and the other one independent variable) indicates that both species tend to aggregate in the same grid cells in the landscape. Because an obvious factor causing the aggregation of coccinellids might be the spatial distribution of the preferred habitat in the landscape, the presence of alfalfa in the grid cell was included in the model as another independent variable. Abundances of exotic species were relatively low in comparison to the native ones and responded similarly to fragmentation (see below), so their densities were pooled for these analyses. The spatial regression analyses was performed using the SpatialStats module of Splus (Kaluzny *et al.*, 1998). Graphical representations of spatial patterns of coccinellid abundance were carried out using the Kernel Smoothed Intensity of Point Pattern (ksmooth.ppp) procedure of the Spatstat package (Baddeley & Turner, 2004) for the R system (Ihaka & Gentleman, 1996).

Results

In total 8420 adults of the four most abundant species were counted: 6363 *Eriopsis connexa* (Germ.), 908 *Hippodamia variegata* (Goeze), 614 *Hyperaspis sphaeridioides* Mulsant, and 535 *Hippodamia convergens* Guerin. Three other species were found in very low numbers (see below). *Eriopsis connexa* and *H. sphaeridioides* are considered native to Chile, while both species of *Hippodamia* are exotic and were imported into the country in the 1960s and 1970s for biological control of aphids (Zúñiga, 1985).

Coccinellid density

Habitat loss, fragmentation, and isolation differentially affected the population density of the different species of coccinellids in alfalfa fragments (Fig. 2; Table 1). Only the native species were affected by habitat loss, with *E. connexa* being negatively affected 1 week after fragmentation, but positively on week 7. On the other hand, *H. sphaeridioides* abundance was always favoured by habitat loss, being more abundant in the fragmented landscapes (Table 2; Fig. 2). Both native species were positively affected by the degree of fragmentation, with

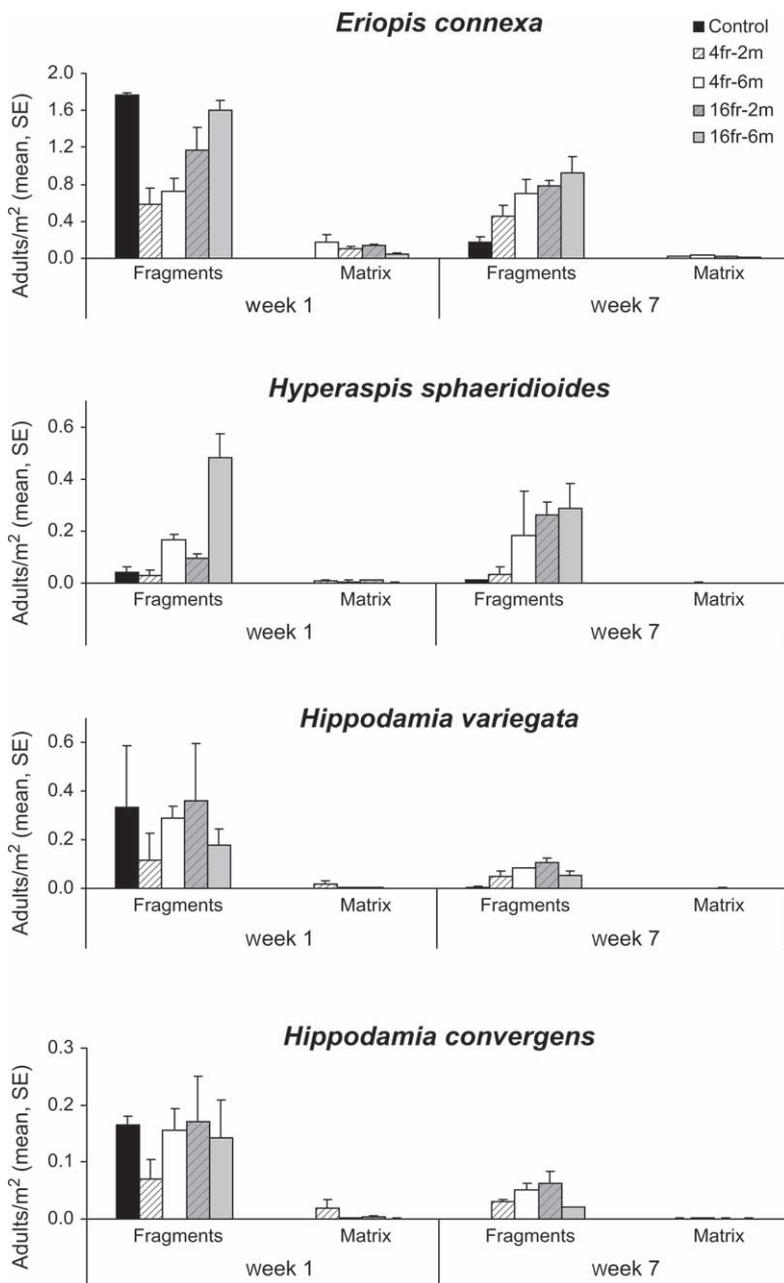


Fig. 2. Density (mean adults per $m^2 \pm 1$ SE) of the four most abundant coccinellid species in different landscapes, in fragments and matrix, 1 week (week 1) and 7 weeks (week 7) after fragmentation.

higher densities in the more fragmented landscapes (Table 2; Fig. 2; repeated measures ANOVA fragmented landscapes, fragmentation effect: $F_{1,3} = 20.99$, $P = 0.020$ for *E. connexa* and $F_{1,3} = 26.73$, $P = 0.014$ for *H. sphaeridioides*). In addition, *H. sphaeridioides* population densities were positively affected by an increase in isolation (Table 2; Fig. 2; repeated measures ANOVA fragmented landscapes, isolation effect: $F_{1,3} = 24.34$, $P = 0.016$), but there was no interaction between fragmentation and isolation (repeated measures ANOVA fragmented landscapes, fragmentation \times isolation effect: $F_{1,3} < 0.01$, $P = 0.950$ for *E. connexa* and $F_{1,3} = 0.28$, $P = 0.633$ for *H. sphaeridioides*). None of the exotic species was affected by any of the factors,

and they were less abundant 7 weeks after fragmentation, together with *E. connexa* (Table 1).

At the landscape scale, coccinellids were mainly found in the remaining fragments with very low abundance in the bare ground matrix of fragmented landscapes (Figs 1 and 2). One week after fragmentation, *E. connexa* and *H. convergens* were negatively affected by habitat loss, with the unfragmented control having six to nine times the densities of the fragmented landscapes, when abundances in the matrix were included in the mean (unfragmented vs. 84% habitat loss landscapes: $F_{1,4} = 168.76$, $P < 0.0001$ for *E. connexa*, and $F_{1,4} = 36.14$, $P = 0.002$ for *H. convergens*). But 7 weeks after fragmentation densities in

Table 1. Repeated measures ANOVA for the effect of landscape on the population density (log-transformed) of the four most abundant species of coccinellid in alfalfa fragments. The repeated measures was time after fragmentation (week 1 and week 7).

Source	d.f.	F	P
<i>E. connexa</i>			
Block	1	0.55	0.490
Landscape	4	8.54	0.031
Block × Landscape	4	0.69	0.632
Week	1	29.35	0.003
Landscape × Week	4	7.73	0.023
<i>H. sphaeridioides</i>			
Block	1	8.22	0.035
Landscape	4	10.56	0.021
Block × Landscape	4	1.62	0.301
Week	1	0.03	0.869
Landscape × Week	4	2.94	0.134
<i>H. variegata</i>			
Block	1	0.00	0.998
Landscape	4	0.44	0.777
Block × Landscape	4	1.21	0.411
Week	1	7.24	0.043
Landscape × Week	4	0.37	0.821
<i>H. convergens</i>			
Block	1	0.01	0.933
Landscape	4	0.97	0.511
Block × Landscape	4	0.82	0.565
Week	1	18.19	0.008
Landscape × Week	4	0.67	0.638

the unfragmented control dropped and were similar to the fragmented landscapes (repeated measures ANOVA, Landscape × Week effect: $F_{4,4} = 37.18$, $P < 0.001$ and $F_{4,4} = 8.08$, $P = 0.021$ for *E. connexa* and *H. convergens* respectively). Densities of *H. variegata* were also higher in the unfragmented control, but did not significantly differ from the fragmented landscapes (unfragmented vs. 84% habitat loss landscapes: $F_{1,4} = 4.49$, $P = 0.088$). At the landscape scale, *H. sphaeridioides* was still positively affected by an increase in fragmentation scale and distance between fragments ($F_{1,4} = 27.42$, $P = 0.003$ and $F_{1,4} = 21.57$, $P = 0.006$ respectively).

Species richness

During the whole experiment, 57 individuals of the three rare species of coccinellids were found: the exotics *Adalia bipunctata* (L.) and *Scymnus loewi* Germain, and the native *Scymnus bicolor* Mulsant. The mean number of species per m² was affected by kind of landscape and date, but there was no interaction between these factors (Table 3). The mean number of species per m² was higher in the fragmented landscapes with 84% habitat loss than in the unfragmented control, in the more fragmented landscapes, and 1 week after fragmentation (Table 3, Fig. 3). Also, species richness differed among 4fr–6m and 16fr–2m landscapes, being larger in the more fragmented landscapes (Table 3, Fig. 3). Analyses with only fragmented landscapes also showed a positive effect of fragmentation ($F_{1,3} =$

Table 2. Planned comparisons for the effect of habitat loss (unfragmented vs. 84% habitat loss), fragmentation scale (16 vs. 4 fragments), isolation degree (2 vs. 6 m), and landscape spatial extent (4fr–6m vs. 16fr–2m landscapes) on the population density (log-transformed) of the four most abundant species of coccinellid in alfalfa fragments.

Source	d.f.	F	P
<i>E. connexa</i>			
Unfragmented vs. 84% habitat loss	1	0.06	0.809
4 vs. 16 fragments	1	19.18	0.007
2 vs. 6 m	1	4.19	0.096
4fr–6m vs. 16fr–2m	1	2.72	0.160
<i>H. sphaeridioides</i>			
Unfragmented vs. 84% habitat loss	1	18.68	0.008
4 vs. 16 fragments	1	25.98	0.004
2 vs. 6 m	1	23.65	0.005
4fr–6m vs. 16fr–2m	1	0.03	0.876
<i>H. variegata</i>			
Unfragmented vs. 84% habitat loss	1	<0.01	0.993
4 vs. 16 fragments	1	0.24	0.648
2 vs. 6 m	1	<0.01	0.993
4fr–6m vs. 16fr–2m	1	0.11	0.750
<i>H. convergens</i>			
Unfragmented vs. 84% habitat loss	1	0.05	0.837
4 vs. 16 fragments	1	0.53	0.499
2 vs. 6 m	1	0.12	0.747
4fr–6m vs. 16fr–2m	1	0.08	0.799

37.76, $P = 0.010$), and no interaction among fragmentation and isolation ($F_{1,3} = 1.26$, $P = 0.357$). In contrast to the above, at the landscape scale, the number of species per m² was negatively affected by habitat loss (unfragmented vs. 84% habitat loss landscapes: $F_{1,4} = 35.10$, $P = 0.002$), with the unfragmented control having the highest species richness, especially 1 week after fragmentation, when it was three times larger than in the fragmented landscapes.

Table 3. Repeated measures ANOVA for the effects of landscape on coccinellid species richness (number of species per m² log-transformed) in alfalfa fragments and planned comparisons for the effect of habitat loss (unfragmented vs. 84% habitat loss), fragmentation scale (16 vs. 4 fragments), isolation degree (2 vs. 6 m), and landscape spatial extent (4fr–6m vs. 16fr–2m landscapes).

Source	d.f.	F	P
Species richness			
Block	1	0.21	0.669
Landscape	4	16.70	0.009
Block × Landscape	4	0.84	0.553
Week	1	16.89	0.009
Landscape × Week	4	1.10	0.446
Planned comparisons			
Unfragmented vs. 84% habitat loss	1	14.55	0.012
4 vs. 16 fragments	1	37.34	0.002
2 vs. 6 m	1	3.18	0.135
4fr–6m vs. 16fr–2m	1	9.37	0.028

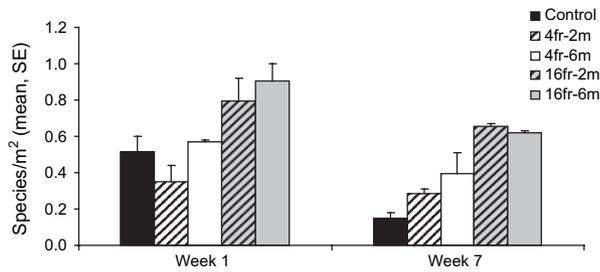


Fig. 3. Species richness (mean number of species per $m^2 \pm 1$ SE) in alfalfa habitat of the different landscapes, 1 week (week 1) and 7 weeks (week 7) after fragmentation.

Spatial distribution

Both the native and exotic species of coccinellids never showed negative spatial associations in any of the landscapes, even after having accounted for the effect of the presence of the preferred habitat. From the 60 possible combinations (three groups of species \times five landscapes \times two weeks \times two blocks), positive associations were found in 27, and in the remaining 33 no significant association was found (Table 4). In the unfragmented control landscapes and in the 4fr–6m, four out of 12 combinations showed positive associations, while in the 4fr–2m this occurred six times, in the 16fr–2m five times, and in the 16fr–6m eight times. More highly significant associations ($P < 0.01$ or < 0.001), and a lower number of non-significant ones were found in the 16fr–6m landscapes (seven vs. four), while the opposite was observed in the unfragmented control (one vs. eight). The rest of the landscapes showed intermediate situations, but always with more associations being non-significant

than significant (Table 4). This can be seen in Fig. 1, where the distributions of all coccinellid species in block 2, week 7 are shown. In that case all the species were highly positively associated in the 16fr–6m landscape, while in the rest of the landscapes they were rarely significantly associated.

Discussion

In general, when considering only the individuals in the alfalfa habitat, there was a variable effect of habitat loss, a positive effect of habitat fragmentation, and rarely a positive effect of isolation on the density of coccinellids. Nevertheless, these effects varied among species and over time. One week after fragmentation, when densities of *E. connexa* were high in the unfragmented landscape, habitat loss negatively affected its density only on the less fragmented landscapes with closer fragments, but this effect disappeared on week 7, when the unfragmented landscapes had the lowest densities. The ideal experimental design for evaluating the effects of habitat loss, independent of fragmentation, should have considered a landscape with less habitat distributed in only one fragment, which was not included in this study. Nevertheless, the 4fr–2m landscape, with the least fragmentation and distance between fragments, might mimic a landscape with only habitat loss. Then, the lower abundances of *E. connexa* in the 4fr–2m landscapes compared with the unfragmented control, 1 week after fragmentation, might be interpreted as the negative effect of habitat loss only. In the case of *H. sphaeridioides*, densities in the unfragmented control were always among the lower ones, along with the 4fr–2m landscapes, suggesting that habitat loss alone was not an important factor determining the abundance of this species.

Table 4. Probabilities of spatial association between native and exotic species of coccinellid in the different landscapes, in blocks 1 (B1) and 2 (B2), 1 and 7 weeks after fragmentation. All significant associations were positive. Spatial associations were calculated after spatial regression models.

	Week 1				Week 7			
	Exotics		<i>H. sphaeridioides</i>		Exotics		<i>H. sphaeridioides</i>	
	B1	B2	B1	B2	B1	B2	B1	B2
Control								
<i>E. connexa</i>	***	NS	*	*	NS	*	NS	NS
Exotics			NS	NS			NS	NS
4fr–2m								
<i>E. connexa</i>	*	***	**	***	NS	NS	NS	NS
Exotics			NS	*			**	NS
4fr–6m								
<i>E. connexa</i>	***	NS	***	NS	NS	NS	***	NS
Exotics			***	NS			NS	NS
16fr–2m								
<i>E. connexa</i>	NS	NS	*	***	***	NS	NS	NS
Exotics			NS	NS			***	*
16fr–6m								
<i>E. connexa</i>	**	NS	***	***	NS	***	*	***
Exotics			***	NS			NS	***

* $P < 0.05$, ** $P \leq 0.01$, *** $P \leq 0.001$. NS, non-significant.

The experimental design allowed testing for the effect of fragmentation *per se* only when comparing fragmented landscapes that have the same amount of habitat loss, but distributed in a different number of fragments. Habitat fragmentation (expressed as the total number of fragments in the landscape) had a consistent positive effect on the local density of *E. connexa* and *H. sphaeridioides*, an effect that was still detected 7 weeks after fragmentation. On the other hand, neither habitat loss nor fragmentation affected the density of either *Hippodamia* species. In a previous paper reporting the effects of habitat fragmentation on coccinellid densities in the same experimental landscapes (Gre \acute{z} *et al.*, 2004a), a transient, marginally significant positive effect of fragmentation on *H. variegata* and *H. convergens* was detected, but in this paper no effects for these species was found. No effects were found for *E. connexa* and *H. sphaeridioides* (Gre \acute{z} *et al.*, 2004a), in contrast here these species were positively affected by fragmentation. The differences between these studies are probably related to the different sampling protocols, and also because in this paper sampling was not carried out at the time of peak abundances of both *Hippodamia* species, when the effects were more evident (Gre \acute{z} *et al.*, 2004a). Nonetheless, both papers agree that fragmentation *per se* does not negatively affect the local densities of coccinellids, as most theory suggests, and moreover give empirical support to the more recent idea that fragmentation, when isolated from habitat loss, may have nil or positive effects on population densities (Collins & Barret, 1997; Wolff *et al.*, 1997; Caley *et al.*, 2001; Fahrig, 2003; Gre \acute{z} *et al.*, 2004a). Isolation, in general, did not affect the density of coccinellid species, except for *H. sphaeridioides*, which contrary to the prediction, reached higher densities in those fragmented landscapes with more isolated fragments. Even when negative effects of isolation in population density are reported (e.g. Thomas *et al.*, 2001), the lack of significant effects of isolation on three of the species of coccinellids studied suggests that the distances used were not different enough to greatly impact coccinellid movement. The nil effect of isolation on population abundance has been observed also in other type of organisms and habitats, like butterflies in calcareous grasslands in Germany (Krauss *et al.*, 2003) and squirrels in fragmented forest in Belgium (Verbeylen *et al.*, 2003). Probably, for species highly vagile, like the ones mentioned and at the scales studied, fragmented landscapes still retain high connectivity, and therefore densities would not be greatly affected (Ricketts, 2001). Also, even though the bare ground matrix represents a hostile environment for coccinellids, it offers little resistance to their movement (Gre \acute{z} *et al.*, 2005), diminishing effective isolation (Ricketts, 2001).

The higher densities observed in more fragmented landscapes for some coccinellids could have been the result of how they perceive the spatial extent of the landscapes after fragmentation, even though initially it was equal for all, 30 \times 30 m. If coccinellids perceive as effective landscape only that contained by the outer fragments, different treatments vary in their extent based on the distribution of habitat fragments, which is unavoidable given the experimental design used. The landscape with the least spatial extent was 4fr–2 m (14 \times 14 m), followed by 4fr–6 m and 16fr–2 m landscapes (18 \times 18 m) and then by 16fr–6 m and unfragmented control landscapes (30 \times 30 m). Therefore,

the number, size, and distribution of the fragments could have an effect on immigration, due to the extent perceived by individuals before landing, and on within-landscape dispersal, after they have landed. Which of these characteristics of fragmented landscape was more important in determining coccinellid abundance and richness is not clear. Nevertheless, several of the results obtained could not be explained by spatial extent alone. For example, densities of *E. connexa* (on week 7) and *H. sphaeridioides* (on both dates), as well as species richness, differed among the 16fr–6 m and unfragmented control landscapes, both with the same spatial extent (Tables 1 and 2). Moreover, coccinellid species richness also differed among 4fr–6 m and 16fr–2 m landscapes, which also had the same spatial extent (Table 3). These differences could not be attributed to landscape spatial extent, and it cannot explain why many landscapes that differed in spatial extent had similar densities and richness, suggesting that if there were any effects of spatial extent, they were weak compared with the ones of fragmentation. Similar results have also been found for other species, like grey-tailed voles, where higher densities were found in experimentally fragmented alfalfa landscapes in relation to the unfragmented control of the same spatial extent (Wolff *et al.*, 1997).

At the landscape scale, the negative effects of habitat loss became more apparent for three of the four most abundant species of coccinellids. But this effect was transient, and by week 7 the densities of these species in the unfragmented control were similar to the fragmented ones. One possible explanation for the decrease in the densities of these coccinellids would be low prey availability. However aphid abundance increased from December to January in the experimental site, and it was similar in all landscapes (Gre \acute{z} *et al.*, 2005), suggesting that some other factor, such as high daily temperatures, might have been more important in driving coccinellid dynamics at this time of the year. The one exception to the patterns observed was *H. sphaeridioides*, which never reached high densities in the control, and therefore at the landscape scale, habitat loss had no effects on its abundance. The positive effect of habitat fragmentation on density observed for *E. connexa* at the habitat scale was lost when considering the whole landscape, but this was not the case for *H. sphaeridioides*. This is explained by the difference in the relative abundances of these species in landscapes with more fragments compared with landscapes with fewer fragments. In the case of *E. connexa*, average densities were 1.7 times higher in the more fragmented landscapes when considering only fragments, but in *H. sphaeridioides* they were 2.8 times higher. Therefore in the latter case, the differences did not get completely lost when taking the matrix into account.

Similar to population density, coccinellid species richness was positively affected by habitat loss and fragmentation, and was usually not affected by isolation. An increase of species richness with fragmentation *per se* has been reported before. For example, when considering the same amount of habitat, Tschamtko *et al.* (2002) found higher number of butterflies in more fragmented agricultural landscapes than in less fragmented ones. Collinge and Forman (1998) found the same pattern in insect assemblages associated with grasslands in North America, and also a higher species richness of ground-dwelling beetles

was found in the same experimental landscapes used in this paper (Grez *et al.*, 2004b).

The higher species richness of coccinellids observed in more fragmented landscapes could have been the result of species' spatial segregation, in different fragments, as initially predicted. Nevertheless, the spatial analysis did not show any evidence of segregation between species of coccinellids in any landscape. Either they were not associated or, in many cases, they were positively associated. Moreover, stronger positive associations occurred more frequently in landscapes with a high degree of fragmentation and isolation (16fr–6 m). Apparently, individuals of different species would have been forced, at least temporarily, to coexist inside fragments because of the low habitat availability after extensive habitat loss and fragmentation. This is interesting because two of the most abundant species, *H. variegata* and *H. convergens*, imported into the country in the 1960s and 1970s as biological control agents, could have displaced the native species, *E. connexa* and *H. sphaeridioides*, such as has occurred for other coccinellids (e.g. Obrycki & Tauber, 1985; Obrycki *et al.*, 1998; Turnock *et al.*, 2003; Evans, 2004; but see Kajita *et al.*, 2006a). Because there is no spatial segregation of native and exotic species, there is no evidence of displacement, at least at the short temporal scale of this study. But at longer temporal scales, interspecific competition or intra-guild predation could occur and may explain why the peak abundances of different species are detected at slightly different times of the year. *Eriopis connexa* has its peak abundance in spring or early summer while *H. variegata* has its peak abundance in summer and autumn (Grez, 1997; Grez *et al.*, 2004a). *Adalia bipunctata*, an introduced species, is scarce during summer, but is the most abundant species during winter (T. Zaviezo & A. A. Grez, unpublished data). Interestingly, it has been proposed recently that *A. bipunctata* has not been able to establish and spread successfully in Japan because it suffers high intra-guild predation from native species and because the population that arrived there is univoltine (Kajita *et al.*, 2006a, b). The temporal abundance pattern observed for this species in Chile gives supports to this hypothesis, and might help to understand why coccinellid species establish more successfully in some regions than others.

Even when the effects of habitat loss, fragmentation, and isolation reported in this paper are at relatively short timescales, these effects may be relevant for insects like coccinellids that can complete one generation in 1 month (Etchégaray & Barrios, 1979) and feed on aphids, which also vary greatly in their abundance over short time spans (Evans, 2003). In fact, in ephemeral agro-ecosystems, such as many field crops, pest control exerted by natural enemies may be rapidly disrupted in fragmented landscapes (Kareiva, 1987; Roland, 1993; Roland & Taylor, 1997; With *et al.*, 2002).

In summary, our results with coccinellids support the more recent ideas that population abundance and species richness are generally positively affected by habitat fragmentation, with isolation usually having insignificant effects (Fahrig, 2003; Borne & Bowers, 2004). This is important to take into account, because the negative effects of habitat loss in density and species richness at the landscape scale may be compensated by the positive effects of fragmentation, resulting in a nil global effect. Therefore, it is critical to more deeply comprehend their inde-

pendent impacts on population density and species richness in order to design sound strategies of pest control or conservation (McGarigal & Cushman, 2002; Fahrig, 2003).

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