Desintegración fenotípica y relajo selectivo mediado por cambios en polinizadores y herbívoros sobre la enredadera *Bomarea salsilla* en ambientes fragmentados

Tesis
entregada a la
Universidad de Chile
en cumplimiento parcial de los requisitos
para optar al grado de

Doctor en Ciencias con Mención en Ecología y Biología Evolutiva

Facultad de Ciencias

Por

Carlos Eduardo Valdivia Prats

Agosto, 2008

Director de Tesis
Dr. Javier A. Simonetti

AGRADECIMIENTOS

Quiero expresar mi gratitud a todos aquellos que de una u otra forma contribuyeron a que yo pudiese llegar a esta instancia. En primer lugar vayan mis agradecimientos a mi familia, especialmente a mis padres y hermanas, quienes supieron enseñarme y estimular el amor por la naturaleza y con ello darme una vocación. Su irrestricto apoyo no solo ha sido el aliciente, sino también el gran sostén emocional en el cual tantas veces me he cobijado. De igual manera quiero agradecer el incondicional apoyo recibido por parte de mis amigos, quienes entre tantas risas y uno que otro llanto y, por que no decirlo, entre una y otra cerveza, me han brindado el más cálido de los apoyos en todo el amplio sentido de la palabra. Finalmente, quiero agradecer a las personas que han sido mis maestros por haberme dado las herramientas para observar y fascinarme con el maravilloso mundo natural y sus muchos secretos. Especialmente quiero agradecer a Javier, mi tutor, por la infinita paciencia y confianza que me ha dado en estos ya varios años. Ciertamente, el apoyo brindado no se restringe solo al ámbito académico, en cuyo contexto me ha enseñado la responsabilidad social de nuestro quehacer, sino que también abarca los muchos y variados aspectos del ámbito personal. En forma especial quiero agradecer también a Alejandra Bahamondez y Álvaro Rivera quienes, aunque de maneras distintas, colaboraron arduamente en la ejecución del presente trabajo de tesis. Sin su generoso apoyo las dificultades habrían sido bastante mayores.

Quiero señalar que deliberadamente omití mencionar más personas ya que la lista sería demasiado extensa y probablemente injusta en la mayoría de los casos. Espero así honrar el incondicional apoyo recibido en estos años por tantas y tan distintas personas quienes, con su compañía, han contribuido generosamente en mi crecimiento profesional y personal.

Muchas gracias a todos.

INDICE DE MATERIAS

Capítulo III:

Capítulo IV:

LISTA DE TABLAS

Capítulo I:
Herbivory release does not offset pollinator-mediated decreased reproductive success of *Bomarea salsilla* in a fragmented forest

Capítulo II:
Herbivore-independent pollinator-mediated evolutionary potential of *Bomarea salsilla* in a fragmented forest

Capítulo III:
Pollinator-mediated phenotypic selection on reproductive and vegetative traits in a fragmented forest: an inquiry on the microevolutionary present of *Bomarea salsilla*

Capítulo IV:
Phenotypic integration between reproductive and vegetative traits: an inquiry on the microevolutionary past of *Bomarea salsilla* in a fragmented forest

LISTA DE FIGURAS

Antecedentes generales

Capítulo I:
Herbivory release does not offset pollinator-mediated decreased reproductive success of *Bomarea salsilla* in a fragmented forest

Capítulo II: Herbivore-independent pollinator-mediated evolutionary potential of *Bomarea salsilla* in a fragmented forest

Capítulo III: Pollinator-mediated phenotypic selection on reproductive and vegetative traits in a fragmented forest: an inquiry on the microevolutionary present of *Bomarea salsilla*

Capítulo IV:

Phenotypic integration between reproductive and vegetative traits: an inquiry on the microevolutionary past of *Bomarea salsilla* in a fragmented forest

Conclusión general

RESUMEN

El alcance que tienen las modificaciones de las interacciones planta-animal debido a la fragmentación del hábitat es de importancia crucial en biología de la conservación. La reducción del tamaño del hábitat e incremento en el aislamiento en los fragmentos remanentes acoplado a la creación de hábitat de borde puede reducir la diversidad y abundancia de las especies interactuantes. Reducciones en la diversidad y abundancia de los animales pueden conducir a efectos contrastantes sobre las plantas con las cuales interactúan dependiendo si ellas afectan a mutualistas (*e.g.*, polinizadores) o antagonistas (*e.g.*, herbívoros). Acoplado a una disminución en la diversidad y abundancia de polinizadores hay usualmente una disminución en la frecuencia de visitas de éstos a las flores y, consecuentemente, una disminución en el éxito reproductivo de las plantas. No obstante, cuando los herbívoros también son escasos en los fragmentos y bordes, una suerte de compenación puede expresarse en el éxito reproductivo de las plantas dependiendo de la fuerza de las modificaciones de los mutualismos y antagonismos (*i.e.*, la simetría de las relaciones).

Actualmente, las evaluaciones de los efectos de la fragmentación del hábitat sobre la reproducción de las plantas han estado mayormente orientadas a develar los procesos demográficos más que aquellos evolutivos. Modificaciones en las interacciones planta-mutualista y planta-antagonista dado la fragmentación del hábitat pueden tener consecuencias de mucho más largo alcance que sólo aquellas usualmente invocadas en términos de la persistencia poblacional al afectar, por ejemplo, las dinámicas microevolutivas. En fragmentos y bordes, la reducción en las interacciones planta-polinizador y planta-herbívoro puede probablemente reducir la

cantidad y magnitud de rasgos enfrentando selección fenotípica mediada por polinizadores y herbívoros. Por lo tanto, no es raro esperar que tales reducciones se traduscan en desacoplamientos fenotípicos en los rasgos ligados a los polinizadores y herbívoros (*i.e.*, rasgos reproductivos y vegetativos).

En la Reserva Nacional Los Queules y sitios aledaños evalue los efectos de la fragmentación del bosque y la creación de hábitats de borde sobre las dinámicas microevolutivas de *Bomarea salsilla*, una enredadera que habita los bosques altamente fragmentados del centro y sur de Chile. Para ello, contraste la condición de centro de bosque continuo (hábitat prístino) con las condiciones de borde de bosque continuo, centro de fragmento y borde de fragmento (habitats perturbados). Primero, evalue el alcance al cual la fragmentación y los bordes afectaron las interacciones planta-polinizador y planta-herbívoro con el propósito de develar las modificaciones en la simetría de ambas relaciones (ver Capítulo I). Segundo, evalue el alcance al cual los polinizadores y herbívoros son capaces de ejercer presiones de selección correlacional sobre *B. salsilla* modificando directamente ambos tipos de interacciones, y como estas interacciones son modificadas por la fragmentación del hábitat (ver Capítulo II). Tercero, evalue los efectos de la fragmentación y los bordes sobre la selección fenotípica mediada por polinizadores que es ejercida sobre un conjunto de rasgos reprodutivos y vegetativos (*i.e.*, por un lado: número de flores, largo de las flores, largos de los rayos florales y complejidad de la inflorescencia; por el otro, número de hojas, área foliar, tamaño de la planta y resistencia a la herbivoría) (ver Capítulo III). Finalmente, evalue los efectos de la fragmentación del bosque y la creación de hábitat de borde sobre la magnitud y patrones de integración fenotípica de los rasgos reproductivos y vegetativos de *B. salsilla* (ver Capítulo IV).

La fragmentación y la creación de hábitat de borde afectaron negativamente las interacciones planta-polinizador y planta-herbívoro y, consecuentemente, la producción de semillas en las plantas. La relación entre la polinización y la herbivoría fue asimétrica: los polinizadores ejercieron un impacto mucho mayor que los herbívoros sobre la adecuación de las plantas en todos los sitios estudiados. Consecuentemente, debido a ésta asimetría, los polinizadores y herbívoros no ejercieron presiones de selección correlacional en ningún sitio, indicando por tanto, que la evolución de *B. salsilla* esta mucho más fuertemente conducida por sus polinizadores. En los fragmentos y bordes la cantidad de rasgos, o combinaciones de éstos, bajo selección por parte de los polinizadores fue significativamente menor que en el centro del bosque continuo. Por el contrario, la fragmentación del bosque y la creación de hábitats de borde no redujieron la magnitud de los eventos selectivos. Finalmente, acoplado a la reducción en el número de eventos selectivos, los rasgos estuvieron más desintegrados en los fragmentos y bordes que en el centro del bosque continuo. Colectivamente considerados, los resultados aquí reportados indican que la fragmentación del bosque y la creación de hábitats de borde deterioraron fuertemente las dinámicas microevolutivas de *B. salsilla.* Por lo tanto, el presente trabajo enfatiza la capacidad de la fragmentación del hábitat mediada por las actividades humanas de deteriorar la evolución Darwiniana, peligrando de esta manera la persistencia en el largo plazo de la identidad de las especies y, quizas más importantemente, la futura persistencia de las poblaciones. Desarrollar estrategias de manejo evolutivamente informadas es, por lo tanto, un hecho de suma importancia para conservar la integridad de las especies y los procesos evolutivos en el más amplio sentido.

ABSTRACT

The extent to which plant-animal interactions are modified by habitat fragmentation is of paramount importance in conservation biology. The reduction in habitat size and increase in isolation in the remnant fragments coupled to the creation of edge habitats may reduce the diversity and abundance of interacting species. A reduction in animal interactors may lead to contrasting effects on their plant counterparts depending on whether they are mutualists (*e.g.*, pollinators) or antagonists (*e.g.*, herbivores). Coupled to a decrease in diversity and abundance of pollinators there is usually a decrease in the frequency of pollinator visits and, consequently, a decrease in the reproductive success of plants. When herbivores are also scarce in fragments and edges, however, a some sort of compensation may arise in the reproductive success of plants which depends on the strength of modifications of mutualisms and antagonisms (*i.e.*, the symmetry of relationships). Currently, assessements addressing the effects of habitat fragmentation have mostly been oriented to disclose demographic rather than evolutionary processess. Modifications in plant-mutualistic and plant-anatagonistic interactions given by habitat fragmentation may have far reaching consequences than those usually claimed in terms of population persistence by affecting, for instance, microevolutionary dynamics. In fragments and edges, the reductions in plant-pollinator and plant-herbivore interactions most likely would lead to reductions in the amount and magnitude of traits, and combinations thereof, facing pollinator- and herbivore-mediated phenotypic selection. Such reductions are expected to be translated into decoupling of pollinator- and herbivore-linked traits (*i.e.*, reproductive and vegetative traits).

In Los Queules National Reserve and nearby sites I assessed the effects of forest fragmentation and creation of edge habitats on the microevolutionary dynamics of *Bomarea salsilla*, a vine occurring at the highly fragmented forests of south-central Chile. In order to achieve this goal I compared the core of a continuous forest (pristine habitat) with an edge of continuos forest, core of forest fragment and an edge of forest fragment (disturbed habitats). First, I evaluated the extent to which forest fragmentation and edges affected plant-pollinator and plant-herbivore interactions in order to disclose modifications on the symmetry of both relatioships (see Chapter I). Second, I assessed the extent to which pollinators and herbivore are capable of exerting correlational selection pressures on *B. salsilla* by directly modifying both types of interactions, and how these interactions are modified by forest fragmentation (see Chapter II). Third, I evaluated the effects of forest fragmentation and edges on pollinator-mediated phenotypic selection exerted on reproductive and vegetative traits (*i.e.,* on one side: flower number, flower length, ray length, inflorescence complexity; on the other: leaf number, foliar area, plant height, and herbivory resistance) (see Chapter III). Finally, I evaluated the effects of forest fragmentation on the magnitude and patterns of phenotypic integration on such traits (see Chapter IV).

Forest fragmentation and the creation of edge habitats negatively affected plant-pollinator and plant-herbivore interactions and, consequently, plant reproduction. The relationship between pollination and herbivory was asymmetrical: pollinators had a much higher impact than herbivores on plant fitness at all sites studied. Consequently, due to this asymmetrical relationship, at all sites studied, pollinators and herbivores did not exert correlational selection pressures, thereby indicating that the evolution of *B. salsilla* is mostly conducted by pollinators. In fragments and edges the amount of traits and combinations thereof facing pollinator-mediated selection was significantly lower than at the core of continuous forest. By contrast, forest fragmentation and the creation of edge habitats did not reduce the magnitude of selection events. Coupled to a decrease in the amount of selection events, traits were more decoupled at fragments and edges than at the core of continuous forest. Taken together, the results herein reported indicate that forest fragmentation and the creation of edge habitats mostly impaired the microevolutionary dynamics of *B. salsilla.* Therefore, the present report stress how human-mediated habitat fragmentation has the property of imparing Darwinian evolution, thus endangering the long-term persistence of species identity and, perhaps more importantly, the long-term population persistence. Developing evolutionary enlightened management schedules appears as a fact of mandatory importance for conserving species and evolutionary processes in a broader sense.

ANTECEDENTES GENERALES

Disrupciones en las interacciones planta-animal debido a la fragmentación del hábitat están entre los temas claves en biología de la conservación. La reducción en el tamaño del hábitat y el incremento en el aislamiento de los fragmentos remanentes pueden reducir la diversidad y la abundancia de las especies interactuantes (Gross, 2001; Kery *et al.*, 2001; Samejima *et al.*, 2004). Tales reducciones en las abundancias de estos animales pueden generar efectos contrastantes sobre la adecuación de las plantas dependiendo si ellos afectan a mutualistas o antagonistas. Así, reducciones en la abundancia de polinizadores (*i.e.*, mutualistas) pueden reducir su frecuencia de visitas a las flores y, consecuentemente, reducir la adecuación de las plantas (Aguilar *et al.*, 2006). Por ejemplo, en 89 especies de plantas que exhiben diversas formas de vida (*i.e.*, árboles, arbustos, hierbas), diversos sistemas reproductivos (*i.e.*, autocompatibles, autoincompatibles), diversos grados de especialización hacia sus polinizadores (*i.e.*, especialistas, generalistas) y habitantes de diversos ambientes (*i.e.*, praderas, bosques tropicales, subtropicales y templados) existe un efecto negativo, aunque variable, de la fragmentación del hábitat sobre su adecuación biológica (Aguilar *et al.*, 1996). La depresión por endogamia y la deriva génica causadas por el aumento en el número de apareamientos entre individuos emparentados en los fragmentos de pequeño tamaño junto con el reducido tamaño poblacional de las plantas en estos fragmentos, respectivamente, también pueden contribuir a tal reducción (Cruzan, 2001; Severns, 2003).

En lo concerniente a los antagonismos, sin embargo, una reducción en la abundancia de herbívoros (*i.e.*, antagonistas) permitiría a las plantas escapar de las consecuencias negativas de la remoción de la superficie foliar e incrementar, por tanto, la adecuación de las plantas (Hendrix, 1988; Strauss & Zangerl, 2002). Reducciones en las abundancias de herbívoros en fragmentos y, consecuentemente, en los niveles de herbivoría pueden permitir a las plantas compensar los efectos negativos de la fragmentación sobre polinizadores y, por consiguiente, sobre la adecuación de las plantas (*e.g.*, Groom, 2001; Kery *et al.*, 2001; Colling & Matthies, 2004). Tales compensaciones en la adecuación de las plantas dependerán de la magnitud de las interacciones y de la magnitud en que son modificadas por la fragmentación del hábitat. De hecho, desde un punto de vista teórico, podrían existir efectos negativos, neutros o positivos de la fragmentación del hábitat sobre la adecuación de las plantas dependiendo si éstas sub-compensan, compensan o sobre-compensan la acción de los herbívoros sobre el efecto mediado por los polinizadores en la adecuación de las plantas.

El incremento en los habitats de borde debido a la fragmentación puede también afectar el éxito reproductivo de las plantas. Plantas creciendo en habitats de borde podrían enfrentar una reducción en la fuerza de las interacciones acoplado a cambios en la abundancia de mutualistas y antagonistas afectando, por lo tanto, positiva o negativamente el éxito reproductivo (Murcia, 1995).

Los cambios en abundancia en grupos funcionales, tales como polinizadores y herbívoros, podrían tener más impactos que las meras modificaciones de las interacciones en las cuales participan directamente al gatillar, por ejemplo, variaciones en las presiones selectivas sobre las plantas remanentes en los fragmentos y habitats de borde (Herrera & Pellmyr, 2002; Simonetti *et al.*, 2006). De este modo, en poblaciones grandes tanto los polinizadores como los herbívoros jugarían un papel central en la evolución de rasgos fenotípicos en las plantas (Herrera & Pellmyr, 2002). Los polinizadores, por ejemplo, representan agentes selectivos que dirigen la evolución de rasgos reproductivos como el tamaño de la corola (*e.g.*, Campbell, 1989; Galen, 1989; Campbell *et al.*, 1991; 1996; 1997; Herrera, 1993a; Conner *et al.*, 1996a; Caruso, 2000), exerción del estigma (*e.g.*, Conner *et al.*, 1996b), distancia entre el nectario y el estigma (Johnston 1991), longitud de la vara floral (*e.g.*, O'Connell & Johnston, 1998; Maad, 2000), fecha de floración (*e.g.*, Campbell, 1989; Johnston, 1991; Widén, 1991; Kelly, 1992; Gómez, 1993), color de la corola (*e.g.*, Nagy, 1997; Campbell *et al.*, 1997; Gómez, 2000), forma de la corola (*e.g.*, Herrera 1993a, b; Nagy, 1997; Galen & Cuba, 2001), y tamaño y forma de las guías de néctar (Medel *et al.*, 2003).

Los herbívoros, por su parte, constituyen agentes selectivos importantes que dan cuenta de la evolución de rasgos vegetativos como el área foliar (Rausher & Simms, 1989), grosor del tallo (Agrawal & van Zandt, 2003), tamaño de la planta (Núñez-Farfán & Dirzo, 1994), tasa de crecimiento (Monro & Poore, 2004) y densidad de tricomas (Valverde *et al.*, 2001). Los herbívoros, además, pueden actuar como agentes selectivos sobre los mismos rasgos sujetos a selección por los polinizadores (*i.e.*, sobre rasgos reproductivos) y con ello promover, por ejemplo, la mantención en la variabilidad de los colores de las flores (Frey, 2004); o bien, neutralizar los efectos selectivos ejercidos por los polinizadores (*e.g.*, Gómez, 2003).

Tanto los rasgos reproductivos como vegetativos en las plantas pueden encontrarse asociados o correlacionados de un modo tal que su accionar coordinado permitiría incrementar la adecuación de las plantas al ser más eficientes en la atracción de polinizadores y la expulsión (o resistencia) de los herbívoros (*i.e.*, hipótesis de las pléyades correlacionadas de Berg) (Berg, 1960; Armbruster, 1991; Fenster, 1991; Armbruster & Schwaegerle, 1996; Herrera, 1996; Cresswell, 1998; Armbruster *et al.*, 1999; Herrera *et al.*, 2002a). Si bien, la correlación de estos rasgos puede deberse a factores genéticos (*e.g.*, pleiotropía, ligamiento) y de desarrollo (Lande, 1980; Falconer, 1989), la selección correlacional impuesta por polinizadores y herbívoros puede ser otra de las vías que da cuenta de integraciones o correlaciones en rasgos fenotípicos (en adelante hipótesis de Herrera) (Herrera, 2000; Herrera *et al.*, 2002b; Gómez, 2005; Valdivia & Niemeyer, 2005).

Variaciones en los ensambles de animales interactuantes en los rangos de distribución de las plantas pueden gatillar variaciones interpoblacionales en los regimenes selectivos determinando la existencia de puntos evolutivamente cálidos o fríos (*i.e.*, donde ocurre o no ocurre selección de ciertos atributos fenotípicos) y, por lo tanto, de mayores o menores niveles de integración fenotípica (Thompson, 1997; Gómez & Zamora, 2000; Herrera *et al.*, 2002, 2006; Aspi *et al.*, 2003).

En poblaciones pequeñas, como las remanentes en los fragmentos o hábitats de borde, el debilitamientos en las interacciones planta-polinizador y planta-herbívoro y, consecuentemente, en las presiones selectivas ejercidas por polinizadores y herbívoros, podrían dar cuenta de la pérdida de integración en rasgos fenotípicos (*e.g.*, Anderson & Busch, 2006), así como podrían, además, dar cuenta de evolución no correlacionada para rasgos ligados a polinizadores y herbívoros (*e.g.*, Valdivia & Niemeyer, 2007). Los fragmentos y hábitats de borde remanentes podrían constituir, por tanto, puntos evolutivamente fríos (Thompson, 1997; Gómez & Zamora, 2000; Herrera *et al.* 2002; Aspi *et al.*, 2003). Además, considerando que en poblaciones pequeñas, como las remanentes en los fragmentos y bordes, fuerzas evolutivas como las mutaciones y la deriva génica aumentan en importancia relativa a la selección o al flujo génico, la fragmentación del hábitat no solo disminuiría la fuerza de la selección impuesta por polinizadores y herbívoros, sino además contribuiría a disminuir aun más la importancia relativa de la selección en este tipo de hábitats con respecto a las otras fuerzas evolutivas (Wright, 1932). De hecho, la menor fuerza de la selección, concomitantemente con la mayor importancia relativa de la deriva génica y las mutaciones, harían que las plantas remanentes en los fragmentos y bordes se encuentren fuera de los picos adaptativos en que se encontrarían en las poblaciones continuas de mayor tamaño (Wright, 1932). Las variaciones de magnitud en las interacciones planta-animal podrían estar determinando, por consiguiente, cambios en el teatro ecológico en el cual las especies remanentes están inmersas (Hutchinson, 1968), con consecuencias aún ignoradas en sus dinámicas microevolutivas (Bronstein *et al.*, 2004).

Si bien, en los últimos años el análisis de las interacciones planta-polinizador y planta-herbívoro ha sido progresivo y crecientemente sofisticado tanto desde la perspectiva de la biología de la conservación como desde la perspectiva de la ecología evolutiva, la determinación de los patrones de variabilidad e integración fenotípica y los regimenes selectivos impuestos por polinizadores y herbívoros sobre las plantas, que podrían dar cuenta de dicha variabilidad, no ha sido aún formalmente incorporada a evaluaciones que consideren el contexto de un paisaje modificado antropogénicamente (*e.g.*, en ambientes fragmentados). Dicha omisión representa un inconveniente serio considerando la necesidad de incorporar la dimensión evolutiva dentro de la biología de la conservación (Ferrière *et al.*, 2004). Existe, además, información empírica y teórica sobre la variabilidad en los regimenes selectivos y las respuestas evolutivas en un contexto espacialmente explícito (Thompson, 1994, 2005; Gómez & Zamora, 2000; Herrera *et al.*, 2002, 2006; Aspi *et al.*, 2003). Por lo tanto, considerando que la fragmentación es un escenario espacialmente explícito donde la fuerza de las interacciones varía, resulta indispensable evaluar los cambios microevolutivos que sufre la biota remanente en comparación con la situación original (Ferrière *et al.*, 2004; Simonetti *et al.*, 2006). Por ello, el objetivo general de la presente tesis es evaluar la magnitud de las interacciones planta-polinizador y planta-herbívoro en un ambiente fragmentado, junto con el efecto de la fragmentación del bosque sobre los patrones de variabilidad e integración fenotípica para rasgos reproductivos y vegetativos, así como los regímenes de selección fenotípica ejercidos por polinizadores y herbívoros sobre estos rasgos en la enredadera perenne *Bomarea salsilla*, una planta nativa de los bosques del centro y sur de Chile (Fig. A.1).

Figura A.1. *Bomarea salsilla* (Alstroemeriaceae) se distribuye en la zona centro-sur de Chile, entre las regiones de Valparaíso y la Araucanía (rojo) donde ha habido una severa perdida y fragmentación de hábitat (Luebert & Pliscoff, 2006); mientras que sus congéneres lo hacen desde el norte de Méjico hasta al sur de Argentina (gris) (Modificado de Alzate *et al.*, 2008).

Dado que la fragmentación del bosque reduce la interacción planta-polinizador y planta-herbívoro, se espera que en plantas que habitan fragmentos y bordes de bosque, polinizadores y herbívoros no representen fuerzas selectivas importantes sobre rasgos reproductivos y vegetativos, en relación al centro del bosque continuo (*i.e.*, evaluación del efecto de rasgos reproductivos y vegetativos sobre la adecuación de *B. salsilla*) (Fig. A.2). Dado, además, que la fragmentación del bosque reduce la interacción planta-polinizador y planta-herbívoro, se espera que en plantas que habitan fragmentos y bordes de bosque, polinizadores y herbívoros presenten efectos aditivos sobre la adecuación de *B. salsilla,* es decir, evidencia de evolución no-correlacionada; mientras que en el centro del bosque continuo presentes efectos no-aditivos, es decir, evidencia de evolución correlacionada (*i.e.*, evaluación de la hipótesis de Herrera) (Fig. A.2). Finalmente, ya que la fragmentación del bosque reduce la fuerza de la interacción planta-polinizador y planta-herbívoro, se espera que plantas que habitan fragmentos y bordes de bosque presenten una menor correlación entre rasgos reproductivos y rasgos vegetativos con respecto a plantas que habitan el bosque continuo (*i.e.*, evaluación de la hipótesis modificada de Berg) (Fig. A.2).

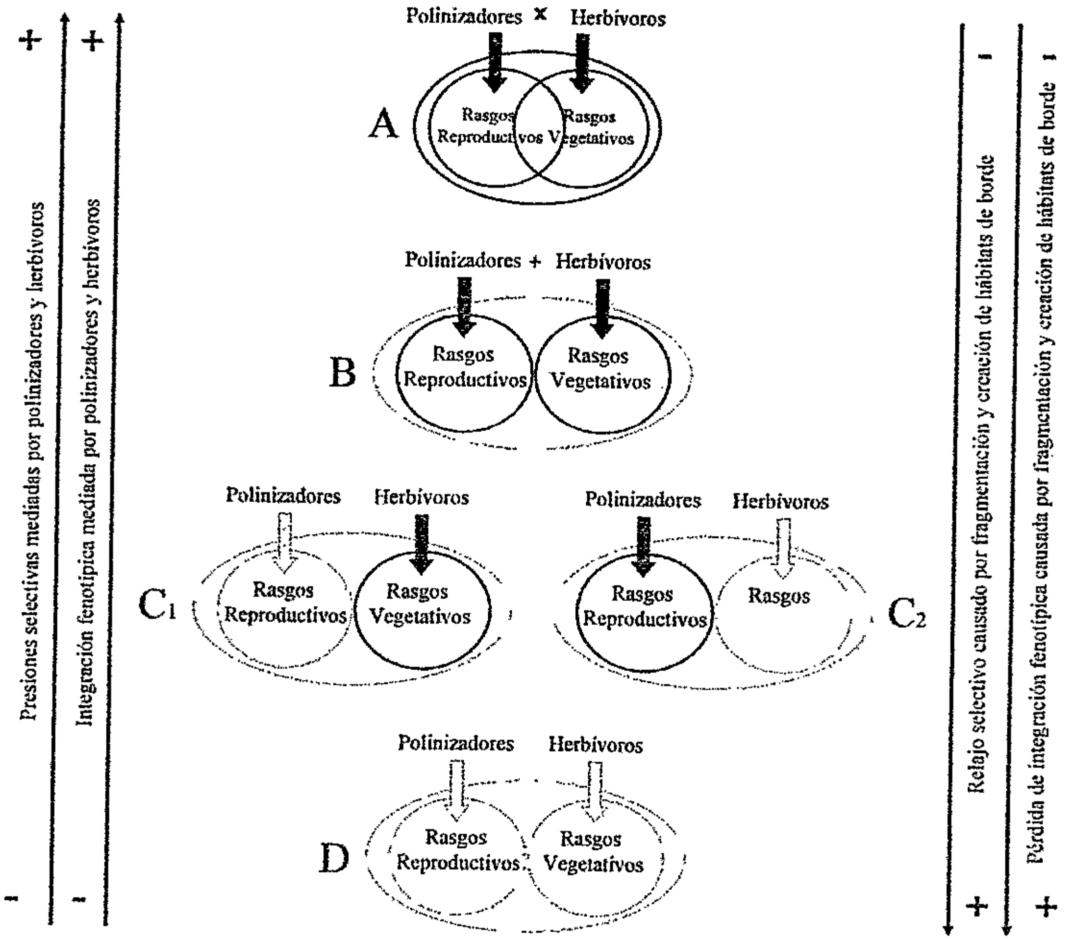

Figura A.2. Polinizadores y herbívoros pueden constituir agentes selectivos que dirigen la evolución de los rasgos fenotípicos de las plantas si su accionar impacta la adecuación biológica de éstas (flechas negras). Si polinizadores y herbívoros prefieren el mismo conjunto de rasgos y en rangos similares de su expresión, ellos ejercerán selección correlacional (**x**, no aditividad de efectos) provocando un alto nivel de integración fenotípica sobre el conjunto total de rasgos en la planta (**A**). Si el accionar de ambos tipos de animales ocurre sobre rasgos o rangos disimiles (+, aditividad de efectos), la selección impuesta afectará sólo a aquel conjunto funcional de rasgos provocando altos niveles de integración local, pero no general (**B**). Si sólo uno de los dos grupos de animales ejerce un efecto significativo sobre la adecuación de las plantas, solo aquel conjunto de rasgos vinculados funcionalmente a este grupo de animales exhibirá altos niveles de integración fenotípica (**C$_1$** o **C$_2$**). Si ningún grupo de animal impacta la adecuación de las plantas los rasgos se encontrarán desacoplados (**D**). La fragmentación y creación de hábitats bordes, al reducir las interacciones planta-polinizador y planta-herbívoro, pueden conducir a las plantas hacia una situación de completo desacoplamiento fenotípico llevándolas desde estados superiores de complejidad hacia otro(s) de menor complejidad fenotípica.

Específicamente, los objetivos son evaluar la fuerza y naturaleza (*i.e.*, simetría) de la interacción planta-polinizador y planta-herbívoro en *B. salsilla* comparando la condición de bosque continuo con fragmentos de bosque en habitats de centro y borde (Capítulo I). Evaluar la hipótesis de la evolución correlacionada para mutualistas y antagonistas de Herrera (2002) (*i.e.*, potencial microevolutivo actual) comparando la condición de bosque continuo con fragmentos de bosque en habitats de centro y borde (Capítulo II). Evaluar la selección fenotípica mediada por polinizadores y herbívoros sobre rasgos reproductivos y vegetativos en *B. salsilla* (*i.e.*, procesos microevolutivos actuales) comparando la condición de bosque continuo con fragmentos de bosque en habitats de centro y borde (Capítulo III) y, finalmente, evaluar la hipótesis de las pléyades correlacionadas de Berg (1960) (*i.e.*, procesos microevolutivos pasados y restricciones futuras) comparando la condición de bosque continuo con fragmentos de bosque en habitats de centro y borde (Capítulo IV). Estos objetivos específicos son abordados en capítulos independientes y constituyen, cada uno, un artículo en sí mismo.

Capítulo I

HERBIVORY RELEASE DOES NOT OFFSET POLLINATOR-MEDIATED DECREASED REPRODUCTIVE SUCCESS OF *BOMAREA SALSILLA* IN A FRAGMENTED FOREST

CARLOS E. VALDIVIA, ALEJANDRA BAHAMONDEZ AND JAVIER A. SIMONETTI

Departamento de Ciencias Ecológicas, Facultad de Ciencias, Universidad de Chile.
Casilla 653, Santiago, Chile.

ABSTRACT

Habitat fragmentation may have detrimental effects on plant-mutualistic (*i.e.*, pollination) and plant-antagonistic interactions (*i.e.*, foliar herbivory). Such negative effects may lead plants to a trade-off in their fitness because the negative effects of a loss of a mutualism may be offset by the positive effects of a loss of an antagonistism. In spite of being of great importance, however, the assessment of such ecological relationships has traditionally been performed independently. We evaluated the fragmentation and edge effects on floral and foliar display, frequency of pollinator visits, foliar herbivory, and reproductive success, assessed as number of fruits per plant, number of seeds per fruit, and number of seeds per plant, on *Bomarea salsilla*, a xenogamous vine from southern Chile. Plants thriving at fragments and edges exhibited a smaller floral display and a larger foliar display, and were meagerly visited and preyed by pollinators and herbivores, respectively. Number of fruits per plant, number of seeds per fruit, and number of seeds per plant were lower at fragments and edges than at the core of continuous forest. Despite forest fragmentation had a detrimental effect on hebivory, herbivores did not play a modulating role on the lowered pollinator-mediated reproductive success of *B. salsilla* probably due to the scarcity of foliar attack. Therefore, decreased reproductive success of *Bomarea salsilla* plants due to forest fragmentation and the creation of edge habitats was not offset by foliar herbivory as expected. The ecological scenario faced by *B. salsilla* may be a representative phenomenon of numerous other species of the fragmented forests of South America.

Key words: pollination, herbivory, forest fragmentation, fitness trade-offs.

INTRODUCTION

Disruptions in plant-animal interactions due to habitat fragmentation are among the key issues in conservation biology. As far as mutualistic interactions are concerned, since the pioneer publication on pollination by Jennersten (1988) increasing evidence has been gathered about the relevance of habitat fragmentation as a detrimental factor conducting plant reproduction (Aguilar *et al.*, 2006; Aizen & Vázquez, 2006). Concerning antagonistic interactions, however, negative relationships, such as foliar herbivory, have received much less attention than pollination in spite of being equally important in leading plant reproductive success (*e.g.*, Groom, 2001; Kery *et al.*, 2001; Colling & Matthies, 2004).

In short, the reduction in habitat size and increase in isolation of fragments can reduce the diversity and abundance of remaining plant animal-interactors (Kery *et al.*, 2001; Steffan-Dewenter *et al.*, 2006). Such reductions in animal adundances may lead to contrasting effects on plant reproduction depending on whether they affect mutualistic or antagonistic animals. Thus, a reduction in the abundance of pollinators may lead to a reduction in the frequency of pollinator visits and, consequently, a reduction in the reproductive success of plants (Aguilar *et al.*, 2006; Aizen & Vázquez, 2006). Inbreeding depression, triggered by the increased number of mates between close relatives in small-sized fragments, may also contribute to such a reduction (Cruzan, 2001; Severns, 2003). Nevertheless, a reduction in abundance in herbivores may allow plants to escape from the negative consequences of foliar removal, therefore increasing the reproductive success (Hendrix, 1988; Strauss & Zangerl, 2002).

Furthermore, habitat fragmentation might significantly modify plant traits related to floral and foliar display due to changes in abiotic or biotic conditions (Aizen & Vázquez, 2006). Reductions in floral and foliar displays may significantly reduce plant attrativeness to pollinators and herbivores repectively, thus negatively or positively affecting plant reproductive success (Hendrix, 1988; Pellmyr, 2002; Strauss & Zangerl, 2002). The net effect on plant reproductive success of a decrease in a mutualistic and an antagonistic interaction should depend on the strength of such interactions and the extent to which they are modified by habitat fragmentation. Modifications in plant traits might also be contributing to modulate plant reproductive success. Thus, plants facing asymmetrical relationships, in terms of mutualisms and antagonisms, might face negative or positive effects on reproductive success given by habitat fragmentation. On the contrary, if plants face symmetrical relationships in mutualistic and antagonistic interactions, and they are equally modified by habitat fragmentation, plants occurring at fragmented habitats might face a trade-off in their fitness because a loss of a mutualism (*i.e.*, pollination) may be offset by a loss of an antagonism (*i.e.*, herbivory) (*e.g.*, Kery *et al.*, 2001; Colling & Matthies, 2004).

The increase of edge habitats due to habitat fragmentation might also impinge upon plant reproduction. Plants thriving in edge habitats might face a reduction in the strength of the interactions coupled to changes in the abundance of their mutualistic and antagonistic animal counterparts, therefore positively or negatively affecting plant reproductive success (Murcia, 1995).

Although habitat fragmentation and the increase of edge habitats may simultaneously affect plant-pollinator and plant-herbivore interactions, the assessment of such ecological relationships has traditionally been performed independently (but see Groom, 2001). This approach, albeit convenient, may lead to misunderstand the isolated and combined effects of such types of animals on plant performance, thereby hindering our understanding of such ecological scenarios. The aim of this work is to evaluate the effect of forest fragmentation, including the effect of occurring in edge habitats, on the pollinator-mediated reproductive success of *Bomarea salsilla*, a native vine-type monocot from the temperate rainforests from southern Chile, yet considering the effect of its herbivores on plant performance. If forest fragmentation has a negative effect on pollinator visits, a lowered reproductive success is expected in fragments with respect to continuous forests. If there is an edge effect, plants occurring at edges might also be facing negative effects with respect to the core of such habitats. Nevertheless, if forest fragmentation also negatively affect plant-herbivore interactions, a some sort of compensation is expected in fragments and edges.

MATERIALS AND METHODS

Natural history

Fieldwork was conducted in Maulino forest in the northernmost zone of the temperate rainforest from Chile (35°59'S, 72°41'W; Bustamante *et al.*, 2005). Specifically, the study was performed in Los Queules National Reserve and two neighbouring forest fragments. Los Queules is a protected area of 145 ha embedded in a large tract of 600 ha of continuous forest. Forest fragments, ranging from 1 to 6 ha, are patches surrounded by commercial plantations of *Pinus radiata*. Here, four sites were defined taking into account the spatial arrangement of plant populations: one core and one edge of continuous forest, both sites placed in Los Queules National Reserve, and one core and one edge of forest fragment, both sites placed in two different fragments nearby Los Queules National Reserve. While cores were defined as sites placed ≥ 50 m inwards the border of each site, edges were defined as sites placed ≤ 10 m inwards the forest margin. Distance between sites ranges from 0.5 up to 2.5 km.

Bomarea salsilla (Alstroemeriaceae) is a protandrous, small-sized red-climbing perennial vine, inhabiting the sclerophyllous and temperate forests of Chile from 33°S to 40°S (Muñoz & Moreira, 2003). In the study site, it flowers from *ca.* November to January. Its breeding system is unknown; however, two pilot pollinator-exclusion experiments in the study site, devised to test for autogamy and agamospermy, demostrated that *B. salsilla* is a totally pollinator-dependent plant for seed set (*i.e.*, a xenogamous plant). In the study site, floral visitors are hummingbirds, *Sephanoides sephaniodes* (Trochilidae); bumblebees and bees, *Bombus dahlbomii, Bombus terrestris,* and *Manuelia gayatina* (Apidae); butterflies, *Mathania leucothea* (Pieridae); and flies, *Acrophthalmyda paulseni* (Bombyliidae) (pers. obs.). Its herbivores are unknown; however, several leaf-eating herbivores have been reported for this forest (Simonetti *et al.*, 2006).

Plant density

To assess the overall plant density and density of reproductive individuals, twenty 4

m^2 plots were randomly set up at each site. These plots were stablished at the peak of reproductive season recording the total number of plants and if they were in either a vegetative or a reproductive stage.

Floral and foliar display

To determine floral display, assessed as the number of flowers per plant and perigony length, 50 plants were randomly chosen at each site and the total number of flowers produced by each plant counted at the end of the reproductive season, in January 2006. In these plants, tepal length was determined in two flowers per plant, and then averaged, in order to estimate perigony length in a per-plant basis.

To determine foliar display, assessed as the total number of leaves per plant and leaf length, in the same plants of the assessment of floral display, the total number of leaves per plant were counted at the end of the reproductive season. Leaf length was estimated in two leaves borne by single plants, and then averaged, for obtaining a single per-plant estimate.

Frequency of pollinator visits and herbivory

To evaluate the frequency of pollinator visits, the number of visits per pollinator to randomly chosen plants was determined during 10-min periods over 50 plants at each site, throughout the day from 8:30 to 17:30 h. A visit was considered as such when the floral visitor touched a reproductive structure (*i.e.*, pistil or anthers). One observation period for every half hour for each plant was performed at the peak of flowering season in December 2005, and at each site simultaneously by different observers, in order to avoid any weather-mediated bias. Total number of observations were 3,800 periods (950 periods at each site). Total number of periods per plant were averaged for obtaining a single estimate for each plant.

To evaluate foliar herbivory on adult plants, 50 individuals were randomly selected at each site at the end of the reproductive season in January 2006. In these plants, the cumulative herbivory by chewing herbivores was recorded using a Herbivory Index, HI (Dirzo and Domínguez, 1995). Twenty-five randomly chosen leaves per plant were checked, assessing the degree of foliar surface lost due to herbivores. Each leaf was assigned to one of the following six categories: 0, 1, 2, 3, 4, or 5, depending on whether 0%, 1-6%, 7-12%, 13-25%, 26-50%, or 51-100% of foliar surface was absent. The HI was determined as follows:

$$\mathrm{HI} = \sum_{i=0}^{5} x_i n / \mathrm{N}$$

where n is the frequency of observations, x_i is the damage category, and N is the total number of observations. A single per-plant estimate of HI was thus obtained.

Reproductive success

At the end of the reproductive season, fruits per plant (w_1) and number of seeds per fruit (w_2) were determined and then multiplyed (w_1 x w_2) in order to estimate the overall reproductive success for each plant. Plants were the same as those of foliar herbivory assessment. To assess w_1, all flowers borne by single plants were tagged and followed until finishing the reproductive season for determining if they did or did not set fruits. Thereafter, to estimate w_2, just prior to seed dispersal, one to two ripe fruits per plant were severed and the total number of seeds counted; these values were then averaged for attaining a single estimation for each plant.

Statistical analyses

A rigorous research devised to test for the effects of forest fragmentation and edge habitats on a given set of variables must include several sites of each category. In the present report, however, only one site per each category was included because no other site was found harbouring *B. salsilla* populations. Despite this statistical caveat all the following analyses were performed assessing the effects of forest fragmentation and edge habitats. Caution must therefore keep in mind. Thus, plant density, floral display, and foliar display were evaluated by using two-way ANOVAs taking into account if plants grow either at continuous forest or forest fragments (factor 1) and if they grow at the core or edge of such habitats (factor 2). Frequency of pollinator visits and foliar herbivory were assessed by using two-way ANCOVAs including as co-variables all parameters of floral and foliar displays exhibiting significant fragmentation or edge effects. Furthermore, because frequency of pollinator visits and foliar herbivory may exhibit density-dependent responses (Aizen & Vázquez, 2006), the population mean values of overall plant density and density of reproductive plants were correlated with the population mean values of foliar herbivory and frequency of pollinator visits respectively, by using non-parametric Sperman rank correlation tests. Fruits per plant (w_1), number of seeds per fruit (w_2), and total number of seeds per plant (w_1 x w_2) were assessed by using two-way

ANCOVAs considering foliar herbivory per plant as a covariate for evaluating a possible modulating role of herbivores on plant reproductive success. Because number of flowers per plant may also affect fruit and seed set, this was included in the analyses as a second covariate. Furthermore, in order to discard a possible spatial self-correlation and, therefore, statistical dependency, product-moment Pearson regression tests were performed by relating geographical distances with absolut values of biological distances for the mean values of all parameters herein assessed. There was no spatial self-correlation for none of the variables analysed (data not shown).

RESULTS

Plant density

There was a significant fragmentation and edge effect on the overall plant density (Table 1.1a). In fact, overall plant density was significantly higher at the edge of the continuous forest (Fig. 1.1A). Density of reproductive plants however did not differ among sites (Fig. 1.1B, Table 1.1b).

Table 1.1. Summary of two-way ANOVAs testing the fragmentation and edge effect on: (a) overall plant density, and (b) plant density at the reproductive stage, of *Bomarea salsilla* in the fragmented Maulino forest.

Source	df	MS	*F*	*P*
(a) Overall plant density:				
Fragmentation effect (FE)	1	1001.11	16.71	<0.001
Edge effect (EE)	1	357.01	5.96	0.017
FE x EE	1	456.01	7.61	0.008
Error	76	59.91		
(b) Density of reproductive plants:				
Fragmentation effect (FE)	1	5.51	0.68	0.412
Edge effect (EE)	1	25.31	3.12	0.081
FE x EE	1	10.51	1.30	0.258
Error	76	8.10		

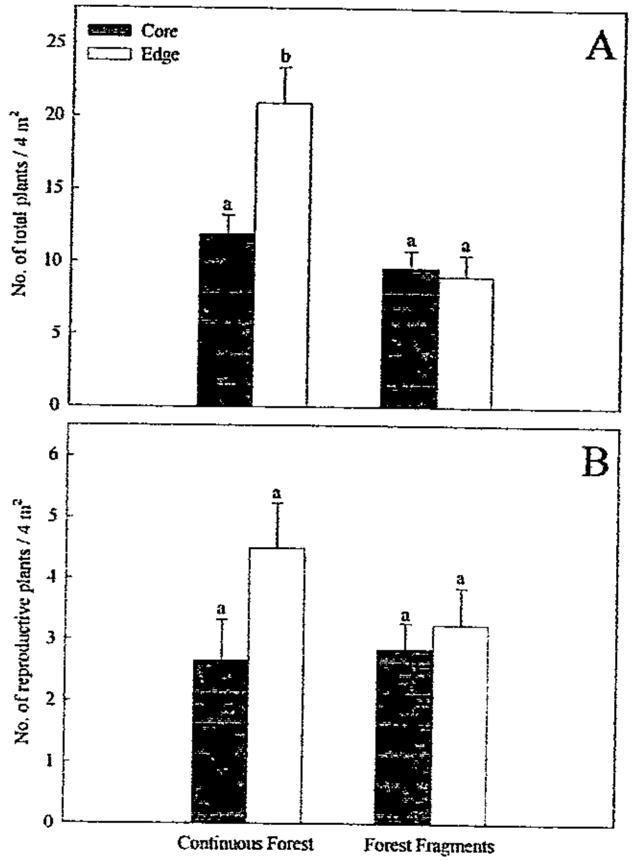

Figure 1.1. Overall plant density (A) and density of reproductive plants (B) of *Bomarea salsilla* in the fragmented Maulino forest. Dissimilar letters depict significant differences ($P < 0.05$) following Tukey HSD tests (mean ± 1SE are depicted).

Floral and foliar display

There was a significant fragmentation and edge effect on floral display (Table 1.2a). In fact, plants at forest fragments exhibited a significantly lower floral display than those from continuous forest (Fig. 1.2A, B).

There was a significant edge effect, albeit not a fragmentation effect, on foliar display (Table 1.2c, d). Plants at the edge of forest fragments exhibited a significantly larger foliar display than those from both core and edge of the continuous forest in term of leaf length, but not in term of number of leaves per plant (Fig. 1.2C, D).

Table 1.2. Summary of two-way ANOVAs testing the fragmentation and edge effect on: (a) number of flowers per plant, (b) perigony length, (c) number of leaves per plant, and (d) leaf length in *Bomarea salsilla* in the fragmented Maulino forest.

Source	df	MS	*F*	*P*
(a) Number of flowers per plant:				
Fragmentation effect (FE)	1	169.68	8.37	<0.001
Edge effect (EE)	1	176.72	8.73	<0.001
FE x EE	1	147.92	7.31	<0.001
Error	196	20.22		
(b) Perigony length:				
Fragmentation effect (FE)	1	0.12	3.54	0.062
Edge effect (EE)	1	0.78	22.37	<0.001
FE x EE	1	0.05	1.34	0.248
Error	196	0.04		
(c) Number of leaves per plant:				
Fragmentation effect (FE)	1	84.40	0.17	0.681
Edge effect (EE)	1	1080.30	2.17	0.143
FE x EE	1	696.70	1.40	0.239
Error	196	498.70		
(d) Leaf length:				
Fragmentation effect (FE)	1	0.16	0.11	0.744
Edge effect (EE)	1	31.44	21.41	<0.001
FE x EE	1	107.17	72.97	<0.001
Error	196	1.47		

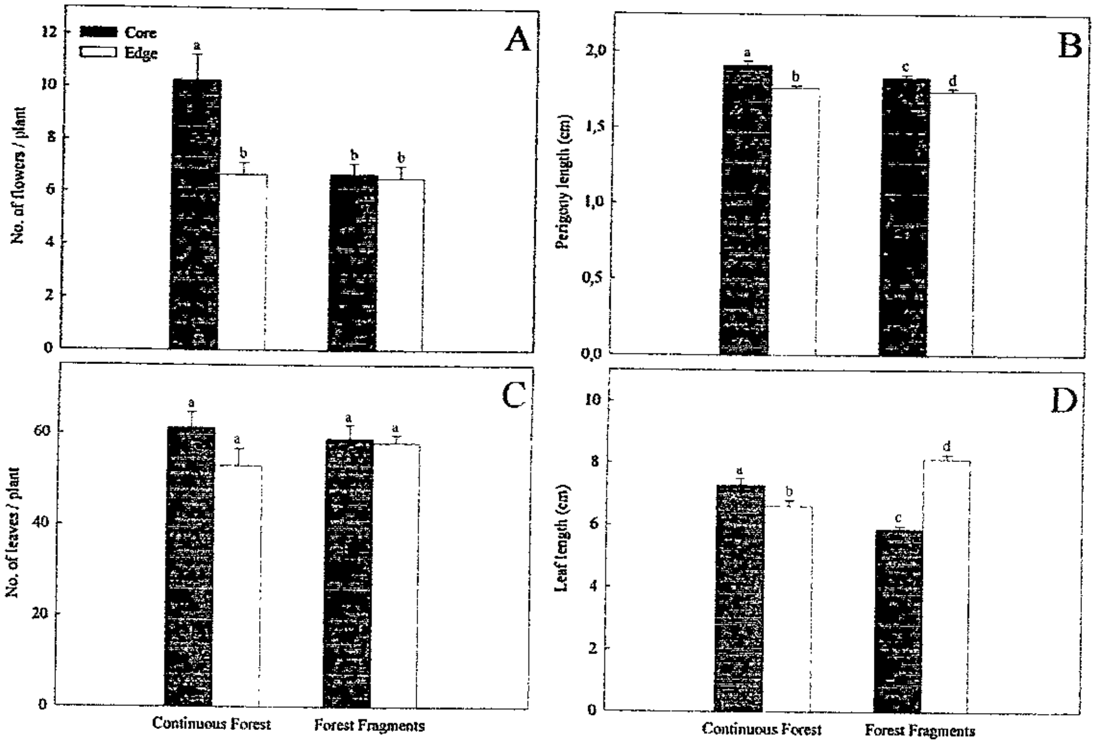

Figure 1.2. Floral display, assessed through number of flowers per plant (A) and perigony length (B), and foliar display, assessed through number of leaves per plant (C) and leaf length (D), on *Bomarea salsilla* in the fragmented Maulino forest. Dissimilar letters depict significant differences ($P < 0.05$) following Tukey HSD tests (mean ± 1SE are depicted).

Frequency of pollinator visits and herbivory

There was a significant fragmentation and edge effect on the frequency of pollinator visits (Table 1.3a). In fact, plants at the edge of forest fragments were significantly less visited by pollinators than those from continuous forest (Fig. 1.3A). Furthermore, frequency of pollinator visits was marginally modulated by floral display (Table 1.3a), and was unrelated to reproductive plant density ($r_s = -0.800$, $P =$

0.200).

Similarly, there was a significant fragmentation and edge effect on foliar herbivory which, however, was unrelated to foliar display (Table 1.3b). In fact, plants at forest fragments were significantly less preyed by herbivores than those from both the core and edge of the continuous forest (Fig. 1.3B). Foliar herbivory was unrelated to the overall plant density ($r_s = -0.400$, $P = 0.600$).

Table 1.3. Summary of two-way ANCOVAs testing the fragmentation and edge effect on: (a) frequency of pollinator visits to *Bomarea salsilla* plants, and (b) foliar herbivory in the fragmented Maulino forest. Number of flowers per plant, perigony length, and leaf length were considered as covariates because they significantly differ among sites (see Table 2).

Source	df	MS	*F*	*P*
(a) Frequency of pollinator visits:				
Number of flowers per plant	1	0.04	2.85	0.093
Perigony length	1	<0.01	0.10	0.075
Fragmentation effect (FE)	1	<0.01	4.95	0.027
Edge effect (EE)	1	0.13	9.50	0.002
FE x EE	1	<0.01	0.01	0.917
Error	194	0.01		
(b) Foliar herbivory:				
Leaf length	1	0.04	0.22	0.638
Fragmentation effect (FE)	1	1.39	8.51	0.004
Edge effect (EE)	1	1.39	8.54	0.004
FE x EE	1	1.82	11.14	0.001
Error	195	0.16		

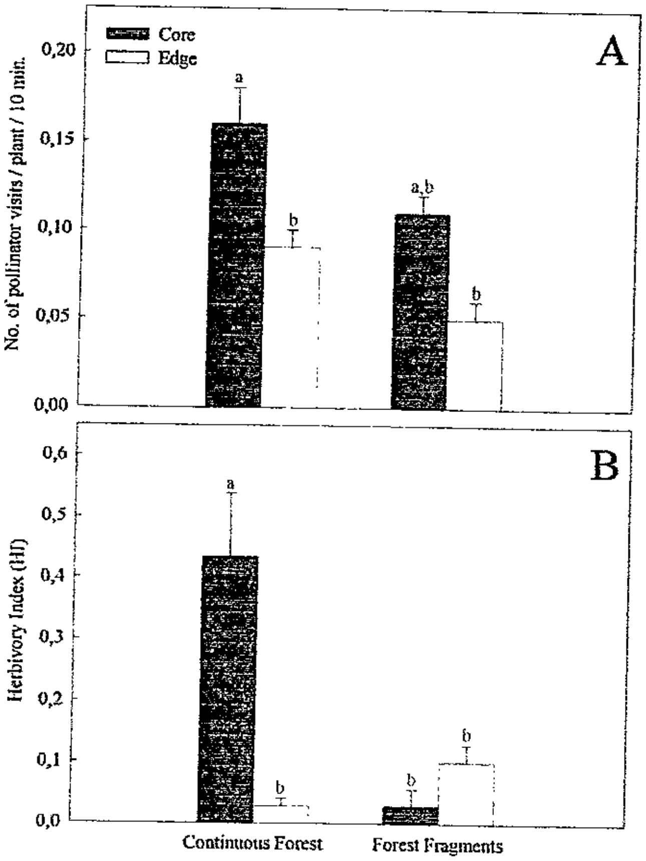

Figure 1.3. Frequency of pollinator visits (A) and foliar herbivory (B) in *Bomarea salsilla* in the fragmented Maulino forest. Dissimilar letters depict significant differences ($P < 0.05$) following Tukey HSD tests (mean ± 1SE are depicted).

Reproductive success

There was both significant fragmentation and edge effects on the number of seeds per fruit and seeds per plant, albeit only a fragmentation effect on the number of fruits per plant (Table 1.4). Plants at forest fragments and at the edge of continuous forest produced significantly less fruits per plant, seeds per fruit, and seeds per plant than those from the core of continuous forest (Fig. 1.4). Furthermore, the number of flowers per plant had a significant effect on the reproductive success of *B. salsilla* (Table 1.4). Herbivores, however, did not play a modulating role (Table 1.4, Table 1.5).

Table 1.4. Summary of two-way ANCOVAs testing the fragmentation and edge effect on: (a) number of fruits per plant (w_1), (b) number of seeds per fruit (w_2), and (c) number of seeds per plant (w_1 x w_2) in *Bomarea salsilla* in the fragmented Maulino forest. Number of flowers per plant and foliar herbivory were considered as covariates.

Source	df	MS	*F*	*P*
(a) Number of fruits per plant (w_1):				
Number of flowers per plant	1	489.91	121.22	<0.001
Foliar herbivory	1	0.05	0.01	0.914
Fragmentation effect (FE)	1	57.03	14.11	<0.001
Edge effect (EE)	1	13.44	3.33	0.070
FE x EE	1	0.46	0.11	0.736
Error	194	4.04		
(b) Number of seeds per fruit (w_2):				
Number of flowers per plant	1	1662.95	58.38	<0.001
Foliar herbivory	1	0.11	<0.01	0.950
Fragmentation effect (FE)	1	543.50	19.08	<0.001
Edge effect (EE)	1	281.01	9.86	0.002
FE x EE	1	14.38	0.50	0.478
Error	194	28.59		
(c) Number of seeds per plant (w_1 x w_2):				
Number of flowers per plant	1	179049.70	237.86	<0.001
Foliar herbivory	1	1410.10	1.87	0.173
Fragmentation effect (FE)	1	18362.80	24.39	<0.001
Edge effect (EE)	1	7034.60	9.35	0.002
FE x EE	1	4978.20	6.61	0.011
Error	194	752.80		

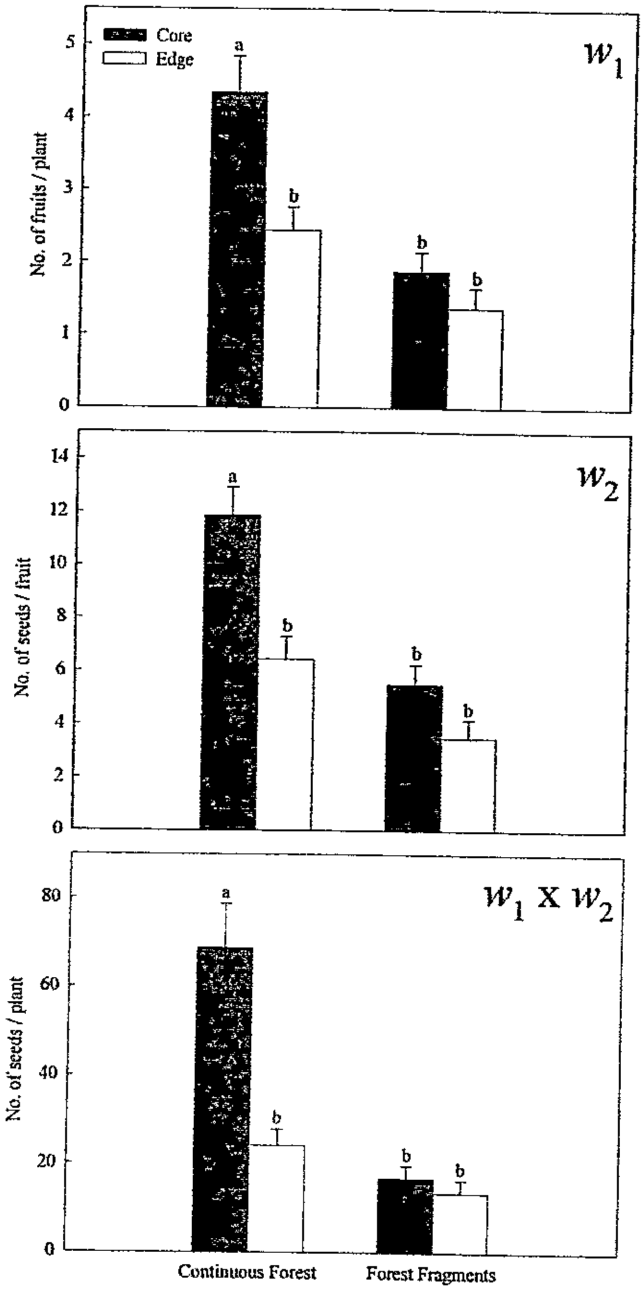

Figure 1.4. Reproductive success, assessed through number of fruits per plant (w_1), number of seeds per fruit (w_2), and number of seeds per plant (w_1 x w_2), in *Bomarea salsilla* in the fragmented Maulino forest. Dissimilar letters depict significant differences ($P < 0.05$) following Tukey HSD tests (mean ± 1SE are depicted).

Table 1.5. Pearson product-moment regression coefficients for the number of fruits per plant (w_1), number of seeds per fruit (w_2), and number of seeds per plant (w_1 x w_2) in *Bomarea salsilla* related to foliar herbivory in (a) continuous forest and (b) forest fragments and at the core and edge of both types of habitats.

Reproductive success	Core			Edge		
	β	R^2	*P*	β	R^2	*P*
(a) Continuous forest:						
w_1	-0.487	0.010	0.482	4.550	0.034	0.202
w_2	-1.338	0.017	0.363	8.220	0.017	0.352
w_1 x w_2	-15.822	0.027	0.250	37.587	0.018	0.482
(b) Forest fragments:						
w_1	-0.681	0.005	0.632	0.297	<0.001	0.837
w_2	3.166	0.014	0.420	4.503	0.035	0.195
w_1 x w_2	-5.248	0.002	0.756	3.828	0.001	0.809

DISCUSSION

Bomarea salsilla plants thriving at forest fragments and edge habitats exhibited a smaller floral display, were less visited and preyed on by pollinators and herbivores respectively, and produced a significantly lower fruit and seed set. Consequently, forest fragmentation and the increase in edge habitats significantly affected plant reproductive success by modifying morphological features as well as plant-mutualistic and plant-antagonistic relationships.

Forest fragmentation, including its edge effect, significantly modified morphological aspects related to floral and foliar display. Plants occurring at forest fragments exhibited a smaller floral display in term of number of flowers per plant and perigony length. Decreased values of these traits related to floral display may significantly decrease the frequency of pollinator visits, thereby decreasing fruit and seed set (Pellmyr, 2002). A similar pattern, however, was not observed with regard to foliar display. In fact, plants inhabiting the edge of forest fragments exhibited a larger foliar display, yet herbivory was lower, with regard to the core of continuous forest. Taken together, both floral and foliar display patterns suggest differences in the investment in vegetative and reproductive structures and, therefore, in energy budgets, given by forest fragmentation (Hendrix, 1988). Energy budgets and morphological trait patterns related to forest fragmentation and their consequences on reproductive success and even upon the microevolutionary trends remain still to be clarified.

Coupled to the lowered frequency of pollinator vists, which was unrelated to density in reproductive plants, fruit and seed set was lower in the core and edge of fragments and in the edge of continuous forest with respect to the core of continuous forest on account of the lowered pollination service, which was further modulated by the total amount of flowers per plant. This fact is of a more general occurrence because in the same fragmented forests, the hummingbird-pollinated vine *Lapageria rosea*, similar to *B. salsilla* mostly pollinated by insects, exhibits a lowered reproductive success on account of a lowered pollination service triggered by forest fragmentation (Valdivia *et al.*, 2006). Given that in these forests vines depict 14.5% of all native plants with several hummingbird- and insect-pollinated endemic genera (*e.g.*, *Boquila, Hydrangea, Lardizabala, Luzuriaga*; Aizen *et al.*, 2002), an important number of plants facing a reduced fitness due to forest fragmentation, including its edge effect, is indeed to be expected.

Herbivores, however, did not play a modulating role on plant fitness and did not offset the pollinator-mediated decreased reproductive success of *B. salsilla*. This might be owing to the scarcity in foliar attack which did not exceed five percent of foliar surface removal. In the fragmented Maulino forest, an increase in insectivorous animals in fragments of compared to continuous forest might has triggered an increase in predation on herbivorous larvae and, therefore, a reduction in herbivory through cascading effects (González-Gómez *et al.*, 2006; Simonetti *et al.*, 2006). Certainly, the lack of effects by herbivores strongly contrasts with other plants exhibiting a trade-off in pollinator- and herbivore-mediated reproductive success in fragmented habitats (*e.g.*, Kery *et al.*, 2001; Colling & Matthies, 2004). Nevertheless, because herbivores might play a modulating role in other phenological stages (*e.g.*, seedling stage), an assessment takes into account the overall life cycle would be desirable (*e.g.*, Ledergerber *et al.*, 2002).

In the *B. salsilla* case the net effect of mutualists and antagonists on reproductive success was negative due to the asymmetrical relationship between both types of interactions: the mutualism was stronger than the antagonism. Assessments taking into account both types of relationships on plant reproductive success are certainly required to fully understand the effects of human-mediated habitat disturbances on such types of interactions and their consequences on plant fitness in the highly diverse temperate rainforest from South America.

ACKNOWLEDGEMENTS

We thank Yuri Zúñiga, Fernando Campos, Fernanda Valdovinos, and Christian González who provided valuable logistic and technical support. We also thank Forestal Millalemu and Chilean Forestry Service (CONAF) for permission to work in their lands. Research funded by Fondecyt 1050745 to J.A.S.

REFERENCES

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., Vázquez, D.P. 2006. Flower performance in human-altered habitats. In: Harder, L.D., Barrett, S.C.H. (Eds.). Ecology and Evolution of Flowers, pp: 159-179. Oxford University Press.

Aizen, M.A., Vázquez, D.P., Smith-Ramírez, C. 2002. Historia natural y conservación de los mutualismos planta-animal del bosque templado de Sudamérica austral. Revista Chilena de Historia Natural 75, 79-97.

Bustamante, R.O., Simonetti, J.A., Grez, A.A., San Martín, J. 2005. Fragmentación y dinámica de regeneración del bosque Maulino: diagnóstico actual y perspectivas futuras. In: Smith-Ramírez, C.S., Armesto, J.J., Valdovinos, C. (Eds.), Historia, biodiversidad y ecología de los bosques costeros de Chile, pp: 555–564. Editorial Universitaria, Santiago.

Colling, G., Matthies, D. 2004. The effects of plant population size on the interactions between the endangered plant *Scorzonera humilis*, a specialised herbivore, and a phytopathogenic fungus. Oikos 105, 71-78.

Cruzan, M.B. 2001. Population size and fragmentation thresholds for the maintenance of genetic diversity in the herbaceous endemic *Scutellaria montana* (Lamiaceae). Evolution 55, 1569-1580.

Dirzo, R., Domínguez, C. 1995. Plant-herbivore interactions in Mesoamerican tropical dry forests. In: Bullock, S., Mooney, S., Medina, E. (Eds.), Seasonally dry tropical forests. Massachussets, pp: 304-345. Cambridge University Press.

González-Gómez, P.L., Estades, C.F., Simonetti, J.A. 2006. Strengthened insectivory in a temperate fragmented forest. Oecologia 148, 137-143.

Groom, M.J. 2001. Consequences of subpopulation isolation for pollination, herbivory, and population growth in *Clarkia concinna concinna* (Onagraceae). Biological Conservation 100, 55-63.

Hendrix, S.D. 1988. Herbivory and its impact on plant reproduction. In: Lovett Doust, J., Lovett Doust, L. (Eds.), Plant reproductive ecology. Patterns and strategies, pp 246-263. Oxford University Press.

Jennersten, O. 1988. Pollination in *Dianthus deltoides* (Caryophyllaceae). Effects of habitat fragmentation on visitation and seed set. Conservation Biology 2, 359-366.

Kery, M., Matthies, D., Fischer, M. 2001. The effect of plant population size on the interactions between the rare plant *Gentiana cruciata* and its specialized herbivore *Maculinea rebeli*. Journal of Ecology 89, 418-427.

Ledergerber, S., Dolt, C., Zschokke, S., Baur, B. 2002. Effects of experimental small-scale grassland fragmentation on the extent of grazing damage in *Trifolium repens* seedlings. Acta Oecologica 23, 329-336.

Muñoz, M., Moreira, A. 2002. *Alstroemerias* de Chile. Diversidad, distribución y conservación. Taller La Era, Santiago, Chile.

Murcia, C. 1995. Edge effects in fragmented forests. Implications for conservation.

Trends in Ecology and Evolution 10, 58-62.

Pellmyr, O. 2002. Pollination by animals. In: Herrera, C.M., Pellmyr, O. (Eds.), Plant-animal interactions, an evolutionary approach, pp: 157–184. Blackwell Publishing.

Severns, P. 2003. Inbreeding and small population size reduce seed set in a threatened and fragmented plant species, *Lupinus sulphureus* ssp *kincaidii* (Fabaceae). Biological Conservation 110, 221-229.

Simonetti, J.A., Grez, A.A., Bustamante, R.O. 2006. Interacciones y procesos en bosque Maulino fragmentado. In: Grez, A.A., Simonetti, J.A., Bustamante, R.O. (Eds.), Biodiversidad en ambientes fragmentados de Chile: Patrones y procesos a diferentes escalas, pp: 99-114. Editorial Universitaria. Santiago.

Steffan-Dewenter, I., Klein, A.M., Gaebele, V., Alfert, T., Tscharntke, T. 2006. Bee diversity and plant-pollinator interactions in fragmented landscapes. In: Waser, N.M., Ollerton, J. (Eds.), Plant-Pollinator Interactions, from Specialization to Generalization, pp: 387-407. The University of Chicago Press.

Strauss, S.Y., Zangerl, A.R. 2002. Plant-insect interactions in terestrial ecosystems. In: Herrera, C.M., Pellmyr, O. (Eds.), Plant-animal interactions, an evolutionary approach, pp: 77-106. Blackwell Publishing.

Valdivia, C.E., Simonetti, J.A., Henríquez, C.A. 2006. Depressed pollination of *Lapageria rosea* Ruiz et Pav. (Philesiaceae) in the fragmented temperate rainforest of southern South America. Biodiversity and Conservation 15, 1845-1856.

Capítulo II

HERBIVORE-INDEPENDENT POLLINATOR-MEDIATED EVOLUTIONARY POTENTIAL OF *BOMAREA SALSILLA* IN A FRAGMENTED FOREST

CARLOS E. VALDIVIA AND JAVIER A. SIMONETTI

Departamento de Ciencias Ecológicas, Facultad de Ciencias, Universidad de Chile.
Casilla 653, Santiago, Chile.

ABSTRACT

Modifications in plant-mutualistic and plant-anatagonistic interactions given by habitat fragmentation may have far reaching consequences by affecting plant reproductive success and their microevolutionary dynamics. Mutualists (*i.e.* pollinators) and antagonists (*i.e.* herbivores) can exert non-additive effects on plant fitness, which is interpreted as evidence for correlated evolution on mutualist- and antagonist-linked traits, respectively. This situation would prevail in plants exhibiting larger floral (*i.e.*, reproductive traits) and foliar displays (*i.e.*, vegetative traits). We suggest that a decrease in pollination and herbivory due to habitat fragmentation may lead plants to face noncorrelated (*i.e.*, non-additivity) selection pressures exerted by pollinators and herbivores. We assessed the effects of pollinators and herbivores on *B. salsilla* seed set by separately and simultaneously excluding pollinators and herbivores in a factorial design. These exclusion were performed in the core and edge of a continuous forest, and in the core and edge of forest fragments. We also assessed a suite of reproductive and vegetative traits for disclosing pollinator and herbivore preferences at each site. Pollinators and herbivores exhibited similar and dissimilar preferences for reproductive and vegetative traits in *B. salsilla* depending on whether which trait and which population is considered. However, in *B. salsilla* thriving at all sites studied, pollinators, albeit not herbivores, significantly affected plant fitness (*i.e.*, noncorrelated selection pressures). Consequently, forest fragmentation and the creation of edge habitats significantly affected the pollinator-mediated evolutionary potential, but in a mostly herbivore-independent fashion.

Key words: Forest fragmentation, pollination, herbivory, noncorrelated evolution.

INTRODUCTION

The extent to which plant-animal interactions are modified by habitat fragmentation is of paramount importance in conservation biology. The reduction in habitat size, increase in isolation, and creation of edge habitats in the remnant fragments may reduce the diversity and abundance of interacting species (Aguilar *et al.*, 2006; Steffan-Dewenter *et al.*, 2006). A reduction in animal interactors may lead to contrasting effects on their plant counterparts depending on whether they are mutualists (*e.g.*, pollinators) or antagonists (*e.g.*, herbivores). Coupled to a decrease in diversity and abundance of pollinators there is usually a decrease in the frequency of pollinator visits and, consequently, a decrease in the reproductive success of plants (Aguilar *et al.*, 2006). When herbivores are also scarce in fragments and edges, however, a some sort of compensation may arise in the reproductive success of plants depending on the strength of modifications of mutualisms and antagonisms (*i.e.*, the symmetry of relationships).

Modifications in plant-mutualistic and plant-anatagonistic interactions given by habitat fragmentation may have far reaching consequences than those usually claimed in terms of population persistence by affecting, for instance, microevolutionary dynamics (Bronstein *et al.*, 2004). Plants may face numerous selective pressures exerted by pollinators and herbivores, which may influence their ecological and evolutionary responses (Zamora, 2000; Herrera & Pellmyr, 2002). Herbivores may modify plant survivorship and reproductive success since their action may have a significant impact on plants by directly reducing seed production, or by indirectly modifying floral attractiveness (*i.e.*, plant traits) to pollinators, which in turn may also reduce seed production (Hendrix, 1987; Herrera, 2000; Herrera *et al.*, 2002b; Strauss & Zangerl, 2002; Danell & Bergström, 2002). Despite the pivotal role played by herbivores on the evolution of pollination-related traits in plants, however, assessments take into account the selection pressures jointly exerted by herbivores and pollinators have usually been overlooked (Strauss & Whitall, 2006). When fitness-reducing herbivores and fitness-enhancing pollinators have opposing preferences for a given plant trait (or combination of traits), they exert coincident selection favouring the same trait optimum, or favouring monomorphic populations when plant traits are discrete. When pollinators and herbivores share the same preference for a given plant trait, plants may exhibit traits that reflect a compromise between one or more than one value that maximizes fitness through interactions with mutualists and antagonists. Thus, conflicting selection pressures jointly exerted by pollinators and herbivores may contribute to maintain polymorphisms and additive genetic variance (Strauss & Whitall, 2006). Therefore, non-additivity of effects on plant fitness triggered by mutualists and antagonists, given by conflicting selection pressures, can be interpreted as evidence for correlated evolution on traits tending to increase and decrease the interaction of plants with their mutualists and antagonists, respectively (*sensu* Herrera, 2000; Herrera *et al.*, 2002b; Gómez, 2005). For instance, Herrera (2000) and Herrera *et al.* (2002b) proposed a herbivore-dependent selection pressure led by pollinators based on the non-additive effects elicited by mutualists and antagonists on seed production and seedling recruitment in the perennial herbs *Paeonia broteroi* and *Helleborus foetidus*, respectively. Similar conclusions were arrived at by Gómez (2005) for *Erysimum mediohispanicum*, and by Valdivia & Niemeyer (2005) for *Alstroemeria umbellata*.

Correlational selection elicited by pollinators and herbivores should be particularly frequent if pollinators and herbivores forage on plants bearing large floral (*i.e.*, reproductive traits) and foliar displays (*i.e.*, vegetative traits). Accordingly, reproductive traits such as number of flowers per plant, flower length, inflorescence length, and inflorescence complexity may altogether contribute to increase the reproductive success of plants and, therefore, to facing pollinator-mediated selection pressures (Geber, 1985; Schoen & Dubuc, 1990; Fishbein & Venable, 1996). Vegetative traits, such as number of leaves, foliar area, plant height, and herbivory resistance may also contribute to increase reproductive success of plants facing herbivore-mediated selection pressures (Simms, 1990; Marquis, 1992; Nuñez-Farfán & Dirzo, 1994).

Nevertheless, plants may undergo a reduction in the strength of interactions with their mutualists and antagonists which in turn may promote an evolutionary pathway in which traits linked to them run along dissimilar lanes. This fact may occur when plants naturally exhibit a strongly limited seed production due to their pollination mechanism (*i.e.*, pollination-related intrinsec factor) (*e.g.*, Valdivia & Niemeyer, 2007), when plants exhibit a high resistance to herbivores (*i.e.*, herbivory-related intrinsec factor), or when pollinators and herbivores are scarce as may occur in human-mediated fragmented habitats (*i.e.*, pollinator- and/or herbivore-mediated extrinsec factors). Unfortunately, no previous work has stressed this latter possibility. This is particularly important because correlational selection may be a mechanism for the functional integration of one trait with another, most likely promoting genetic integration (Lande, 1980; Brodie, 1992). Thus, regarding that a reduction in the strength of one or both interactions may lead plants towards an ecological scenario in which they do not face correlational selection pressures, thereby impinging upon trait correlations and even upon genetic correlations, the human-mediated habitat fragmentation might settle conditions for a lacking of phenotypic and genotypic integration of plants.

The aim of this work is to evaluate correlational selection pressures exerted by pollinators and herbivores in *Bomarea salsilla*, a vine endemic to the fragmented forest from south-central Chile, by modifying plant-pollinator and plant-herbivore interactions. Additionally, a suite of reproductive and vegetative traits was also evaluated for disclosing which traits are prone to be selected by pollinators and herbivores. If forest fragmentation and increase of edge habitats negatively affected pollination and herbivory on *B. salsilla*, additive effects (*i.e.*, non-correlated evolution) on plant fitness is indeed to be expected in these disturbed habitats, whereas in the core of continuous forest non-additivity (*i.e.*, correlational evolution) should be expected.

MATERIALS AND METHODS

Natural history

Fieldwork was conducted in Maulino forest in the northernmost zone of the temperate rainforest from Chile, from September 2006 to January 2007, and from October 2007 to February 2008, during the austral spring-summer seasons (35°59'S, 72°41'W; Bustamante *et al.*, 2005). Specifically, the study was performed in Los Queules National Reserve and two neighbouring forest fragments. Los Queules is a protected area of 145 ha embedded in a large tract of 600 ha of continuous forest. Forest fragments, ranging from 1 to 6 ha, are patches surrounded by commercial plantations of *Pinus radiata*. Here, four sites were defined taking into account the spatial arrangement of plant populations: one core and one edge of continuous forest, both sites placed in Los Queules National Reserve, and one core and one edge of forest fragment, both sites placed in two different fragments nearby Los Queules National Reserve. While cores were defined as sites placed ≥ 50 m inwards the border of each site, edges were defined as sites placed ≤ 10 m inwards the forest margin. Distance between sites ranges from 0.5 up to 2.5 km.

Bomarea salsilla (Alstroemeriaceae) is a protandrous, small-sized red-coloured climbing perennial vine, inhabiting the sclerophyllous and temperate forests of Chile from 33°S to 40°S (Muñoz and Moreira, 2003). In the study site, it flowers from *ca.* November to January. Its breeding system is unknown; however, two pilot pollinator-exclusion experiments in the study site, devised to test for autogamy and agamospermy, demostrated that *B. salsilla* is a totally pollinator-dependent plant for seed set (*i.e.*, a xenogamous plant). In the study site, floral visitors are hummingbirds, *Sephanoides sephaniodes* (Trochilidae); bumblebees and bees, *Bombus dahlbomii, Bombus terrestris*, and *Manuelia gayatina* (Apidae); butterflies, *Mathania leucothea* (Pieridae); and flies, *Acrophthalmyda paulseni* (Bombyliidae) (pers. obs.). Its herbivores are unknown; however, several leaf-eating herbivores have been reported for this forest (Simonetti *et al.*, 2006).

Correlated evolution on pollinator- and herbivore-linked traits

In order to determine a possible pathway for correlated evolution on pollinator- and herbivore-linked traits, during the austral spring-summer season, from September 2006 to January 2007, an experiment with a 2x2 factorial design following Herrera (2000) by excluding separately and simultaneously pollinators and herbivores was conducted. In short, this methodological approach, rather than modifying a specific set of traits, allowed for a statistical dissection of plant fitness following the experimental exclusions. Thus, pollinator exclusions mimicked an ancestral situation whereby plants exhibited a suite of traits which reduced the optimal mutualistic relationship between plants and pollinators, which in turn led to a decreased fitness of plants. On the other hand, herbivore exclusions mimicked an ancestral situation in which plants presented a suite of traits that allowed them to escape from herbivores (or show resistance to them), which in turn produced an increased fitness of plants. Therefore, this artificial array allows the dissection of the isolated effects of herbivores and pollinators, as well as the combined actions of both, and further the detection of non-additive effects of herbivores and pollinators, the obligate condition for the ocurrence of correlated evolution on traits related to them (*sensu* Herrera *et al.*, 2002b).

Herbivores were excluded by monthly spraying plants with Fastac, thus ensuring that no insect larvae nor mollusc fed on plants. Pollinators were excluded by enclosing inflorescences at the floral-bud stage with a tulle-mesh bag, albeit allowing herbivore access to leaves. Both treatment levels were factorially combined to conform the following four combinations: 1) control plants, *i.e.*, exposed to herbivores and pollinators, 2) herbivore treatment, *i.e.*, only exposed to pollinators, 3) pollinator treatment, *i.e.*, only exposed to herbivores, and 4) simultaneous herbivore and pollinator treatment, *i.e.*, excluded to both herbivores and pollinators.

For each combination, 30 plants were selected and monitored from September 2006 to February 2007 at each study site: core of continuous forest, edge of continuous forest, core of forest fragment, and edge of forest fragment. When the reproductive season was over, developing fruits were enclosed in tulle-mesh bags for collecting all seeds produced by each plant at the end of the reproductive season after experiencing the four treatments described above.

Furthermore, during the next reproductive season, from October 2007 to February 2008, in an extensive assessment comprising nearly all plants growing at each population (196, 153, 341, and 173 individual plants at the core of the continuous forest, edge of the continuous forest, core of the forest fragment, and edge of forest fragment, respectively), the naturally-occurring interactions between *B. salsilla* and its pollinators and herbivores were recorded. At each site, plants were categorized depending on whether they did or did not interact with pollinators and herbivores. A plant significantly interacted with pollinators when exhibited fruit production immediately after flowering period just when fruits were distinctly swollen. A plant significantly interacted with herbivores when at the end of the reproductive season, *i.e.*, when seed dispersal took place, exhibited a significant loss of vegetative tissue unequivocally owing to the action of leaf-chewing and leaf-sucking animals. Thus, plants that significantly interacted with pollinators, herbivores, none or boths were registered and tagged for disclosing the reproductive success after naturally experienced the action of both kind of animals, and then a possible pathway for pollinator- and herbivore-mediated correlational evolution on pollinator- and herbivore-linked traits.

Pollinator and herbivore preferences for reproductive and vegetative traits

In all plants naturally excluded or exposed to pollinators and herbivores two suites of reproductive and vegetative traits were assessed, from October 2007 to February 2008, at each study site (196, 153, 341, and 173 individual plants at the core of the continuous forest, edge of the continuous forest, core of the forest fragment, and edge of forest fragment, respectively) in order to disclose which traits and phenotypic values are attracting or repelling pollinators and herbivores. Reproductive traits assessed were i) flower number, ii) flower length, iii) ray length, and iv) inflorescence complexity. Flower number was determined by directly counting all flowers borne on inflorescences (there is a single inflorescence per invidual plant). Flower length was estimated from the tepal insertion to the end of this with a hand-held ruler. Similarly, ray length was estimated in the inflorescence portion ranging from the inflorescence base to the flower calix. Because rays may be arranged, in an increasing order, as monoseried, biseried, and triseried, ray length was estimated from the inflorescence base up to the flower calyx of the bigger series (Fig. 2.1). Inflorescence complexity was estimated by scoring plants as one, two, or three depending on whether they exhibited monoseried, biseried, or triseried rays, respectively; when plants showed more than one type of ray (*i.e.*, exhibit irregular shapes), they were scored with intermediate values (Fig. 2.1). Vegetative traits assessed were v) leaf number, vi) foliar area, vii) plant height, and viii) herbivory resistance. Leaf number was determined by directly counting and tagging all leaves on plant spikes (there is a single spike per invidual plant) at the start of the flowering period when no new leaf develops. Foliar area was estimated by measuring length and wide of leaves and then computing area by using the oval area equation because this figure closely resembles leaf shape (data not shown). Plant height was estimated in elongated spikes from the soil surface up to the inflorescence base with a hand-held metric band (Fig. 2.1). Herbivory resistance, assessed as the percentage of undamaged foliar tissue, was estimated at the start of seed dispersal period by checking all leaves borne by each plant. In each leaf, a transparent paper grid (2-mm spaced) was put on the leaf surface for then counting the total squares occupied by foliar tissue and the total squares exhibiting a lack of foliar tissue but inside the projection of the leaf margin.

In all those cases in which more than one measure is attainable for each individual plant (*i.e.*, flower length, ray length, and foliar area) two measures per each trait were performed and then averaged in order to obtain a representative sample in a per-plant basis.

Statistical analyses

Non-additivity for the effects of pollinators and herbivores on plant fitness in the manipulative and natural experiments was analysed by non-parametric two-way ANOVAs for ranked data (Scheirer-Ray-Hare extension of Kruskal-Wallis Test) because the original data set did not meet the normality assumptions required for the traditionl ANOVAs. Mann-Whitney tests were further applied for pairwise comparisons between groups with significant *P*-values obtained after Bonferroni corrections. Thereafter, medians instead of means were reported.

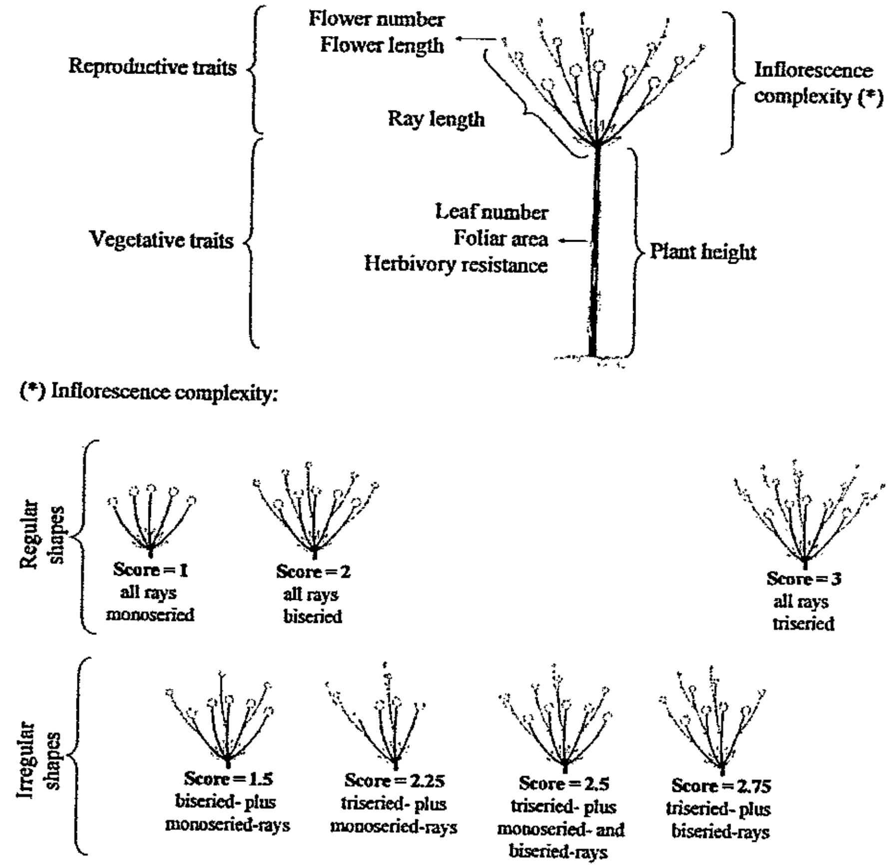

Figure 2.1. Non-scaled schematic representation of reproductive and vegetative traits in an elongated plant of *Bomarea salsilla.*

RESULTS

Correlated evolution on pollinator- and herbivore-linked traits

In plants experimentally exposed or excluded to pollinators and herbivores no seed was produced in absence of pollinators; whereas, seed set was significantly higher when pollinators were present in plants exposed and excluded to herbivores at all sites studied (Fig. 2.2). Herbivores, however, did not reduce seed set significantly neither in those plants excluded from pollinators (*i.e.*, a direct effect) (Fig. 2.2), nor in the presence of pollinators (*i.e.*, an indirect pollinator-mediated effect) at all sites herein assessed (Fig. 2.2). Consequently, pollinators, but not herbivores, had a significant effect of seed set of *B. salsilla* by exerting additive effects (*i.e.*, non-correlated selection pressures) on seed production at all sites studied (Table 2.1).

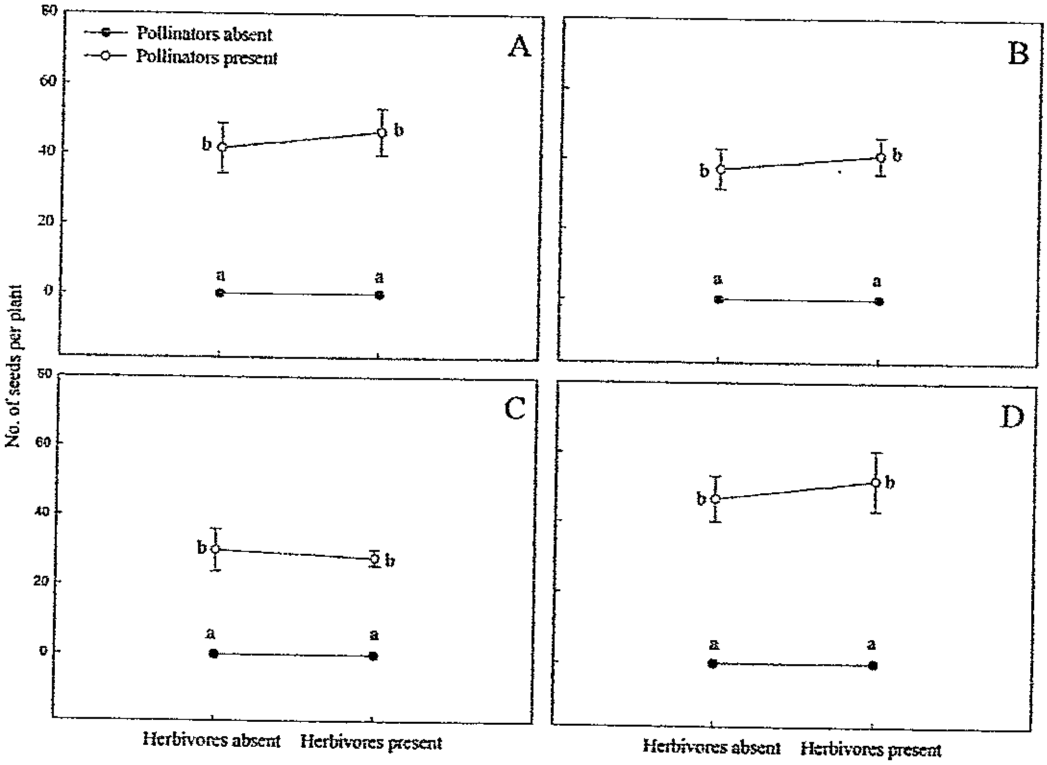

Figure 2.2. Experimental manipulations testing for the effects of pollinators and herbivores on seed set of *Bomarea salsilla* in the core of continuous forest (A), core of forest fragment (B), edge of continuous forest (C), and edge of forest fragment (D) (Dissimilar letter depict significant differences ($P < 0.008$) following Mann-Whitney *U*-Tests for pairwise comparisons after Bonferroni corrections; means ± 1SE are depicted).

Table 2.1. Summary of the non-parametric two-way ANOVAs for ranked data (Scheirer-Ray-Hare extension of Kruskal-Wallis Test) testing for the effects of pollinators and herbivores on the reproductive success of *Bomarea salsilla* experimentally exposed and excluded to pollinators and herbivores in (a) core of continuous forest, (b) edge of continuous forest, (c) core of forest fragment, and (d) edge of forest fragment.

Source	df	MS	*H*	*P*
(a) Core of Continuous Forest:				
Pollinator Effect (PE)	1	97,470.0	20.798	<0.001
Herbivore Effect (HE)	1	197.6	0.042	0.838
PE x HE	1	197.6	0.042	0.838
Error	116	217.9		
(b) Edge of Continuous Forest:				
Pollinator Effect (PE)	1	108,000.0	22.933	<0.001
Herbivore Effect (HE)	1	525.0	0.111	0.739
PE x HE	1	525.0	0.111	0.739
Error	116	145.1		
(c) Core of Forest Fragment:				
Pollinator Effect (PE)	1	108,000.0	22.930	<0.001
Herbivore Effect (HE)	1	110.2	0.023	0.879
PE x HE	1	110.2	0.023	0.879
Error	116	153.0		
(d) Edge of Forest Fragment:				
Pollinator Effect (PE)	1	108,000.0	22.930	<0.001
Herbivore Effect (HE)	1	5.6	0.001	0.975
PE x HE	1	5.6	0.001	0.975
Error	116	154.8		

In plants naturally exposed or excluded to pollinators and herbivores, the proportion of plants interacting with none, one, or both types of animals significantly differed from a random distribution in the core of continuous forest and edge of forest fragment, but not in the core of forest fragment and edge of continuous forest (Fig. 2.3). In plants excluded from pollinators, there was a reduction of 32.8%, 92.6%, and 83.3% in the amount of plants interacting with herbivores in the edge of continuous forest, core of forest fragment, and edge of forest fragment with respect to the core of continuous forest, respectively (Fig. 2.3). Similarly, in plants exposed to pollinators, there was a reduction of 85.0%, 69.2% and 60.9% in the amount of plants interacting with herbivores in the edge of continuous forest, core of forest fragment, and edge of forest fragment with respect to the core of continuous forest, respectively (Fig. 2.3). In contrast, in plants excluded from herbivores, there was an increase of 60.7%, 688.5% and 98.4% in the amount of plants interacting with pollinators in the edge of continuous forest, core of forest fragment, and edge of forest fragment with respect to the core of continuous forest, respectively (Fig. 2.3). Furthermore, there was an increase of 36.0% and 56.6% in the amount of plants which did not interact with pollinators and herbivores in the edges of continuous forest and forest fragment with respect to the core of continuous forest, respectively; and a decrease of 8.1% in the amount of plants which did not interact with pollinators or herbivores in the core of forest fragment with respect to the core of continuous forest (Fig. 2.3).

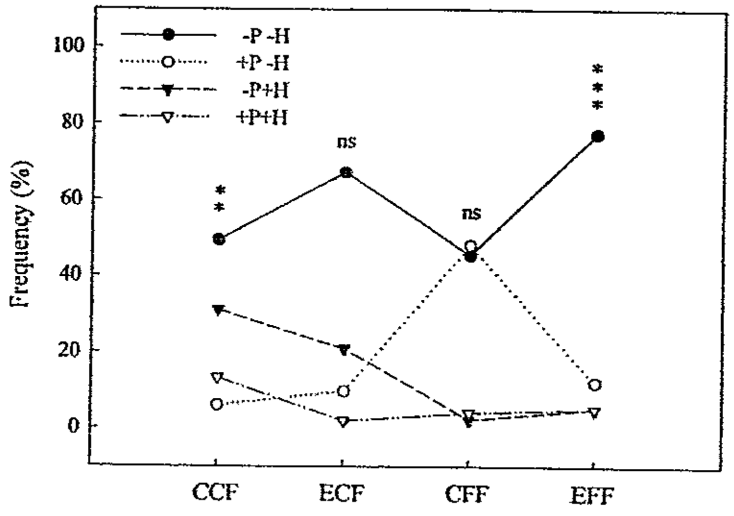

Figure 2.3. Proportion of plants interacting (+) or not (-) with pollinators (P) and herbivores (H) in the core of continuos forest (CCF), edge of continuous forest (ECF), core of forest fragment (CFF), and edge of forest fragment (EFF) (ns: not significant differences, **: $P < 0.01$; ***: $P < 0.001$; following two-tailed Fisher Exact Tests for each site).

In plants naturally exposed or excluded to pollinators and herbivores no seed was produced in absence of pollinators; whereas, seed set was significantly higher when pollinators were present in plants exposed and excluded to herbivores at all sites studied (Fig. 2.3). On the contrary, herbivores did not reduce seed set significantly neither in those plants excluded from pollinators (*i.e.*, a direct effect) (Fig. 2.3), nor in the presence of pollinators (*i.e.*, an indirect pollinator-mediated effect) at all sites herein assessed (Fig. 2.3). Therefore, pollinators, but not herbivores, had a significant effect of seed set of *B. salsilla* (*i.e.*, non-correlated selection pressures) on seed production at all sites studied (Table 2.2, Fig. 2.3).

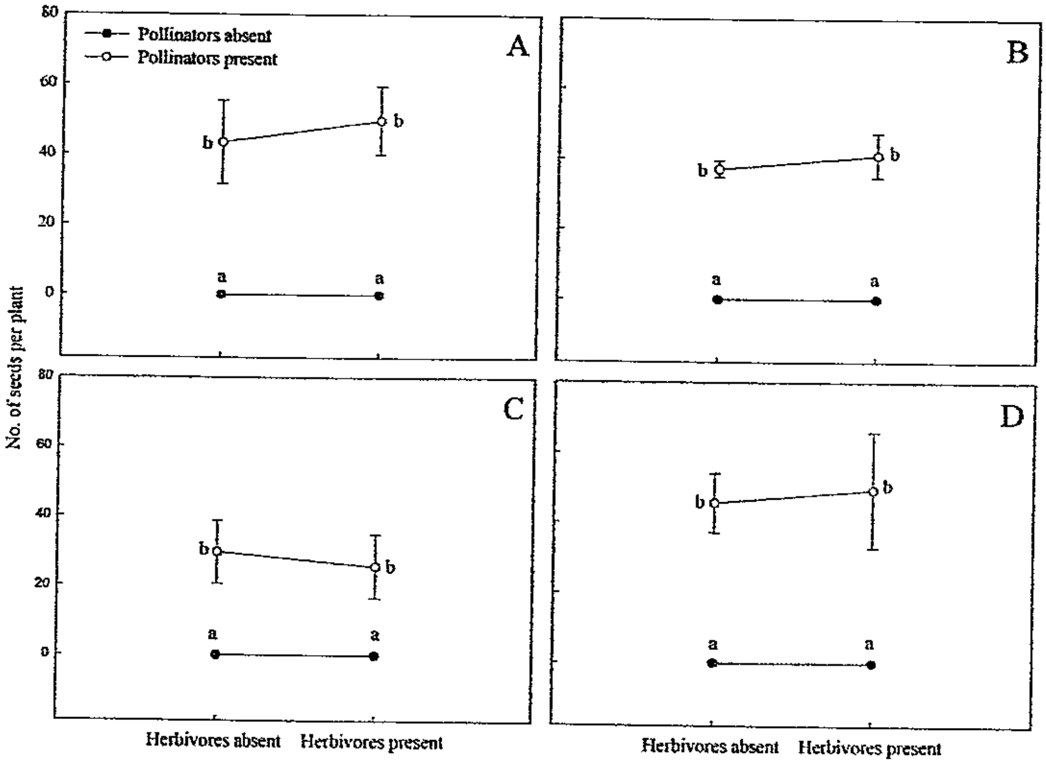

Figure 2.4. Pollinator- and herbivore-mediated effects on seed set of *Bomarea salsilla* in the core of continuous forest (A), core of forest fragment (B), edge of continuous forest (C), and edge of forest fragment (D) following naturally excluded or exposed plants (Dissimilar letter depict significant differences ($P < 0.008$) following Mann-Whitney *U*-Tests for pairwise comparisons after Bonferroni corrections; means ± 1SE).

Table 2.2. Summary of the non-parametric two-way ANOVAs for ranked data (Scheirer-Ray-Hare extension of Kruskal-Wallis Test) testing for the effect of pollinators and herbivores on the reproductive success of *Bomarea salsilla* naturally exposed and/or excluded to pollinators and herbivores in (a) core of continuous forest, (b) edge of continuous forest, (c) core of forest fragment, and (d) edge of forest fragment.

Source	df	MS	*H*	*P*
(a) Core of Continuous Forest:				
Pollinator Effect (PE)	1	237,589.0	23.422	<0.001
Herbivore Effect (HE)	1	449.0	0.044	0.833
PE x HE	1	449.0	0.044	0.833
Error	192	68.0		
(b) Edge of Continuous Forest:				
Pollinator Effect (PE)	1	54,015.9	17.708	<0.001
Herbivore Effect (HE)	1	9.1	0.003	0.956
PE x HE	1	9.1	0.003	0.956
Error	149	3.2		
(c) Core of Forest Fragment:				
Pollinator Effect (PE)	1	75.4	2.179	0.140
Herbivore Effect (HE)	1	51.1	1.477	0.224
PE x HE	1	19.2	0.556	0.456
Error	337	17.9		
(d) Edge of Forest Fragment:				
Pollinator Effect (PE)	1	107,888.9	21.297	<0.001
Herbivore Effect (HE)	1	0.0	<0.001	0.998
PE x HE	1	0.0	<0.001	0.998
Error	169	13.3		

Pollinator and herbivore preferences for reproductive and vegetative traits

Pollinators and herbivores exhibited similar and dissimilar preferences for reproductive and vegetative traits in *B. salsilla* depending on whether which trait and which population is considered (Fig. 2.4 and 2.5). As far as reproductive traits are concerned, at the core of continuous forest, pollinators preferred plants with higher number of flowers in the presence and absence of herbivores; by contrast, at the edge of continuous forest equally than at the core of forest fragment no preference was recorded, while at the edge of forest fragment pollinators preferred plants with higher number of flowers, but only in the absence of herbivores (Fig. 2.4). In terms of flower length, pollinators preferred plants exhibiting longer flowers only at the edge of forest fragment and in the absence of herbivores (Fig. 2.4). In terms of ray length, pollinators preferred longer inflorescences in the absence of herbivores at all sites studied, while in the presence of herbivores, pollinators preferred longer inflorescences only at the core of continuous forest (Fig. 2.4). In terms of inflorescence complexity, pollinators preferred more complex inflorescences at the core of continuous forest, core of forest fragment, and edge of forest fragment while herbivores were absents, while present, however, pollinators preferred more complex inflorescences only at the core of continuous forest (Fig. 2.4). Taken together, pollinators exhibited preferences for 3, 2, 2, and 4 reproductive traits in the absence of herbivores; and for 3, 0, 0, and 0 in the presence of herbivores at the core of continuous forest, edge of continuous forest, core of forest fragment, and edge of forest fragment, respectively.

Concerning vegetative traits, while pollinators were absents, herbivores preferred plants bearing higher number of leaves only at the edge of continuous forest; whereas pollinators were present, herbivores did not exhibit preferences (Fig. 2.5). In terms of foliar area and plant height, herbivores did not exhibit preferences for higher values, both in the presence and absence of pollinators, at all sites studied (Fig. 2.5). In terms of herbivory resistance, herbivores preferred plants exhibiting a lower herbivory resistance while pollinators were present or absent (Fig. 2.5). Taken together, herbivores exhibited preferences for 1, 2, 1, and 1 vegetative traits in the absence of pollinators at the core of continuous forest, edge of continuous forest, core of forest fragment, and edge of forest fragment, respectively; and for only one trait in the presence of pollinators at all sites studied.

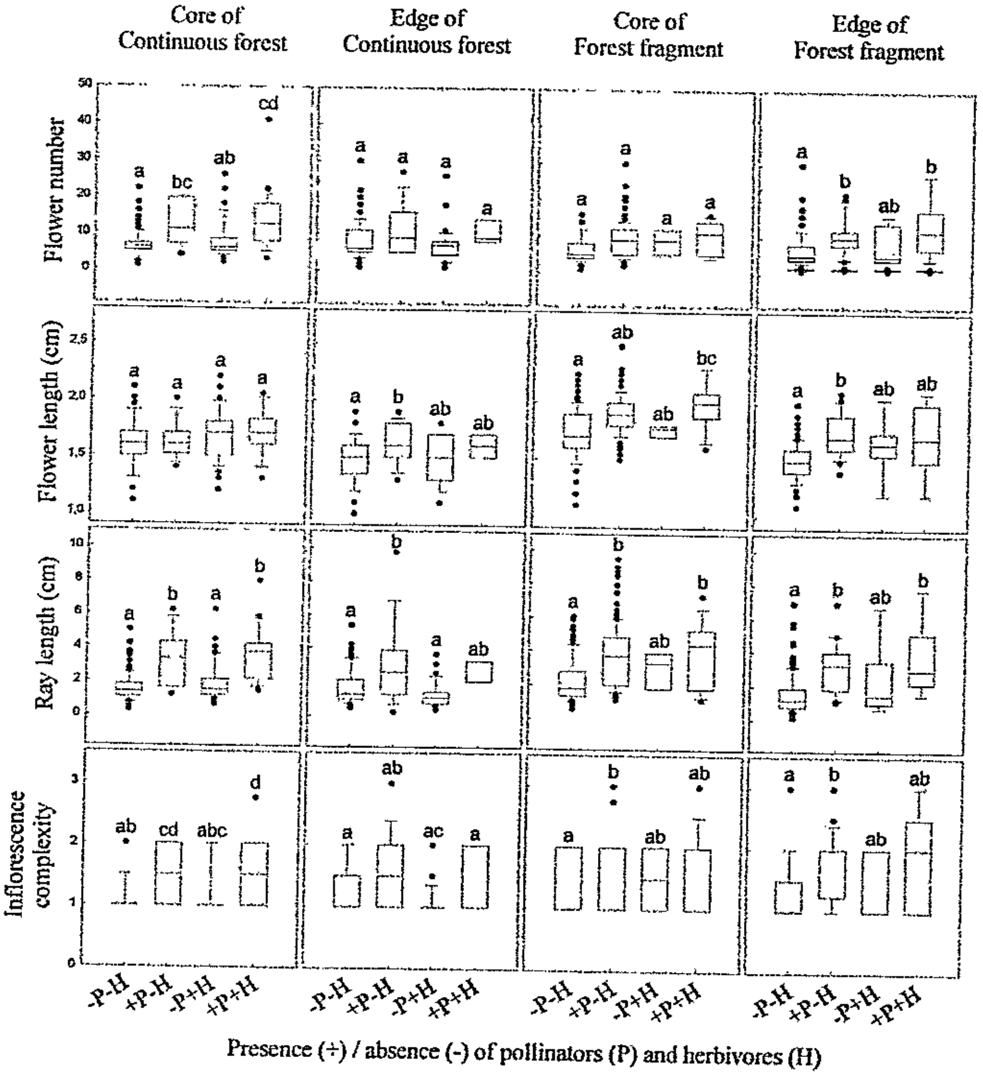

Figure 2.5. Pollinator and herbivore preferences for reproductive traits in *Bomarea salsilla* at the fragmented Maulino forest (Dissimilar letters depict significant differences ($P < 0.008$) following pairwise comparisons through Mann-Whitney *U*-Tests after Bonferroni corrections at each site. Medians plus the percentils 25% and 75% are depicted in plots, bars depict non-outlier minimum and maximum values, whereas dopts depict outliers values).

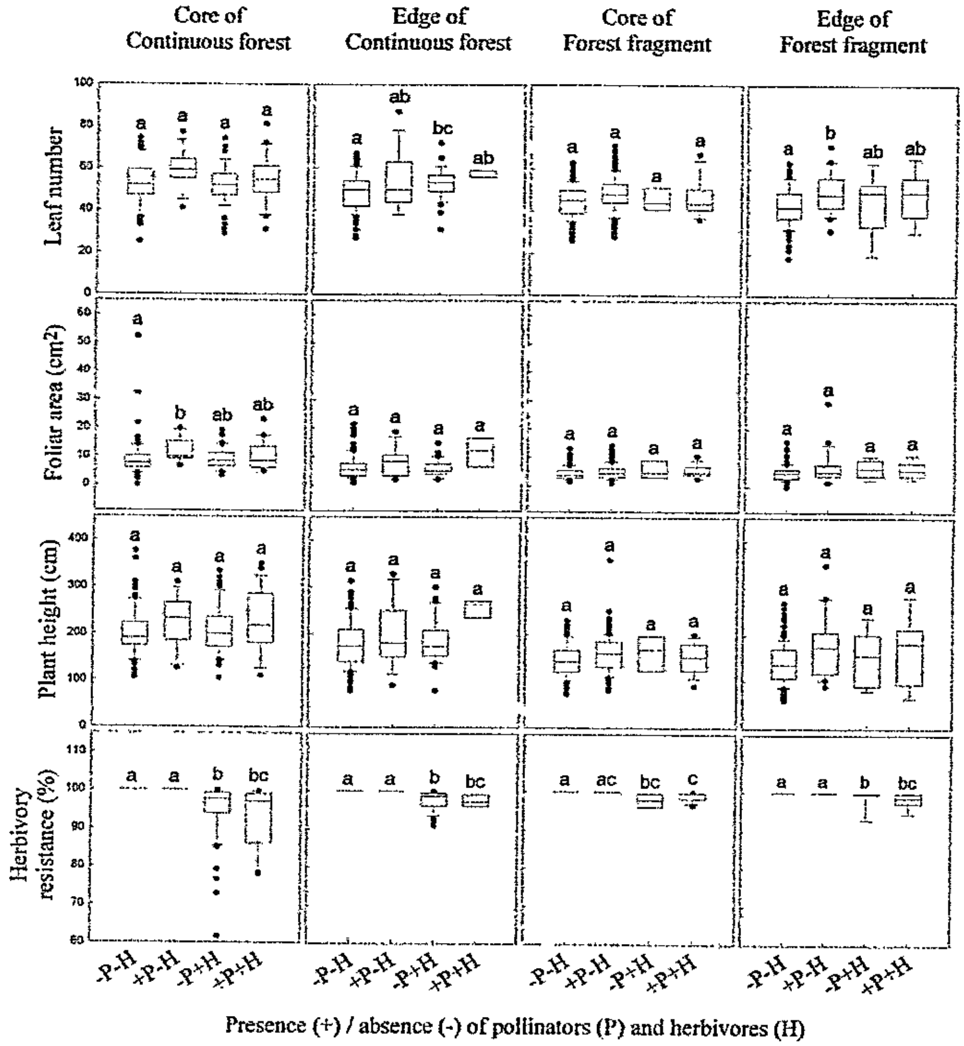

Figure 2.6. Pollinator and herbivore preferences for vegetative traits in *Bomarea salsilla* at the fragmented Maulino forest (Dissimilar letters depict significant differences ($P < 0.008$) following pairwise comparisons through Mann-Whitney *U*-Tests after Bonferroni corrections at each site. Medians plus the percentils 25% and 75% are depicted in plots, bars depict non-outlier minimum and maximum values, whereas dopts depict outliers values).

DISCUSSION

Pollinators and herbivores did not exert correlational selection pressures in *B. salsilla* at the core of continuous forest as well as at the disturbed sites (*i.e.*, edges and fragments), thus suggesting that pollinator- and herbivore-linked traits currently run along dissimilar lanes (Herrera *et al.*, 2002b). Therefore, there was not a negative effect of forest fragmentation and the increase in edge habitats on the possibility of coupling reproductive and vegetative traits through pollinator- and herbivore-mediated correlational selection pressures. This fact contrasts with our original expectations by which herbivores should impair *B. salsilla* reproductive success in the core of continuous forest. The most plausible explanation for this situation arises from the evolutionary trajectory of plant-herbivore relationships. Plants currently unaffected by herbivores most likely loss their additive genetic variance that confer susceptibility to the action of herbivores throughout the evolutionary time (*i.e.*, a herbivory-related intrinsec factor) due to herbivore-mediated positive selection pressures on resistance. Such plants, therefore, may currently "escape" from the negative impact of herbivory on fitness by avoiding to be preyed by herbivores. In fact, at the core of continuous forest, herbivores accounted for by 2.6% of foliar surface removal and preyed on 44.4% of *B. salsilla* plants, thus rendering unlikely the possibility of a fitness reduction. For instance, Mothershead & Marquis (2000) found that herbivores accounted for by 6.5% of leaf area loss in *Oenothera macrocarpa* (*i.e.*, a 2.5-fold higher herbivory with respect to *B. sasilla*) which translated into a narrow decrease in seed production. In fact, only when herbivory was experimentally increased up to 33.4% of foliar surface loss, plants experienced an important reduction in seed production (Mothershead & Marquis, 2000). On the other hand, Nuñez-Farfán & Dirzo (1994) in spite of finding a significant herbivore-mediated phenotypic selection pressure on plant size and herbivory resistance in the alien plant *Datura stramonium* from central Mexico, heritabilities exhibited by both traits were very small and not significantly different from zero. Taken together, both examples point to consider that a loss in proclivity of plants to be preyed on by herbivores and a loss of additive genetic variance accounting for such a proclivity is not an uncommon situation.

Herbivores may also modify reproductive traits in plants which may translate into modifications in pollinator behaviours and, consequently, in plant reproductive success (Mothershead & Marquis, 2000). In fact, despite pollinators were the only animals significantly affecting plant reproductive success in *B. salsilla*, their actions seem to be partially mediated by herbivores which determined pollinator preferences for plants bearing a given suite of traits. This indicates that herbivores played an indirect role in determining a pollinator-driven evolutionary potential which also differ among sites. Plants inhabiting continuous forest attracted pollinators in a non-random fashion regardless of whether herbivores were present or absent. In this site, pollinators selected for plants bearing a higher number of flowers, and longer and complex inflorescences. In disturbed sites, however, pollinators randomly visited plants when herbivores were present, but when absent, pollinators preferred plants bearing longer flowers and inflorescences in the edge of continuous forest and core of forest fragment, and higher number of flowers, longer flowers and inflorescences, and more complex inflorescences in the edge of forest fragment. This points to consider that, in plants inhabiting disturbed sites, herbivores might impair pollinator preferences and, therefore, pollinator-mediated selection pressures by modifying reproductive traits. Nevertheless, in these disturbed sites, herbivores preyed on very few plants of compared with the core of continuous forest and, therefore, very few plants may exhibit an indirectly herbivore-dependent pollinator-mediated reproductive success. In fact, while the proportion of plants significantly interacting with herbivores and pollinators differed from a random distribution in the core of continuous forest, in fragments and edges -excepting the edge of forest fragment- the proportion of plants interacting with both types of animals did not differ from a random expectation.

Taken together, the effect of herbivores on seed production and on pollinator preferences seems contradictory. On one hand, herbivores did not impair plant fitness. On the other hand, herbivores modified pollinator preferences but only in those sites where herbivores meagerly preyed on plants. If herbivores really modified pollinator choice with no effect on seeds production, a some sort of tolerance could had arose in such disturbed habitats. Tolerance is an important trait in plants that compensates the negative effects of herbivores on plant fitness (Marquis, 1992). The very low herbivory faced by plants in fragments and edges, however, points to consider that tolerance is not the most parsimonious explanation. In fact, correlations between vegetative and reproductive traits, instead of herbivore-mediated modifications on plant reproductive traits, may account for such an apparently indirect herbivore-dependent pollinator-mediated preferences on reproductive traits in disturbed habitats. Plants bearing vegetative traits preferred by herbivores may simultaneously exhibit lowered values of reproductive traits which are not preferred by pollinators. Reproductive and vegetative traits, however, are not usually highly integrated (Berg, 1960; Armbruster *et al.*, 1999; Herrera *et al.*, 2002a). An assessment takes into consideration tolerance and phenotypic integration would be desirable.

The present report fails to disclose correlational selection pressures on pollinator- and herbivore-linked traits in *B. salsilla*, and a lack thereof in disturbed habitats due to herbivory-related intrinsec factors. Nowaday, however, many plants grows in highly fragmented habitats and are likely to face a disruption on mutualist- and antagonist-related selection pressures (*i.e.*, a herbivore-related extrinsec factor). In this regard, the incorporation of the more progressive and sofisticated assessments given by evolutionary ecology into conservation biology is of a mandatory importance.

ACKNOWLEDGEMENTS

Numerous persons, in several ways, kindly contributed to perform this work: Brenda Valdivia, Carlos O. Valdivia, Florencia Prats, Sandra Valdivia, Fernando Campos, Luciano Silva, Patricio Molina, and Sergio Hernández. The Chilean Forestry Service (CONAF) and Forestal Millalemu partially supported fieldwork. This work was funded by Beca de Apoyo a la Realización de Tesis Doctoral, Conicyt 23070138 (C.E.V.).

REFERENCES

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.

Armbruster, W.S., di Stilio, V.S., Tuxill, J.D., Flores, T.C., Velásquez Runk, J.L. 1999. Covariance and decoupling of floral and vegetative traits in nine Neotropical plants: a re-evaluation of Berg's correlation-pleiades concept. American Journal of Botany 86, 39-55.

Berg, R.L. 1960. The ecological significance of correlation pleiades. Evolution 14, 171-180.

Brodie, E.D. 1992. Correlational selection for color pattern and antipredator behavior in the garter snake *Thamnophis ordinoides*. Evolution 46, 1284–1298.

Bronstein, J.L., Dieckmann, U., Ferrière, R. 2004. Coevolutionary dynamics and the conservation of mutualisms. In: Ferrière, R., Dieckmann, U., Couvet, D. (Eds.). Evolutionary Conservation Biology, pp: 305-326, Cambridge University Press.

Bustamante, R.O., Simonetti, J.A., Grez, A.A., San Martín, J. 2005. Fragmentación y dinámica de regeneración del bosque Maulino: diagnóstico actual y perspectivas futuras. In: Smith-Ramírez, C.S., Armesto, J.J., Valdovinos, C. (Eds.), Historia, biodiversidad y ecología de los bosques costeros de Chile. Editorial Universitaria, Santiago, Chile, pp: 555–564.

Danell, K., Bergström, R. 2002. Mammalian herbivory in terrestrial environments. In: Herrera, C.M., Pellmyr, O. (Eds.). Plant-animal interactions, an evolutionary approach, pp: 107-131. Blackwell Science. Oxford, UK.

Fishbein, M., Venable, D.L. 1996. Evolution of inflorescence design: theory and data. Evolution 50, 2165–77.

Geber, M. 1985. The relationship of plant size to self-pollination in *Mertensia ciliata*. Ecology 64, 762–72.

Gómez, J.M. 2005. Non-additive effects of herbivores and pollinators on *Erysimum mediohispanicum* (Cruciferae) fitness. Oecologia 143, 412–418.

Herrera, C.M. 2000. Measuring the effects of pollinators and herbivores: evidence for non-additivity in a perennial herb. Ecology 81, 2170–2176.

Herrera, C.M., Cerdá, X., García, M.B., Guitián, J., Medrano, M., Rey, P.J., Sánchez-Lafuente, A.M. 2002a. Floral integration, phenotypic covariance structure and pollinator variation in bumblebee-pollinated *Helleborus foetidus*. Journal of Evolutionary Biology 15, 108-121.

Herrera, C.M., Medrano, M., Rey, P.J., Sánchez-Lafuente, A.M., García, M.B., Guitián, J., Manzaneda, A.J. 2002b. Interactions of pollinators and herbivores on plant fitness suggests a pathway for correlated evolution of mutualism- and antagonism-related traits. Proceedings of the National Academy of Sciences of the United States of America (PNAS) 99, 16823–16828.

Herrera, C.M., Pellmyr, O. (Eds.). 2002. Plant–Animal Interactions. An evolutionary approach. Oxford: Blackwell Publishing.

Hendrix, S.D. 1987. Herbivory and its impact on plant reproduction. In: Lovett-Doust, J., Lovett-Doust, L. (Eds.). Plant Reproductive Ecology, Patterns and Strategies, pp: 246-263. Oxford University Press, Oxford, U.K.

Lande, R. 1980. The genetic covariance between characters maintained by pleiotropic mutations. Genetics 94, 203–215.

Marquis, R. 1992. Selective impact of herbivores. In: Fritz, R.S., Simms, E.L. (Eds.). Plant Resistance to Herbivores. Ecology, Evolution, and Genetics, pp: 301-325. University of Chicago Press.

Mothershead, K., Marquis, R.J. 2000. Fitness impacts of herbivory through indirect effects on plant-pollinator interactions in *Oenothera macrocarpa*. Ecology 81, 30-40.

Muñoz, M., Moreira, A. 2002. *Alstroemerias* de Chile. Diversidad, distribución y conservación. Taller La Era, Santiago, Chile.

Nuñez-Farfán, J., Dirzo, R. 1994. Evolutionary ecology of *Datura stramonium* L. in central Mexico: natural selection for resistance to herbivorous insects. Evolution 48, 423-436.

Schoen, D.J., Dubuc, M. 1990. The evolution of inflorescence size and number: a gamete-packaging strategy in plants. American Naturalist 135, 841–57.

Simms, E.L. 1990. Examining selection on multivariate phenotype: plant resitance to herbivores. Evolution 44, 1177-1188.

Simonetti, J.A., Grez, A.A., Bustamante, R.O. 2006. Interacciones y procesos en bosque Maulino fragmentado. In: Grez, A.A., Simonetti, J.A., Bustamante, R.O. (Eds.), Biodiversidad en ambientes fragmentados de Chile: Patrones y procesos a diferentes escalas, pp: 99-114. Editorial Universitaria. Santiago.

Steffan-Dewenter, I., Klein, A.M., Gaebele, V., Alfert, T., Tscharntke, T. 2006. Bee diversity and plant-pollinator interactions in fragmented landscapes. In: Waser, N.M., Ollerton, J. (Eds.). Plant-Pollinator Interactions, from Specialization to Generalization, pp: 387-407. The University of Chicago

Press.

Strauss, S.Y., Whitall, J.B. 2006. Non-pollinator agents of selection on floral traits. In: Harder, L.D., Barrett, S.C.H. (Eds.). Ecology and Evolution of Flowers, pp: 120-138. Oxford University Press.

Strauss, S.Y., Zangerl, A.R. 2002. Plant-insect interactions in terestrial ecosystems. In: Herrera, C.M., Pellmyr, O. (Eds.). Plant-animal interactions, an evolutionary approach, pp: 77-106. Blackwell Science. Oxford, U.K.

Valdivia, C.E., Niemeyer, H.M. 2005. Reduced maternal fecundity of the high-Andean perennial herb *Alstroemeria umbellata* (Alstroemeriaceae) by aphid herbivory. New Zealand Journal of Ecology 29, 321–324.

Valdivia, C.E., Niemeyer, H.M. 2007. Noncorrelated evolution between herbivore- and pollinator-linked features in *Aristolochia chilensis* (Aristolochiaceae). Biological Journal of the Linnean Society 91, 239-245.

Zamora, R. 2000. Functional equivalence in plant–animal interactions: ecological and evolutionary consequences. Oikos 88, 442–447.

Capítulo III

POLLINATOR-MEDIATED PHENOTYPIC SELECTION ON REPRODUCTIVE AND VEGETATIVE TRAITS IN A FRAGMENTED FOREST: ASSESSING THE MICROEVOLUTIONARY PRESENT OF *BOMAREA SALSILLA*

CARLOS E. VALDIVIA AND JAVIER A. SIMONETTI

Departamento de Ciencias Ecológicas, Facultad de Ciencias, Universidad de Chile.
Casilla 653, Santiago, Chile.

ABSTRACT

Habitat fragmentation and the creation of edge habitats negatively affect pollination and plant reproductive success. Nevertheless, no previous work has stressed microevolutionary processes in fragmented habitats. In fragments and edges a reduction in frequency and magnitude of traits, and combinations thereof, facing pollinator-mediated phenotypic selection is indeed to be expected. Such a reduction coupled to changes in identity of traits under selection in fragments and edges may translate into changes of means, variances, and covariances of characters. Collectively, changes in character expression and selective pression may render plants into the pathway of evolutionary trapping or suicide, thereby endangering the long-term population persistence. In the fragmented Maulino forest, we assessed pollinator-mediated phenotypic selection acting on eight reproductive and vegetative traits (*i.e.*, flower number, flower length, ray length, inflorescence complexity, leaf number, foliar area, plant height, and herbivory resistance) of *Bomarea salsilla.* In fragments and edges there was a reduction in frequency and identity, albeit not magnitude, of traits and combinations thereof, to be selected by pollinators. Therefore, plants occurring at these disturbed sites might most likely be in the pathway of evolutionary trapping and even suicide, thus endangering the long-term population persistence. A long-term research devised to test for the effects of habitat fragmentation on population persistence is of mandatory importance for disclosing such posibilities.

Key words: Pollination, habitat fragmentation, phenotypic selection, population persistence

INTRODUCTION

The extent to which plant-animal interactions are disrupted by habitat fragmentation and the creation of edge habitats is of pivotal importance in conservation biology. The reduction in habitat size, and increase in isolation in the remnant fragments may reduce the diversity and abundance of interacting species as, for instance, mutualists (*e.g.*, pollinators) (Aguilar *et al.*, 2006; Steffan-Dewenter *et al.*, 2006). A reduction in abundance and diversity of pollinators may lead to a decrease in the frequency of pollinator visits and, consequently, a decrease in the reproductive success of plants (Aguilar *et al.*, 2006). Such a reduction, however, varies according to plant traits and pollinator types (Aguilar *et al.*, 2006).

From the evolutionary viewpoint, pollinators have provided convincing evidence for the evolution on a variety of traits. For instance, reproductive traits such as number of flowers per plant, flower length, inflorescence length, and inflorescence complexity may altogether contribute to increase the reproductive success of plants (Geber, 1985; Schoen & Dubuc, 1990; Fishbein & Venable, 1996). Vegetative traits, such as number of leaves, foliar area, plant height, and herbivory resistance may also contribute to increase reproductive success of plants if they closely correlates with reproductive traits (Simms, 1990; Marquis, 1992; Nuñez-Farfán & Dirzo, 1994). Nevertheless, although pollinator-mediated evolution and diversification of plants have undobtely occurred in an ever-changing world that includes habitat fragmenation and creation of edge habitats, the rate, scale, and intensity of such disturbances when humans mediated propably exceed that previously experienced by plants (Kearns *et al.*, 1998; Aizen and Vázquez, 2006). Despite this fact, however, throughout the last two decades fragmentation studies of plant populations have mainly focused on demographic processes, instead of evolutionary ones, with particular emphasis in evaluating the effects of fragmentation on pollination and plant fecundity (Hobbs & Yates, 2003; Ghazoul, 2005; Honnay *et al.*, 2005; Aguilar *et al.*, 2006). This situation may certainly hinder our understanding of plant evolution in "the real world" which strongly encourages the incorporation of evolutionary ecology into conservation biology (Bronstein *et al.*, 2004).

In human-mediated fragmented habitats, as in edge habitats, plants might experience three evolutionary pathways depending on whether which type of selection is operating, namely, evolutionary rescue, evolutionary trapping, or evolutionary suicide (Ferrière *et al.*, 2004). The former would occur when fragmentation reduce the viability range of a population to an extent that, in the absence of evolution, the population would go extinct, but simultaneously induces directional selection pressures that population to escape extinction through evolutionary adaptation. Evolutionary trapping would happen when stabilizing selection pressures prevent a population from responding evolutionarily to fragmentation, that is, populations maintain their phenotypic states until they cease to be viable. Evolutionary suicide amounts to a gradual decline, driven by directional selection, of a population's phenotypic state towards extinction. Such a tendency can be trigger and/or exacerbated by environmetal changes (Ferrière *et al.*, 2004).

In short, Ferrière *et al.* (2004) point to consider that directional, disruptive, and correlational selection pressures may lead plants to an either evolutionary rescue (*i.e.*, population persistence) or evolutionary suicide (*i.e.*, population extinction) depending on whether the nature and intensity of fragmentation as well as the population capacities of fitting to these fragmented habitats. On the contrary, stabilizing selection pressures might lead plants to an evolutionary trapping (*i.e.*, popultion extinction) because populations would be unable of adapting to new conditions exhibited by fragmented habitats. In this regard, however, it is worthwhile highlighting that stabilizing selection pressures might conduct population to extinction if environmental conditions of fragmented habitats (*e.g.*, successional stage) are out of an equilibrium stage with respect to the original conditions. If not so, stabilizing selection might by contrast contribute to population persistence as well as to maintain the mean character values. Regarding that plants facing a lack of selection pressures may wander throughout their fitness landscapes, with a high probability of falling into fitness valleys, when disturbed populations undergo such a lack of selection or maintain only stabilizing selection in still-changing disturbed habitats, population extinction due to evolutionary trapping is indeed to be expected.

Directional selection is the most frequent type of selection found in nature (Kingsolver & Pfennig, 2007). Nevertheless, it is commonly assumed for many traits that an optimal value is determined by the trade-off between fitness and costs imposed by the trait (de Jong & Klinkhamer, 2005; Reekie & Bazzaz, 2005). The cost-benefit models usually employed to understand the evolution of such traits generally predict that cost and benefits interact to produce stabilizing selection, which maintain intermediate equilibrium values (Simms, 1990). Thus, regarding that stabilizing selection pressures should be the rule rather than exception, plants inhabiting disturbed habitats such as human-mediated fragmented habitats, most likely would be in the pathway of evolutionary trapping and, therefore, population extinction.

The aim of this work is to evaluate pollinator-mediated phenotypic selection on reproductive (*i.e.*, number of flowers per plant, flower length, inflorescence length, and inflorescence complexity) and vegetative traits (*i.e.*, number of leaves, foliar area, plant height, and herbivory resistance) on *Bomarea salsilla*, a vine endemic to forests from central Chile. These forest are highly fragmented and comprise fragments in still-changing successional stages (*i.e.*, out of equilibrium) as well as continuous forests exhibiting a climax stage (Bustamante *et al.*, 2005; Ramos *et al.*, 2008). If habitat fragmentation and the creation of edge habitats reduced plant-pollinator interactions and, consequently, seed output, few traits –mostly vegetative traits- would face less pollinator-mediated selection pressures in fragments and edges of compared to the core of continuous forest. Similarly, a reduction in the strength of selection in fragments and edges with respect to the core of continuous forest is to be expected. Finally, stabilizing selection pressures are to be expected as the most frequent remaining selection pressures in fragments and edges.

MATERIALS AND METHODS

Natural history

Fieldwork was conducted in Maulino forest in the northernmost zone of the temperate rainforest from Chile (35°59'S, 72°41'W; Bustamante *et al.*, 2005). Specifically, the study was performed in Los Queules National Reserve and two neighbouring forest fragments. Los Queules is a protected area of 145 ha embedded in a large tract of 600 ha of continuous forest. Forest fragments, ranging from 1 to 6 ha, are patches surrounded by commercial plantations of *Pinus radiata*. Here, four sites were defined taking into account the spatial arrangement of plant populations: one core and one edge of continuous forest, both sites placed in Los Queules National Reserve, and one core and one edge of forest fragment, both sites placed in two different fragments nearby Los Queules National Reserve. While cores were defined as sites placed ≥ 50 m inwards the border of each site, edges were defined as sites placed ≤ 10 m inwards the forest margin. Distance between sites ranges from 0.5 up to 2.5 km.

Bomarea salsilla (Alstroemeriaceae) is a protandrous, small-sized red-coloured climbing perennial vine, inhabiting the sclerophyllous and temperate forests of Chile from 33°S to 40°S (Muñoz and Moreira, 2003). In the study site, it flowers from *ca.* November to January. Its breeding system is unknown; however, two pilot pollinator-exclusion experiments in the study site, devised to test for autogamy and agamospermy, demostrated that *B. salsilla* is a totally pollinator-dependent plant for seed set (*i.e.*, a xenogamous plant). In the study site, floral visitors are hummingbirds, *Sephanoides sephaniodes* (Trochilidae); bumblebees and bees, *Bombus dahlbomii*, *Bombus terrestris*, and *Manuelia gayatina* (Apidae); butterflies, *Mathania leucothea* (Pieridae); and flies, *Acrophthalmyda paulseni* (Bombyliidae) (pers. obs.).

Traits and reproductive success

In order to estimate reproductive and vegetative traits in *B. salsilla* and further the action of pollinators as selective agents, 196, 153, 341, and 173 individual plants were chosen in the core of the continuous forest, edge of the continuous forest, core of the forest fragment, and edge of forest fragment, respectively, from October 2007 to February 2008. In these plants, reproductive traits assessed were i) flower number, ii) flower length, iii) ray length, and iv) inflorescence complexity. Flower number was determined by directly counting all flowers borne on inflorescences (there is a single inflorescence per invidual plant). Flower length was estimated from the tepal insertion to the end of this with a hand-held ruler. Similarly, ray length was estimated in the inflorescence portion ranging from the inflorescence base to the flower calix. Because rays may be arranged, in an increasing order, as monoseried, biseried, and triseried, ray length was estimated from the inflorescence base up to the flower calyx of the bigger serie (Fig. 3.1). Inflorescence complexity was estimated by scoring plants as one, two, or three depending on whether they exhibited monoseried, biseried, or triseried rays, respectively; when plants showed more than one type of ray (*i.e.*, exhibit irregular shapes), they were scored with intermediate values (Fig. 3.1). Vegetative traits assessed were v) leaf number, vi) foliar area, vii) plant height, and viii) herbivory resistance. Leaf number was determined by directly counting and tagging all leaves on plant spikes (there is a single spike per invidual plant) at the start of the flowering period when no new leaf develops. Foliar area was estimated by measuring length and wide of leaves and then computing area by using the oval area equation because this figure closely resembles leaf shape (data not shown). Plant height was estimated in elongated spikes from the soil surface up to the inflorescence base with a metric band (Fig. 3.1). Herbivory resistance, assessed as the percentage of undamaged foliar tissue, was estimated at the start of seed dispersal period by checking all leaves borne by each plant. In each leaf, a transparent paper grid (2-mm spaced) was put on the leaf surface for then counting the total squares occupied by foliar tissue and the total squares exhibiting a lack of foliar tissue but inside the projection of the leaf margin.

In all those cases in which more than one measure is attainable for each individual plant (*i.e.*, flower length, ray length, and foliar area) two measures per each trait were performed and then averaged in order to obtain a representative sample in a per-plant basis.

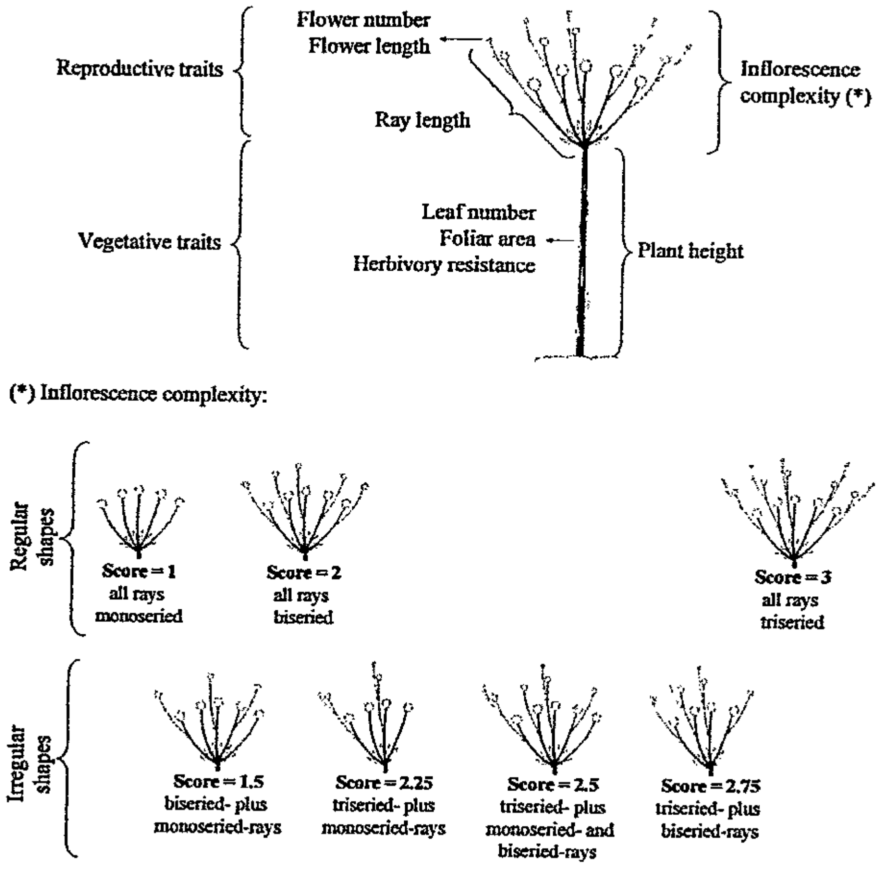

Figure 3.1. Non-scaled schematic representation of reproductive and vegetative traits in an elongated plant of *Bomarea salsilla*.

Pollinator-mediated phenotypic selection on reproductive and vegetative traits

In order to describe pollinator-mediated selection pressures acting on reproductive and vegetative traits of *B. salsilla*, estimations of quantitative selection coefficients were used within generation (Lande & Arnold, 1983; Brodie *et al.*, 1995). Thereafter, to estimate the overall strength of selection, linear (S'_i) and non-linear (C'_{ii} and C'_{ij}) selection differentials were determined according to Brodie *et al.* (1995) as follows:

$$S'_i = \mathrm{COV}\ (w, x) \qquad \text{(equation 1)}$$

$$C'_{ii} = \mathrm{COV}\ (w, x'^2) \qquad \text{(equation 2)}$$

$$C'_{ij} = \mathrm{COV}\ (w, x'_i x'_j) \qquad \text{(equation 3)}$$

where w is the relative fitness expressed as the absolute individual fitness divided by the population mean fitness, and x is the trait value of the i and j phenotypes, which was standardised by substracting the population mean value and then divided by the standard deviation of the trait (*i.e.*, a z-transformation procedure). While linear selection differentials (S'_i) give insights into the total change of the mean phenotype within a generation, the bivariate non-linear selection differentials account for by the total change in the variance of single traits (C'_{ii}) and the total change in the covariance of both traits (C'_{ij}) within a generation after adjusting for directional selection (Brodie III *et al.*, 1995).

In order to estimate partial changes in phenotypic means (*i.e.*, direct linear selection), partial changes in the variance of a single trait (*i.e.*, direct non-linear selection), and partial changes in the covariance of paired traits (*i.e.*, direct non-linear selection on combinations of reproductive and vegetative traits), linear (β'_i) and non-linear (γ'_{ii} and γ'_{ij}) selection gradients were respectively determined according to Lande & Arnold (1983) through the following linear (equation 4) and non-linear (equation 5) equations:

$$w = \alpha + \sum_{i=1}^{n} \beta'_i x_i + \varepsilon \qquad \text{(equation 4)}$$

$$w = \alpha + \sum_{i=1}^{n} \beta'_i x_i + \tfrac{1}{2}\sum_{i=1}^{n} \gamma'_{ii} x_i^2 + \sum_{i=1}^{n} \sum_{i \neq j}^{n} \gamma'_{ij} x_i x_j + \varepsilon \qquad \text{(equation 5)}$$

where α is a constant, β'_i represents the average slope of the selection surface in the plane of the character i, and ε is an error term. In the equation 4, the linear gradient of selection (β') provides information of the direction and magnitude of change expected after current selection. The non-linear selection gradients (γ') in the equation 5 describe the nature of selection on quadratic deviations from the mean for both single and pairwise combinations of characters. While non-linear gradients were estimated from the second order coefficients of the equation 5, linear gradients of selection were only estimated from the first order coefficients of the equation 4 because if characters are multivariate non-normally distributed, x_i and x_i^2 in the equation 5 are intercorrelated (Lande & Arnold, 1983). In the equation 5, γ'_{ii} depicts the curvature of the relationship between the character i and relative fitness. When $\gamma'_{ii} < 0$, downward concavity and stabilizing selection acting on the character i occurs, whereas $\gamma'_{ii} > 0$ reflects upward concavity and, therefore, disruptive selection. The correlational selection gradients (γ'_{ij}) reflect the extent to which selection acts on the correlation of traits i and j. The significance of β' and γ' in the equations 4 and 5, respectively, was determined directly from the regression coefficients after the effects of all other parameters had been removed.

RESULTS

Traits and reproductive success

There were not significant effects on the mean number of flowers per plant for those inhabiting fragments or continuous forest (hereafter FE), and edges or cores (hereafter EE) (Non-parametric Scheirer-Ray-Hare extension of Kruskal-Wallis Tests for two-way ANOVAs on ranked data, FE: $H_{1,859} = 2.882$, $P = 0.090$; EE: $H_{1,859} = 2.800$, $P = 0.094$; FE x EE: $H_{1,859} = 0.896$, $P = 0.344$). Mean flower length was 10.8% and 5.8% lower in the edge of continuous forest and edge of forest fragment with respect to the core of the continuous forest, respectively, yet it was 10.4% higher in the core of forest fragment (FE: $H_{1,859} = 57.347$, $P < 0.001$; EE: $H_{1,859} = 170.773$, $P < 0.001$; FE x EE: $H_{1,859} = 13.300$, $P < 0.001$). Mean ray length did not differ significantly in the edges of continuous forest and forest fragment with respect to the core of continuous forest, but it was 34.1% higher in the core of forest fragment with respect to the core of continuous forest (FE: $H_{1,859} = 29.904$, $P < 0.001$; EE: $H_{1,859} = 57.750$, $P < 0.001$; FE x EE: $H_{1,859} = 17.079$, $P < 0.001$). Mean inflorescence complexity did not differ significantly in the edges of continuous forest and forest fragment with respect to the core of continuous forest, but it was 18.0% higher in the core of forest fragment with respect to the core of continuous forest (FE: $H_{1,859} = 29.446$, $P < 0.001$; EE: $H_{1,859} = 4.768$, $P = 0.023$; FE x EE: $H_{1,859} = 5.671$, $P = 0.017$).

Concerning vegetative traits, mean leaf number did not differ significantly between the edge of continuous forest and the core of continuous forest, albeit it was 14.3% and 21.0% lower in the core of continuous forest with respect to the core and edge of forest fragment (FE: $H_{1,859} = 91.44$, $P < 0.001$; EE: $H_{1,859} = 12.970$, $P < 0.001$; FE x EE: $H_{1,859} = 0.018$, $P = 0.893$). Mean foliar area was 27.3%, 48.5%, and 41.6% lower in the edge of continuous forest, and core and edge of forest fragments, with respect to the core of continuous forest, respetively (FE: $H_{1,859} = 109.567$, $P < 0.001$; EE: $H_{1,859} = 11.585$, $P < 0.001$; FE x EE: $H_{1,859} = 37.643$, $P < 0.001$). Similarly, mean plant height was 11.6%, 26.2%, and 29.1% lower in the edge of continuous forest, and core and edge of forest fragments with respect to the core of continuous forest, respetively (FE: $H_{1,859} = 147.203$, $P < 0.001$; EE: $H_{1,859} = 17.741$, $P < 0.001$; FE x EE: $H_{1,859} = 4.905$, $P = 0.027$). Conversely, mean herbivory resistance was 1.8%, 2.6%, and 2.6% higher in the edge of continuous forest, and core and edge of forest fragments with respect to the core of continuous forest, respetively (FE: $H_{1,859} = 89.560$, $P < 0.001$; EE: $H_{1,859} = 12.467$, $P < 0.001$; FE x EE: $H_{1,859} = 22.546$, $P < 0.001$).

Seed set did not differ significantly between the core of continuous forest and edges of continuous forest and forest fragment, yet it was 110.9% higher in the core of forest fragment with respect to the core of continuous forest (FE: $H_{1,859} = 39.915$, $P < 0.001$; EE: $H_{1,859} = 47.150$, $P < 0.001$; FE x EE: $H_{1,859} = 17.027$, $P < 0.001$).

Pollinator-mediated phenotypic selection on reproductive and vegetative traits

In edges and fragments there were eleven to five less significant selection events (β_i,

γ_{ii}, and γ_{ij}) than in the core of continuous forest (One-tailed Exact-Fisher test P = 0.025). On the contrary, there were not significant differences in magnitude of selection in the edge of continuous forest, core of forest fragment, and edge of forest fragment with respect to the core of continuous forest, although there were significant differences between the core and edge of forest fragments (ANOVA for Log-transformed data, FE: $F_{1,859} = 0.019$, $P = 0.892$; EE: $F_{1,859} = 9.080$, $P = 0.003$; FE x EE: $F_{1,859} = 4.853$, $P = 0.029$).

As far as directional selection pressures are concerned, there were not significant differences in the amount of traits facing selective pressures in fragments and edges with respect to the core of continuous forest (One-tailed Exact Fisher-test $P = 0.608$) (Fig. 3.2A). Similarly, the magnitude of directional selection did not differ significantly among these sites (ANOVA for Log-transformed data, FE: $F_{1,859} = 0.188$, $P = 0.668$; EE: $F_{1,859} = 0.029$, $P = 0.865$; FE x EE: $F_{1,859} = 1.059$, $P = 0.312$) (Fig. 3.2B).

Concerning disruptive/stabilizing selection pressures, in the edge of continuous forest, core of forest fragment, and edge of forest fragment there were one, three, and two less traits, respectively, facing pollinator-mediated phenotypic selection on reproductive and vegetative traits with respect to the core of continuous forest, albeit these differences were not significant (One-tailed Exact-Fisher test P = 0.500) (Fig. 3.2C). The magnitude of disruptive/stabilizing selection, however, did not differ significantly among these sites, albeit there was a marginally significant ($0.05 < P < 0.10$) edge effect (ANOVA for Log-transformed data, FE: $F_{1,859} = 0.820$, $P = 0.373$; EE: $F_{1,859} = 3.482$, $P = 0.073$; FE x EE: $F_{1,859} = 0.676$, $P = 0.418$) (Fig. 3.2D).

In terms of correlational selection pressures, in the edge of continuous forest, core of forest fragment, and edge of forest fragment there were five, eight, and three less trait pairs, respectively, facing pollinator-mediated phenotypic selection on reproductive and vegetative traits with respect to the core of continuous forest, albeit these differences were not significant (One-tailed Exact-Fisher test $P = 0.295$) (Fig. 3.2E). The magnitude of correlational selection pressures, however, did not differ significantly between the core of continuous forest and the edge of forest fragment, core of forest fragment, and edge of forest fragment, respectively; although it was significantly higher in the edge of forest fragment with respect to the core of forest fragment and the edge of continuous forest (ANOVA for Log-transformed data, FE: $F_{1,859} = 0.121$, $P = 0.729$; EE: $F_{1,859} = 10.695$, $P = 0.001$; FE x EE: $F_{1,859} = 13.703$, $P < 0.001$) (Fig. 3.2F).

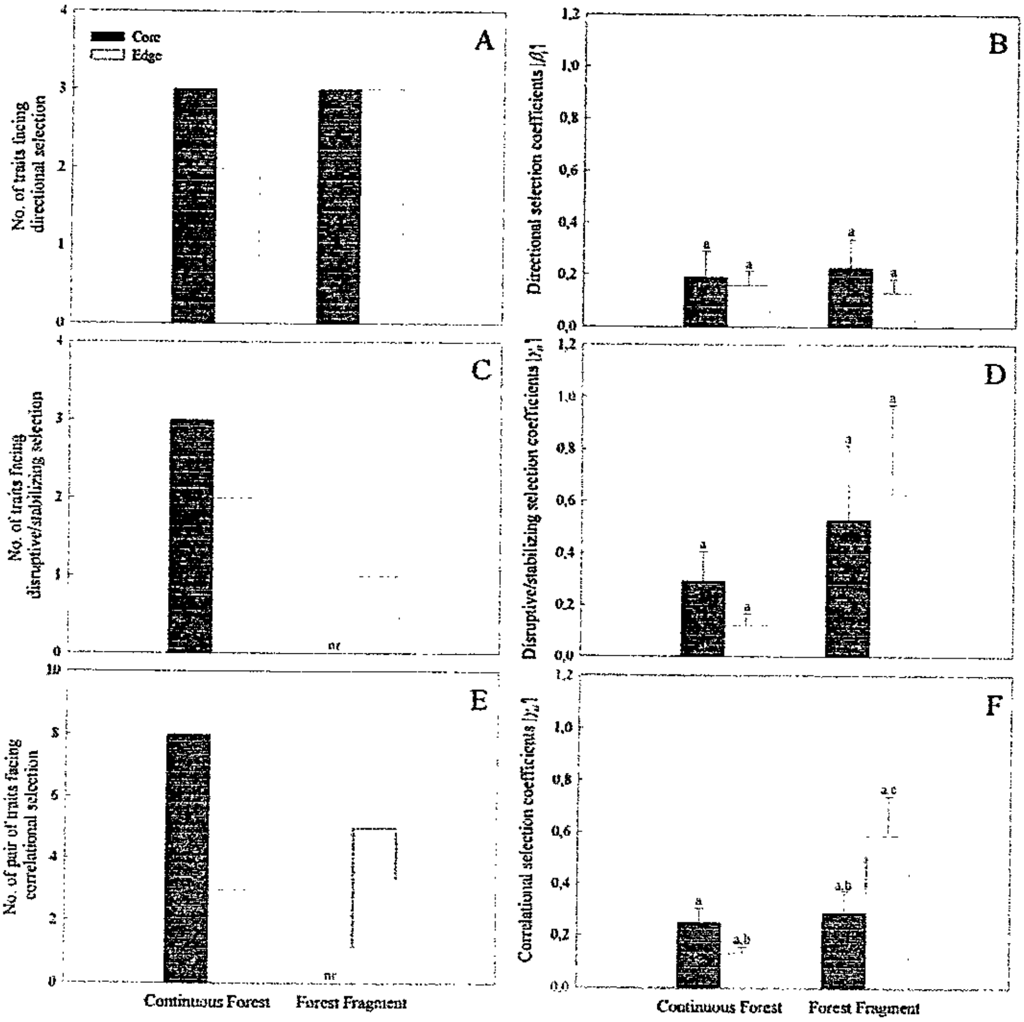

Figure 3.2. Frequency and magnitude of directional (A, B), disruptive/stabilizing (C, D), and correlational selection pressures (E, F) on *Bomarea salsilla* in the fragmented Maulino forest (Dissimilar letters depict significant differences ($P < 0.05$) following Tukey HSD Tests for post hoc comparisons; means ± 1SE are depicted; nr: not recorded).

In the core of continuous forest, *B. salsilla* faced selection pressures for increased mean-values of flower number and ray length, albeit a negative selection pressure on inflorescence complexity (Table 3.1). In the edge of continuous forest, only ray length was a trait significantly conducting plant reproduction facing a positive selection pressure by pollinators (Table 3.1). In the core of forest fragment, flower number, flower length, and ray length significantly affected reproductive success of *B. salsilla* by facing positive selection pressures (Table 3.1). In the edge of forest fragments, flower length and ray length faced selection by pollinator for increased values on such traits (Table 3.1). Taken together, mean characters and pollinator-mediated directional phenotypic selection partially correlates (Fig. 3.3)

In the core of continuous forest, inflorescence complexity, foliar area, and herbivory resistance faced a significant stabilizing selection pressure (Table 3.2). In the edge of continuous forest, ray length faced a significant disruptive selection pressure, while foliar area faced a significant stabilizing selection pressure (Table 3.2). In the core of forest fragment, no trait faced disruptive or stabilizing selection pressures (Table 3.2). In the edge of forest fragment herbivory resistance faced a strong and significant stabilizing selection pressure (Table 3.2). Taken together, variance of characters and disruptive/stabilizing pollinator-mediated phenotypic selection partially correlates (Fig. 3.3)

In the core of continuous forest, the pairwise correlations: flower number/inflorescence complexity, plant height/herbivory resistance, flower number/herbivory resistance, inflorescence complexity/foliar area, and inflorescence complexity/herbivory resistance faced significant positive correlational selection pressures, while correlations flower length/ray length, flower length/herbivory resistance, and ray length/herbivory resistance faced significant negative correlational selection pressures (Table 3.3). Taken together, covariances of characters and correlational pollinator-mediated phenotypic selection partially correlates (Fig. 3.5)

Table 3.1. Summary of directional selection coefficients for pollinator-mediated phenotypic selection on reproductive and vegetative traits in *B. salsilla* in the fragmented Maulino forest from south-central Chile (model fits (R^2) and means ± 1SE are depicted).

Traits	Core of Continuous Forest ($R^2 = 0.500$**)		Edge of Continuous Forest ($R^2 = 0.350$***)		Core of Forest Fragment ($R^2 = 0.462$**)		Edge of Forest Fragment ($R^2 = 0.344$***)	
	S'_i	β'_i	S'_i	β'_i	S'_i	β'_i	S'_i	β'_i
Reproductive Traits								
Flower number	1.806	0.299 ± 0.108**	1.347	-0.254 ± 0.151	0.873	0.419 ± 0.090***	1.329	0.001 ± 0.142
Flower length	0.239	-0.018 ± 0.053	0.769	-0.123 ± 0.081	0.444	0.184 ± 0.044***	1.087	0.181 ± 0.071*
Ray length	2.002	0.734 ± 0.107***	2.130	0.909 ± 0.146***	0.888	0.334 ± 0.082***	1.527	0.422 ± 0.119***
Inflor. complexity	1.409	-0.377 ± 0.103***	1.463	-0.270 ± 0.166	0.757	-0.142 ± 0.088	1.330	0.059 ± 0.117
Vegetative Traits								
Leaf number	0.769	0.070 ± 0.077	1.297	0.211 ± 0.105*	0.425	-0.050 ± 0.057	0.754	0.141 ± 0.103
Foliar area	0.703	0.003 ± 0.062	1.121	0.039 ± 0.107	0.522	0.063 ± 0.052	0.313	-0.161 ± 0.078*
Plant height	0.974	-0.036 ± 0.085	1.209	0.011 ± 0.132	0.526	0.026 ± 0.065	0.782	0.067 ± 0.123
Herb. resistance	-0.621	-0.001 ± 0.057	0.121	-0.007 ± 0.071	0.045	0.061 ± 0.040	-0.224	0.035 ± 0.065

*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$

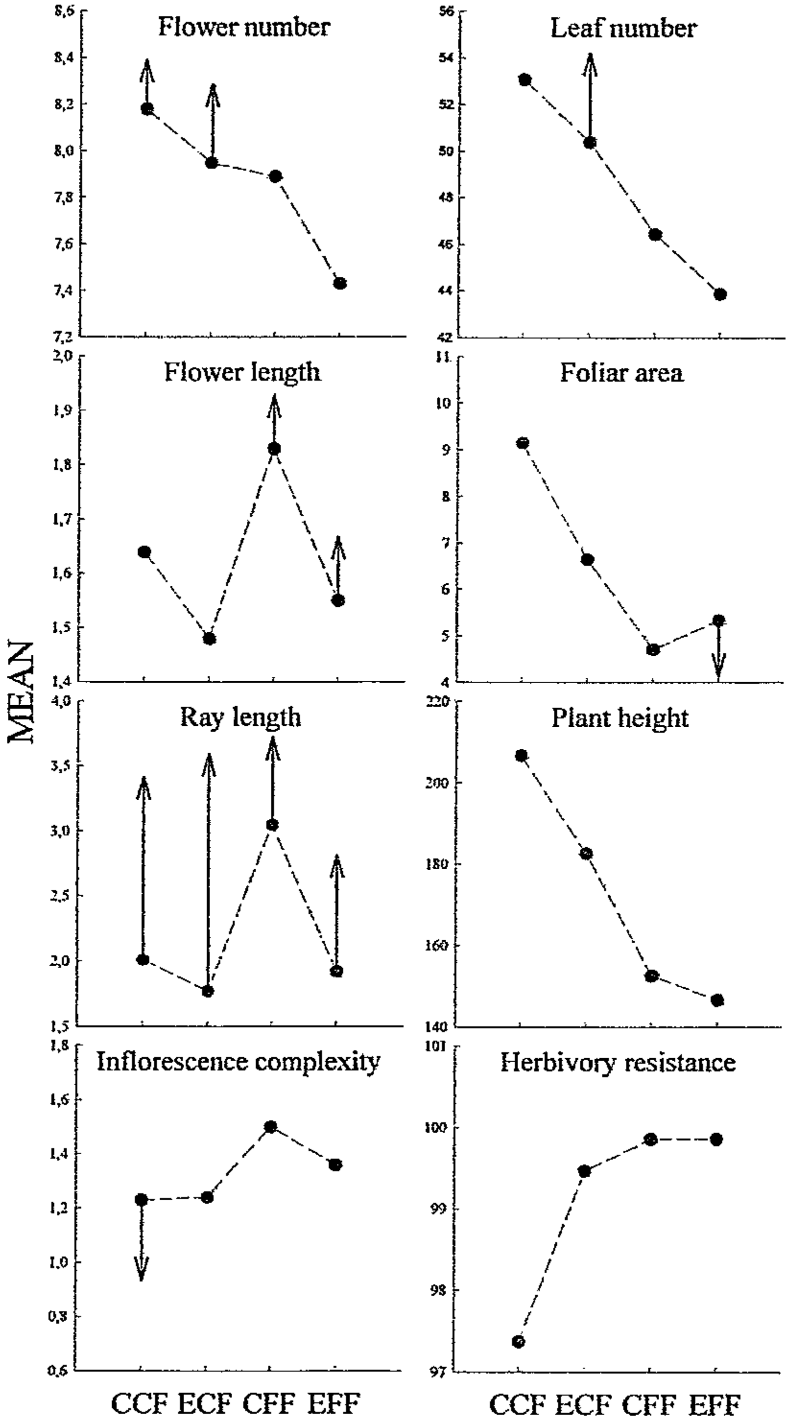

Figure 3.3. Mean traits (dots) and significant pollinator-mediated directional selection on such traits (arrows) in *Bomarea salsilla* at the core of continuous forest (CCF), edge of continuous forest (ECF), core of forest frgament (CFF), and edge of forest fragment (EFF).

Table 3.2. Summary of disruptive/stabilizing selection coefficients for pollinator-mediated phenotypic selection on reproductive and vegetative traits in *B. salsilla* in the fragmented Maulino forest from south-central Chile. Positive γ'_{ii} values indicates stabilizing selection while negative γ'_{ii} values indicates disruptive selection (model fits (R^2) and means ± 1SE are depicted).

Traits	Core of Continuous Forest ($R^2 = 0.810^{***}$)		Edge of Continuous Forest ($R^2 = 0.758^{***}$)		Core of Forest Fragment ($R^2 = 0.533^{**}$)		Edge of Forest Fragment ($R^2 = 0.641^{***}$)	
	C'_{ii}	γ'_{ii}	C'_{ii}	γ'_{ii}	C'_{ii}	γ'_{ii}	C'_{ii}	γ'_{ii}
Reproductive Traits								
Flower number	4.829	-0.135 ± 0.412	3.585	0.150 ± 0.481	1.405	-0.391 ± 0,314	2.413	0.628 ± 0.460
Flower length	-0.167	0.051 ± 0.042	0.798	0.091 ± 0.082	-0.198	-0.024 ± 0,049	1.389	-0.018 ± 0.090
Ray length	4.666	0.164 ± 0.232	10.232	2.281 ± 0.713**	0.979	-0.076 ± 0,131	2.793	-0.723 ± 0.388
Inflor. complexity	1.977	-0.870 ± 0.206***	4.352	0.854 ± 0.534	0.656	0.047 ± 0,166	2.279	0.449 ± 0.265
Vegetative Traits								
Leaf number	0.786	-0.086 ± 0.107	3.007	0.107 ± 0.175	0.373	0.128 ± 0,091	0.583	-0.086 ± 0.177
Foliar area	0.405	-0.377 ± 0.154*	2.044	-0.410 ± 0.200*	0.702	-0.063 ± 0,086	0.278	0.162 ± 0.184
Plant height	1.244	0.051 ± 0.122	2.061	-0.109 ± 0.196	0.532	-0.059 ± 0,151	1.103	-0.172 ± 0.350
Herbiv. resistance	0.943	-0.602 ± 0.134***	-0.549	0.255 ± 0.268	-0.866	-0.187 ± 0,703	0.242	-2.786 ± 1.337*

$^*P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$

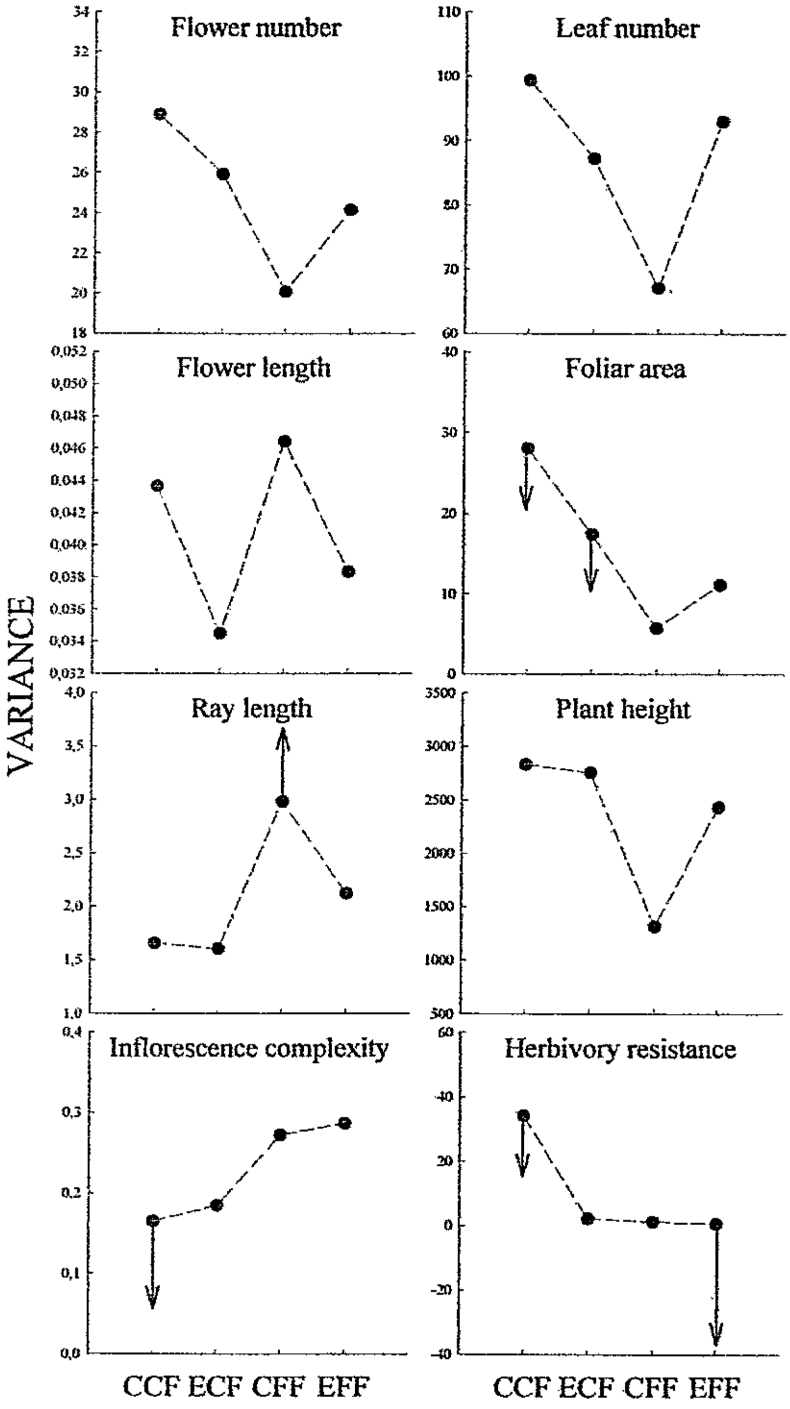

Figure 3.4. Trait variances (dots) and significant pollinator-mediated disruptive (upward arrows) or stabilizing selection (downward arrows) on such traits in *Bomarea salsilla* at the core of continuous forest (CCF), edge of continuous forest (ECF), core of forest fragment (CFF), and edge of forest fragment (EFF).

Table 3.3. Summary of correlational selection coefficients for pollinator-mediated phenotypic selection on reproductive and vegetative traits in *B. salsilla* in the fragmented Maulino forest from south-central Chile (model fits (R^2) and means ± 1SE are depicted; FN: flower number, FL: flower length, RL: pedicel length, IC: inflorescence complexity, LN: leaf number, FA: foliar area, PH: plant height, HR: herbivory resistance).

Paired Traits	Core of Continuous Forest ($R^2 = 0.810$***)		Edge of Continuous Forest ($R^2 = 0.758$***)		Core of Forest Fragment ($R^2 = 0.533$**)		Edge of Forest Fragment ($R^2 = 0.641$***)	
	C'_{ij}	γ'_{ij}	C'_{ij}	γ'_{ij}	C'_{ij}	γ'_{ij}	C'_{ij}	γ'_{ij}
Within Reproductive Traits								
FN x FL	0.582	-0.112 ± 0.098	1.836	0.135 ± 0.227	0.309	0.187 ± 0,109	1.606	0.034 ± 0.231
FN x RL	4.636	0.283 ± 0.464	6.194	-0.260 ± 0.710	1.081	0.473 ± 0,310	2.345	0.033 ± 0.544
FN x IC	3.062	0.542 ± 0.261*	3.989	-0.091 ± 0.714	0.925	-0.099 ± 0,329	2.139	-1.294 ± 0.455**
FL x RL	0.524	-0.199 ± 0.091*	3.068	-0.992 ± 0.255***	0.235	0.046 ± 0,086	1.768	0.328 ± 0.229
FL x IC	0.272	0.087 ± 0.092	2.196	0.434 ± 0.210*	0.191	-0.170 ± 0,107	1.506	-0.170 ± 0.199
RL x IC	3.088	-0.060 ± 0.239	6.695	-2.057 ± 0.906*	0.787	-0.023 ± 0,239	2.498	0.999 ± 0.394*
Within Vegetative Traits								
LN x FA	0.786	-0.325 ± 0.210	2.107	-0.014 ± 0.131	0.340	0.009 ± 0,076	0.508	-0.179 ± 0.185
LN x PH	0.876	0.061 ± 0.158	2.637	0.322 ± 0.267	0.370	0.036 ± 0,131	0.815	0.145 ± 0.388
LN x HR	-0.169	-0.226 ± 0.126	0.407	0.124 ± 0.169	0.025	0.084 ± 0,096	-0.017	-0.316 ± 1.290
FA x PH	1.016	0.025 ± 0.155	1.794	0.082 ± 0.233	0.574	-0.073 ± 0,161	0.374	0.210 ± 0.231
FA x HR	-0.174	-0.109 ± 0.071	-0.073	-0.091 ± 0.123	0.035	0.031 ± 0,108	-0.031	0.478 ± 0.355
PH x HR	-0.434	0.528 ± 0.157***	0.064	-0.095 ± 0.181	0.098	-0.130 ± 0,439	-0.219	0.678 ± 0.966

*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$

Table 3.3. (Continuation) Summary of correlational selection coefficients for pollinator-mediated phenotypic selection on reproductive and vegetative traits in *B. salsilla* in the fragmented Maulino forest from south-central Chile (model fits (R^2) and means ± 1SE are depicted; FN: flower number, FL: flower length, RL: pedicel length, IC: inflorescence complexity, LN: leaf number, FA: foliar area, PH: plant height, HR: herbivory resistance).

Paired Traits	Core of Continuous Forest ($R^2 = 0.810^{***}$)		Edge of Continuous Forest ($R^2 = 0.758^{***}$)		Core of Forest Fragment ($R^2 = 0.533^{**}$)		Edge of Forest Fragment ($R^2 = 0.641^{***}$)	
	C'_{ij}	γ'_{ij}	C'_{ij}	γ'_{ij}	C'_{ij}	γ'_{ij}	C'_{ij}	γ'_{ij}
Between Reproductive and Vegetative Traits								
FN x LN	2.175	0.161 ± 0.266	2.913	-0.424 ± 0.353	0.570	-0.205 ± 0,177	1.345	0.811 ± 0.325*
FN x FA	1.897	-0.325 ± 0.210	2.705	-0.101 ± 0.408	0.911	0.316 ± 0,198	0.437	0.440 ± 0.381
FN x PH	2.346	-0.159 ± 0.283	2.520	0.213 ± 0.458	0.698	0.240 ± 0,212	1.120	-1.073 ± 0.478*
FN x HR	-0.756	0.539 ± 0.129***	0.203	-0.017 ± 0.123	0.107	-0.264 ± 0,706	-0.034	-4.047 ± 2.138
FL x LN	0.339	0.027 ± 0.072	0.934	-0.088 ± 0.105	-0.023	-0.121 ± 0,067	0.694	0.132 ± 0.161
FL x FA	0.004	-0.085 ± 0.058	1.499	0.108 ± 0.138	0.136	-0.014 ± 0,057	0.432	0.226 ± 0.147
FL x PH	0.296	0.051 ± 0.075	0.945	-0.061 ± 0.147	0.050	0.064 ± 0,077	0.912	-0.212 ± 0.238
FL x HR	-0.095	-0.198 ± 0.062***	0.068	0.152 ± 0.111	0.013	-0.007 ± 0,078	0.025	0.163 ± 0.320
RL x LN	2.022	0.069 ± 0.212	4.448	0.573 ± 0.409	0.389	0.027 ± 0,131	1.056	-0.325 ± 0.230
RL x FA	1.863	-0.107 ± 0.168	4.574	0.653 ± 0.379	0.673	-0.030 ± 0,149	0.233	-0.230 ± 0.284
RL x PH	2.434	0.406 ± 0.247	4.001	-0.448 ± 0.464	0.464	-0.222 ± 0,146	0.814	0.513 ± 0.244*
RL x HR	-0.977	-1.422 ± 0.196***	0.439	0.006 ± 0.245	0.085	-0.111 ± 0,269	0.044	1.635 ± 0.840
IC x LN	1.265	-0.131 ± 0.213	2.885	-0.105 ± 0.286	0,411	0.079 ± 0,152	1.037	-0.166 ± 0.187
IC x FA	1.083	0.486 ± 0.183**	3.090	0.132 ± 0.348	0.549	-0.070 ± 0,153	0.227	-0.464 ± 0.252
IC x PH	1.434	-0.224 ± 0.208	2.628	-0.132 ± 0.360	0.455	0.157 ± 0,155	0.707	0.513 ± 0.279
IC x HR	-0.680	0.300 ± 0.135*	0.195	-0.229 ± 0.211	0.096	0.417 ± 0,554	0.176	0.694 ± 1.038

$^*P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$

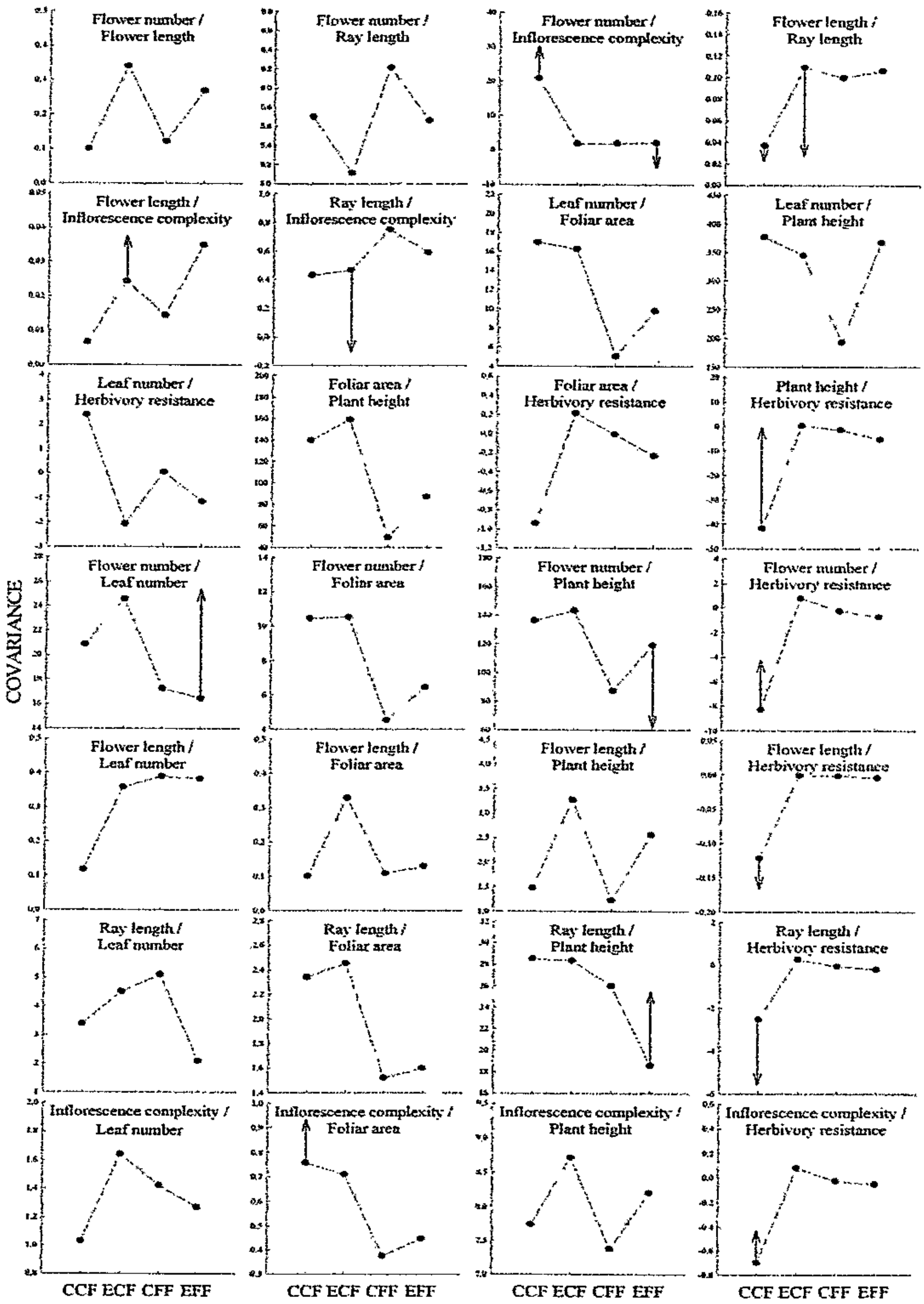

Figure 3.5. Paired trait covariances (dots) and significant pollinator-mediated correlational selection (arrows) on such traits in *Bomarea salsilla* at the core of continuous forest (CCF), edge of continuous forest (ECF), core of forest fragment (CFF), and edge of forest fragment (EFF).

DISCUSSION

Forest fragmentation and the creation of edge habitats reduced the frequency, albeit not magnitude, of traits and combinations thereof to be selected by pollinators in *B. salsilla*. Regarding that fragments and edges of the fragmented Maulino forest are in a still-changing sucessional stage as compared to continuous forest (Bustamante *et al.*, 2005; Ramos *et al.*, 2008), a reduction in the number of traits facing pollinator-mediated selection may allow plants to warrant throughout the fitness surface and probably to fall into fitness valleys, thereby compromising the long-term population persistence.

An important feature determining the reproductive success of plants is the number of flowers per plant. Factors described as favouring increased floral displays are inter and intraspecific competition for pollinators, potential for higher seed production, and saturation of seed-eating predators (Geber, 1985; Snow *et al.*, 1996; and references therein). In the *B. salsilla* case, plants inhabiting the core and edge of continuous forest faced only a positive pollinator-mediated directional selection pressure for increased number of flowers per plant which is congruent with many other cases previously reported (Snow *et al.*, 1996 and references therein). In the core and edge of forest fragments, however, there was a lack of selection which points to consider that the reproductive success of plants inhabiting these disturbed sites is mostly conducted by random rather than deterministic processes as pollinator-mediated selection is.

Flower size is another important trait controlling plant reproductive success which has been reported for a variety of plants (*e.g.*, Campbell, 1989; Galen, 1989;

Campbell *et al.*, 1991; 1996; 1997; Herrera, 1993a; Conner *et al.*, 1996a; Caruso, 2000). At the core and edge of continuous forest, however, flower length -a surrogate of flower size- was not selected by pollinators and, coupled to it, the population mean values were lower than at the core of fragment, but not at the edge of fragment, where this trait was positively selected by pollinators. Collectively, these results suggest that plants occurring at the core of forest fragment allocate higher resources for exhibiting a higher floral display, through increased flower sizes, in order to ensure plant reproductive success. Such an allocation mostly reflects the action of pollinators acting as selective agents. At the edge of forest fragments, however, despite plants exhibited a positive selection pressure by pollinators, plants do not bear higher flower sizes. Environmental constraints may contribute to explain such a discrepancy. Water stress may highly constrain flower size and, therefore, plant reproductive success by altering floral reward and/or flowering phenology (Galen, 2005). In fragments and more likely in edges, a lower humidity has been observed (Simonetti, unpublished data), which might likely preclude the trait expression in spite of selection pressures exerted by pollinators.

The ray length, a trait that contributes to increase floral display, faced positive selection pressure by pollinators at all sites studied but at the core of forest fragment also faced a disruptive selection pressure. This may partially explain the fact that character expression, in term of their means, was not associated with the magnitude of selection because at the core of forest fragment there is a high variance for ray length. Disruptive selection pressures exerted by pollinators usually reflects the action of two types of pollinators preferring contrasting expressions of any given trait (*e.g.*, Medel *et al.*, 2003). In the *B. salsilla* case, it appears that one set of pollinators select plants bearing short rays while others select longer rays. Unfortunately, no pollinator census is available to disclose which species are grouped into these two distictive suites of pollinators.

Inflorescence complexity, by contrast, ceased to be selected by pollinators at all disturbed sites while at the core of continuous forest faced a negative pollinator-mediated directional selection pressure coupled to a stabilizing selection. These results suggest that inflorescence complexity is a costly trait that not enhance reproductive success in a manner that benefits can be greater than costs. Accordingly, both theoretical models (Schoen & Dubuc 1990) and empirical assessments (Fishbein & Venable 1996) have pointed that inflorescences exhibiting lower or mid stages of complexity may be favoured over large ones in a variety of ways.

As far as vegetative traits are concerned, while plants at the core of continuous forest only faced stabilizing selection pressures for foliar area and herbivory resistance, in disturbed sites, stabilizing selection for foliar area was maintained in the edge of continuous forest, while the same type of selection for herbivory resistance was only maintained at the edge of forest fragment. Likewise, in disturbed sites there was a positive directional selection for leaf number at the edge of continuous forest and negative directional selection at the edge of forest fragment. Taken together, the expression of means and variances for vegetative traits was partially coupled to directional and disruptive/stabilizing selection pressures, respectively. In fact, while the mean expression of foliar display was consistently lower in disturbed sites, variance patterns are less clear. Such reductions in foliar display in disturbed sites may certainly impair the resource and energy intake by reducing photosynthesis, thus altering the expression of reproductive traits (Reekie & Bazzaz, 2005).

In terms of the coordinate action of traits, correlational selection pressures was frequently observed at the core of continuous forest. At disturbed sites, however, the same was not true. This is an important fact because correlational selection is considered as one of the main factors promoting genetic and phenotypic integrations (Herrera *et al.*, 2002). Furthermore, closely related traits might enhance efficiency of pollinators and, therefore, plant reproductive success (Berg, 1960; Stebbins, 1974; Armbruster *et al.*, 1999; Herrera *et al.*, 2002).

A rigorous assessment devised to test for the impact of pollinators as agents conducting plant diversification must demostrate correlations or associations between plant traits and selection pressures exerted by such pollinators on these traits (Herrera *et al.*, 2006). This viewpoint, albeit convenient, seems too simple regarding that, coupled to selection pressures, the stage of any character also reflects genetic, developmental, and environmental constraints. In the present report it appears that a set of factors other than pollinator-mediated selection pressures may collectivelly contribute to the expression of population means, variances, and covariances. Further studies should stress this subject.

Taking into consideration the variability of environmental conditions, changes in pollinator-mediated directional selection, lack of stabilizing, disruptive, and correlational selection, and changes in character stages in edges and fragments, plants occurring at these disturbed sites might most likely be in pathways of evolutionary trapping and even suicide, thus endangering the long-term population persistence of *B. salsilla* (*cf.* Ferrière *et al.*, 2004). Regarding that many plants mostly depends on pollinators for either ensuring reproductive success and change throughout the evolutionary time in the highly fragmented temperate rainforest from souther south America (Aizen *et al.*, 2002) numerous species might be suffering the evolutionary consequences of evolutionary disruptions. Therefore, a long-term research devised to test for the effects of habitat fragmentation on population persistence of such species is of mandatory importance in order to conserve and manage populations in a process- rather than a species-oriented manner.

ACKNOWLEDGEMENTS

Numerous persons, in several ways, kindly contributed to perform this work: Brenda Valdivia, Carlos O. Valdivia, Florencia Prats, Sandra Valdivia, Fernando Campos, Luciano Silva, Patricio Molina, and Sergio Hernández. The Chilean Forestry Service (CONAF) and Forestal Millalemu partially supported fieldwork. This work was funded by Beca de Apoyo a la Realización de Tesis Doctoral, Conicyt 23070138 (C.E.V.).

REFERENCES

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., Vázquez, D.P. 2006. Flower performance in human-altered habitats. In: Harder, L.D., Barrett, S.C.H. (Eds.), Ecology and Evolution of Flowers, pp:

159-179. Oxford University Press.

Aizen, M.A., Vázquez, D.P., Smith-Ramírez, C. 2002. Historia natural y conservación de los mutualismos planta-animal del bosque templado de Sudamérica austral. Revista Chilena de Historia Natural 75, 79-97.

Armbruster, W.S., di Stilio, V.S., Tuxill, J.D., Flores, T.C., Velásquez Runk, J.L. 1999. Covariance and decoupling of floral and vegetative traits in nine Neotropical plants: a re-evaluation of Berg's correlation-pleiades concept. American Journal of Botany 86, 39-55.

Berg, R.L. 1960. The ecological significance of correlation pleiades. Evolution 14, 171-180.

Brodie, E.D., Moore, A.J., Janzen, F.J. 1995. Visualizing and quantifying natural selection. Trends in Ecology and Evolution 10, 313–318.

Bronstein, J.L., Dieckmann, U., Ferrière, R. 2004. Coevolutionary dynamics and the conservation of mutualisms. In: Ferrière, R., Dieckmann, U., Couvet, D. (Eds.). Evolutionary Conservation Biology, pp: 305-326, Cambridge University Press.

Bustamante, R.O., Simonetti, J.A., Grez, A.A., San Martín, J. 2005. Fragmentación y dinámica de regeneración del bosque Maulino: diagnóstico actual y perspectivas futuras. In: Smith-Ramírez, C.S., Armesto, J.J., Valdovinos, C. (Eds.), Historia, Biodiversidad y Ecología de los Bosques Costeros de Chile. Editorial Universitaria, Santiago, Chile, pp: 555–564.

Campbell, D.R. 1989. Measurements of selection in a hermaphroditic plant: variation in male and female pollination success. Evolution 43, 318-334.

Campbell, D.R., Waser, N.M., Meléndez-Ackerman, E.J.. 1997. Analyzing pollinator-mediated selection in a plant hybrid zone: hummingbird visitation patterns on three spatial scales. American Naturalist 149, 295-315.

Campbell, D.R., Waser, N.M., Price, M.V. 1996. Mechanisms of hummingbird-mediated selection for flower width in *Ipomopsis aggregata.* Ecology 77, 1463-1472.

Campbell, D.R., Waser, N.M., Price, M.V., Lynch, E.A., Mitchell, R.J. 1991. Components of phenotypic selection: pollen export and flower corolla width in *Ipomopsis aggregata.* Evolution 45, 1458-1467.

Caruso, C.M. 2000. Competition for pollination influences selection on floral traits of *Ipomopsis aggregata.* Evolution 54, 1546–1557.

Conner, J.K., Rush, S., Kercher, S., Jenneten, P. 1996. Measurements of natural selection on floral traits in wild radish (*Raphanus raphanistrum*). II. Selection through lifetime male and total fitness. Evolution 50, 1137-1146.

de Jong, T., Klinkhamer, P. 2005. Evolutionary Ecology of Plant Reproductive Strategies. Cambridge University Press.

Ferrière, R., Dieckmann, U., Couvet, D. (Eds.). 2004. Evolutionary Conservation Biology. Cambridge University Press.

Fishbein, M., Venable, D.L. 1996. Evolution of inflorescence design: theory and data. Evolution 50, 2165–77.

Galen, C. 1989. Measuring pollinator-mediated selection on morphometric floral traits: bumblebees and the alpine sky pilot, *Polemonium viscosum.* Evolution 43, 882–890.

Galen, C. 2005. It never rains but then it pours: the diverse effects of water on flower integrity and function. In: Reekie, *E.G.*, Bazzaz, F.A. (Eds.), Reproductive

Allocation in Plants, pp: 77-95. Elsevier Academic Press.

Ghazoul, J. 2005. Buzzines as usual? Questioning the global pollination crisis. Trends in Ecology and Evolution 20, 367-373.

Geber, M. 1985. The relationship of plant size to self-pollination in *Mertensia ciliata*. Ecology 64, 762–72.

Herrera, C.M. 1993. Selection on floral morphology and environmental determinants of fecundity in a hawkmoth-pollinated violet. Ecological Monographs 63, 251-275.

Herrera, C.M., Castellanos, M.C., Medrano, M. 2006. Geographical context of floral evolution: towards an improved research programme in floral diversification. In: Harder, L.D., Barrett, S.C.H. (Eds.), Ecology and Evolution of Flowers, pp: 278-294. Oxford University Press.

Herrera, C.M., Cerdá, X., García, M.B., Guitián, J., Medrano, M., Rey, P.J., Sánchez-Lafuente, A.M. 2002. Floral integration, phenotypic covariance structure and pollinator variation in bumblebee-pollinated *Helleborus foetidus*. Journal of Evolutionary Biology 15, 108-121.

Hobbs, R.J., Yates, C.J. 2003. Impacts of ecosystem fragmentation on plant populations: generalising the idiosyncratic. Australian Journal of Botany 51, 471–488.

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.

Kearns, C.A., Inouye, D.W., Waser, N.M. 1998. Endangered mutualisms: the conservation of plant-pollinator interactions. Annual Review of Ecology and

Systematics 29, 83-112.

Kingsolver, J.G., Pfennig, D.W. 2007. Patterns and power of phenotypic selection in nature. Bioscience 57, 561-572.

Lande, R., Arnold, S.J. 1983. The measurement of selection on correlated characters. Evolution 37, 1210-1226.

Marquis, R. 1992. Selective impact of herbivores. In: Fritz, R.S., Simms, E.L. (Eds.). Plant Resistance to Herbivores. Ecology, Evolution, and Genetics, pp: 301-325. University of Chicago Press.

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.

Muñoz, M., Moreira, A. 2002. *Alstroemerias* de Chile. Diversidad, distribución y conservación. Taller La Era, Santiago, Chile.

Nuñez-Farfán, J., Dirzo, R. 1994. Evolutionary ecology of *Datura stramonium* L. in central Mexico: natural selection for resistance to herbivorous insects. Evolution 48, 423-436.

Ramos, C., Simonetti, J.A., Flores, J.D., Ramos-Jiliberto, R. 2008. Modelling the management of fragmented forests: Is it possible to recover the original tree composition? The case of the Maulino forest in Central Chile. Forest Ecology and Management 255, 2236-2243.

Reekie, *E.G.*, Bazzaz, F.A. (Eds.) 2005. Reproductive Allocation in Plants. Elsevier Academic Press.

Schoen, D.J., Dubuc, M. 1990. The evolution of inflorescence size and number: a gamete-packaging strategy in plants. American Naturalist 135, 841–57.

Simms, E.L. 1990. Examining selection on multivariate phenotype: plant resitance to herbivores. Evolution 44, 1177-1188.

Snow, A.A., Spira, T.P., Simpson, R., Klips, R.A. 1996. The ecology of geitonogamous pollination. In: Lloyd, D.G., Barrett, S.C.H. (Eds.), Floral Biology: Studies in Floral Evolution in Animal-Pollinated Plants, pp: 191–216. Chapman & Hall, New York.

Stebbins, G.L. 1974. Flowering Plants. Evolution Above the Species Level. Belknap Press, Cambridge.

Steffan-Dewenter, I., Klein, A.M., Gaebele, V., Alfert, T., Tscharntke, T. 2006. Bee diversity and plant-pollinator interactions in fragmented landscapes. In: Waser, N.M., Ollerton, J. (Eds.). Plant-Pollinator Interactions, from Specialization to Generalization, pp: 387-407. The University of Chicago Press.

Capítulo IV

PHENOTYPIC INTEGRATION BETWEEN REPRODUCTIVE AND VEGETATIVE TRAITS: AN INQUIRY ON THE MICROEVOLUTIONARY PAST OF *BOMAREA SALSILLA* IN A FRAGMENTED FOREST

CARLOS E. VALDIVIA, ÁLVARO A. RIVERA-REI AND JAVIER A. SIMONETTI

Departamento de Ciencias Ecológicas, Facultad de Ciencias, Universidad de Chile.
Casilla 653, Santiago, Chile.

ABSTRACT

Habitat fragmentation and the creation of edge habitats negatively affect pollination and plant reproductive success. Nevertheless, no previous work has stressed microevolutionary processes in fragmented habitats. In fragments and edges a reduction in frequency and magnitude of traits, and combinations thereof, facing pollinator-mediated phenotypic selection and, consequently, a decoupling in variance/coavariance matrices, are indeed to be expected. We assessed covariance matrices for reproductive (*i.e.*, flower number, flower length, ray length, and inflorescence complexity) and vegetative traits (*i.e.*, leaf number, foliar area, plant height, and herbivory resistance) of *Bomarea salsilla*, a pollinator-dependent plant from south-central Chile, inhabiting a fragmented forest. *Bomarea salsilla* plants thriving at fragments and edges exhibited roughly similar levels of integration in overall traits (ranging from 12.5 up to 26.4%), showed dissimilar structures of correlation pleiades, mostly due to reproductive traits, and partially lose the coordinate expression of reproductive characters, as compared with the core of continuous forest. This agrees with previous results indicating a reduction and a change in pollinator-mediated selection pressures due to habitat fragmentation and the creatoin of edge habitats. Therefore, habitat fragmentation impaired the covariance expression of traits in *B. salsilla*.

Key words: phenotypic matrix covariance, pollinator- and herbivore-linked traits, fragmentation, edges, coevolution.

INTRODUCTION

The extent to which plant-animal interactions are disrupted by habitat fragmentation is a key subject in conservation biology. The reduction in habitat size, increase in isolation, and creation of edge habitats in the remnant fragments may reduce the diversity and abundance of interacting species (Aguilar *et al.*, 2006; Steffan-Dewenter *et al.*, 2006). A reduction in animal interactors may lead to contrasting effects on their plant counterparts depending on whether they are mutualists (*e.g.*, pollinators) or antagonists (*e.g.*, herbivores). Coupled to a decrease in diversity and abundance of pollinators there is usually a decrease in the frequency of pollinator visits and, consequently, a decrease in the reproductive success of plants (Aguilar *et al.*, 2006). When herbivores are also scarce in fragments and edges, however, a some sort of compensation may arise in the reproductive success of plants depending on the strength of modifications of mutualisms and antagonisms (*i.e.*, the symmetry of relationships).

Modifications in plant-mutualistic and plant-anatagonistic interactions given by habitat fragmentation might also translate into modifications in microevolutionary dynamics (sensu Bronstein *et al.*, 2004). Currently, it is widely accepted that the rate, scale, and intensity of habitat fragmentation when humans mediated propably exceed that previously experienced by plants (Kearns *et al.*, 1998; Aizen and Vázquez, 2006). To date, however, the vast majority of reports have mostly addressed the impact of habitat fragmentation on demographic processes, rather than evolutionary ones, with particular emphasis in evaluating the effects of fragmentation on pollination and plant fecundity (Hobbs & Yates, 2003; Ghazoul, 2005; Honnay *et al.*, 2005; Aguilar *et al.*, 2006). Much less atention has been put on plant-herbivore interactions. Unfortunately, these demographic-biased approaches preclude advancing in our understanding of plant evolutionary dynamics in the increasingly fragmented habitats, thereby strongly encouraging the incorporation of evolutionary ecology into conservation biology (Bronstein *et al.*, 2004).

Correlational selection exerted by pollinators and probably herbivores on plant reproductive and vegetative traits may play an important role in conducting populations to exhibit closely related traits (Stebbins, 1950, 1974; Conner & Via, 1993; Conner & Sterling, 1995; Waitt & Levin, 1998; Herrera *et al.*, 2002). The assumption that pollinators can exert correlational selection on reproductive traits lies at the core of Berg's (1959, 1960) classical hypothesis on correlation pleiades. Berg noted that many plants have specialized relationships with pollinators and have evolved precise correspondence between flower and pollinator morphology. She hypothesized that selection against covariation of floral morphology with vegetative traits would be generated by reduced reproduction in plants with flowers of unusual shape or size that place pollen in "improper" places on pollinators (*i.e.*, places not contacting stigmas) or whose stigmas contact pollinators in places where there is no pollen. Thus the floral morphology of plants with specialized pollination ecology should have evolved to be decoupled from the large phenotypic variation usually exhibited by vegetative traits. Thus plants with specialized pollination (*i.e.*, precise and consistent fit between flowers and pollinators) should exhibit floral characters that strongly covary with one another but not with vegetative traits. Or, as Berg described it, in these species floral and vegetative traits should form two distinct "correlation pleiades".

Patterns of phenotypic variation and covariation depict a pivotal subject in the evolutionary context because they can yield insights into the processes controlling past evolution of traits and probable trajectories of future evolution (Lande & Arnold, 1983). This is due to the phenotypic variance/covariance matrix mostly, but not totally, reflects the genetic variance/covariance matrix (Roff, 1995; Waitt & Levin, 1998). Trait variation and covariation reflect the interaction between phenotypic response to the environment, genetic response to natural selection, and the limits to such responses imposed by genetic and developmental constraints (Schlichting & Pigliucci, 1998). In this regard, it is worthwhile highlighting that rather than reflecting the result of adaptive evolution, the tight integration of floral parts and partial decoupling of floral from vegetative traits could be by-products of the genetic/developmental architecture of the plants (Armbruster *et al.*, 2004). Traits that are developmentally more closely related to one another tend to be more tightly correlated phenotypically and genetically, probably because of overlapping genetic control and developmental regulation. Therefore, strong correlations among functionally related traits may be owing to genetic or developmental factors or, alternatively, to pollinator-mediated correlational selection (*e.g.*, Herrera *et al.*, 2002; Armbruster *et al.*, 2004).

Reproductive traits such as number of flowers per plant, flower length, inflorescence length, and inflorescence complexity may altogether contribute to increase the reproductive success of plants and are likely to be selected by pollinators (Geber, 1985; Schoen & Dubuc, 1990; Fishbein & Venable, 1996). Similarly, vegetative traits, such as number of leaves, foliar area, plant height, and herbivory resistance may also contribute to increase reproductive success of plants (Simms, 1990; Marquis, 1992; Nuñez-Farfán & Dirzo, 1994). In fragmented and edge habitats, however, reductions in plant-animal interactions might translate into a reduction in pollinator-mediated selection events, particularly in terms of correlational selection. If such a situation has prevailed during more than one generation of plants, a decoupled expression of traits is indeed to be expected in plants from fragments and edges. The aim of this work is to evaluate patterns and magnitudes of phenotypic integration in reproductive and vegetative traits of *Bomarea salsilla*, a vine endemic to the human-mediated fragmented forest from south-central Chile. Because reproductive traits in a per flower basis are more likely to be constrained by genetic than ecological factors (Glover, 2007), an inflorescence-based approach was chose by selecting the number of flowers per plant, flower length, inflorescence length, and inflorescence complexity as reproductive traits. This approach lies at the core of the current suggestions stressing the need of considering inflorescences instead of flowers as reproductive units, which depict an extension of the correlation pleiades hypothesis of Berg. As vegetative traits, the number of leaves, foliar area, plant height, and herbivory resistance were selected. If a reduction in correlational selection events has occurred, as currently do (see Chapter III), during more than one generation of *B. salsilla* a decoupled expression of traits, mostly reproductive traits, is to be expected in plants from fragments and edges.

MATERIALS AND METHODS

Natural history

Fieldwork was conducted in Maulino forest in the northernmost zone of the temperate rainforest from Chile (35°59'S, 72°41'W; Bustamante *et al.*, 2005). Specifically, the study was performed in Los Queules National Reserve and two neighbouring forest fragments. Los Queules is a protected area of 145 ha embedded in a large tract of 600 ha of continuous forest. Forest fragments, ranging from 1 to 6 ha, are patches surrounded by commercial plantations of *Pinus radiata*. Here, four sites were defined taking into account the spatial arrangement of plant populations: one core and one edge of continuous forest, both sites placed in Los Queules National Reserve, and one core and one edge of forest fragment, both sites placed in two different fragments nearby Los Queules National Reserve. While cores were defined as sites placed ≥ 50 m inwards the border of each site, edges were defined as sites placed ≤ 10 m inwards the forest margin. Distance between sites ranges from 0.5 up to 2.5 km.

Bomarea salsilla (Alstroemeriaceae) is a fully protandrous, small-sized red-climbing perennial vine, inhabiting the sclerophyllous and temperate forests of Chile from 33°S to 40°S (Muñoz & Moreira, 2003). In the study site, it flowers from *ca.* November to January. Its breeding system is unknown; however, two pilot pollinator-exclusion experiments in the study site, devised to test for autogamy and agamospermy, demostrated that *B. salsilla* is a totally pollinator-dependent plant for seed set (*i.e.*, a xenogamous plant). In the study site, floral visitors are the nectarseeking hummingbirds, *Sephanoides sephaniodes* (Trochilidae); bumblebees, *Bombus dahlbomii* and *Bombus terrestris* (Apidae); butterflies, *Mathania leucothea* (Pieridae); and flies, *Acrophthalmyda paulseni* (Bombyliidae) (pers. obs.). *Manuelia gayatina* (Apidae) acts as a pollen-gathering floral vistor and, therefore, a poor positive effect on the reproductive success of *B. salsilla* is to be expected regarding the protandrous nature of this plant. Its herbivores are unknown, although several leaf-eating herbivores have been reported for this forest (Simonetti *et al.*, 2006).

Reproductive and vegetative traits

In order to estimate reproductive and vegetative traits in *B. salsilla* and their covariance matrices, 196, 153, 341, and 173 individual plants were chosen in the core of the continuous forest, edge of the continuous forest, core of the forest fragment, and edge of forest fragment, respectively, from October 2007 to February 2008. In these plants, reproductive traits assessed were i) flower number, ii) flower length, iii) ray length, and iv) inflorescence complexity. Flower number was determined by directly counting all flowers borne on inflorescences (there is a single inflorescence per invidual plant). Flower length was estimated from the tepal insertion to the end of this with a hand-held caliper. Similarly, ray length was estimated in the inflorescence portion ranging from the inflorescence base to the flower calix. Because rays may be arranged, in an increasing order, as monoseried, biseried, and triseried, ray length was estimated from the inflorescence base up to the flower calyx of the bigger serie (Fig. 4.1). Inflorescence complexity was estimated by scoring plants as one, two, or three depending on whether they exhibited monoseried, biseried, or triseried rays, respectively; when plants showed more than one type of ray (*i.e.*, exhibit irregular shapes), they were scored with intermediate values (Fig. 4.1). Vegetative traits assessed were v) leaf number, vi) foliar area, vii) plant height, and viii) herbivory resistance. Leaf number was determined by directly counting and tagging all leaves on plant spikes (there is a single spike per invidual plant) at the start of the flowering period when no new leaf develops. Foliar area was estimated by measuring length and wide of leaves and then computing area by using the oval area equation because this figure closely resembles leaf shape (data not shown). Plant height was estimated in elongated spikes from the soil surface up to the inflorescence base with a metric band (Fig. 4.1). Herbivory resistance, assessed as the percentage of undamaged foliar tissue, was estimated at the start of seed dispersal period by checking all leaves borne by each plant. In each leaf, a transparent paper grid (2-mm spaced) was put on the leaf surface for then counting the total squares occupied by foliar tissue and the total squares exhibiting a lack of foliar tissue but inside the projection of the leaf margin.

In all those cases in which more than one measure is attainable for each individual plant (*i.e.*, flower length, ray length, and foliar area) two measures per each trait were performed and then averaged in order to obtain a representative sample in a per-plant basis.

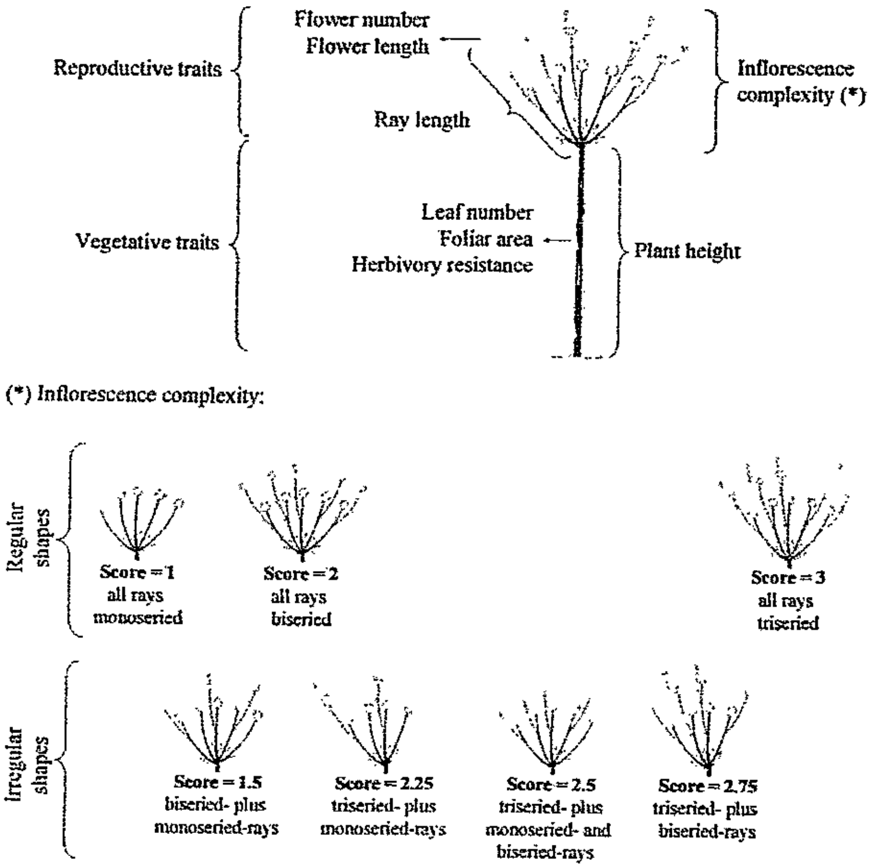

Figure 4.1. Non-scaled schematic representation of reproductive and vegetative traits in an elongated plant of *Bomarea salsilla.*

Magnitudes and patterns of integration in phenotypic covariance matrices

The magnitude and statistical significance of trait integration was assessed for each population using the variance of the eigenvalues (λ_i, i = 1, 8) of its character correlation matrix (Integration index = Var [λ_i], INT hereafter) (Wagner, 1984; Cheverud *et al.*, 1989). Standard errors and confidence intervals of INT were obtained by bootstrapping the original data set. Character correlation matrices for different populations were based on a different number of plants, thus the expected eigenvalue variance on the hypothesis of random covariation among characters [Exp(INT) = (number of characters ± 1)/number of plants; Wagner, 1984] varied among populations. For the purpose of comparisons among populations in the magnitude of integration, empirical integration indices were thus corrected by substracting the population-specific expected value. The (corrected) INT for a population was considered to reject significant trait integration if its 95% confidence interval includes zero. Character covariance matrices can share more complex relationships between one another than just being equal or unequal (*e.g.*, Flury, 1988; Steppan, 1997), hence we tested the similarity of within-population trait covariance structures by applying Flury's (1988) hierarchical method in a planned manner by comparing the core of continuous forest with all disturbed sites in terms of reproductive and vegetative traits as well as all traits pooled. This approach can reveal shared similarities across covariance matrices that go beyond the simple question of matrix equality (Phillips & Arnold, 1999). The "jump-up" approach of Phillips & Arnold (1999) was adopted. The model assuming heterogeneity of covariance matrices was sequentially compared using log-likelihood ratio tests with models that specify different relationships among the populations covariance matrices (*i.e.*, common principal components, partial principal components, proportionality, and equality), until a statistically significant deviation was encountered.

Boostraps procedures were performed by using R-program available at http://www.R-project.com. Tests related to Flury's hierarchy were carried out using the CPC program provided by P. Phillips (available at http://www.uoregon.edu/~pphil/software.html).

Developmental vs. functional integration hypotheses

In the absence of correlational selection from pollinators and herbivores favouring coordinated variation of reproductive and vegetative traits, thus enhancing the overall plant integration, reproductive and vegetative traits should tend to vary as relatively independent suites of characters because of their origin from different whorls and distinct developmental origins. Alternatively, if correlational selection exerted by pollinators and herbivores has enhanced the functional integration of both set of traits, it may be hypothesized that reproductive and vegetative traits should vary more coordinately. The first alternative (developmental hypothesis) may be seen as a null-type hypothesis, while the second one (functional hypothesis) represents a pollinator- and herbivore-mediated adaptive hypothesis. Dow & Cheverud's (1985) approach for comparing the observed correlation structure (R matrix) with alternative hypothetical integration patterns was used to discriminate between the two possibilities (see also Cheverud *et al.*, 1989; Waitt & Levin, 1993; for details and applications). An hypothetical integration matrix corresponding to the expectation under the developmental hypothesis (T_d matrix) was constructed by entering 1's at matrix cells corresponding to correlations among variables related to the same correlation pleiade (flower number, flower length, ray length, and inflorescence complexity, on one side; leaf number, foliar area, plant height, and herbivory resistance, on the other), and entering 0's at cells corresponding to correlations across the two sets. The hypothetical matrix under the functional hypothesis (T_f matrix) was constructed by entering 1's at matrix positions corresponding to correlations within subsets, and 0.5's at positions corresponding to correlations between subsets. To determine which of the two hypotheses best fitted the observed data and whether their difference in fit was statistically significant, the difference matrix $Z = T_d - T_f$ was obtained, and a matrix permutation test was then performed to compare similarity in structure between Z and R. Thereafter, a significant positive matrix correlation would indicate that observations fit T_d better than T_f, while a significant negative matrix correlation would indicate that T_f fits significantly better than T_d (Cheverud *et al.*, 1989).

RESULTS

Reproductive and vegetative traits

There were not significant effects on the mean number of flowers per plant for those inhabiting fragments or continuous forest (hereafter FE), and edges or cores (hereafter EE) (Non-parametric Scheirer-Ray-Hare extension of Kruskal-Wallis Tests for two-way ANOVAs on ranked data, FE: $H_{1,859} = 2.882$, $P = 0.090$; EE: $H_{1,859} = 2.800$, $P = 0.094$; FE x EE: $H_{1,859} = 0.896$, $P = 0.344$). Mean flower length was 10.8% and 5.8% lower in the edge of continuous forest and edge of forest fragment with respect to the core of the continuous forest, respectively, yet it was 10.4% higher in the core of forest fragment (FE: $H_{1,859} = 57.347$, $P < 0.001$; EE: $H_{1,859} = 170.773$, $P < 0.001$; FE x EE: $H_{1,859} = 13.300$, $P < 0.001$). Mean ray length did not differ significantly in the edges of continuous forest and forest fragment with respect to the core of continuous forest, but it was 34.1% higher in the core of forest fragment with respect to the core of continuous forest (FE: $H_{1,859} = 29.904$, $P < 0.001$; EE: $H_{1,859} = 57.750$, $P < 0.001$; FE x EE: $H_{1,859} = 17.079$, $P < 0.001$). Mean inflorescence complexity did not differ significantly in the edges of continuous forest and forest fragment with respect to the core of continuous forest, but it was 18.0% higher in the core of forest fragment with respect to the core of continuous forest (FE: $H_{1,859} = 29.446$, $P < 0.001$; EE: $H_{1,859} = 4.768$, $P = 0.023$; FE x EE: $H_{1,859} = 5.671$, $P = 0.017$) (Table 4.1).

Concerning vegetative traits, mean leaf number did not differ significantly between the edge of continuous forest and the core of continuous forest, albeit it was 14.3% and 21.0% lower in the core of continuous forest with respect to the core and edge of forest fragment (FE: $H_{1,859} = 91.44$, $P < 0.001$; EE: $H_{1,859} = 12.970$, $P < 0.001$; FE x EE: $H_{1,859} = 0.018$, $P = 0.893$). Mean foliar area was 27.3%, 48.5%, and 41.6% lower in the edge of continuous forest, and core and edge of forest fragments, with respect to the core of continuous forest, respetively (FE: $H_{1,859} = 109.567$, $P < 0.001$; EE: $H_{1,859} = 11.585$, $P < 0.001$; FE x EE: $H_{1,859} = 37.643$, $P < 0.001$). Similarly, mean plant height was 11.6%, 26.2%, and 29.1% lower in the edge of continuous forest, and core and edge of forest fragments with respect to the core of continuous forest, respetively (FE: $H_{1,859} = 147.203$, $P < 0.001$; EE: $H_{1,859} = 17.741$, $P < 0.001$; FE x EE: $H_{1,859} = 4.905$, $P = 0.027$). Conversely, mean herbivory resistance was 1.8%, 2.6%, and 2.6% higher in the edge of continuous forest, and core and edge of forest fragments with respect to the core of continuous forest, respetively (FE: $H_{1,859} = 89.560$, $P < 0.001$; EE: $H_{1,859} = 12.467$, $P < 0.001$; FE x EE: $H_{1,859} = 22.546$, $P < 0.001$) (Table 4.1).

Table 4.1. Summary of population mean values and coefficients of variation (CV) for reproductive and vegetative traits in *B. salsilla* in the fragmented Maulino forest from south-central Chile (Dissimilar letters depict significant differences ($P<0.05$) following Tukey HSD tests).

Traits and reproductive traits	Core of Continuous Forest		Edge of Continuous Forest		Core of Forest Fragment		Edge of Forest Fragment	
	CV (%)	Mean ± 1SE	CV (%)	Mean ± 1SE	CV (%)	Mean ± 1SE	CV (%)	Mean ± 1SE
Reproductive Traits								
Flower number	65.8	8.18 ± 0.38^{a}	64.0	7.95 ± 0.41^{a}	56.8	7.89 ± 0.24^{a}	66.2	7.43 ± 0.37^{a}
Flower length	12.8	1.64 ± 0.01^{a}	12.5	1.48 ± 0.02^{b}	11.8	1.83 ± 0.01^{c}	12.6	1.55 ± 0.01^{d}
Ray length	64.1	2.01 ± 0.09^{a}	71.6	1.77 ± 0.10^{a}	56.7	3.05 ± 0.09^{b}	75.7	1.92 ± 0.11^{a}
Inflor. complexity	33.0	1.23 ± 0.03^{a}	34.6	1.24 ± 0.03^{a}	34.8	1.50 ± 0.03^{b}	39.3	$1.36 \pm 0.04^{a,c}$
Vegetative Traits								
Leaf number	18.8	53.08 ± 0.71^{a}	18.5	50.40 ± 0.76^{a}	17.6	46.45 ± 0.44^{b}	22.0	43.87 ± 0.73^{b}
Foliar area	57.9	9.15 ± 0.38^{a}	62.8	6.65 ± 0.34^{b}	50.9	4.71 ± 0.13^{c}	62.6	5.34 ± 0.25^{d}
Plant height	25.7	206.77 ± 3.79^{a}	28.7	182.72 ± 4.24^{b}	23.8	152.50 ± 1.97^{c}	33.7	146.60 ± 3.75^{c}
Herb. resistance	6.0	97.37 ± 0.42^{a}	1.5	99.47 ± 0.12^{b}	1.1	99.86 ± 0.06^{b}	0.8	99.86 ± 0.06^{c}

Magnitudes and patterns of integration in phenotypic covariance matrices

Integration indices did not include zero, thus indicating sifnificant levels of integration at all populations studied. Similarly, they did not differ significantly among populations as revealed by the partial overlap of their confidence intervals, hence suggesting that overall traits are equally integrated at all populations (Table 4.2). In fact, while at the core of continuous forest trait integration varied from 12.8 to 20.0%, at the edge of continuous forest, core of forest fragment, and edge of forest fragment the INT ranges were 16.4-26.4, 13.9-18.7, and 12.5-19.2% respectively (maximum INT = 8) (Table 4.2).

Table 4.2. Between-population variation in the extent of phenotypic integration among morphometric traits, as measured with the variance of eigenvalues of phenotypic correlation matrices. Standard errors and confidence intervals were obtained through the percentil methods by bootstrapping 10.000 times the originl data set.

Population	Integration Index (mean ± 1se)	95% confidence interval
Core of continuous forest	1.241 ± 0.146	1.026 – 1.600
Edge of continuous forest	1.672 ± 0.208	1.308 – 2.114
Core of forest fragment	1.288 ± 0.098	1.110 – 1.495
Edge of forest fragment	1.219 ± 0.136	1.001 – 1.539

Nevertheless, the structure of correlation matrices totally differed among sites for all traits pooled (Table 4.3). A similar situation was observed when reproductive, but not when vegetative traits are compared (Table 4.3). In fact, the structure of correlation matrices of the core of continuous forest as compared with the core and edge of forest fragment did not differ significantly, as revealed for comparisons of CPC (1) with an unrelated model (Significant differences attained at $P = 0.02$ after Bonferroni corrections) (Table 4.3).

Table 4.3. Summary of Flury's hierarchical analyses (jump-up approach) for population-based pairwise comparisons between covariance matrices of reproductive and vegetative traits for *Bomarea salsilla* in the fragmented Maulino forest (CCF: core of continuous forest, CCB: edge of continuous forest, CFF: core of forest fragment, EFF: edge of forest fragment; CPC: common principal component).

Paired comparisons between populations	Higher	Lower	χ^2	df	P
CCF vs. ECF					
All traits pooled	Equality	Unrelated	393.2	36	<0.001
	Proportionality	Unrelated	313.9	35	<0.001
	CPC	Unrelated	64.4	28	<0.001
	CPC (6)	Unrelated	65.4	27	<0.001
	CPC (5)	Unrelated	63.8	25	<0.001
	CPC (4)	Unrelated	60.6	22	<0.001
Reproductive traits	Equality	Unrelated	73.4	10	<0.001
	Proportionality	Unrelated	58.8	9	<0.001
	CPC	Unrelated	21.5	6	0.002
	CPC (2)	Unrelated	20.2	5	0.001
	CPC (1)	Unrelated	19.8	3	<0.001
Vegetative traits	Equality	Unrelated	938.4	10	<0.001
	Proportionality	Unrelated	738.9	9	<0.001
	CPC	Unrelated	74.6	6	<0.001
	CPC (2)	Unrelated	74.6	5	<0.001
	CPC (1)	Unrelated	58.2	3	<0.001
CCF vs. CFF					
All traits pooled	Equality	Unrelated	1036.0	36	<0.001
	Proportionality	Unrelated	816.8	35	<0.001
	CPC	Unrelated	160.8	28	<0.001
	CPC (6)	Unrelated	160.8	27	<0.001
	CPC (5)	Unrelated	158.9	25	<0.001
	CPC (4)	Unrelated	158.8	22	<0.001
Reproductive traits	Equality	Unrelated	120.7	10	<0.001
	Proportionality	Unrelated	115.0	9	<0.001
	CPC	Unrelated	82.7	6	<0.001
	CPC (2)	Unrelated	82.7	5	<0.001
	CPC (1)	Unrelated	81.2	3	<0.001
Vegetative traits	Equality	Unrelated	865.9	10	<0.001

Paired comparisons between populations	Higher	Lower	χ^2	df	*P*
	Proportionality	Unrelated	345.6	9	<0.001
	CPC	Unrelated	15.5	6	0.017
	CPC (2)	Unrelated	14.9	5	0.011
	CPC (1)	Unrelated	6.9	3	0.076
CCF vs. EFF					
All traits pooled	Equality	Unrelated	637.5	36	<0.001
	Proportionality	Unrelated	603.2	35	<0.001
	CPC	Unrelated	88.3	28	<0.001
	CPC (6)	Unrelated	88.1	27	<0.001
	CPC (5)	Unrelated	85.4	25	<0.001
	CPC (4)	Unrelated	83.1	22	<0.001
Reproductive traits	Equality	Unrelated	71.0	10	<0.001
	Proportionality	Unrelated	61.1	9	<0.001
	CPC	Unrelated	23.3	6	<0.001
	CPC (2)	Unrelated	23.2	5	<0.001
	CPC (1)	Unrelated	22.1	3	<0.001
Vegetative traits	Equality	Unrelated	533.3	10	<0.001
	Proportionality	Unrelated	383.8	9	<0.001
	CPC	Unrelated	15.8	6	0.015
	CPC (2)	Unrelated	15.1	5	0.010
	CPC (1)	Unrelated	6.7	3	0.081

Continuation of Table 4.2.

Reproductive traits covary more closely than vegetative ones. In fact, while mean correlation coefficients for reproductive traits vary from 0.46 to 0.61, for vegetative traits they varied from 0.20 to 0.29 at all sites studied.

As far as correlations within reproductive traits are concerned, correlations between flower number and flower length, flower number and inflorescence complexity, and flower length and inflorescence complexity were higher in disturbed sites with respect to the core of continuous forest, with increase ranges of 38.4 up to 295.7%, 95.8-241.0%, and 65.1-328.0% in correlation coefficients, respectively. With respect to the core of continuous forest, correlations between flower number and inflorescence complexity, and ray length and inflorescence complexity were higher at the edge of continuous forest and the core of forest fragment with increase range of 2.8-5.1 and 1.3-3.9% in magnitudes of correlation coefficients, respectively; yet lower with respect to the edge of forest fragment with a decrease of 1.4 and 8.0%. Correlations between flower number and ray length were always lower at disturbed sites, ranging from 2.8 to 4.0% (Fig. 4.2).

Concerning correlations within vegetative traits, correlations between leaf number and herbivory resistance were always lower at disturbed sites with respect to the core of continuous forest, with a decrease range of 89.8-489.4%. By contrast, correlations between foliar area and plant height were always higher at disturbed sites, with an increase range of 6.6-46.8%. Correlations between leaf number and plant height, foliar area and herbivory resistance, and plant height and herbivory resistance exhibited a decrease in the strength of correlation, with respect to the core of continuous forest, that collectively ranges from 215.2 to 0.9% at the edge of continuous forest and core of forest fragment, respectively; while at the edge of forest fragment there was an increase ranging from 6.9 to 203.5% (Fig. 4.2).

In terms of correlations between reproductive and vegetative traits, all reproductive traits correlating with herbivory resistance exhibited a decrease in the strength of correlation at disturbed sites, with respect to the core of continuous forest, that ranges from 25.7 to 143.8%. Conversely, correlations of flower number with foliar area and plant height, and flower length with leaf number, foliar area and plant height were always higher at disturbed sites, with an increase ranging from 3.3 up to 288.7%. Moreover, correlations of flower number with leaf number, ray length with leaf number and foliar area, and infloresence complexity with leaf numer and plant height were always higher at the edge of continuous forest and core of forest fragment (range: 6.9-60.4%), yet lower at the edge of forest fragment, ranging from 3.4-43.5%. Correlations of ray length with plant height, and inflorescence complexity with foliar area were higher at the edge of continuous forest (range: 2.6-8.0%), but lower at both the core and edge of forest fragment (range: 0.6-37.8%) with respect to the core of continuous forest (Fig. 4.2).

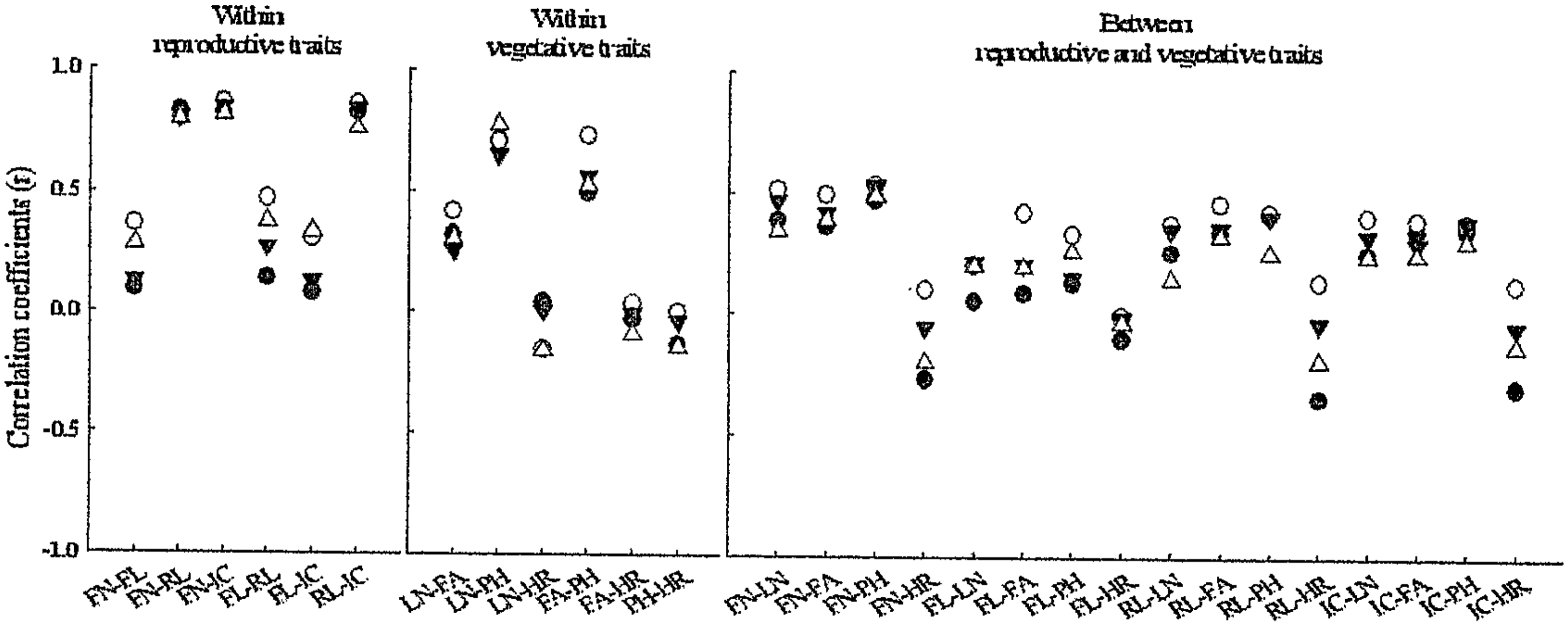

Figure 4.2. The sign and magnitude of correlations both within and between reproductive and vegetative traits of *Bomarea salsilla* in the fragmented Maulino forest (closed circles: core of continuous forest, closed triangles: edge of continuous forest, open circles: core of forest fragment, closed triangles: edge of forest fragment). Reproductive traits, FN: flower number, FL: flower length, RL: ray length, IC: inflorescence complexity. Vegetative traits, LN: leaf number, FA: foliar area, PH: plant height, HR: herbivory resistance.

Developmental vs. functional integration hypotheses

There was no evidence of extensive integration among the whole traits considered at all population herein assessed as revealed by rejecting the functional hypothesis. Conversely, while at the core of continuous forest and edge of forest fragment reproductive and vegetative traits covaried independently, but grouped into their respective subsets, mostly in terms of reproductive traits (see Fig. 4.2), at the edge of continuous forest and core of forest fragment traits assigned to reproductive and vegetative functions covary fully independently both within and between classes (Table 4.4).

Table 4.4. Comparisons of observed character correlation matrices with two different hypotheses of trait integration. The "developmental hypothesis" considers two groups of independently covarying traits defined on the basis of membership to reproductive and vegetative traits. The "functional hypothesis" postulates extensive integration among all traits. r_D, r_F and r_{diff} = element-wise Pearson correlation coeficients between the observed character correlation matrix (R matrix) and hypothetical matrices D and F, and their difference matrix (Z matrix), respectively. Statistical significance of the difference between hypotheses was tested by comparing R and Z matrices using permutation tests.

Population	Developmental Hypothesis r_d	Functional Hypothesis r_f	Comparison between hypotheses $H_0: r_d = r_f;$ $H_1: r_d > r_f$	
			r_z	P
Core of Continuous Forest	0.297	0.297	0.297	0.063
Edge of Continuous Forest	0.217	0.217	0.217	0.134
Core of Forest Fragment	0.217	0.217	0.216	0.126
Edge of Forest Fragment	0.335	0.335	0.335	0.042

DISCUSSION

Bomarea salsilla plants thriving at the fragmented Maulino forest exhibit roughly similar levels of integration in overall traits, show dissimilar structures of correlation pleiades, mostly due to reproductive traits, and partially loose the coordinate expression of reproductive characters. Integration indices depicted moderate levels of phenotypic integration ranging from 12.5 up to 26.4% of trait integration. This finding seems to be a common situation. For instance, assessments on a variety of traits borne by several plant species have indicated that integration indices ranging from 0 to 20% are more likely to be disclosed than those of higher values (Herrera *et al.*, 2002). Nevertheless, variation in the integration levels varied depending on which set of traits is considered. Thus, at all populations studied, vegetative traits were up to 67% less integrated than reproductive ones which is in agreement with the correlation pleiades hypothesis early addressed by Berg (1960). Nevertheless, while reproductive traits act as a functionally coordinate group at the core of continuous forest, at disturbed sites -excepting the edge of forest fragment- the same is not true. This indicates a thoroughly decoupling expression of both within and between reproductive and vegetative trait correlations triggered by habitat fragmentation and the creation of edge habitats.

The pairs of traits of *B. salsilla* exhibiting negative correlation coefficients may represent the trade-off expression of the traits involved (Reekie & Bazzaz, 2005). Traits exhibiting such a trade-off varied in identity and magnitude across sites, yet in an unclear fashion, thus pointing to consider the existence of dissimilar context-dependent compromises at each population (Reekie & Bazzaz, 2005).

In terms of patterns and magnitudes of trait integrations, it is whorthwhile highlighting that there are two divergent views concerning how quantitative traits evolve: while some authors hold that the developmental and genetic architecture of organisms (*e.g.*, pleiotropy) constrains morphological evolution, others claim that natural selection quickly overwhelms developmental and genetic constraints, thus allowing adaptive evolution to proceed, but largely unconstrained by these factors (see Lande, 1979, 1980; Riska, 1989; Houle, 1991). This leads to consider, for the former, that multivariate patterns of genetic and phenotypic correlations constitute evidence of developmental and genetic constraints on evolution, and for the latters that they reflect the action of natural selection generating adaptive patterns of genetic correlations (*e.g.*, Lande, 1979, 1982; Cheverud, 1982, 1984, 1996; Zeng, 1988; Riska, 1989; Diggle, 1992; Wagner, 1996). Nevertheless, despite numerous reports have been gathered in the last time addressing this subject, it is far from resolved as Armbruster *et al.* (1999) stressed. The *B. salsilla* case partially agrees with both viewpoints. On one hand, the fact that there is not an extensive trait integration at all population studied, as revealed by rejection of the functional hypothesis, is in accordance with the finding that pollinators and herbivores do not exert correlational selection pressures on pollinator- and herbivore-linked traits, that is, on reproductive and vegetative traits, at all populations studied (see Chapter II). Likewise, the consideration that pollinators, instead of herbivores, were the main animals capable of conducting trait diverification of *B. salsilla* (see Chapter II), renders out of odds the importance of such an animals in shapping the coordinate expression of reproductive traits. On the other hand, however, the expression of means, variances, and covariances of *B. salsilla* traits weakly reflects directional, disruptive/stabilizing, and correlational selection pressures exerted by its pollinators, respectively, as should be in the frame of an adaptive hypothesis (see Chapter III) (sensu Herrera *et al.*, 2006). Hence, genetic and developmental factors seem also play an important role in conducting the evolution of *B. salsilla*. Determining how patterns of trait integrations constrain and arise from pollinator-mediated selective processes remain still to be clarified.

Beyond theoretical discrepancies, the fact that habitat fragmentation impair the coordinate expression of traits imposes serious restrictions to the evolutionary fate of *B. salsilla*. Patterns of phenotypic variation and covariation do not depict only processes controlling past evolution of traits, but also guide probable trajectories of future evolution (Lande & Arnold, 1983). Regarding that tight coordinate systems of traits have the property of enhancing plant fitness, plants inhabiting fragments and edges are probably facing a positive feed-back with their pollinators: the fragmentation-mediated reduction in plant-pollinator interactions and hence in plant reproductive success impairs plant trait integrations and, as a consequence of it, lowered levels of plant trait integration may also reduce the plant reproductive success by precluding a proper fit between plants and pollinators, and so on.

The putative positive feed-back coupled to dissimilar patterns of trait correlations -and most likely in the G-matrix- together with a pollinator-mediated selection relaxation in fragments and edges may by all means put *B. salsilla* populations in the pathways of evolutionary trapping or suicide in the sense of Ferrière *et al.* (2004), thus endangering the maintenance of characters conferring the distinctive identity of plants and, more importantly, endangering the long-term population persistence in such disturbed habitats. Certainly, the case of *B. salsilla* may be representative of numerous plant-pollinator systems in the currently increasingly fragmented habitats. Therefore, regarding that pollination is an extensive and seriously threatened ecological interaction (Kearns et al., 1998; Aizen & Vázquez, 2006), evolutionary enlightened management schedules are required in order to restore plant-pollinator interactions and the coordinate expression of traits (Ashley *et al.*, 2003), thus allowing the persistence of coevolutionary processes.

ACKNOWLEDGEMENTS

Numerous persons, in several ways, kindly contributed to perform this work: Brenda Valdivia, Carlos O. Valdivia, Florencia Prats, Sandra Valdivia, Fernando Campos, Luciano Silva, Patricio Molina, and Sergio Hernández. The Chilean Forestry Service (CONAF) and Forestal Millalemu partially supported fieldwork. This work was funded by Beca de Apoyo a la Realización de Tesis Doctoral, Conicyt 23070138 (C.E.V.).

REFERENCES

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., Vázquez, D.P. 2006. Flower performance in human-altered habitats. In: Harder, L.D., Barrett, S.C.H. (Eds.), Ecology and Evolution of Flowers, pp: 159-179. Oxford University Press.

Armbruster, W.S., di Stilio, V.S., Tuxill, J.D., Flores, T.C., Velásquez Runk, J.L. 1999. Covariance and decoupling of floral and vegetative traits in nine Neotropical plants: a re-evaluation of Berg's correlation-pleiades concept. American Journal of Botany 86, 39-55.

Armbruster, W.S., Pélabon, C., Hansen, T.F., Muldr, C.P.H. 2004. Floral integration, modularity, and accuracy. Distinguishing complex adaptations from genetic constraints. In: Pigliucci, M., Preston, K. (Eds.), Phenotypic Integration, Studying the Ecology and Evolution of Complex Phenotypes. Pp: 23-49. Oxford University Press.

Ashley, M.V., Willson, M.F., Pergamsa, O.R.W., O'Dowd, D.J., Gended, S.M., Brown, J.S. 2003. Evolutionarily enlightened management. Biological Conservation 111, 115-123.

Berg, R.L. 1959. A general evolutionary principle underlying the origin of developmental homeostasis. American Naturalist 93, 103–105.

Berg, R.L. 1960. The ecological significance of correlation pleiades. Evolution 14, 171-180.

Bronstein, J.L., Dieckmann, U., Ferrière, R. 2004. Coevolutionary dynamics and the conservation of mutualisms. In: Ferrière, R., Dieckmann, U., Couvet, D. (Eds.). Evolutionary Conservation Biology, pp: 305-326, Cambridge University Press.

Bustamante, R.O., Simonetti, J.A., Grez, A.A., San Martín, J. 2005. Fragmentación y dinámica de regeneración del bosque Maulino: diagnóstico actual y perspectivas futuras. In: Smith-Ramírez, C.S., Armesto, J.J., Valdovinos, C. (Eds.), Historia, Biodiversidad y Ecología de los Bosques Costeros de Chile.

Editorial Universitaria, Santiago, Chile, pp: 555–564.

Conner, J.K., Sterling, A. 1995. Testing hypotheses of functional relationships: a comparative survey of correlation patterns among floral traits in five insect-pollinated plants. American Journal of Botany 82, 1399–1406.

Conner, J.K., Via, S. 1993. Patterns of phenotypic and genetic correlations among morphological and life-history traits in wild radish. Evolution 47, 704–711.

Cheverud, J. M. 1982. Phenotypic, genetic, and environmental morphological integration of the cranium. Evolution 36: 499-516.

Cheverud, J.M. 1984. Quantitative genetics and developmental constraints on evolution by selection. Journal of Theoretical Biology 110, 155-172.

Cheverud, J.M. 1996. Developmental integration and the evolution of pleiotropy. American Zoologist. 36, 44–50.

Cheverud, J.M., Wagner, G.P., Dow, M.M. 1989. Methods for the comparative analysis of variation patterns. Systematics Zoology 38, 201-213.

Diggle, P.K. 1992. Development and the evolution of plant reproductive characters. In: Wyatt, R. (Ed.), Ecology and evolution of plant reproduction, pp: 326–355. Chapman and Hall, New York, NY.

Dow, M.M., Cheverud, J.M. 1985. Comparison of distance matrices in studies of population structure and genetic microdifferentiation: quadratic assignment. American Journal of Phyical Anthropology 68, 367-374.

Ferrière, R., Dieckmann, U., Couvet, D. (Eds.). 2004. Evolutionary Conservation Biology. Cambridge University Press.

Fishbein, M., Venable, D.L. 1996. Evolution of inflorescence design: theory and data. Evolution 50, 2165–77.

Flury, B. 1988. Common Principal Components and Related Multivariate Models. Wiley, New York, NY.

Ghazoul, J. 2005. Buzzines as usual? Questioning the global pollination crisis. Trends in Ecology and Evolution 20, 367-373.

Geber, M. 1985. The relationship of plant size to self-pollination in *Mertensia ciliata*. Ecology 64, 762–72.

Glover, B. 2007. Understanding Flowers and Flowering. An Integrated Approach. Oxford University Press.

Herrera, C.M., Castellanos, M.C., Medrano, M. 2006. Geographical context of floral evolution: towards an improved research programme in floral diversification. In: Harder, L.D., Barrett, S.C.H. (Eds.), Ecology and Evolution of Flowers, pp: 278-294. Oxford University Press.

Herrera, C.M., Cerdá, X., García, M.B., Guitián, J., Medrano, M., Rey, P.J., Sánchez-Lafuente, A.M. 2002. Floral integration, phenotypic covariance structure and pollinator variation in bumblebee-pollinated *Helleborus foetidus*. Journal of Evolutionary Biology 15, 108-121.

Hobbs, R.J., Yates, C.J. 2003. Impacts of ecosystem fragmentation on plant populations: generalising the idiosyncratic. Australian Journal of Botany 51, 471–488.

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.

Houle, D. 1991. Genetic covariance of fitness correlates: what genetic correlations are made of and why it matters. Evolution 45, 630-648.

Kearns, C.A., Inouye, D.W., Waser, N.M. 1998. Endangered mutualisms: the conservation of plant-pollinator interactions. Annual Review of Ecology and Systematics 29, 83-112.

Lande, R. 1979. Quantitative genetic analysis of multivariate evolution, applied to brain-body size allometry. Evolution 33, 402-416.

Lande, R. 1980. The genetic covariance between characters maintained by pleiotropic mutations. Genetics 94, 203-215.

Lande, R. 1982. A quantitative genetic theory of life history evolution. Ecology 63, 607-615.

Lande, R., Arnold, S.J. 1983. The measurement of selection on correlated characters. Evolution 37, 1210-1226.

Marquis, R. 1992. Selective impact of herbivores. In: Fritz, R.S., Simms, E.L. (Eds.). Plant Resistance to Herbivores. Ecology, Evolution, and Genetics, pp: 301-325. University of Chicago Press.

Muñoz, M., Moreira, A. 2002. *Alstroemerias* de Chile. Diversidad, distribución y conservación. Taller La Era, Santiago, Chile.

Nuñez-Farfán, J., Dirzo, R. 1994. Evolutionary ecology of *Datura stramonium* L. in central Mexico: natural selection for resistance to herbivorous insects. Evolution 48, 423-436.

Phillips, P.C., Arnold, S.J. 1999. Hierarchical comparison of genetic variance-covariance matrices. I. Using the Flury hierarchy. Evolution 53, 1506-1515.

Reekie, E.G., Bazzaz, F.A. (Eds.) 2005. Reproductive Allocation in Plants. Elsevier Academic Press.

Riska, B. 1989. Composite traits, selection response, and evolution. Evolution 43, 1172-1191.

Roff, D. 1995. The estimation of genetic correlations from phenotypic correlations: a test of Cheverud's conjecture. Heredity 74, 481-490.

Schlighting, C.D., Pigliucci, M. 1998. Phenotypic Evolution: A Reaction Norm Perspective. Sinauer, Sunderland.

Schoen, D.J., Dubuc, M. 1990. The evolution of inflorescence size and number: a gamete-packaging strategy in plants. American Naturalist 135, 841–57.

Simms, E.L. 1990. Examining selection on multivariate phenotype: plant resitance to herbivores. Evolution 44, 1177-1188.

Simonetti, J.A., Grez, A.A., Bustamante, R.O. 2006. Interacciones y procesos en bosque Maulino fragmentado. En: Grez, A.A., Simonetti, J.A., Bustamante, R.O. (Eds.) Biodiversidad en ambientes fragmentados de Chile: patrones y procesos a diferentes escalas, pp: 99-114. Editorial Universitaria, Santiago.

Stebbins, G.L. 1974. Flowering Plants. Evolution Above the Species Level. Belknap Press, Cambridge.

Steffan-Dewenter, I., Klein, A.M., Gaebele, V., Alfert, T., Tscharntke, T. 2006. Bee diversity and plant-pollinator interactions in fragmented landscapes. In: Waser, N.M., Ollerton, J. (Eds.). Plant-Pollinator Interactions, from Specialization to Generalization, pp: 387-407. The University of Chicago Press.

Steppan, S.J. 1997. Phylogenetic analysis of phenotypic covariance structure. I. Contrasting results from matrix correlation and common principal component analyses. Evolution 51, 571-586.

Waitt, D.E., Levin, D.A. 1998. Genetic and phenotypic correlations in plants: a botanical test of Cheverud's conjecture. Heredity 80, 310–319.

Wagner, G.P. 1984. On the eigenvalue distribution of genetic and phenotypic dispersion matrices: evidence for a nonrandom organization of quantitative character variation. Journal of Mathematic Biology 21, 77-95.

Wagner, G.P. 1996. Homologues, natural kinds and the evolution of modularity. American Zoologist 36, 36-43.

Zeng, Z.-B. 1988. Long-term correlated response, interpopulation covariation, and interspecific allometry. Evolution 42, 363-374.

CONCLUSION GENERAL

Las poblaciones de *B. salsilla* que habitan el fragmentado bosque Maulino del sur de Chile exhiben patrones de variabilidad espacial y temporal en términos de las interacciones con polinizadores y herbívoros, expresión de rasgos reproductivos y vegetativos, éxito reproductivo y, consecuentemente, en los procesos microevolutivos dirigidos por polinizadores y herbívoros. Estos, sin embargo, concuerdan con las predicciones originales. En bordes y fragmentos hubo una reducción en las interacciones planta-polinizador y planta-herbívoro, disminuyó el número, aunque no la magnitud, de los eventos selectivos mediados mayormente por los polinizadores, y consecuentemente hubo un desacoplamiento en los niveles de integración fenotípica de los rasgos considerados. Mientras la herbivoría resultó negativamente afectada por la fragmentación del bosque y la consecuente creación de hábitats de borde durante las dos temporadas evaluadas en el presente estudio, la polinización se vió negativamente afectada sólo en la primera temporada. De hecho, durante la segunda temporada de estudio la polinización experimentó un incremento significativo en el centro del fragmento con respecto al centro del bosque continuo. Desafortunadamente se carece de un registro completo de los polinizadores que permita señalar con precisión cual o cuales especies dieron cuenta de tal incremento. No obstante, en la segunda temporada fue posible constatar un aumento en la proporción de visitas del abejorro exótico *Bombus terrestris*. La intrusión de polinizadores exóticos y el concomitante incremento en la producción de semillas en las plantas que visitan parece ser un fenómeno usual en los hábitats fragmentados (Aizen & Feinsinger 1994a,b). Así, acoplado al patrón de variabilidad espaciotemporal en la interacción planta-polinizador, sean éstos últimos nativos o exóticos, la producción de semillas en *B. salsilla* también vario, aunque de manera independiente a la intensidad de la herbivoría.

Los herbívoros del fragmentado bosque Maulino parecen no tener un efecto significativo sobre la producción de semillas al no ejercer ningún efecto aparente sobre el éxito reproductivo de *B. salsilla* (ver Capítulos I y II). Sin embargo, tal aceveración debe tomarse con cautela ya que, por un lado, los herbívoros parecieron modificar el accionar de los polinizadores (Capítulo II), mientras que por otro, la resistencia a la herbivoría fue un rasgo que participó de manera importante en los procesos de selección correlacional y estabilizadora en el centro del bosque continuo. Mecanismos de tolerancia así como restricciones genéticas, de desarrollo o ambientales pueden estar enmascarando la real importancia de los herbívoros a la hora de conducir el éxito reproductivo de *B. salsilla*.

A pesar de la variabilidad en la producción de semillas, debido probablemente a la intrusión de *B. terrestris*, los regímenes selectivos fueron menores en los hábitats de borde y fragmentos de bosque confirmando la hipótesis incial que señalaba a esta tipo de hábitats como puntos evolutivamente fríos. De hecho, la tendencia a exhibir una menor presión de selección estabilizadora, disruptiva y correlacional incidió en la caída en el número total de eventos selectivos registrados en los hábitats perturbados. Esta situación sienta las bases para que las poblaciones vaguen por el paisaje adaptativo aumentando así la probabilidad de caer en valles desadaptativos con la consecuente mayor probabilidad de extinción poblacional. De hecho, la menor cantidad de eventos de selección correlacional sugiere que las presiones de selección remanentes condicirían a las poblaciones hacia el suicidio o entrampamiento evolutivo (*sensu* Ferrière *et al.*, 2004). Ciertamente, esta situación se agrava considerando que las poblaciones perturbadas se encuentran en estados sucecionales tempranos a diferencia del centro del bosque continuo donde las comunidades vegetales se encuentran en un estado de climax sucesional (Bustamante *et al.*, 2005; Ramos *et al.*, 2008).

Los menores eventos selectivos registrados en los hábitats perturbados que son dirigidos por los herbívoros y, principalmente, por los polinizadores, plantean que es factible esperar menores niveles de integración fenotípica en estas poblaciones si es que tales reducciones en los eventos selectivos han sido consistentes a traves de las últimas generaciones de la planta. De hecho, esto resultó ser así en los niveles subordinados de integración. En orden decreciente, todas las poblaciones evaluadas exhibieron un significativo e igual nivel de integración fenotípica en lo referente a la totalidad de los rasgos considerados. No obstante, considerando los rasgos reproductivos y vegetativos de manera independiente (*i.e.*, niveles subordinados de integración), en los fragmentos y borde -excepto el borde de fragmento- hubo una desintegración en la matriz de correlaciones.

Colectivamente, los resultados obtenidos concuerdan con las predicciones inicialmente planteadas, aunque de un modo más restringido. Tanto los regimenes selectivos como los patrones de integración fenotípica indican un moderado nivel de integración coevolutiva entre *B. salsilla* y sus herbívoros y, principalmente, sus polinizadores, como fue revelado en el centro del bosque continuo. La fragmentación y la consecuente creación de hábitats de borde, por tanto, no produjieron un desacoplamiento extensivo como al comienzo se sugirió. Desde una perspectiva gráfica y considerando el esquema de la figura A2 (ver *Antecedentes generales* página 14), la fragmentación y creación de hábitats de borde condujieron a las poblacioes desde el estado C2 al D, en el caso del borde del bosque continuo y centro de fragmento, y a una situación intermedia entre estos dos estado en el caso del borde del fragmento. La figura B1 representa esquemáticamente dichas variaciones.

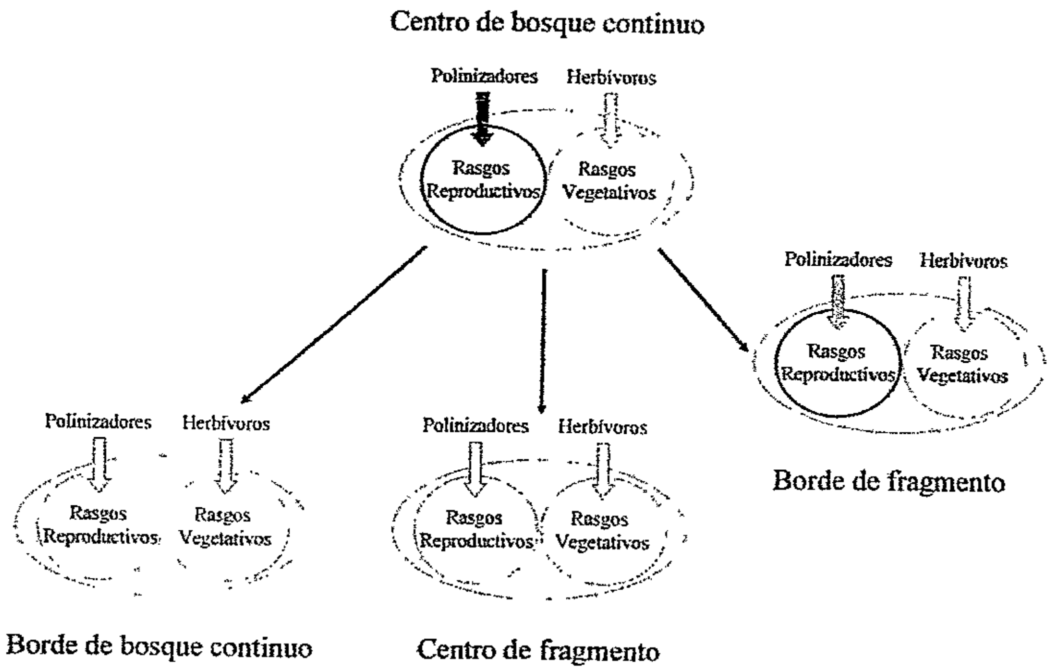

Figura B1. Niveles de integración fenotípica (ovalos) y presiones selectivas (flechas) mediado por los polinizadores y herbívoros de *Bomarea salsilla* en el fragmentado bosque Maulino de sur de Chile. Líneas delgadas y flechas grises representa relajamiento o pérdida del patrón o interacción considerada.

Las determinaciones de los patrones de variabilidad poblacional en la expresión de rasgos relevantes para la adecuación biológica de los organismos, junto con los cambios en las presiones selectivas y los posibles cambios en los patrones de integración genética y fenotípica, son primordiales para el establecimiento de planes de manejo evolutivamente informados (Ashley *et al.*, 2004). De hecho, actualmente es cada vez más ampliamente reconocido que las poblaciones tienen el potencial de divergir evolutivamente en plazos breves (*i.e.*, pocas décadas) y que las modificaciones antropogénicas del hábitat juegan un rol primordial en dicho proceso (Kettlewell, 1955; Palumbi, 2001; Stockwell *et al.*, 2003). Desafortunadamente, en pocas y destacadas instancias las acciones de manejo han sido elaboradas con el propósito explícito de mantener el potencial evolutivo de los organismos en ambientes cambiantes (Ferrière *et al.*, 2004). A nivel específico el único ejemplo existente es el plan de manejo del puma de la Florida (*Felis concolor coryi*). Luego de haber determinado que la depresión por endogamia es la mayor amenaza enfrentada por las poblaciones de esta subespecie de puma, los esquemas de conservación fueron implementados con el propósito de manejar la diversidad genética. El objetivo fue reducir los efectos deletereos de corto plazo que se estaban expresando en las poblaciones de *F. c. coryi*, pero al mismo tiempo mantener las características genéticas que le confieren su identidad y adaptabilidad a las peculiares condiciones de su hábitat. El reforzamiento genético se esta subespecie se realizó utilizando otra subespecie de puma, *F. c. stanleyana*. Ambos taxas, sin embargo, no son ni genética ni ecológicamente "intercambiables" en el sentido de Crandall *et al.* (2000) lo cual implica que están genéticamente aislados y adaptados a diferentes condiciones ecológicas. El principal desafío para el plan de conservación evolutivamente informado fue, por lo tanto, evitar la pérdida de la identidad genética así como la adaptación a las condiciones locales logradas por *F. c. coryi* durante el proceso de divergencia evolutiva del resto de las subespecies de pumas. Para solucionar este problema, Hedrick (1995) construyó un modelo matemático que evaluó la proporción de individuos introducidos de *F. c. stanleyana* que eliminarían los genes responsables de la depresión por endogamia en *F. c. coryi* y que además mantendrían tanto los genes responables de la adecuación local como aquellos genes neutros que caracterizan a la subespecie de Florida. El posterior manejo se efectúo de acuerdo a lo predicho por el modelo.

Al nivel poblacional-comunitario destaca el plan de manejo y conservación evolutivamente informado de la provincia florística de El Cabo en Sudáfrica. Un rasgo evolutivo distintivo de esta provincia es la reciente (post-Plioceno) y masiva diversificación de unos pocos linajes de plantas, para el cual existe evidencia de un fuerte componente ecológico en los procesos de diversificación el que involucra gradientes ambientales de meso y macroescala en las dinámicas coevolutivas de los sistemas planta-polinizador. Los planes de conservación de esta región consideran la importancia de los gradientes ambientales como un componente fundamental de la diversificación de plantas y animales. De hecho, éstos sugieren la necesidad de preservar al menos una instancia de gradiente para cada una de las zonas climáticas de la región. Complementa esta aproximación la identificación de las zonas geográficas que concentran el mayor número de especies y que, por lo tanto, requieren irrestrictamente ser protegidas (Ferrière *et al.*, 2004).

El caso de *B. salsilla* desarrollado en la presente tesis ratifica la magnitud y direccionalidad de los cambios eco-evolutivos esperados en las plantas con mecanismos de polinización biótica en el contexto de un paisaje fragmentado. El caso del puma de la Florida ilustra la factibilidad de determinar e implementar planes de manejo capaces de restaurar las poblaciones o taxas amenazados por los cambios antropogénicos. Finalmente, el caso de la provincia florística de El Cabo delimita la forma de establecer el ordenamiento territorial con el propósito de conservar no sólo las especies que actualmente se desarrollan allí, sino también el potencial futuro para adaptarse a los cada vez mayores impactos antropogénicos. Estos ejemplos, en conjunto, validan la capacidad de evaluar, restaurar y conservar la biota a la luz del creciente y cada vez más sofisticado marco teórico dado por la biología evolutiva. De hecho, dado que la conservación especie a especie puede ser difícil de implementar, existe un acuerdo cada vez más amplio en señalar que la conservación de las interacciones ecológicas y los procesos evolutivos sería más eficiente y costo efectiva que la aproximación especie específica (Noss, 1996; Thompson, 1998; 1999; Myers & Knoll, 2001; Ferrière *et al.*, 2004).

A modo de ejemplo, un plan de manejo evolutivamente informado para *B. salsilla* debiera incluir diversos puntos dependiendo de la escala geográfica a tratar. A un nivel de micro o mesoescala, como lo es la presente evaluación, los esquemas de conservación debieran implementarse con el propósito de conectar funcionalmente las poblaciones ubicadas en los fragmentos y bordes con aquellas ubicadas en los centros de bosque continuo. Esto se lograría permitiendo el movimiento de los polinizadores, para lo cual se requiere de un sistema de corredores biológicos a través de la matriz, además de un proceso de restauración eco-evolutiva como la realizada con el puma de la Florida para manejar inicialmente las poblaciones de los fragmentos y bordes.

A un nivel de macroescala las acciones son más complejas. Primero, es necesario identificar las diversas poblaciones de esta planta a lo largo de todo su rango de distribución. Considerando que en Chile Luebert & Pliscoff (2006) identificaron una serie de pisos vegetacionales caracterizados por presentar condiciones ecológicas distintivas, es factible establecer cuales son los pisos vegetacionales donde se desarrolla *B. salsilla* y en cada uno de éstos seleccionar una población focal así como poblaciones periféricas. Esto permitiría contar con una muestra representativa de las diversas condiciones ecológicas que contextualizan el rango de distribución de *B. salsilla* al mismo tiempo que evitaría caer en el sinsentido de proteger la totalidad de las poblaciones identificadas. En este esquema las poblaciones focales actuarían como reservorios de la diversidad genética y ecológica, mientras que las poblaciones periféricas servirían como corredores biológicos que conecten las poblaciones focales seleccionadas en cada piso vegetacional. Una vez identificados ambos tipos de poblaciones, las condiciones ecológica de cada una de ellas debiera ser manejada con el propósito de mantener las características distintivas de cada comunidad en la cual participan las poblaciones de nuestra especie focal. Por ejemplo, en el caso del fragmentado bosque Maulino, Ramos *et al.* (2008) establecieron el manejo del árbol *Aristotelia chilensis* como la mejor forma de aproximarse a las características distintivas del bosque Maulino y evitar que los fragmentos viren hacia condiciones esclerófilas, perdiendo así su identidad. Una vez identificadas las poblaciones y establecidos los respectivos protocolos de manejo para mantener las características ecológicas en las cuales están inmersas las poblaciones debiera establecerce un sistema de corredores biológicos que conecten esta poblaciones. Para ello probablemente es necesario restaurar algunas poblaciones de *B. salsilla* que se encuentren en dichos corredores así como las poblaciones focales. Un esquema de trabajo como el desarrollado para el puma de la Florida puede dar la pauta de tal procedimiento. Cabe señalar además que el plan de manejo planteado para *B. salsilla* podría involucrar más especies si se consideran los sitios de mayor riqueza existentes al interior de los diferentes pisos vegetacionales.

En Chile ha ocurrido un deterioro creciente de la biodiversidad en todos sus niveles y atributos en los últimos cien años (Hoffmann, 1998; Castro, 2002; Camus, 2006; Luebert & Pliscoff, 2006). No obstante, el actual sistema de areas silvestres protegidas no asegura la persistencia de las especies nativas del territorio a pesar de existir las capacidades económicas de financiar, al menos en parte, la conservación *in situ* de éstas (Asmüssen & Simonetti, 2007). Es, por tanto, de importancia crucial evaluar e implementar planes de manejo evolutivamente informados con el propósito de conservar tanto las especies como el potencial evolutivo de una fracción importante de la flora nacional. El caso de *B. salsilla* ratifica que las actuales poblaciones se encuentran en un proceso de divergencia evolutiva por causas antropogénicas. Sin embargo, esto no representa el ingreso de las poblaciones a un callejón sin salida, por el contrario, ilustra y delimita las futuras acciones a seguir con tal de evaluar y establecer las acciones de manejo pertinentes que aseguren la conservación actual y futura de las especies a la luz de los posibles cambios evolutivos que ocurren en hábitats fragmentados.

REFERENCIAS

Agrawal, A.A., van Zandt, P.A. 2003. Ecological play in the coevolutionary theatre: genetics and environmental determinants of attacks by a specialist weevil on milkweed. Journal of Ecology 91, 1049-1059.

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., Feinsinger, P. 1994a. Forest fragmentation, pollination, and plant reproduction in a Chaco dry forest, Argentina. Ecology 75, 330-51.

Aizen, M.A. Feinsinger, P. 1994b. Habitat fragmentation, native insect pollinators, and feral honeybees in Argentine "Chaco Serrano." Ecological Applications 4, 378-92.

Alzate, F., Quijano-Abril, M.A., Morrone, J.J. 2008. Panbiogeographical analysis of the genus *Bomarea*. Journal of Biogeography 35, 1250-1257.

Anderson, I.A., Busch, J.W. 2006. Relaxationed pollinator-mediated selection weakens floral integration in self-compatible taxa of *Leavenworthia* (Brassicaceae). American Journal of Botany 93, 860-867.

Armbruster, W.S. 1991. Multilevel analysis of morphometric data from natural plant populations: insights into ontogenetic, genetic, and selective correlations in *Dalechampia scandens*. Evolution 45, 1229-1244.

Armbruster, W.S., di Stilio, V.S., Tuxill, J.D., Flores, T.C., Velásquez Runk, J.L. 1999. Covariance and decoupling of floral and vegetative traits in nine Neotropical plants: a re-evaluation of Berg's correlation-pleiades concept.

American Journal of Botany 86, 39-55.

Armbruster, W.S., Schwaegerle, K.E. 1996. Causes of covariation of phenotypic traits among populations. Journal of Evolutionary Biology 9, 261-276.

Ashley, M.V., Willson, M.F., Pergamsa, O.R.W., O'Dowd, D.J., Gended, S.M., Brown, J.S. 2003. Evolutionarily enlightened management. Biological Conservation 111, 115-123.

Asmüssen, M.V., Simonetti, J.A. 2007. Can a developing country like Chile invest in biodiversity conservation? Environmental Conservation 34, 183-185.

Aspi, J., Jakalaniemi, A., Tuomi, J., Siikamaki, P. 2003. Multilevel phenotypic selection on morphological characters in a metapopulation of *Silene tatarica*. Evolution 57, 509-517.

Berg, R.L. 1960. The ecological significance of correlation pleiades. Evolution 14, 171-180.

Bronstein, J.L., Dieckmann, U., Ferrière, R. 2004. Coevolutionary dynamics and the conservation of mutualisms. In: Ferrière, R., Dieckmann, U., Couvet, D. (Eds.). Evolutionary Conservation Biology, pp: 305-326, Cambridge University Press.

Bustamante, R.O., Simonetti, J.A., Grez, A.A., San Martín, J. 2005. Fragmentación y dinámica de regeneración del bosque Maulino: diagnóstico actual y perspectivas futuras. In: Smith-Ramírez, C.S., Armesto, J.J., Valdovinos, C. (Eds.), Historia, Biodiversidad y Ecología de los Bosques Costeros de Chile, pp: 555–564. Editorial Universitaria, Santiago, Chile.

Campbell, D.R. 1989. Measurements of selection in a hermaphroditic plant: variation in male and female pollination success. Evolution 43, 318-334.

Campbell, D.R., Waser, N.M., Meléndez-Ackerman, E.J.. 1997. Analyzing pollinator-mediated selection in a plant hybrid zone: hummingbird visitation patterns on three spatial scales. American Naturalist 149, 295-315.

Campbell, D.R., Waser, N.M., Price, M.V. 1996. Mechanisms of hummingbird-mediated selection for flower width in *Ipomopsis aggregata*. Ecology 77, 1463-1472.

Campbell, D.R., Waser, N.M., Price, M.V., Lynch, E.A., Mitchell, R.J. 1991. Components of phenotypic selection: pollen export and flower corolla width in *Ipomopsis aggregata*. Evolution 45, 1458-1467.

Camus, P. 2006. Ambiente, Bosques y Gestión Forestal en Chile. 1541-2005. Dirección de Bibliotecas, Archivos y Museos y Lom Ediciones, Santiago.

Caruso, C.M. 2000. Competition for pollination influences selection on floral traits of *Ipomopsis aggregata*. Evolution 54, 1546–1557.

Castro, E. 2002. Réquiem por el Bosque Nativo. Salesianos S.A., Santiago.

Conner, J.K., Rush, S., Jennetten, P. 1996a. Measurements of natural selection on floral traits in wild radish (*Raphanus raphanistrum*). I. Selection through lifetime female fitness. Evolution 50, 1127-1136.

Conner, J.K., Rush, S., Kercher, S., Jenneten, P. 1996b. Measurements of natural selection on floral traits in wild radish (*Raphanus raphanistrum*). II. Selection through lifetime male and total fitness. Evolution 50, 1137-1146.

Colling, G., Matthies, D. 2004. The effects of plant population size on the interactions between the endangered plant *Scorzonera humilis*, a specialised herbivore, and a phytopathogenic fungus. Oikos 105, 71-78.

Crandall, K.A., Bininda-Emonds, O.R.P., Mace, G.M., Wayne, R.K. 2000.

Considering evolutionary processes in conservation biology. Trends in Ecology and Evolution 15, 290-295.

Cresswell, J.E. 1998. Stabilizing selection and the structural variability of flowers within species. Annals of Botany 81, 463-473.

Cruzan, M.B. 2001. Population size and fragmentation thresholds for the maintenance of genetic diversity in the herbaceous endemic *Scutellaria montana* (Lamiaceae). Evolution 55, 1569-1580.

Falconer, D.S. 1989. Introduction to Quantitative Genetics. Longman, Harlow, UK.

Fenster, C.B. 1991. Selection on floral morphology by hummingbirds. Biotropica 23, 98-101.

Ferrière, R., Dieckmann, U., Couvet, D. (Eds.). 2004. Evolutionary Conservation Biology. Cambridge University Press.

Frey, F.M. 2004. Opposing natural selection from herbivores and pathogens may maintain floral-color variation in *Claytonia virginica* (Portulacaceae). Evolution 58, 2426-2437.

Galen, C. 1989. Measuring pollinator-mediated selection on morphometric floral traits: bumblebees and the alpine sky pilot, *Polemonium viscosum*. Evolution 43, 882–890.

Galen, C., Cuba, J. 2001. Down the tube: pollinators, predators, and the evolution of flower shape in the alpine skypilot, *Polemonium viscosum*. Evolution 55, 1963-1971.

Gómez, J.M. 1993. Phenotypic selection on flowering synchrony in a high mountain plant. Journal of Ecology 81, 605-613.

Gómez, J.M. 2000. Phenotypic selection and response to selection in *Lobularia* *maritima*: importance of direct and correlational components of natural selection. Journal of Evolutionary Biology 13, 689-699.

Gómez, J.M. 2005. Long-term effects of ungulates on performance, abundance, and spatial distribution of two montane herbs. Ecological Monographs, 75: 231-258.

Gómez, J.M. 2003. Herbivory reduces the strength of pollinator-mediated selection in the Mediterranean herb *Erysimum mediohispanicum*: consequences for plant-specialization. American Naturalist 162, 242-256.

Gómez, J.M., Zamora, R. 2000. Spatial variation in the selective scenarios of *Hormathophylla spinosa* (Cruciferae). American Naturalist 155, 657-668.

Groom, M.J. 2001. Consequences of subpopulation isolation for pollination, herbivory, and population growth in *Clarkia concinna concinna* (Onagraceae). Biological Conservation 100, 55-63.

Gross, C.L. 2001. The effect of introduced honeybees on native bee visitation and fruit-set in *Dillwynia juniperina* (Fabaceae) in a fragmented ecosystem. Biological Conservation 102, 89-95.

Hedrick, P.W. 1995. Gene flow and genetic restoration: the Florida panther as a case study. Conservation Biology 9, 996-1007.

Hendrix, S.D. 1988. Herbivory and its impact on plant reproduction. In: Lovett Doust, J., Lovett Doust, L. (Eds.), Plant reproductive ecology. Patterns and strategies, pp. 246-263. Oxford University Press.

Herrera, C.M. 1993a. Selection on floral morphology and environmental determinants of fecundity in a hawkmoth-pollinated violet. Ecological Monographs 63, 251-275.

Herrera, C.M. 1993b. Selection on complexity of corolla outline in a hawkmoth-pollinated violet. Evolutionary Trends in Plants 7, 9-13.

Herrera, C.M. 1996. Floral traits and plant adaptation to insect pollinators: a devil's advocate approach. In: Lloyd, D.G., Barrett, S.C.H. (Eds.), Floral Biology. Studies on Floral Evolution in Animal-Pollinated Plants, pp: 65-87. Chapman & Hall, New York.

Herrera, C.M. 2000. Measuring the effects of pollinators and herbivores: evidence for non-additivity in a perennial herb. Ecology 81, 2170-2176.

Herrera, C.M., Cerdá, X., García, M.B., Guitián, J., Medrano, M., Rey, P.J., Sánchez-Lafuente, A.M. 2002a. Floral integration, phenotypic covariance structure and pollinator variation in bumblebee-pollinated *Helleborus foetidus*. Journal of Evolutionary Biology 15, 108-121.

Herrera, C.M., Medrano, M., Rey, P.J., Sánchez-Lafuente, A.M., García, M.B., Guitián, J., Manzaneda, A.J. 2002b. Interaction of pollinators and herbivores on plant fitness suggests a pathway for correlated evolution of mutualism- and antagonism-related traits. Proceedings of the National Academy of Science, USA 99, 16823-16828.

Herrera, C.M., Pellmyr, O. (Eds.) 2002. Plant-animal interactions, an evolutionary approach. Blackwell Publishing.

Herrera, C.M., Castellanos, M.C., Medrano, M. 2006. Geographical context of floral evolution: towards an improved research programme in floral diversification. En: Harder, L.D., Barrett, S.C.H. (Eds.) Ecology and Evolution of Flowers, pp: 278-294. Oxford University Press.

Hoffmann, A.E. 1998. La Tragedia del Bosque Chileno. Ocho Libros Editores, Santiago.

Hutchinson, G.E. 1968. The ecological theater and the evolutionary play. Yale University Press, New Haven.

Johnston, M.O. 1991. Natural selection on floral traits in two species of *Lobelia* with different pollinators. Evolution 45, 1468-1479.

Kelly, C.A. 1992. Spatial and temporal variation in selection on correlated life-history traits and plant size in *Chamaecrista fasciculata*. Evolution 46, 1658-1673.

Kery, M., Matthies, D., Fischer, M. 2001. The effect of plant population size on the interactions between the rare plant *Gentiana cruciata* and its specialized herbivore *Maculinea rebeli*. Journal of Ecology 89, 418-427.

Kettlewell, H.B.D. 1955. Selection experiments on industrial melanism in the Lepidoptera. Heredity 9, 323-342.

Lande, R. 1980. The genetic covariance between characters maintained by pleiotropic mutations. Genetics 94, 203-215.

Luebert, F., Pliscoff, P. 2006. Sinópsis Bioclimática y Vegetacional de Chile. Editorial Universitaria, Santiago, Chile.

Maad, J. 2000. Phenotypic selection in hawkmoth-pollinated *Platanthera bifolia*: targets and fitness surfaces. Evolution 54, 112-123.

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.

Monro, K., Poore, A.G.B. 2004. Selection in modular organisms: is intraclonal variation in macroalgae evolutionarily important? American Naturalist 163, 564-578.

Murcia, C. 1995. Edge effects in fragmented forests. Implications for conservation. Trends in Ecology and Evolution 10, 58-62.

Myers, N., Knoll, A.H. 2001. The biotic crisis and the future evolution. Proceedings of the National Academy of Science of the USA 98, 5389-5392.

Nagy, E.S. 1997. Selection for native characters in hybrids between two locally adapted plant subspecies. Evolution 51, 1469-1480.

Noss, R.F. 1996. Ecosystem as conservation targets. Trends in Ecology and Evolution 11, 351.

Núñez-Farfán, J., Dirzo, R. 1994. Evolutionary ecology of *Datura stramonium* L. in central Mexico: natural selection for resistance to herbivorous insects. Evolution 48, 423-436.

O'Connell, L.M., Johnston, M.O. 1998. Male and female pollination success in a deceptive orchid: a selection study. Ecology 79, 1246-1260.

Palumbi, S.R. 2001. Humans as the world's greatest evolutionary force. Science 293, 1786-1790.

Ramos, C., Simonetti, J.A., Flores, J.D., Ramos-Jiliberto, R. 2008. Modelling the management of fragmented forests: Is it possible to recover the original tree composition? The case of the Maulino forest in Central Chile. Forest Ecology and Management 255, 2236-2243.

Rausher, M.D., Simms, E.L. 1989. The evolution of resistance to herbivory in *Ipomea purpurea*, I. Attemps to detect selection. Evolution 43, 563-572.

Samejima, H., Marzuki, M., Nagamitsu, T., Nakasizuka, T. 2004. The effects of human disturbance on a stingless bee community in a tropical rainforest.

Biological Conservation 120, 577-587.

Severns, P. 2003. Inbreeding and small population size reduce seed set in a threatened and fragmented plant species, *Lupinus sulphureus* ssp *kincaidii* (Fabaceae). Biological Conservation 110, 221-229.

Simonetti, J.A., Grez, A.A., Bustamante, R.O. 2006. Interacciones y procesos en bosque Maulino fragmentado. En: Grez, A.A., Simonetti, J.A., Bustamante, R.O. (Eds.) Biodiversidad en ambientes fragmentados de Chile: patrones y procesos a diferentes escalas, pp: 99-114. Editorial Universitaria, Santiago.

Stockwell, C.A., Hedry, A.P., Kinnison, M.T. 2003. Contemporary evolution meets conservation biology. Trends in Ecology and Evolution 18, 94-101.

Strauss, S.Y., Zangerl, A.R. 2002. Plant-insect interactions in terrestrial ecosystems. In: Herrera, C.M., Pellmyr, O. (Eds.), Plant-animal interactions, an evolutionary approach, pp. 77-106. Blackwell Publishing.

Thompson, J.N. 1994. The Coevolutionary Process. Chicago, IL, USA. University of Chicago Press.

Thompson, J.N. 1997. Evaluating the dynamics of coevolution among geographically structured populations. Ecology 78, 1619-1623.

Thompson, J.N. 1998. Rapid evolution as an ecological process. Trends in Ecology and Evolution 13, 329-332.

Thompson, J.N. 1999. The evolution of species interactions. Science 284, 2116-2118.

Thompson, J.N. 2005. The Geographic Mosaic of Coevolution. The University of Chicago Press.

Valdivia, C.E., Niemeyer, H.M. 2005. Reduced maternal fecundity of the high

Andean perennial herb *Alstroemeria umbellata* (Alstroemeriaceae) by aphid herbivory. New Zealand Journal of Ecology 29, 321-324.

Valdivia, C.E., Niemeyer, H.M. 2007. Non-correlated evolution between herbivore- and pollinator-linked features on *Aristolochia chilensis* (Aristolochiaceae). Biological Journal of the Linnean Society.

Valverde, P.L., Fornoni, J., Nuñez-Farfán, J. 2001. Defensive role of leaf trichomes in resistance to herbivorous insects in *Datura stramonium*. Journal of Evolutionary Biology 14, 424-432.

Widén, B. 1991. Phenotypic selection on flowering phenology in *Senecio integrifolius*, a perennial herb. Oikos 61, 205-215.

Wright, S. 1932. The roles of mutation, inbreeding, crossbreeding and selection in evolution. Proceedings of the 6th international congress of genetics 1, 356-366.