

Does human-induced habitat transformation modify pollinator-mediated selection? A case study in *Viola portalesia* (Violaceae)

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Abstract Pollinator-mediated selection is one of the most important factors driving adaptation in flowering plants. However, as ecological conditions change through habitat loss and fragmentation, the interactions among species may evolve in new and unexpected directions. Human-induced environmental variation is likely to affect selection regimes, but as yet no empirical examples have been reported. In the study reported here, we examined the influence of human-induced habitat transformation on the composition of pollinator assemblages and, hence, pollinator-mediated selection on the flower phenotype of *Viola portalesia* (Violaceae). Our results indicate that pollinator assemblages differed substantially in terms of species composition and visitation rate between nearby native and transformed habitats. Similarly, the insect species that contributed most to visitation rates differed between plant populations. While the magnitude and sign of pollinator-mediated selection on flower length and width did not differ between sites, selection for flower number lost significance in the transformed habitat, and a significant pattern of disruptive selection for flower shape, undetected in the native habitat, was present in the transformed one. Overall, the results of this study suggest that human-induced habitat change may not only modify the species

composition of pollinator assemblages, relaxing the selection process on some flower characters, but they may also create new opportunities for fitness-trait covariation not present in pristine conditions.

Keywords Chile · Flower · Phenotypic selection · Pollination · Ruil forest

Introduction

Pollinator-mediated selection is one of the main factors driving adaptation and diversification in angiosperms (Hodges and Arnold 1994; Maad 2000; Medel et al. 2007). The preference shown by pollinators for specific floral phenotypes and the subsequent fitness advantage obtained by a number of phenotypic variants relative to the remaining individuals in the population is the proximal factor by which natural selection influences the evolution of flower phenotypes. Several studies have reported that pollinator preference for some floral variants within populations often creates a significant covariation between floral characters and fitness in a wide variety of floral traits. For example, significant short-term phenotypic selection has been described for corolla size (e.g., Campbell 1991; Campbell et al. 1996; Ambruster et al. 2005), corolla color (e.g., Nagy 1997; Campbell et al. 1997), corolla shape (e.g., Herrera 1993; Gómez et al. 2006), nectar guides (e.g., Medel et al. 2003), stigma exertion (e.g., Conner et al. 1996), spur length (e.g., Herrera 1993; Maad 2000), and flowering date (e.g., Johnston 1991; Gómez 1993). Studies of selection performed in a range of populations have also shown variable results as populations often face variation in the species composition of the pollinator assemblage (e.g., Campbell et al. 1997; Totland 2001; Caruso et al.

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2003; Anderson and Johnson 2008). Since plants may adapt to different pollinator species across populations, the identification of the relevant floral traits involved in local adaptation represents an important step towards understanding the mechanisms that determine the formation of ecotypes and incipient pollinator-mediated speciation (see reviews in Waser and Campbell 2004; Herrera et al. 2006). However, in addition to the profuse literature documenting pollinator-mediated selection, it has becoming increasingly clear that the selective impact of pollination on flower characters depends not only on factors inherent to the pollination process, such as the composition of the pollinator species assemblage (e.g., Totland 2001; Anderson and Johnson 2008), but also on factors extrinsic to the pollination process, such as habitat variables (e.g., Totland 2001) and flower and foliar herbivory (e.g., Gómez and Zamora 2000; Gómez 2003), and the ecological context provided by the composition of the accompanying flora (e.g., Rathcke 1983; Jennersten 1988).

Contemporary human-induced landscapes consist of heterogeneous patches that often provide new habitats to the native biota. Habitat degradation due to human activities, such as deforestation and farming, may modify the composition of the pollinator assemblage, which in turn may influence the selective regime experienced by plant populations. While many studies have assessed the impact of human activities on pollination service and plant reproduction (e.g., Saunders et al. 1991; Ghazoul and McLeish 2001; see review in Kearns et al. 1998), the ultimate impact of human-induced habitat degradation on pollinator-mediated selection has not been examined in the literature. This omission is unfortunate because as ecological conditions change through habitat loss and fragmentation, the interactions among the remaining species may evolve rapidly in new directions (Thompson 1998). In the study reported here, we addressed this issue by examining the relationship among the composition of the pollinator species assemblage, visitation rate, and phenotypic selection on the floral traits of *Viola portalesia* in two contrasting habitats.

Maulino forest is a native Chilean evergreen association characterized by a wide spectrum of life forms and tall sclerophyllous trees. This forest is located in the Coastal Mountain Range between 35° and 37°S. It develops under a Mediterranean-type climate (Di Castri and Hajek 1976), with a dry season of 3–5 months and a winter precipitation regime of 7–8 months. Cumulative annual precipitation is 1,150 mm. Maulino forest is currently observed as a mosaic of highly fragmented patches immersed in a matrix of *Pinus radiata* (Bustamante and Castor 1998). In the last decade, the Maulino forest has experienced a profound change in species richness and abundance of different functional groups, seemingly as a

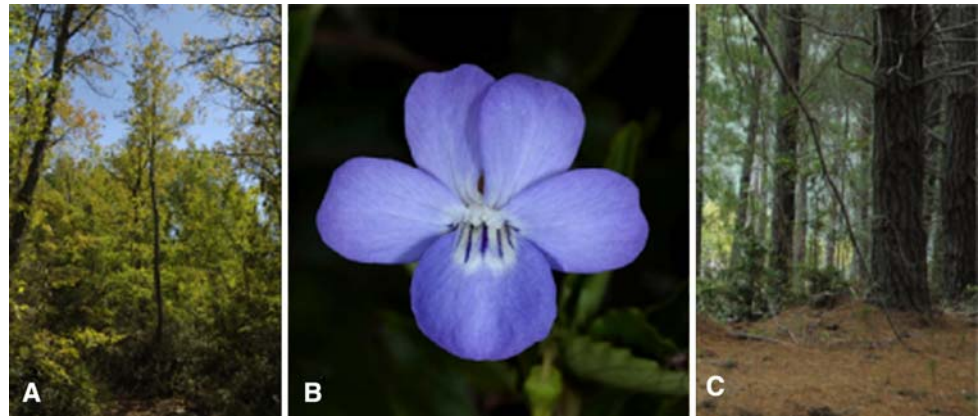
consequence of human-induced habitat change (Simonetti et al. 2006). This transformation has produced a mosaic of habitats with different levels of anthropogenic degradation and microclimatic conditions, which result in a heterogeneous diversity and abundance of pollinator species across the landscape (Bustamante et al. 2006). This scenario raises the question of the effects of habitat transformation on the short-term floral phenotype evolution. We have therefore (1) examined whether the composition of the pollinator assemblages and visitation rate differ between native and transformed habitats, and (2) compared the strength, sign, and curvature of pollinator-mediated selection on floral characters of *V. portalesia* between habitats.

Materials and methods

Natural history and study site

Viola portalesia (Violaceae) is a perennial herb species endemic to Chile that inhabits both the Maulino forest and the matrix of *Pinus radiata* (Fig. 1). Plants produce one to ten flowers during a season. The flowering and fruiting season extends from September to December. Flowers are self-incompatible and thus require cross-pollination by pollen vectors for seed production. The five-petaled blue-purple hermaphroditic flowers (Fig. 1b) produce a tri-valve capsule that contains from 10–30 seeds per capsule. This study was conducted in the Maule Region, Chile, during the Austral spring season, between October and December of 2007. The first site is located at Reserva Nacional Los Ruiles (35°83'S, 72°50'W), in a protected area of approximately 29 ha of native forest (Fig. 1a, native habitat hereafter). The site has a south-east exposure and is situated at an elevation between 160–440 m a.s.l. The dominant species are the caducifolius trees *Nothofagus alessandrii* and *Nothofagus glauca*, which are often associated with the subdominant evergreen *Cryptocarya alba*, *Aextoxicon punctatum*, and *Gevuina avellana*, among others (San Martín and Donoso 1996). The second site is located 3.6 km from the native site and consists of a 20-year-old plantation of *Pinus radiata* (35°50'S, 72°27'W) (Fig. 1c; *Pinus* habitat hereafter). This population occurs at an elevation range of 462–522 m a.s.l. and presents low understory coverage as a consequence of logging and forestry activities. The two sites differ in their vegetational structure, with a species richness of 131 species in the native habitat and 66 in the transformed habitat. Similarly, the native habitat had a higher population density of *V. portalesia* in comparison to the *Pinus radiata* plantation (mean density \pm 1 standard error (SE); native 8.88 \pm 0.97 ind m⁻², *Pinus* 4.16 \pm 0.56 ind m⁻²).

Fig. 1 **a** Native habitat in the Reserva Nacional Los Ruiles, **b** close-up of *Viola portalesia* from a frontal perspective to the corolla plane, **c** transformed habitat in *Pinus radiata* plantations



Pollinator assemblages and visitation rate

We recorded the number and identity of visitors to flowers of *V. portalesia* during 15-min observation periods between 0900 hours and 1700 hours. Censuses were carried out from October 2007 to January 2008, thereby covering the complete flowering season of *V. portalesia*. We performed a total of 190 15-min censuses (47.5 h of observation) in the native habitat and 108 15-min censuses (27 h of observation) in the *Pinus* plantation. Because of the unequal sampling effort between habitats, we performed a rarefaction analysis combined with a fitting method to calculate the expected number of species at each habitat. To this end, we fitted an exponential model to the species accumulation curve at increasing focal samplings. Only species that introduced at least its entire head into the floral tube were considered as pollinators in the analyses. Samples of each pollinator species were taken for subsequent identification to the lowest level of taxonomic resolution in the laboratory. To determine the similarity in the pollinator assemblage between habitats we used a proportional similarity test (PS) calculated by

$$PS = 1 - \frac{1}{2} \sum_{i=1}^n |P_{ai} - P_{bi}|,$$

where, P_{ai} and P_{bi} represent the proportion of total visitation rate performed by taxon “I” in habitats “a” and “b”, respectively (see Schemske and Brokaw 1981). This index ranges between 0 and 1, and take into account both the identity of the pollinator and its relative visitation rate. Values close to zero indicate a low similitude between pollinator assemblages, and values close to one indicate high similitude. Since this index does not provide information on confidence intervals, we performed a Montecarlo test using 5,000 data randomizations using Poptools (available at: <http://www.cse.csiro.au/poptools/download.htm>).

Morphometric analyses

We tagged and measured all open but not withered flowers on 111 and 112 individuals in native and *Pinus* habitats, respectively. Floral measurements were performed from digital pictures taken from a perpendicular orientation to the plane of flowers, using a graded ruler (precision 1 mm) to convert pixel measurements to a commensurate scale. Digital photographs were analyzed using ImageJ software (available in <http://rsb.info.nih.gov/ij/>). To quantify the floral phenotype of *V. portalesia* we recorded (1) plant height, from the ground to the tip of the plant, (2) the number of flowers per plant, (3) flower length, from the base of the peduncle until the tip of the landing petal, (4) flower width, as the longest horizontal distance perpendicular to the plane of symmetry, and (5) flower shape, from geometric morphometrics analysis [see correlation matrix of flower traits in Appendix 1 of the [Electronic Supplementary Material \(ESM\)](#)].

The corolla shape of each population was described using geometric morphometric analysis. The x , y coordinates of 12 homologous landmarks per specimen were digitized using the tpsDig software (package available at: <http://life.bio.sunysb.edu/morph>). Landmarks were set at the tip and base of each petal. The first step of the analysis is a least-squares Procrustes superimposition of all specimens to extract shape coordinates for use in subsequent statistical analyses. The Procrustes method removes non-shape variation by scaling all specimens to unit size, translating them to a common location, and then rotating them so that their corresponding landmarks line up as closely as possible. The resulting shape coordinates can be analyzed with the tools of multivariate statistics. To model shape change, we used the thin-plate spline (TPS), which represents change as a deformation between landmarks, relative to a grand mean consensus form (Zelditch et al. 2004). The net shape change can be decomposed into uniform and nonuniform components. The uniform

component describes affine transformation (stretching or shearing) with no bending energy applied on specific landmarks. The nonuniform component describes transformations that are different for different parts of the form. The degree of change in height of the plate at each landmark as it is deformed to match the reference shape encodes the amount of difference in shape present at that landmark. The bending-energy matrix resulting from the energy applied to every landmark is summarized as eigenvectors (principal warps). Independent trends of nonuniform shape change are obtained for each object (individual) on each principal warp. In this way, the contribution of every individual to the overall shape variation can be quantified as relative warps using the scores of a principal component analysis on the nonuniform fraction of shapes. This analysis was performed using the tpsRelw software.

Phenotypic selection analysis

We collected fruits of every tagged flower to estimate the mean seed production per plant (W_I). Individual relative female fitness (w) was calculated as W_I/W_{mean} , where W_{mean} is the mean population fitness. The variance in relative fitness allows the opportunity for selection (I) to be estimated. This coefficient indicates the upper limit of the selection intensity that can act on any flower character and permits estimation of the chance that traits of *V. portalesia* have to be selected at each population. To determine the magnitude, direction, and shape of selection acting on flower characters, we quantified the selection differentials and linear and nonlinear gradients of selection using the methodology proposed by Lande and Arnold (1983). The selection differential (S) estimates the total expected phenotypic change, including direct and indirect selection through correlated characters. It is calculated as a covariance of each character and fitness, $S_I = \text{COV}(z_I, w)$, where z_I is the character “I”, and w is relative fitness. The statistical significance of S_I values was obtained from Pearson’s correlation analysis. To estimate the strength and direction selection acting directly on characters, that is excluding indirect selection, we used the multivariate linear model of Lande and Arnold (1983),

$$w = \alpha + \sum_{i=1}^n \beta_I z_I + \varepsilon,$$

where w is relative fitness, α is a constant, β_I represents the average slope of the selection surface on the character z_I , and ε is an error term. The linear gradient of selection, β , provides information on the direction and magnitude of change expected after selection. Nonlinear selection describes the nature of selection acting on the quadratic

deviations from mean individual characters and the relationships between combinations of characters. Nonlinear gradients were estimated from the second order coefficients,

$$w = \alpha + \sum_{i=1}^n \beta_I z_I + 1/2 + \sum_{i=1}^n \gamma_{ii} z_I^2 + \sum_{i=1}^n \sum_{i \neq j} \gamma_{z_I z_j} z_I z_j + \varepsilon,$$

where γ_{ii} represents the curvature of the relationship between character “I” and relative fitness. When $\gamma_{ii} < 0$ (downward concavity), stabilizing selection is acting on a given character, and when $\gamma_{ii} > 0$ (upward concavity) the character “I” is under the action of disruptive selection. The directional selection gradients (β) were estimated only from the multivariate linear model because if characters are multivariate nonnormally distributed, z_I and z_I^2 in the quadratic model are intercorrelated (Lande and Arnold 1983). Each character was standardized to zero mean and unit variance before analysis. This transformation allows phenotypic change to be expressed in terms of standard deviation units, therefore enabling comparison between populations. The standardized differential of selection was described as S' and the linear and quadratic standardized gradients as β' and γ' , respectively. The statistical significance of linear and nonlinear selection gradients was obtained directly from the significance of regression coefficients. In order to reduce potential type-I statistical error, we performed a sequential Bonferroni adjustment with a tablewise P value = 0.05 (Rice 1989). To compare selection coefficients between populations, we performed analysis of covariance (ANCOVA) for characters showing statistical significance. Linear and nonlinear relationships and their respective interactions were included in the same ANCOVA model.

Results

Pollinator assemblages and visitation rate

There was a 12.5% increase in the number of pollinator species in the *Pinus* plantation relative to the native habitat. However, habitats did not differ in terms of the number of species in the insect orders responsible for the pollination of *V. portalesia* (G test, $G = 4.01$, $df = 3$, $P = 0.279$). Globally, in terms of species contribution to the total assemblage, Hymenoptera (44%, 7 species) and Diptera (38%, 6 species) were the most important orders in the native habitat, and Hymenoptera (39%, 7 species) and Coleoptera (28%, 5 species) in the transformed habitat (Fig. 2, see the list of species in Appendix 2 of the **ESM**). Rarefaction analysis and exponential fitting indicated that the expected number of species was 19.4 and 19.1 in the

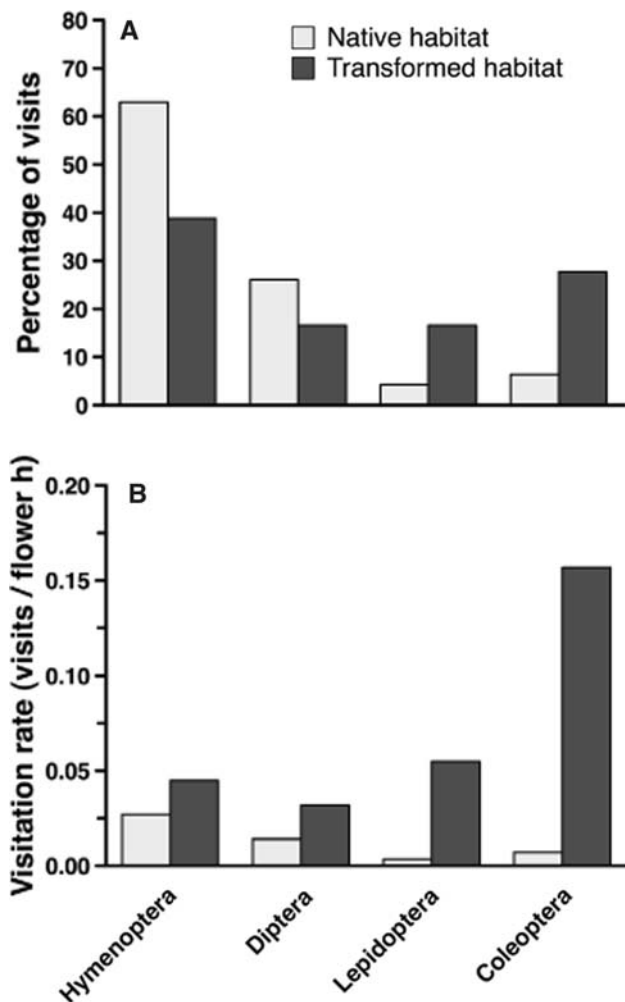


Fig. 2 **a** Composition of pollinator species assemblages in native and transformed habitats. The percentage of the total assemblage that is accounted by different insect orders is indicated. **b** Mean visitation rate of insect orders in native and transformed habitats. Values per order are the grand mean of species visitation rates

native and transformed habitats, respectively. Consequently, our measurements of pollinator species richness may have underestimated the number of species in the native habitat and transformed habitat at the most by 17.5% (3 species) and 5.8% (1 species), respectively. In terms of visitation rate (given as the mean \pm SE), there was a lower overall visitation rate in the native than transformed habitat (native 0.02 ± 0.01 , *Pinus* 0.08 ± 0.03 ; *t* test, $t_{1,28} = 2.75$, $P = 0.010$; Fig. 2b), indicating that flowers inhabiting the transformed habitat had a higher chance of becoming visited. In the native habitat, the bee *Manuelia gayatina* was the pollinator with the highest visitation rate (0.11 ± 0.03 visits flower⁻¹ h⁻¹) (see Appendix 2 of the [ESM](#)). In the transformed habitat, the beetle *Arthrobrachus nigromaculatus* had the highest visitation rate (0.69 ± 0.14 visits flower⁻¹ h⁻¹). Of the 30 insect species, four were shared

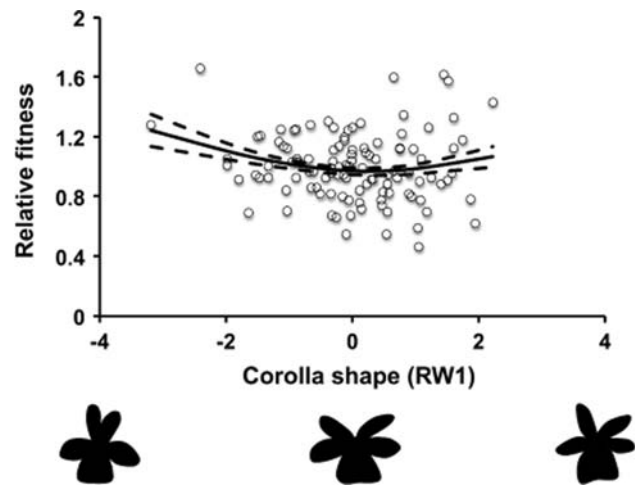


Fig. 3 Cubic spline estimate for corolla shape of *V. portalesia* in the *Pinus* habitat. Dashed lines represent ± 1 SE estimates from 5,000 bootstrap replications. Depicted outlines along the x-axis are mean corolla reconstructions for the lower, medium, and upper 33th percentile of data along the RW1 score distribution

between habitats, which in combination with visitation rate figures rendered a low proportional similarity index between assemblages (0.07 ± 0.08 ; see Schemske and Brokaw 1981).

Pollinator-mediated selection

The opportunity for selection (*I*) did not differ between habitats ($I_{\text{native}} = 0.065$, $I_{\text{Pinus}} = 0.052$, $F_{1,221}$ Bartlett' test = 1.44, $P = 0.229$, $F_{1,221}$ Levene's test = 1.85, $P = 0.175$), indicating that populations have a similar chance to evolve under pollinator-mediated selection. We found significant coefficients for total selection in native and *Pinus* habitats. However, while flower number was the only flower character showing statistical significance in the native habitat, flower length and width were significant in the *Pinus* habitat (Table 1). When traits were analyzed for direct selection, that is, removing the effect of selection through correlated characters, all significant coefficients disappeared, indicating that pollinator-mediated selection acting directly on floral traits was unimportant in the two populations of *V. portalesia* (Table 2; Fig. 3). Interestingly, however, a significant disruptive selection on the first descriptor of flower shape (RW1) was detected in the *Pinus* habitat, indicating the emergence of a selection surface undetected in the pristine habitat (Table 2). Because the aim of this study is to evaluate potential differences in the selection regime between habitats, we performed a comparison of selection coefficients in ANCOVA. The results revealed an important habitat effect on seed production (Table 3), which is consistent with the higher seed production observed in the *Pinus* habitat. Not

Table 1 Plant and floral traits in native and transformed habitats

| Trait | Native | Transformed | <i>t</i> |
|---------------------|--------------|--------------|----------|
| Plant height (cm) | 57.32 (2.07) | 54.19 (1.9) | 1.05 |
| Flower number | 8.77 (0.46) | 10.11 (0.6) | 1.67 |
| Corolla length (cm) | 1.12 (0.01) | 1.35 (0.02) | 41.23*** |
| Corolla width (cm) | 2.13 (0.03) | 2.46 (0.03) | 25.69*** |
| Seeds per fruit | 16.02 (0.39) | 23.74 (0.51) | 11.96*** |

*** $P < 0.001$

Values are given as the mean with the standard error (SE) given in parenthesis. Degrees of freedom for Student's *t* tests are 1,221 in all comparisons. All significant characters retained significance after Bonferroni adjustment

all flower characters under significant selection within habitats showed a different pattern of selection between habitats. For example, selection on flower length and flower width did not differ between native and *Pinus* habitats (Table 3). On the contrary, selection on flower number differed significantly between habitats. Plants with large inflorescences produced fewer seeds per flower than plants with small inflorescences in the National Reserve but not in the transformed habitat, suggesting the presence of an inflorescence size–seed number tradeoff in the pristine habitat only. Likewise, flower shape was under disruptive selection only in *Pinus* (Table 2), and overall selection acting upon RW1 differed between the two environments (Table 3), indicating that significant trait covariation between flower shape and fitness may arise in disturbed habitats where new selective scenarios may occur. Figure 3 indicates that flowers inhabiting the *Pinus* habitat exhibit important variation along the shape axis.

While flowers located at the left of the mean shape show a displacement of the lateral petals toward the lower region and a concomitant movement of the upper petals toward the main symmetry axis, flowers at the right of the mean shape are characterized by an overall loss of corolla symmetry.

Discussion

We have examined the extent to which human-induced habitat modification translates into variation in pollinator-mediated selection on the flower phenotype of the herb *V. portalesia*. To this end, we evaluated the composition of pollinator assemblages, the visitation rate experienced by *V. portalesia*, and the flower characters under pollinator-mediated selection in two adjacent populations that inhabit contrasting habitats. Our results indicate that selection differed in some—but not all—floral characters between habitats. While significant selection differentials were found in the two habitats (Table 2), populations showed a relatively idiosyncratic pattern of selection. For example, selection for flower number was important in the native habitat only, which rendered a significant habitat \times character interaction effect in ANCOVA (Table 3). On the contrary, flower shape was under disruptive selection in *Pinus* but not in the native habitat, where no consistent pattern for this trait was found (Tables 2, 3). These results suggest that while habitat degradation may relax selection on some traits, novel selection patterns, not found in native habitats, can be produced de novo under new habitat conditions.

Table 2 Pollinator-mediated selection coefficients in populations of *Viola portalesia* inhabiting native and *Pinus radiata* (transformed) habitats

| Trait <i>I</i> | Population | S'_I | β'_I | γ'_{ii} |
|----------------|-------------|------------------------|---------------|-----------------------------|
| Plant height | Native | 0.060 | 0.034 (0.03) | −0.004 (0.02) |
| | Transformed | 0.013 | 0.033 (0.03) | −0.015 (0.02) |
| Flower number | Native | −0.317*** ^a | −0.070 (0.04) | −0.0002 (0.01) |
| | Transformed | 0.007 | 0.027 (0.03) | −0.025 (0.02) |
| Flower length | Native | 0.212* | 0.042 (0.03) | −0.024 (0.04) |
| | Transformed | 0.274** ^a | 0.052 (0.04) | −0.051 (0.03) |
| Flower width | Native | 0.227* | 0.011 (0.04) | −0.018 (0.03) |
| | Transformed | 0.287** ^a | 0.006 (0.03) | −0.024 (0.02) |
| Shape 1 (RW1) | Native | 0.215* | 0.043 (0.03) | −0.026 (0.02) |
| | Transformed | −0.088 | −0.013 (0.02) | 0.052 (0.02)** ^a |
| Shape 2 (RW2) | Native | −0.142 | −0.035 (0.02) | 0.013 (0.02) |
| | Transformed | −0.032 | 0.018 (0.02) | −0.021 (0.02) |

* $P < 0.05$; ** $P < 0.01$

S'_I Standardized selection differential; β'_I , γ'_{ii} , standardized directional and stabilizing/disruptive selection gradients, respectively

Values in parenthesis are 1 SE

^a Coefficients that retained statistical significance after Bonferroni adjustment on an habitat basis

Table 3 Analysis of covariance of character impact on female fitness (seed production) in *V. portalesia*

| Source | df | SS | F |
|--|-----|-------|-----------------------|
| Habitat (H) | 1 | 4.988 | 92.88*** ^a |
| Flower number (F) | 1 | 0.339 | 6.32* |
| Flower length (L) | 1 | 0.188 | 3.51 |
| Flower width (W) | 1 | 0.143 | 2.67 |
| Relative warp 1 ² (RW1 ²) | 1 | 0.019 | 0.36 |
| H × F | 1 | 0.454 | 8.46*** ^a |
| H × L | 1 | 0.009 | 0.16 |
| H × W | 1 | 0.012 | 0.22 |
| H × RW1 ² | 1 | 0.513 | 9.56*** ^a |
| Error | 213 | | |

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$

Degrees of freedom (df), sum of squares (SS), and F values are listed. Only characters showing a significant selection coefficient in any habitat type were included in the analyses

^a Source of variation that retained statistical significance after Bonferroni adjustment

As in every comparative study of phenotypic selection, unraveling the causes of the differences in selection between sites is an important challenge. It has been shown that selection often varies between different locally adapted populations as a consequence of different biotic and abiotic environmental factors (e.g., Campbell et al. 1997; Totland 2001; Caruso et al. 2003; Anderson and Johnson 2008). Three non-mutually exclusive factors may account, at least in part, for the observed selection pattern.

First, it is likely that differences in the community context between habitats account for variation in the selection regime (see Totland 2001). Variation in the resource base for pollinators between habitats may have modulated the importance of floral traits involved in pollinator attraction. For example, the almost threefold higher species richness of flowering plants found in the native habitat than in the *Pinus* habitat (161 and 66 species, respectively) may have represented a higher resource basis for pollinator species, resulting in a lower visitation rate and lower seed production per fruit in the native habitat (Table 1). This suggests that traits increasing pollinator attraction may have been under strong selection mostly in the native habitat. One of these traits is flower number. It has been extensively reported that large flower number increases pollinator attraction (e.g., Wyatt 1982; Eckhart 1991; Vaughton and Ramsey 1998; Mitchell et al. 2004), which in turn often results in significant directional selection for large floral displays within populations (e.g., Maad and Alexandersson 2004). In this study, directional selection on flower number was observed only in the native

habitat (Tables 2, 3), which is consistent with the concept of context-dependent selection.

Second, several studies have evaluated whether changes in pollinator assemblages are associated with concomitant changes in flower morphology (e.g., Mitchell 1994; Sazima et al. 1999; Nattero and Cocucci 2007), odor (e.g., Ackerman et al. 1997), pollen and nectar availability (e.g., Stone et al. 1998), phenology (e.g., Utelli and Roy 2000), and color (e.g., Miller 1981; Medel et al. 2007). Comparative studies of phenotypic selection performed on different populations have shown variable results as populations face variation in the species composition of pollinator assemblages (e.g., Campbell et al. 1997; Totland 2001; Caruso et al. 2003; Anderson and Johnson 2008). In our study, despite the 2.3 km proximity between populations, the two habitats differed conspicuously in terms of the composition, visitation rate, and species dominance of pollinator assemblages. Only four of 30 pollinator species were shared between habitats, which together with the low 7% proportional similarity suggest that variation in the composition of pollinator assemblages may have been a relevant factor that shifted the targets of selection between populations. Even though these differences are quite significant, the sampling intensity in the two habitats was not equivalent, being lower in the *Pinus* habitat than in the native habitat (native habitat 47.5 h, transformed habitat 13.25 h). The severe and constant human activity in the *Pinus* plantation prevented us from performing a similar sampling in the two sites, probably leading to an underestimation of the richness of the pollinator species in the transformed habitat. Regardless of differences in sampling effort, the proportional representation of hymenopteran and coleopteran species as flower visitors differed substantially between habitats (Fig. 2). While hymenopterans reduced their visits from 63% in the native habitat to 12% in the *Pinus* habitat, coleopterans showed the reverse pattern, increasing their visits from 7% in the native habitat to 47% in the *Pinus* habitat. Whether this alternative preference translated into the disruptive pattern observed in the *Pinus* habitat is a challenging question. One possibility is that different pollinators differ in their flower shape preferences (see also Medel et al. 2003; Gómez et al. 2006). If coleoptera and diptera visit flowers with different flower shapes, this trait may have been pivotal in determining, at least in part, the selection pattern found in the transformed habitat. While some investigations have reported that dipterous species often use large floral displays as clues that influence the foraging decisions (see review in Weiss 2001), little is known about the flower characters preferred by coleopterans. However, what little information is available indicates that flower size, shape, and symmetry influence landing behavior and visitation rate in *Amphicomma* beetles (Dafni 1997; Dafni and Potts 2004). The

extent to which changes in pollinator composition between habitats is associated with a variable preference for different flower shape phenotypes needs to be assessed in future studies. Studies that identify the flower shape variants preferred by the different pollinator species will be of particular value in this context (e.g., Medel et al. 2003).

Third, it is likely that abiotic conditions external to the plant–pollinator relationship cause a significant covariance between flower characters and fitness in ways unrelated to the pollination process. This possibility was first described by Rausher (1992) to indicate that unless environmental covariance is explicitly taken into account in Lande and Arnold's equations, estimates of selection could be severely biased (see also Scheiner et al. 2002; Stinchcombe et al. 2002; Kruuk et al. 2003). In this case, because the two sites are segregated along an elevation axis by 22 m, it is unlikely that any difference in vegetation and pollinator composition results from elevation differences rather than from human disturbance. Even though we cannot rule out this possibility, the two sites did not differ in temperature [mean \pm SE: native $13.5 \pm 0.14^\circ\text{C}$; *Pinus* $13.3 \pm 0.18^\circ\text{C}$, $t_{1,215} = 0.03$, $P = 0.97$] and humidity at the ground level (native $9.8 \pm 0.8\%$, *Pinus* $11.1 \pm 2.2\%$, $t_{1,83} = 0.54$, $P = 0.59$), which suggests that these abiotic variables are not important in determining the different selection patterns in the two habitats. Notwithstanding, other abiotic factors not measured in this study, such as soil chemistry (Rivas et al. 2009) and light availability, among others, cannot be ruled out as factors that influence the trait–fitness covariation between habitats.

Human-induced habitat change and replacement of the native vegetation by *P. radiata* is the most plausible ultimate explanation for the observed variation in pollinator assemblages and pollinator-mediated selection. Several studies have reported that human-induced habitat change may modify the dominance and composition of species assemblages (e.g., Vinson et al. 1993; Ghazoul and Mcleish 2001; Potts et al. 2001; Burgess et al. 2006) and plant reproductive success (e.g., Aizen and Feinsinger 2003). For example, studies performed in the Argentinean Chaco revealed that habitat fragmentation substantially reduced the diversity and abundance of native pollinator species, which in turn has seemingly permitted the increase in abundance and dominance of the exotic bee *Apis mellifera* in forest fragments (Aizen and Feinsinger 1994). Likewise, Steffan-Dewenter and Tscharntke (1999) observed that farmer activities in Germany increased fragmentation and native habitat loss, hence reducing bee species diversity and pollination service for native flowering plants. In this study, the gradual replacement of the original habitat by *P. radiata* is a cumulative process that has occurred through decades (Bustamante and Castor 1998). Currently, the modified scenario has substantially influenced the structure

of plant–pollinator interaction networks through changes in the main interacting species across the landscape (Medel, unpublished data), probably as a direct consequence of habitat change on plant biomass, light availability, diurnal temperature, and evapotranspiration (e.g., Didham et al. 1996; Ghazoul and Mcleish 2001; Potts et al. 2001; Aizen and Feinsinger 2003). Our results indicate that human-induced habitat change may substantially modify the composition of pollinator assemblages, their pattern of flower visitation and, therefore, their selective impact on flower characters. The extent to which human-induced habitat change influences the evolutionary pathways of native plant populations is a phenomenon that deserves more attention in the literature, especially in a context of global change where evolutionary changes are presumably more rapid than previously thought (Thompson 1998, 2005; Hairston et al. 2005).

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References

- Ackerman JD, Melendez-Ackerman EJ, Sagero-Farias J (1997) Variation in pollinator abundance and selection on fragrance phenotypes in an epiphytic orchid. *Am J Bot* 84:1383–1390
- Aizen M, Feinsinger P (1994) Habitat fragmentation, native insect pollinators, and feral honeybees in Argentine “Chaco Serrano”. *Ecol Appl* 4:378–392
- Aizen M, Feinsinger P (2003) Bees not to be? responses of insect pollinator faunas and flower pollination to habitat fragmentation. In: Bradshaw GA, Marquet PA (eds) How landscapes change: human disturbance and ecosystem fragmentation in the Americas. Springer, Berlin, pp 111–129
- Ambruster WS, Antonsen L, Pelabon C (2005) Phenotypic selection on *Dalechampia* blossoms: honest signaling affects pollination success. *Ecology* 86:3323–3333
- Anderson B, Johnson SD (2008) The geographical mosaic of coevolution in a plant–pollinator mutualism. *Evolution* 62:220–225
- Burgess VJ, Kelly D, Robertson AW, Ladley JJ (2006) Positive effects of forest edges on plant reproduction: literature review and a case study of bee visitation to flowers of *Peraxilla tetrapetala* (Loranthaceae). *N Z J Ecol* 30:179–190
- Bustamante R, Castor C (1998) The decline of an endangered temperate ecosystem: the rui (*Nothofagus alessandrii*) forest in central Chile. *Biodivers Conserv* 7:1607–1626
- Bustamante R, Grez A, Simonetti J (2006) Efectos de la fragmentación del bosque Maulino sobre la abundancia y diversidad de especies nativas. In: Grez A, Simonetti J, Bustamante R (eds) Biodiversidad en ambientes fragmentos de Chile: patrones y procesos a diferentes escalas. Editorial Universitaria, Santiago, pp 83–98
- Campbell D (1991) Effects of floral traits on sequential components of fitness in *Ipomopsis aggregata*. *Am Nat* 137:713–737

- Campbell D, Waser N, Price M (1996) Mechanisms of hummingbird-mediated selection for flower width in *Ipomopsis aggregata*. *Ecology* 77:1463–1472
- Campbell D, Waser M, Price M, Lynch E, Mitchell R (1997) Analysing pollinator-mediated selection in a plant hybrid zone: hummingbird visitation pattern on three spatial scale. *Am Nat* 149:295–315
- Caruso CM, Peterson B, Ridley C (2003) Natural selection on floral traits of *Lobelia* (Lobeliaceae): spatial and temporal variation. *Am J Bot* 90:1333–1340
- Conner J, Rush S, Jennetten P (1996) Measurements of natural selection on floral traits in wild radish (*Raphanus raphanistrum*). I. Selection through lifetime female fitness. *Evolution* 50:1127–1136
- Dafni A (1997) The response of *Amphicoma* spp. (Coleoptera, Glaphyridae) beetles to red models differing in area, shape and symmetry. *Isr J Plant Sci* 45:247–254
- Dafni A, Potts SG (2004) The role of flower inclination, depth, and height in the preferences of a pollinating beetle (Coleoptera: Glaphyridae). *J Insect Behav* 17:823–834
- Di Castri F, Hajek ER (1976) Bioclimatología de Chile. Ediciones de la Universidad Católica de Chile, Santiago, p 128
- Didham R, Ghazoul J, Stork N, Davis A (1996) Insects in fragmented forests: a functional approach. *Trends Ecol Evol* 11:255–260
- Eckhart VM (1991) The effects of floral display on pollinator visitation vary among populations of *Phacelia linearis* (Hydrophyllaceae). *Evol Ecol* 5:370–384
- Ghazoul J, Mcleish M (2001) Reproductive ecology of tropical forest trees in logged and fragmented habitats in Thailand and Costa Rica. *Plant Ecol* 153:335–345
- Gómez J (1993) Phenotypic selection on flowering synchrony in a high mountain plant *Hormathophylla spinosa* (Cruciferae). *J Ecol* 81:605–613
- Gómez JM (2003) Herbivory reduces the strength of pollinator-mediated selection in the Mediterranean herb *Erysimum mediohispanicum*: consequences for plant specialization. *Am Nat* 162:242–256
- Gómez JM, Zamora R (2000) Spatial variation in the selective scenarios of *Hormathophylla spinosa* (Cruciferae). *Am Nat* 155:657–668
- Gómez JM, Perfectti F, Camacho JP (2006) Natural selection of *Erysimum mediohispanicum* flower shape: insights into the evolution of zygomorphy. *Am Nat* 168:531–545
- Hairston NS, Ellner M, Geber T, Fox YJ (2005) Rapid evolution and the convergence of ecological and evolutionary time. *Ecol Lett* 8:1114–1127
- Herrera C (1993) Selection on floral morphology and environmental determinants of fecundity in a hawkmoth-pollinated violet. *Ecol Monogr* 63:251–275
- Herrera CM, Castellanos MC, Medrano M (2006) Geographical context of floral evolution: towards an improved research programme in floral diversification. In: Harder LD, Barrett CH (eds) *Ecology and evolution of flowers*. Oxford University Press, Oxford, pp 278–294
- Hodges S, Arnold M (1994) Floral and ecological isolation between *Aquilegia formosa* and *Aquilegia pubescens*. *Proc Natl Acad Sci USA* 91:2493–2496
- Jennersten O (1988) Pollination in *Dianthus deltooides* (Caryophyllaceae): effects of habitat fragmentation on visitation and seed set. *Conserv Biol* 2:359–366
- Johnston R (1991) Pollen limitation of female reproduction in *Lobelia cardinalis* and *L. siphilitica*. *Ecology* 72:1500–1503
- Kearns CA, Inouye DW, Waser NM (1998) Endangered mutualisms: the conservation of plant–pollinator interactions. *Annu Rev Ecol Syst* 29:83–112
- Kruuk LE, Merila J, Sheldon BC (2003) When environmental variation short-circuits natural selection. *Trends Ecol Evol* 18:207–209
- Lande R, Arnold S (1983) The measurement of selection on correlated characters. *Evolution* 37:1210–1226
- Maad J (2000) Phenotypic selection in hawkmoth-pollinated *Platanthera bifolia*: targets and fitness surfaces. *Evolution* 54:112–113
- Maad J, Alexandersson R (2004) Variable selection in *Platanthera bifolia* (Orchidaceae): phenotypic selection differed between sex functions in a drought year. *J Evol Biol* 17:642–650
- Medel R, Botto-Mahan C, Kalin-Arroyo M (2003) Pollinator-mediated selection on the nectar guide phenotype in the Andean monkey flower, *Mimulus luteus*. *Ecology* 84:1721–1732
- Medel R, Valiente A, Botto-Mahan C, Carvallo G, Pérez F, Pohl N, Navarro L (2007) The influence of insects and hummingbirds on the geographical variation of the flower phenotype in *Mimulus luteus*. *Ecography* 30:812–818
- Miller RB (1981) Hawkmoths and the geographic patterns of floral variation in *Aquilegia caerulea*. *Evolution* 35:763–774
- Mitchell RJ (1994) Effects of floral traits, pollinator visitation, and plant size on *Ipomopsis aggregata* fruit production. *Am Nat* 143:870–889
- Mitchell RJ, Karron JD, Holmquist KG, Bell JM (2004) The influence of *Mimulus ringens* floral display size on pollinator visitation patterns. *Funct Ecol* 18:116–124
- Nagy ES (1997) Selection for native characters in hybrids between two locally adapted plant subspecies. *Evolution* 51:1469–1480
- Nattero J, Cocucci A (2007) Geographical variation in floral traits of the tree tobacco in relation to its hummingbird pollinator fauna. *Biol J Linn Soc* 90:657–667
- Potts S, Dafni A, Ne'eman G (2001) Pollination of a core flowering shrub species in Mediterranean phrygana: variation in pollinator diversity, abundance and effectiveness in response to fire. *Oikos* 92:71–80
- Rathcke BJ (1983) Competition and facilitation among plants for pollination. In: Real L (ed) *Pollination biology*. Academic Press, New York, pp 305–329
- Rausher M (1992) The measurement of selection on quantitative traits: biases due to environmental covariance between traits and fitness. *Evolution* 46:616–626
- Rice W (1989) Analyzing tables of statistical tests. *Evolution* 43:223–225
- Rivas Y, Oyarzún C, Godoy R, Valenzuela E (2009) Mineralización del nitrógeno, carbono y actividad enzimática del suelo en el bosque de *Nothofagus obliqua* (Mirb) Oerst y una plantación de *Pinus radiata* D. Don del centro-sur de Chile. *Rev Chil Hist Nat* 82:119–134
- San Martín J, Donoso C (1996) Estructura florística e impacto antrópico en el bosque Maulino de Chile. In: Armesto J, Villagran C, Arroyo M (eds) *Ecología de los bosques nativos de Chile*. Editorial Universitaria, Santiago, pp 153–168
- Saunders D, Hobbs R, Margules C (1991) Biological consequences of ecosystem fragmentation: a review. *Conserv Biol* 5:18–32
- Sazima M, Buzato S, Sazima I (1999) Bat-pollinated flower assemblages and bat visitors at two Atlantic Forest sites in Brazil. *Annu Bot* 83:705–712
- Scheiner SM, Donohue K, Dorn LA, Mazer SJ, Wolfe LM (2002) Reducing environmental bias when measuring natural selection. *Evolution* 56:2156–2167
- Schemske D, Brokaw N (1981) Treefalls and the distribution of understory birds in a tropical forest. *Ecology* 62:938–945
- Simonetti J, Grez A, Bustamante R (2006) Interacciones y procesos en el bosque Maulino fragmentado. In: Grez A, Simonetti J, Bustamante R (eds) *Biodiversidad en ambientes fragmentados de Chile: patrones y procesos a diferentes escalas*. Editorial Universitaria, Santiago, pp 83–98

- Steffan-Dewenter I, Tschardt T (1999) Effects of habitat isolation on pollinator communities and seed set. *Oecologia* 121:432–440
- Stinchcombe JR, Rutter MT, Burdick DS, Tiffin P, Rausher MD, Rodney M (2002) Testing for environmentally induced bias in phenotypic estimates of natural selection: theory and practice. *Am Nat* 160:511–523
- Stone GN, Willmer P, Rowe JA (1998) Partitioning of pollinators during flowering in an African *Acacia* community. *Ecology* 79:2808–2827
- Thompson JN (1998) Rapid evolution as an ecological process. *Trends Ecol Evol* 13:329–332
- Thompson JN (2005) The geographic mosaic of coevolution. University of Chicago Press, Chicago, p 439
- Totland O (2001) Environment-dependent pollen limitation and selection on floral traits in an alpine species. *Ecology* 82:2233–2244
- Utelli AB, Roy BA (2000) Pollinator abundance and behaviour on *Aconitum lycoctonum* (Ranunculaceae): an analysis of the quantity and quality components of pollination. *Oikos* 89:461–470
- Vaughton G, Ramsey M (1998) Floral display, pollinator visitation and reproductive success in the dioecious perennial herb *Wurmbea dioica* (Liliaceae). *Oecologia* 115:93–101
- Vinson SB, Frankie GW, Barthell J (1993) Threats to the diversity of solitary bees in a neotropical dry forest in Central America. In: LaSalle J, Gauld ID (eds) Hymenoptera and biodiversity. CAB International, Wallingford, pp 53–81
- Waser NM, Campbell DR (2004) Ecological speciation in flowering plants. In: Dieckmann U, Metz H, Doebeli M, Tautz D (eds) Adaptive speciation. Cambridge University Press, Cambridge, pp 264–277
- Weiss M (2001) Vision and learning in some neglected pollinators: beetles, flies, moths, and butterflies. In: Chittka L, Thomson J (eds) Cognitive ecology of pollination: animal behavior and floral evolution. Cambridge University Press, Cambridge, pp 171–190
- Wyatt R (1982) Inflorescence architecture: How flower number, arrangement, and phenology affect pollination and fruit-set. *Am J Bot* 69:585–594
- Zelditch M, Swiderski D, Sheets H, Fink W (2004) Geometric morphometrics for biologists. Elsevier Academic Press, Amsterdam, p 443