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Direct and indirect effects of landscape change on the reproduction of a temperate perennial herb

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Summary

1. The harmful effects of landscape change on species reproduction may be direct when habitat loss and fragmentation affect individual performance within habitat remnants, but also indirect when reproductive collapse derives from the effect of landscape alterations on population traits. Although the distinction between direct and indirect effects is crucial for the effective management of species, studies looking at both are scarce.

2. To assess the mechanisms and the temporal consistency of landscape change effects on reproduction, we quantified flowering, fruiting and seed set of the perennial herb *Primula vulgaris* through a gradient of forest loss and fragmentation, in 2 years with different climatic conditions. We used structural equation modelling to relate, at the landscape scale, forest habitat availability and subdivision, forest edge length, population size and subdivision, and flower, fruit and seed production. We also evaluated the effects of light availability, plant abundance and aggregation on reproduction at the local scale.

3. Flower and fruit production decreased in landscape regions with lower forest habitat availability, and fruit production decreased in areas with a smaller amount of forest edge. There was also a negative indirect effect of habitat loss on seed production, through population size reduction. These effects mostly emerged at the landscape scale and operated in all reproductive stages, but were also transmitted across stages, as flower and fruit production quantitatively influenced seed output.

4. Landscape change effects on reproduction differed between the 2 years, becoming evident after a mild winter that favoured long-lasting flowering, but disappearing, or even changing sign, when winter harshness shortened flowering.

5. *Synthesis and applications*. Disentangling the relative importance of direct and indirect effects of landscape change in plant reproduction is a novel approach to distinguishing between populations and habitats as the required management targets. In our study system, increasing *P. vulgaris* population sizes within small forest patches seems less effective than increasing forest cover around existing populations (even small ones), to enhance individual reproduction. The contrasting effects of the different processes of landscape change and the potential additive role of climatic variability must also be considered in management purposes.

Key-words: Cantabrian range, habitat fragmentation, habitat loss, Northern Spain, *Primula vulgaris*, reproductive success, structural equation modelling, temperate forests, temporal variability

Introduction

Landscape change has been shown to be severely detrimental for the persistence of many species (Lindenmayer & Fischer 2006). Considerable empirical evidence attributes the harmful effects of habitat loss and fragmentation to the collapse of landscape-scale dispersal dynamics (Fahrig 2003). Moreover, population declines may also arise from altered individual reproduction within remnant habitat patches (Kolb 2005). This has been highlighted in the case of plants (e.g. Aguilar *et al.* 2006; Leimu *et al.* 2010), as it has been assumed that local population dynamics may depend more on the net outcomes of reproduction than on immigration and gene flow (Honnay *et al.* 2005; but see Bruna, Fiske & Trager 2009).

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The causal links between landscape change and reproductive disruption are difficult to predict as they derive from three interdependent and simultaneous processes: habitat loss (Andrén 1994), habitat subdivision (Doak 2000) and edge increase (Tomimatsu & Ohara 2002), the latter two usually termed fragmentation processes. Each of these processes may hamper plant reproduction in two ways. First, they may compromise reproduction by directly affecting individual plant performance, irrespective of population traits. For example, the change in microenvironmental conditions associated with the proximity of forest edges may reduce individual reproductive output through decreases in pollination (Jules & Rathcke 1999) or increases in herbivory (Wirth *et al*. 2008). Secondly, landscape change may indirectly affect plant reproduction by modifying population traits such as population size (i.e. Allee effects, Matsumura & Washitani 2000) or the spatial distribution of individuals (Fischer & Matthies 1997), with subsequent effects on reproductive success. For example, in small, subdivided populations, fruit and seed set may be reduced owing to either decreased pollinator visitation, which diminishes the quantity of pollen arriving to flowers (Ågren 1996), or higher inbreeding levels, which reduces the genetic quality of pollen (Chacoff, García & Obeso 2008). Ascertaining the relative contribution of direct and indirect effects on reproductive disruption is an important step in the conservation of species suffering landscape change, as it helps in the complicated balancing act of choosing the appropriate target for management, be that at the habitat, population or individual plant level. For example, population-oriented tools, such as increasing population size, may not prove fruitful when reproduction depends mostly on the effects of landscape features on individual plants.

Besides the diversity of organization levels over which landscape change operates, an additional complexity in the prediction of its effects comes from the temporal dimension of plant reproduction. In this sense, landscape change effects may differ between years owing to the control exerted by large-scale processes, like climatic conditions, on local reproduction. For example, the effects of population size on reproduction may vary greatly between years with different weather conditions (Tomimatsu & Ohara 2002). Ascertaining the interactions between landscape change and climatic variability is a priority in developing management tools for populations under the simultaneous action of different global change drivers (Brook, Sodhi & Bradshaw 2008).

Despite the large body of research demonstrating the negative effects of habitat loss and fragmentation on plant reproduction (e.g. Aguilar *et al*. 2006; but see also Mustajärvi *et al*. 2001 for positive effects), studies discerning the relative importance of direct vs. indirect effects of different landscape change processes, from flowering to seed set, are lacking (but see Ågren, Ehrlén & Solbreck 2008). Similarly, despite the importance of integrating the effects of landscape alteration in a context of global environmental changes (Leimu *et al*. 2010), studies across years are scarce (Rabasa, Gutiérrez & Escudero 2009). In this paper, we used an integrative approach to evaluate the effects of landscape change on the reproduction of the perennial herb *Primula vulgaris* L. (primrose, Primulaceae) in the highly fragmented forests of the Cantabrian range (Northwestern Spain). Over 2 years with contrasted climatic conditions, we linked landscape change gradients to the reproductive success of *P. vulgaris*. Specifically, we sought to answer the following questions: (i) What are the principal processes (habitat loss, habitat subdivision, edge increase) and levels of organization (populations, individuals) through which landscape change affects *P. vulgaris* reproduction? (ii) Does landscape change affect flower production, fruit set, and seed set equally? and (iii) Are landscape change effects consistent across reproductive years with contrasted climatology?

Materials and methods

STUDY SPECIES

We focused on the perennial, early-flowering herb *P. vulgaris*. This species lives in moist open habitats of central Europe (Jacquemyn *et al*. 2009). In our region, it is mainly restricted to the forest understorey, although it is able to live in shady environments such as the banks of roads and paths. It flowers mainly from late winter (February) to late spring (June), with a peak from late March to early April (Alicia Valdés and Daniel García, unpublished data). Flowering is possible in early winter in warm, dry years. It is distylous and self-incompatible, with two floral morphs. Only between-morph pollination is efficient for seed set, although certain levels of self-fertilization are found (Endels *et al*. 2002). Pollinators are Hymenoptera (bumblebees) and Diptera (beeflies and hoverflies). Fruit consumers include ungulates, rodents and Lepidoptera larvae (Jacquemyn *et al*. 2009). Seeds have an elaiosome and are dispersed mainly by barochory (Valverde & Silvertown 1995).

A previous study (Valdés & García 2009) suggested strong effects of landscape structure on the presence and the demography of this species in the Cantabrian forests. Other studies with *Primula* spp. have shown that reductions in population size owing to habitat alteration hamper reproduction (Jacquemyn, Brys & Hermy 2002), which is also thought to depend on habitat quality (Valverde & Silvertown 1995, 1998).

SPATIOTEMPORAL FRAMEWORK

This study was conducted in the Sierra de Peña Mayor, Asturias, Spain (43°17′N–5°30′W, elevation 900 m a.s.l.), a mountainous area of the Cantabrian range. The climate of the region is Atlantic, with a mean annual temperature of 13 °C and annual rainfall of ca. 1300 mm. Historically, as in many other parts of the Cantabrian range (García *et al*. 2005), deforestation for cattle grazing occurred here, and the landscape has suffered considerable change, from the original continuous temperate forests to a heavily fragmented wood pasture habitat.

This study was carried out in an area of 3000 × 1000 m in size (Fig. 1), containing a few large forest fragments with hardwood species (beech *Fagus sylvatica* L. and ash *Fraxinus excelsior* L.), many fringe forest patches dominated by fleshy-fruited, bird-dispersed trees (holly *Ilex aquifolium* L., hawthorn *Crataegus monogyna* Jacq., yew *Taxus baccata* L., rowan *Sorbus aucuparia* and whitebeam *Sorbus aria*) and hazel *Corylus avellana* L., as well as numerous small forest fragments mostly composed of fleshy-fruited trees, embedded in a matrix of stony pastures and heathlands (*Erica* spp., *Ulex europaeus* L.). The matrix covers ca. 60% of the area.

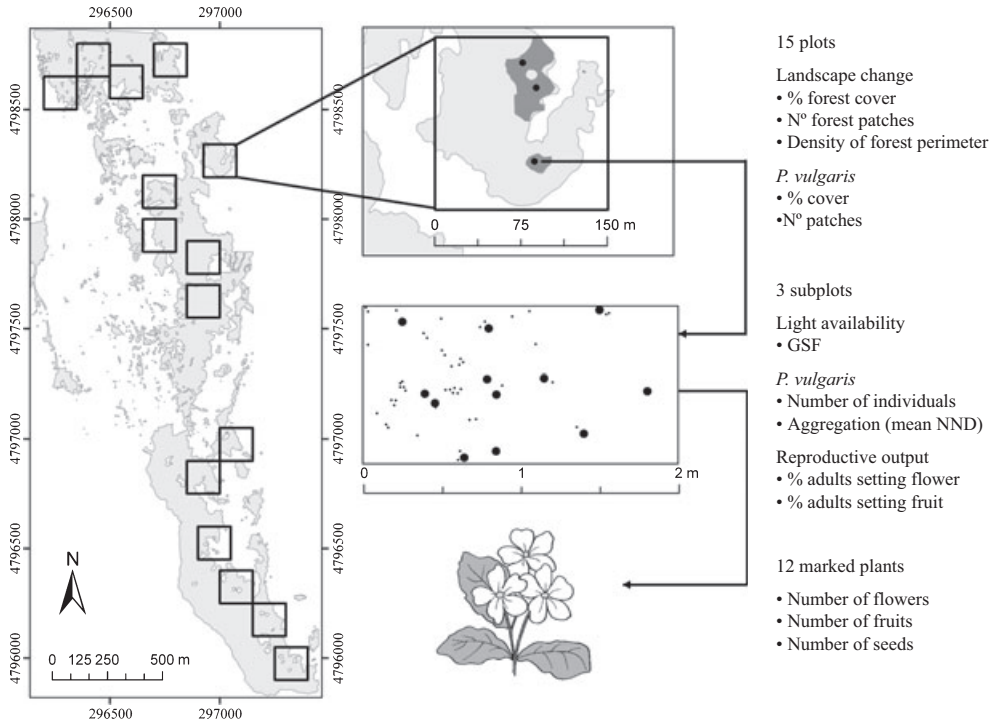


Fig. 1. Outline of the framework for sampling landscape change parameters, population traits and reproductive output at different spatial scales (GSF = global site factor, a measure of light availability; NND = nearest neighbour distance); see text for details on variable calculation. Light grey area represents forest cover and dark grey in the box *Primula vulgaris* cover.

This study took place during 2008 and 2009, 2 years with contrasting weather conditions during winter (Fig. S1 in Supporting Information): the nearest meteorological station (Oviedo, Asturias, 232 m a.s.l.) registered a mean temperature of 9·4 °C and a cumulative precipitation of 243·1 mm for winter 2007–2008 (December–March), and mean temperature of 7·9 °C and cumulate precipitation of 443·5 mm for the same period in 2008–2009. In addition, lower temperatures and heavier precipitation produced much more frequent and abundant snowfalls and an increased persistence of snow cover in winter 2008–2009, resulting in a delayed start to flowering and a much shorter flowering season in 2009 (Fig. S1 in Supporting Information).

LANDSCAPE CHANGE PARAMETERS AND FINE-SCALE HABITAT FEATURES

A previous study (Valdés & García 2009) found that *P. vulgaris* is absent from highly deforested areas (forest cover < 20%), but that it may also be absent from high-cover areas. In fact, large forest patches may contain several groups of isolated *P. vulgaris* populations. With these patterns in mind, for the present study, we delimited, in autumn 2007, 15 square plots (150 × 150 m) for the sampling of landscape structure and *P. vulgaris* population features across the study area (Fig. 1). We selected this sampling grain size (22 500 m^2) as a compromise, enabling us to cover the spatial variability in both population features and processes (e.g. population patchiness, Valdés & García 2009; pollinator range, Osborne *et al.* 1999) and forest landscape structure whilst employing feasible logistical effort. Plot locations were selected arbitrarily to contain the species, to avoid overlapping, to cover a large extent of the local landscape, and to represent sufficiently wide gradients of habitat loss, habitat fragmentation and *P. vulgaris* population features.

The degree of landscape change in these plots was measured from a geographic information system (GIS) of the study area created with ArcGIS 9.1 (see details in Valdés & García 2009), where we included a layer representing forest cover. For each of the plots, we calculated three major landscape change descriptors: the percentage of forest cover (a measure of habitat availability and an inverse measure of habitat loss); the number of forest patches (a measure of habitat subdivision); and the length of forest perimeter (a measure of edge amount). All these parameters showed considerable variation across plots (Table S1 in Supporting Information).

To evaluate habitat features at a fine scale, we established three permanent 2 × 1 m subplots in each of the 15 plots (Fig. 1). In each subplot, we measured light availability, based on the global site factor (GSF, Rich 1990), in July 2008 (the time of year with maximum foliar expansion). We took a hemispherical photograph of the forest canopy in each subplot and analysed all photographs with Hemiview 2.1 (Delta T Devices Ltd, Cambridge, MA, USA). For each subplot, we obtained a GSF value, which is the proportion of global solar radiation at a given location relative to that in the open, varying between 0 (totally closed canopy) and 1 (totally open sites).

ABUNDANCE AND SUBDIVISION OF *P. VULGARIS* POPULATIONS

The GIS also included a layer with *P. vulgaris* cover estimated from schematic maps drawn in the field during a previous study (Valdés & García 2009) which found that the percentage of species cover was strongly positively correlated with plant abundance. In the current study, we therefore used cover as a surrogate of the species' abundance. We also noted the number of *P. vulgaris* patches (defined as clumps of plants separated from each other by more than 20 m) in each plot, as a measure of population subdivision. Both species cover and number of patches showed considerable variation across plots (Table S1 in Supporting Information).

At the fine scale, we estimated abundance as the number of adults in each subplot in late May 2008 and 2009. As a measure of plant aggregation, we also calculated the mean nearest neighbour distance (NND) between adults in each subplot.

SAMPLING OF REPRODUCTIVE OUTPUT

In 2008 and 2009, we sampled reproductive output in reproductive plants (i.e. those showing flower buds), including both early- and late-flowering individuals. In each subplot, we marked eight reproductive plants at the beginning of the flowering season (January 2008, February 2009) and four in the middle of the season (April). Marked plants were surveyed fortnightly during the flowering and fruiting seasons (January–July) and every 2 months for the rest of the year. In each survey, we counted the number of flower buds, open and dead flowers, and intact and preyed fruits per plant. Each year, we took a sample of ripe fruits and counted the number of seeds per fruit.

This species shows a very long (≥15 days) individual flower life span (the amount of time flowers remain receptive and attractive to pollinators) and an extended flowering period. Thus, by the end of the flowering season, a plant may still simultaneously present flower buds, receptive flowers and developing fruits. This is not a handicap in estimating the total number of flowers produced by an individual each year, as flowers that do not set fruit remain attached to the plant for a long time. An exact count of the total number of flowers produced (for example, by labelling individual buds) was logistically unfeasible, so we estimated this as the maximum recorded count, per plant and per year, of the sum of buds, and open and dead flowers. The number of fruits per marked plant and year was estimated as the maximum of the sum of intact and preyed fruits. The number of seeds per marked plant and year was calculated as the number of seeds per fruit times the estimated total number of fruits. A mean value of number of flowers, fruits and seeds in marked plants was obtained for each subplot and year.

During the flowering season, we also calculated the proportion of adults in each subplot setting flower and fruit. Combining these proportions with the average number of flowers, fruits and seeds per marked plant, we were able to estimate the average number of flowers, fruits and seeds per adult per subplot – the three main sequential measures of reproductive output. These small-scale reproductive measures were averaged between subplots to obtain broad-scale measures of reproductive output for each of the 15 plots.

STATISTICAL ANALYSES

Our goal was to evaluate landscape change effects on reproductive output using an integrative approach, i.e. by distinguishing the relative effects of different alteration processes acting through the modification of population traits and/or the change in individual plant performance, across different reproductive stages. For this, we used structural equation modelling (SEM, namely path analysis, Quinn & Keough 2002), which assesses causality without experimental tests and allows the consideration of cascading effects across consecutive reproductive stages. We related landscape change parameters (percentage of forest cover, number of forest patches and density of forest perimeter), *P. vulgaris* population traits (size and subdivision) and reproductive output (mean number of flowers, fruits and seeds per adult) at the plot scale, for each year. Path analysis starts by building an *a priori* path scheme: an analytical model representing all the hypothetic causal links between predictors and response variables, based on previous knowledge of the ecological system. In our case, this saturated model included the links representing expectable effects of fragmentation and population traits on reproduction, which had been proven to operate at the landscape scale. Path analysis enables the exploration of the direct and indirect effects of predictors on response variables, taking into account the possible collinearities among predictors and among responses. Thus, we sought to evaluate the direct effects of landscape change parameters in reproductive output at different stages (i.e. effects on individual performance), the indirect effects of landscape change on final reproductive output (seeds per adult) mediated by previous effects on early reproductive stages (flowers and fruits per adult), as well as the indirect effects of landscape change on reproductive output mediated by the modification of population traits. Direct effects are measured by standardized partial regression coefficients between a predictor and a response, whereas indirect effects are calculated as the sum of the products of all standardized partial regression coefficients over all paths between a predictor and a response. These coefficients were estimated by maximum likelihood procedures, as recommended for small sample sizes (Iriondo, Albert & Escudero 2003).

We conducted separate analyses for each year, considering possible alternatives to the saturated model by constructing nested models sharing the same causal structure. We did this by running a stepwise specification search in Amos 16.0 (SPSS, Chicago, IL, USA) choosing the final model for each of the 2 years on the basis of Akaike Information Criterion (AIC, Akaike 1973). The fit of each model to the data was assessed using a likelihood chi-squared value, which tests the null hypothesis that the covariance matrix implied by the model reproduces the observed covariance matrix. A significant goodness-of-fit test indicates that the model is a poor description of the observed covariance among variables, while a nonsignificant value indicates that the predicted pattern of covariance is not distinguishable from that observed. As our design considered each plot as an experimental replicate to properly represent landscape-scale processes, our sample size was small ($N = 15$). Thus, we also tested goodness-of-fit by means of Bentler's comparative fit index (CFI; Iriondo, Albert & Escudero 2003). CFI $> 0{\cdot}9$ indicates an acceptable fit between the model and the data.

To assess the small-scale effects of habitat suitability and population traits on reproduction, we evaluated the effects of light availability, plant abundance and aggregation on reproductive output at the subplot scale using multiple regression models. We used number of flowers, fruits and seeds per adult as response variables and GSF, adult abundance and mean NND between adults as predictors. The model for the number of fruits also considered the number of flowers as a covariable, as did the model for the number of seeds with respect to the number of flowers and fruits. Given the spatial structure inherent in the empirical design (three subplots within each sampling plot), there could be some bias in the determination of the effects of predictors and covariables owing to spatial autocorrelation. Thus, our multiple regression models incorporated a spatial autoregressive term (simultaneous autoregressive regression, SAR; Keitt *et al.* 2002). We set up a model for each response variable and year, with SAM 3.0 software (Rangel, Felizola Diniz-Filho & Bini 2006).

Prior to analysis, data were transformed to achieve normality and reduce heteroscedasticity (arcsine of square root for proportions and ln $(x + 1)$ for the rest of the data).

Results

Primula vulgaris reproductive output strongly differed between the two study years (Fig. 2). Mean numbers of flowers, fruits and seeds per adult were significantly higher in 2008 than in 2009 (paired *t*-test: $t = -6{\cdot}46$, $P < 0{\cdot}0001$ for flowers;

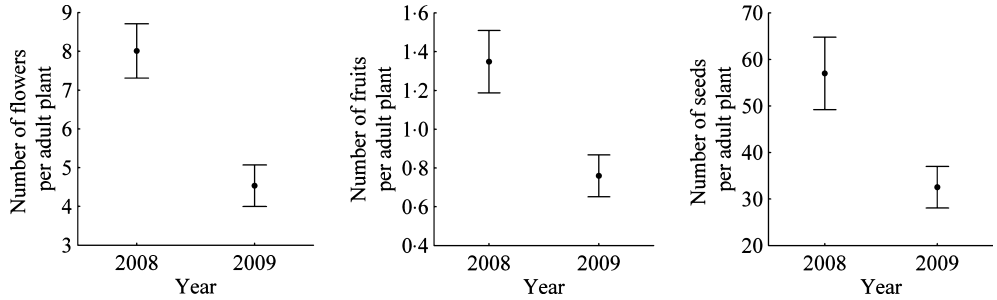


Fig. 2. Average (±SE) of the number of flowers, fruits and seeds per adult *Primula vulgaris* plant in 2008 and 2009 (N = 15 plots).

t = −3·64, P = 0·0027 for fruits; and t = −3·78, P = 0·0020 for seeds). All reproductive parameters varied along the gradient of landscape change, increasing with forest cover, especially in 2008 (Fig. 3).

The best-fit SEMs explained a large amount of the variation in reproductive output (Fig. 4). In 2008, predictors explained 32·5% of the variation in flowers per adult, 73·2% for fruits and 89·2% for seeds. These values were somewhat lower in 2009, explaining 18·8% of variation in flowers, 62·6% in fruits and 58·3% in seeds. In both cases, there was a good fit between the model and the data (2008: χ^2 = 13·19, d.f. = 16, P = 0·65, CFI = 1·00; 2009: χ^2 = 14·14, d.f. = 18, P = 0·72, CFI = 1·00). The best-fit path models (Fig. 4) included negative correlations of forest cover with amount of edge and number of forest patches, suggesting that decreases in habitat cover result in increased edge density and habitat subdivision. In addition, models showed significant effects of landscape change on *P. vulgaris* population traits, as bigger populations occurred in high-cover forest plots, whereas highly subdivided populations occurred in high-cover and edge-rich areas (Fig. 4). These relationships were the same in both years; as landscape change parameters and population traits were constant.

The SEM model for 2008 revealed that landscape change directly affected the different stages of reproduction (Fig. 4), these being the most important effects observed. Production of flowers and fruits per adult was higher in plots with more forest cover. Fruit production was also higher in plots with high forest edge density. Seed production was higher in high-cover plots as a consequence of these plots hosting bigger populations. Additionally, the reproductive stages were interdependent. More fruits per adult were set in plots where more flowers per adult were produced. Similarly, seed production was positively influenced by fruit production. However, more flowers per adult resulted in reduced number of seeds per adult, although the total effect of flower number on seed number (direct plus indirect through fruit number) was positive (see Table S2 in Supporting Information). There were no direct effects of landscape change on seed production, but more seeds per adult were produced in high-cover and edge-rich plots owing to indirect effects of these measures acting through flower and fruit production. Seed production depended mostly on habitat availability, through the effect of forest cover on earlier reproductive stages.

In 2009, forest cover did not influence any of the reproductive stages, either directly or through effects on population traits. Furthermore, the effect of amount of edge in fruit production changed sign: fewer fruits were produced per adult in plots with high forest edge density. This negative effect of edge was also present in flower production, though not significant. Finally, fruit production per adult was higher in plots where the forest habitat was more subdivided. As in the previous year, flower production positively influenced fruit production, although the effect was not significant. More seeds per adult were produced where fruit production was high, but the direct negative effect of flower production on seed production disappeared. Thus, there were no direct effects of landscape change on seed production, but more seeds per adult were produced in plots with low density of forest edge and high forest subdivision, as a result of indirect effects acting through fruit production, with forest edge exerting the major influence.

Small-scale SAR models explained between 13% and 86% of the variance in reproductive output (Table 1). The degree of spatial autocorrelation in the measured parameters was generally low, as revealed by the small difference in the amount of variance explained by the predictor variables and that explained by predictors plus space (see R^2 values in Table 1). The model for number of flowers per adult was never significant, while those for number of fruits and seeds were significant in both years. Number of fruits per adult increased when plants were more sparse (higher NND) in 2008, and, in both years, when more flowers per adult were produced. In addition, number of seeds per adult increased as fruits per adult increased.

Discussion

In this study, we used an integrative approach to demonstrate how *P. vulgaris* reproduction was affected by forest landscape structure in the Cantabrian range. We found that the different processes of landscape change exerted direct effects on individual reproductive potential. To a lesser extent, variations in habitat availability modified plant population size, which also affected individual reproduction. Both direct and indirect habitat alteration effects mostly emerged at a large scale, as habitat features or population traits scarcely affected fine-scale variability in plant reproduction. Moreover, landscape change differentially affected the successive stages of reproduction but also had effects cascading from flowering to seed setting, as

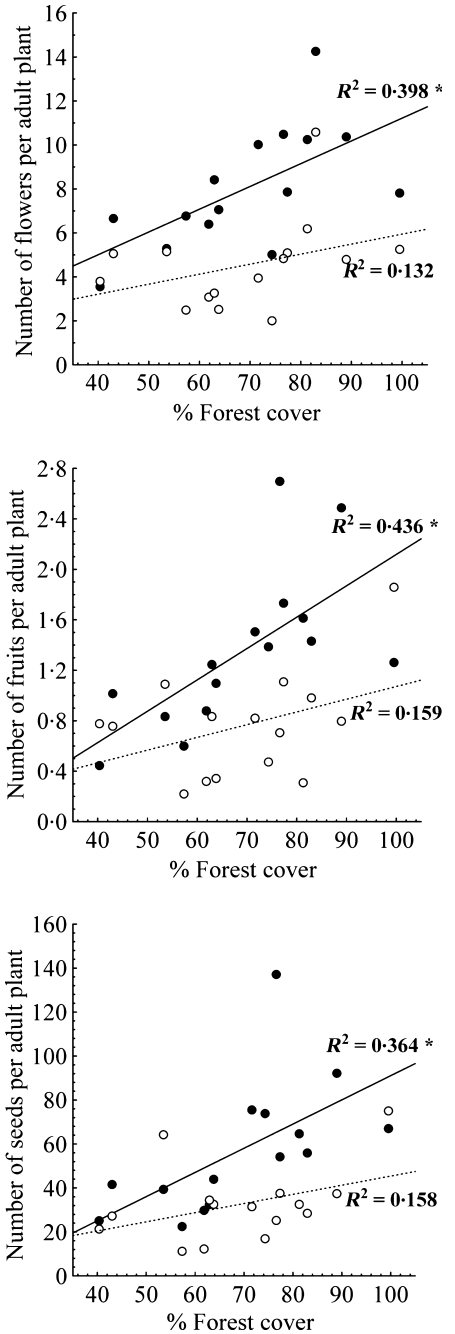


Fig. 3. Effects of percentage of forest cover on the number of flowers, fruits and seeds per adult of *Primula vulgaris* in the 15 plots, with filled circles representing data for 2008 and open circles representing data for 2009. Lines and R^2 values for linear regression fits are shown (asterisks indicate significance at $P < 0{\cdot}05$).

flower and fruit production quantitatively influenced seed output. Finally, landscape change effects were temporally inconsistent, disappearing or even changing sign between the 2 years.

DIRECT, INDIRECT AND CUMULATIVE EFFECTS OF LANDSCAPE CHANGE ON REPRODUCTION

Habitat alteration greatly affected *P. vulgaris* reproduction in its different stages through the direct effect of landscape changes. This was especially evident in 2008, when the amount of forest habitat affected flower, fruit and seed production independently. Sites with low forest availability seem suboptimal for *P. vulgaris* reproduction for several reasons. First, plants in deforested sites may have fewer resources for flowering than plants in highly forested, more humid sites (Jacquemyn *et al.* 2009). Second, the positive effect of forest cover on fruit set, irrespective of flower number, suggests some effect of forest cover favouring pollination and outcrossing (*P. vulgaris* is limited by pollination, Boyd, Silvertown & Tucker 1990). Large forest patches may promote longer pollinator movements than small patches, increasing pollen load quality (Goverde *et al.* 2002). Also, a proportionally greater pollinator abundance in larger patches is expected, as forest habitats account for most floral resources in the late winter and early spring across the fragmented landscape (Hegland & Boeke 2006). Finally, there was also a direct positive effect of edge density on fruit production. These edge effects were found even when considering a reduced analytical variability for edge length in SEM (as correlation with forest cover was significant), indicating that our estimations of edge effects are, in fact, conservative. Although fragmentation paradigms predict negative effects of edges on individual fitness (i.e. edge effects, Murcia 1995), they may benefit some pollinators (Montgomery *et al.* 2003) so enhancing pollen deposition and outcrossing.

Besides these direct effects of habitat loss and fragmentation on *P. vulgaris* reproduction, we also found indirect effects, mediated by modifications in population traits. The links between landscape gradients and population features were, however, complex because forest availability favoured plant abundance but, simultaneously, forest cover and edge density both favoured population subdivision. Thus, the larger the forest habitat availability, the bigger, but more subdivided, the plant populations. Irrespective of the processes linking landscape configuration with population traits, we found reproductive consequences of population variability in the fragmented scenario: namely, plants produced more seeds in large than in small populations, and this effect was independent of fruit production, thus highlighting some mechanism affecting seed development. As suggested above in relation to increased fruit set, improved seed set may be related to quantitatively and qualitatively enhanced pollination in larger populations. For example, higher flower visitation rates and stronger outcrossing are expected in larger populations owing to increased pollinator visitation and higher and more diverse pollen loads (Ågren 1996), leading to increased seed production (Aizen & Harder 2007).

Our integrative approach also revealed the cascading effects of landscape change, from flowering to seed maturation. Greater seed output was ultimately found in less-altered areas, resulting from the overall positive effect of flower production on seed set and explained by the strong effect of fruit production on seed set, counterbalancing the apparent trade-off between flower production and seed set (higher flower production led to higher fruit production, but the number of seeds per fruit decreased; Primack 1987).

Finally, landscape change effects on *P. vulgaris* reproduction were scale dependent, as habitat suitability and population

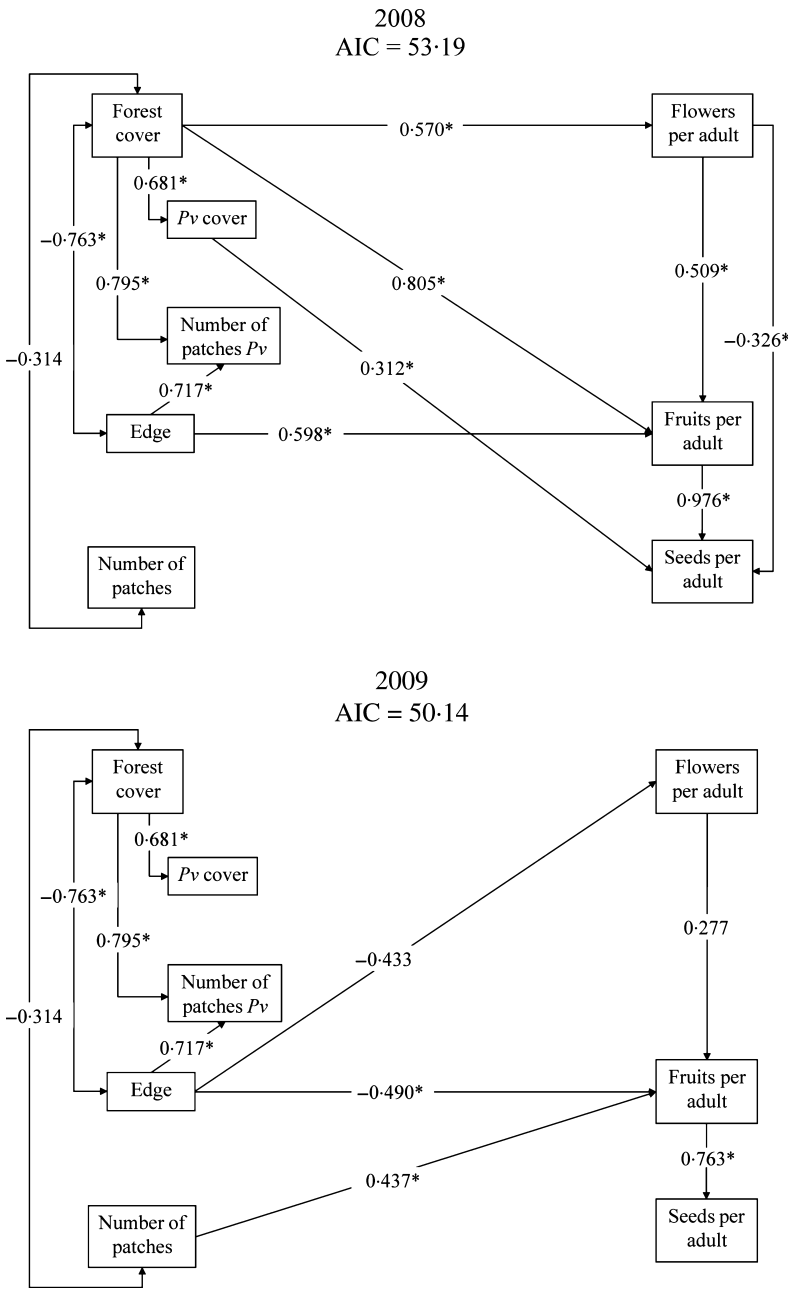


Fig. 4. Path models showing the direct and indirect effects of landscape change variables and population traits on reproductive output of *Primula vulgaris* (*Pv*) per adult in 2008 and 2009. Values of standardized partial regression coefficients and correlation coefficients are shown. Asterisks indicate significance ($P < 0{\cdot}05$). The AIC value for each model is shown.

traits poorly explained the fine-scale variability in reproduction, indicating that these features operated by and large at the landscape scale. In 2008, fruit production per adult was higher in subplots with low plant aggregation, suggesting effects of intraspecific pollinator or resource competition (Zimmerman 1980) but also increased inbreeding (short pollinator movements in clumped patches would lead to low-quality pollen transfer; Goverde *et al.* 2002).

TEMPORAL VARIABILITY IN REPRODUCTION AND LANDSCAPE CHANGE EFFECTS

The magnitude of *P. vulgaris* reproduction was remarkably different in the 2 years studied. As suggested for other forest perennials (De Frenne *et al.* 2010), mild winter temperatures, low precipitation and early snow melt during the first year (Fig. S1 in Supporting Information) facilitated an early start to flowering, which extended over a long period, resulting in higher reproductive output. The extended flowering season in 2008 may well have produced more pollination opportunities, which would have increased fruit and seed production (Alonso 2004). Conversely, the delayed start to, and shortening of, the reproductive season owing to the more adverse meteorological conditions in 2009 resulted in a much lower and more homogeneous reproductive output (see error bars in Fig. 2).

Landscape change effects on reproduction also varied between years, with most disappearing and some changing sign from 2008 to 2009. The lowered reproductive ability imposed by the shorter flowering period seemed to buffer the ability of plants to track for the large-scale environmental heterogeneity

Table 1. Summary of the spatial simultaneous autoregressive (SAR) models considering, as predictors, global site factor (GSF; a measure of light availability), *Primula vulgaris* plant abundance and nearest neighbour distance (NND) and, as response variables, flowers, fruits and seeds per adult in 2 × 1 m subplots. *F* and *P*-values and the coefficients of determination for predictor variables (R^2_p) and for predictor variables plus space (R^2_{p+s}) are shown for each model, and partial regression coefficients (β), *t* and *P*-values are shown for the predictors ($N = 45$; d.f. = 3)

	Dep. vars.	Flowers per adult				Fruits per adult				Seeds per adult			
2008		*F*	*P*	R^2_p	R^2_{p+s}	*F*	*P*	R^2_p	R^2_{p+s}	*F*	*P*	R^2_p	R^2_{p+s}
		2·42	0·080	0·150	0·166	10·99	< 0·001	0·523	0·528	36·32	< 0·001	0·862	0·856
		β	*t*	*P*		β	*t*	*P*		β	*t*	*P*	
	GSF	0·55	1·93	0·061		−0·26	−1·07	0·290		0·28	0·78	0·440	
	Abund.	−0·25	−1·74	0·089		0·15	1·13	0·264		−0·09	−0·42	0·676	
	NND	−1·38	−0·41	0·687		11·97	3·97	< 0·001		−0·96	−0·20	0·845	
	Fl/adult					0·57	4·95	< 0·001		−0·04	−0·18	0·859	
	Fr/adult									2·14	10·34	< 0·001	
2009		*F*	*P*	R^2	R^2_{p+s}	*F*	*P*	R^2	R^2_{p+s}	*F*	*P*	R^2	R^2_{p+s}
		1·74	0·174	0·113	0·129	5·22	0·002	0·343	0·345	35·81	< 0·001	0·852	0·853
		β	*t*	*P*		β	*t*	*P*		β	*t*	*P*	
	GSF	0·48	1·47	0·147		0·14	0·64	0·525		−0·20	−0·57	0·574	
	Abund.	−0·12	−0·76	0·453		0·16	1·35	0·184		0·13	0·68	0·499	
	NND	−4·97	−1·34	0·189		4·55	1·74	0·090		1·00	0·24	0·811	
	Fl/adult					0·46	4·48	< 0·001		−0·13	−0·72	0·480	
	Fr/adult									2·71	11·97	< 0·001	

derived from habitat change. Alternatively, landscape configuration effects might be stronger early in the season and thus mostly emerge when more plants are flowering early, as occurred in 2008. For example, gradients of soil temperature, which affect flowering in vernal plants (Dahlgren, von Zeipel & Ehrlen 2007), may be more marked across the fragmented landscape (with warmer and less variable soils in more forested sectors) in late winter than during spring. Our data support this possibility, as reproductive output was more influenced by habitat availability in plants that flowered early (data not shown).

The positive effect of forest edge on reproduction in 2008 was replaced by the opposite trend in 2009: more fruits per adult were produced in areas with less edge. The same happened with flowers. In this case, the phenology of proximate factors affecting reproduction and its interaction with landscape gradients may also underpin these interannual differences, resulting in detrimental edge effects when the plants flower later. The abundance of edge may favour cattle entering the forest in mid-spring (pers. obs.), resulting in plants suffering from increased herbivory and trampling (e.g. Wirth *et al.* 2008). Herbivores may reduce plant reproduction directly by affecting resource allocation and by consuming reproductive tissue, as well as indirectly by disrupting plant–pollinator interactions (Vázquez & Simberloff 2004). Furthermore, in 2009, more fruits were produced where the forest habitat was scattered through a higher number of forest patches. This could also be related to pollination, as habitat subdivision may make pollinators move between patches and thus carry pollen over longer distances, reducing inbreeding and increasing fruit set (Goverde *et al.* 2002; Aizen & Harder 2007). This effect was probably diluted in 2008 by the high abundance of flowers and flowering individuals, which would favour outcrossing even with short pollinator movements.

In conclusion, we wish to highlight that the temporal variability shown here suggests an interaction between landscape change and the large-scale effects of regional climatic conditions. In this sense, an increased frequency of mild winters, like that established by scenarios of climate warming, seems to enhance *P. vulgaris* reproduction (as reported by De Frenne *et al.* 2010 for *Anemone nemorosa*) but also seems to make fragmentation effects more pronounced, increasing the demographic difference between populations in highly fragmented habitats and those in continuous forests. In other words, if the interaction detected in our short-term study were to be maintained over subsequent years, landscape change could prevent the effects of warming by keeping reproductive output in highly altered sites consistently low (see Fig. 3). Clearly, longer-term studies are needed to confirm this interaction.

IMPLICATIONS FOR MANAGEMENT

Reproduction is a target process when developing conservation or management plans for perennial herbs in fragmented landscapes (Honnay *et al.* 2005; Leimu *et al.* 2010), as it may condition genetic variability within populations, local population growth and regional patch dynamics through seed dispersal. In the case of *P. vulgaris*, reproductive disruption leads to genetic impoverishment (Van Geert, Van Rossum & Triest 2008) and seems to contribute markedly to large-scale dispersal limitation (Valdés & García 2009). Moreover, the contribution of recruitment to the *P. vulgaris* population growth rate in our study site (Alicia Valdés and Daniel García, unpublished data)

suggests that reproduction plays a relevant role in population dynamics and future population size. We therefore consider that our results on *P. vulgaris* reproduction may help to establish guidelines for the conservation of endangered perennial herbs and, ultimately, for the preservation of plant biodiversity in fragmented habitats. Our results may, in addition, be applied to the management of *P. vulgaris* populations in other parts of its distribution range, where extinction risk is much higher (e.g. Endels *et al.* 2002).

We would like to present recommendations to conservation practitioners from three major considerations. First, we would argue that disentangling the relative importance of direct and indirect effects of landscape change in reproduction is a novel way to distinguish between populations and habitats as the required management targets. In the case of *P. vulgaris* in the Cantabrian forests, population-oriented measures, such as increasing population sizes within small forest patches, would be less effective than habitat-oriented management, such as increasing forest cover around existing populations (even small ones), to increase individual reproduction. Secondly, we highlight here the complexity of landscape change effects, given that habitat loss, habitat subdivision and edge increase may have contrasting effects on plant reproduction, which may even be inconsistent over time. In this sense, preventing further loss of forest habitat may be compatible with accepting some degree of forest fragmentation, as increased edge may be beneficial for reproduction. This can be achieved by promoting networks of interconnected and edge-rich forest patches, rather than single, regular forest stands. And thirdly, we urge the consideration of the potential additive role of landscape change and climatic variability, under an integrative framework of the effects of different global change drivers, on species persistence (see also Brook, Sodhi & Bradshaw 2008; Leimu *et al.* 2010). To achieve this goal, simulation modelling based on long-term monitoring of target parameters (i.e. harshness of winter, flowering and fruiting over gradients of landscape modification) would be required to assess the synergistic effects of global warming and habitat fragmentation.

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References

Ågren, J. (1996) Population size, pollinator limitation, and seed set in the self-incompatible herb *Lythrum salicaria*. *Ecology*, **77**, 1779–1790.

Ågren, J., Ehrlén, J. & Solbreck, C. (2008) Spatio-temporal variation in fruit production and seed predation in a perennial herb influenced by habitat quality and population size. *Journal of Ecology*, **96**, 334–345.

Aguilar, R., Ashworth, L., Galetto, L. & Aizen, M.A. (2006) Plant reproductive susceptibility to habitat fragmentation: review and synthesis through a meta analysis. *Ecology Letters*, **9**, 968–980.

Aizen, M.A. & Harder, L.D. (2007) Expanding the limits of the pollen-limitation concept: effects of pollen quantity and quality. *Ecology*, **88**, 271–281.

Akaike, H. (1973) Information theory and an extension of maximum likelihood principle. Second International Symposium on Information Theory, Akademia Kiado.

Alonso, C. (2004) Early blooming's challenges: extended flowering season, diverse pollinator assemblage and the reproductive success of gynodioecious *Daphne laureola*. *Annals of Botany*, **93**, 61–66.

Andrén, H. (1994) Effects of habitat fragmentation on birds and mammals in landscapes with different proportions of suitable habitat: a review. *Oikos*, **71**, 355–366.

Boyd, M., Silvertown, J. & Tucker, C. (1990) Population ecology of heterostyle and homostyle *Primula vulgaris*: growth, survival and reproduction in field populations. *Journal of Ecology*, **78**, 799–813.

Brook, B.W., Sodhi, N.S. & Bradshaw, C.J.A. (2008) Synergies among extinction drivers under global change. *Trends in Ecology & Evolution*, **23**, 453–460.

Bruna, E.M., Fiske, I.J. & Trager, M.D. (2009) Habitat fragmentation and plant populations: is what we know demographically irrelevant? *Journal of Vegetation Science*, **20**, 569–576.

Chacoff, N., García, D. & Obeso, J.R. (2008) Effects of pollen quality and quantity on pollen limitation in *Crataegus monogyna* (Rosaceae) in NW Spain. *Flora*, **203**, 499–507.

Dahlgren, J.P., von Zeipel, H. & Ehrlen, J. (2007) Variation in vegetative and flowering phenology in a forest herb caused by environmental heterogeneity. *American Journal of Botany*, **94**, 1570–1576.

De Frenne, P., Graae, B.J., Kolb, A., Brunet, J., Chabrerie, O., Cousins, S.A.O., Decocq, G., Dhondt, R., Diekmann, M., Eriksson, O., Heinken, T., Hermy, M., Jõgar, Ü., Saguez, R., Shevtsova, A., Stanton, S., Zindel, R., Zobel, M. & Verheyen, K. (2010) Significant effects of temperature on the reproductive output of the forest herb *Anemone nemorosa* L. *Forest Ecology and Management*, **259**, 809–817.

Doak, P. (2000) Population consequences of restricted dispersal for an insect herbivore in a subdivided habitat. *Ecology*, **81**, 1828–1841.

Endels, P., Jacquemyn, H., Brys, R., Hermy, M. & De Blust, G. (2002) Temporal changes (1986–1999) in populations of primrose (*Primula vulgaris* Huds.) in an agricultural landscape and implications for conservation. *Biological Conservation*, **105**, 11–25.

Fahrig, L. (2003) Effects of habitat fragmentation on biodiversity. *Annual Review of Ecology, Evolution and Systematics*, **34**, 487–515.

Fischer, M. & Matthies, D. (1997) Mating structure and inbreeding and outbreeding depression in the rare plant *Gentianella germanica* (Gentianaceae). *American Journal of Botany*, **84**, 1685.

García, D., Quevedo, M., Obeso, J.R. & Abajo, A. (2005) Fragmentation patterns and protection of montane forests in the Cantabrian range (NW Spain). *Forest Ecology and Management*, **208**, 29–43.

Goverde, M., Schweizer, K., Baur, B. & Erhardt, A. (2002) Small-scale habitat fragmentation effects on pollinator behaviour: experimental evidence from the bumblebee *Bombus veteranus* on calcareous grasslands. *Biological Conservation*, **104**, 293–299.

Hegland, S.J. & Boeke, L. (2006) Relationships between the density and diversity of floral resources and flower visitor activity in a temperate grassland community. *Ecological Entomology*, **31**, 532–538.

Honnay, O., Jacquemyn, H., Bossuyt, B. & Hermy, M. (2005) Forest fragmentation effects on patch occupancy and population viability of herbaceous plant species. *New Phytologist*, **166**, 723–736.

Iriondo, J.M., Albert, M.J. & Escudero, A. (2003) Structural equation modelling: an alternative for assessing causal relationships in threatened plant populations. *Biological Conservation*, **113**, 367–377.

Jacquemyn, H., Brys, R. & Hermy, M. (2002) Patch occupancy, population size and reproductive success of a forest herb (*Primula elatior*) in a fragmented landscape. *Oecologia*, **130**, 617–625.

Jacquemyn, H., Endels, P., Brys, R., Hermy, M. & Woodell, S.R.J. (2009) Biological Flora of the British Isles: *Primula vulgaris* Huds (*P. acaulis* (L.) Hill). *Journal of Ecology*, **97**, 812–833.

Jules, E.S. & Rathcke, B.J. (1999) Mechanisms of reduced *Trillium* recruitment along edges of old-growth forest fragments. *Conservation Biology*, **13**, 784–793.

Keitt, T.H., Bjørnstad, O.N., Dixon, P.M. & Citron-Pousty, S. (2002) Accounting for spatial pattern when modelling organism-environment interactions. *Ecography*, **25**, 616–625.

Kolb, A. (2005) Reduced reproductive success and offspring survival in fragmented populations of the forest herb *Phyteuma spicatum*. *Journal of Ecology*, **93**, 1226–1237.

Leimu, R., Vergeer, P., Angeloni, F. & Ouborg, N.J. (2010) Habitat fragmentation, climate change, and inbreeding in plants. *Annals of the New York Academy of Sciences* **1195**(The Year in Ecology and Conservation Biology 2010), 84–98.

Lindenmayer, D.B. & Fischer, J. (2006) Habitat fragmentation and landscape change. *An Ecological and Conservation Synthesis*. Island press, Washington.
Matsumura, C. & Washitani, I. (2000) Effects of population size and pollinator limitation on seed-set of *Primula sieboldii* populations in a fragmented landscape. *Ecological Research*, **15**, 307–322.
Montgomery, B.R., Kelly, D., Robertson, A.W. & Ladley, J.J. (2003) Pollinator behaviour, not increased resources, boosts seed set on forest edges in a New Zealand Loranthaceous mistletoe. *New Zealand Journal of Botany*, **41**, 277–286.
Murcia, C. (1995) Edge effects in fragmented forests: implications for conservation. *Trends in Ecology and Evolution*, **10**, 58–62.
Mustajärvi, K., Siikamäki, P., Rytkönen, S. & Lammi, A. (2001) Consequences of plant population size and density for plant-pollinator interactions and plant performance. *Journal of Ecology*, **89**, 80–87.
Osborne, J.L., Clark, S.J., Morris, R.J., Williams, I.H., Riley, J.R., Smith, A.D., Reynolds, D.R. & Edwards, A.S. (1999) A landscape-scale study of bumble bee foraging range and constancy, using harmonic radar. *Journal of Applied Ecology*, **36**, 519–533.
Primack, R.B. (1987) Relationships among flowers, fruits and seeds. *Annual Review of Ecology & Systematics*, **18**, 409–430.
Quinn, G.P. & Keough, M.J. (2002) *Experimental Design and Data Analysis for Biologists*. New York, Cambridge University Press.
Rabasa, S., Gutiérrez, D. & Escudero, A. (2009) Temporal variation in the effects of habitat fragmentation on reproduction of the Mediterranean shrub *Colutea hispanica*. *Plant Ecology*, **200**, 241–254.
Rangel, T.F.L.V.B., Felizola Diniz-Filho, J.A. & Bini, L.M. (2006) Towards an integrated computational tool for spatial analysis in macroecology and biogeography. *Global Ecology and Biogeography*, **15**, 321–327.
Rich, P.M. (1990) Characterizing plant canopies with hemispherical photographs. *Remote Sensing of Environment*, **5**, 13–29.
Tomimatsu, H. & Ohara, M. (2002) Effects of forest fragmentation on seed production of the understory herb *Trillium camschatcense*. *Conservation Biology*, **16**, 1277–1285.
Valdés, A. & García, D. (2009) Applying a continua landscape approach to evaluate plant response to fragmentation: *Primula vulgaris* in the Cantabrian mountains. *Applied Vegetation Science*, **12**, 504–515.
Valverde, T. & Silvertown, J. (1995) Spatial variation in the seed ecology of a woodland herb (*Primula vulgaris*) in relation to light environment. *Functional Ecology*, **9**, 942–950.
Valverde, T. & Silvertown, J. (1998) Variation in the demography of a woodland understorey herb (*Primula vulgaris*) along the forest regeneration cycle: projection matrix analysis. *Journal of Ecology*, **86**, 545–562.
Van Geert, A., Van Rossum, F. & Triest, L. (2008) Genetic diversity in adult and seedling populations of *Primula vulgaris* in a fragmented agricultural landscape. *Conservation Genetics*, **9**, 845–853.
Vázquez, D.P. & Simberloff, D. (2004) Indirect effects of an introduced ungulate on pollination and plant reproduction. *Ecological Monographs*, **74**, 281–308.
Wirth, R., Meyer, S.T., Leal, I.R. & Tabarelli, M. (2008) Plant herbivore interactions at the forest edge. *Progress in Botany*, **69**, Springer, Berlin Heidelberg, 423–448.
Zimmerman, M. (1980) Reproduction in *Polemonium*: competition for Pollinators. *Ecology*, **61**, 497–501.

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Supporting Information

Additional Supporting Information may be found in the online version of this article.

Figure S1. Climatic diagrams representing the mean monthly temperature, the cumulated precipitation, and the mean monthly percentage of flowering adults.

Table S1. Descriptive statistics for the predictor variables considered in the landscape scale path analysis and in the local scale linear regressions.

Table S2. Direct, indirect and total effects of landscape change variables in reproductive output for 2008 and 2009.

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