LEAF TRAITS AND LEAF HERBIVORY VARIATION BETWEEN CENTRE AND NORTH-EDGE POPULATIONS OF *DRIMYS WINTERI*.

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CANELO

Canelo, planta ancestral
Ícono de nuestra tierra
En el fondo de las sierras
Donde se puede encontrar

No vive con el matorral
Requiere del agua pura
Prospera en su frescura
Lo seco le hace daño
Más la sequia de los años
Amenazan su hermosura

– Ramiro O. Bustamante

Nací en Rancagua en 1989. En el año 2007 inicié mis estudios en la carrera de Biología de la Pontificia Universidad Católica de Chile donde comenzó mi interés por la investigación en ecología, la cual pude desarrollar durante el transcurso de mi maestría en la Facultad de Ciencias en la Universidad de Chile.

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RESUMEN

La hipótesis centro-periferia, postula que las especies encuentran su óptimo ecológico en el centro de su distribución geográfica, el cual decrece hacia los bordes de la distribución. En este trabajo pretendemos probar si las condiciones subóptimas en el borde de distribución afecta a algunos atributos fenotípicos de *Drimys winteri* (atributos morfológicos y fisiológicos) y si estos cambios tienen a su vez implicancias sobre la herbivoría.

Drimys winteri es una especie arbórea de amplia distribución en Chile, localizada desde los 30 ° a 50 ° S. Seleccionamos tres poblaciones del centro (ubicadas entre los 40°-41°S) y tres del borde norte de distribución (32-33°S), a las cuales medimos rasgos foliares sensibles a factores abióticos y relacionados con el rendimiento de las plantas (asignación de masa por área, grosor, contenido de nitrógeno y área), también medimos la frecuencia de daño por herbívoros en las hojas e identificamos el tipo de daño clasificándolo en gremios (masticadores, minadores, succionadores, dobladores). Nuestros principales resultados indican que las hojas del borde norte de distribución son más delgadas, ricas en nitrógeno y presentan una mayor frecuencia de daño por masticadores, en comparación con las poblaciones del centro de distribución. Además, encontramos que el gremio de los masticadores fue el único encontrado en todas las poblaciones estudiadas y el con mayor participación. También encontramos un aumento de la diversidad de gremios en el centro de distribución de *D. winteri*. Este estudio proporciona información acerca de cómo los

factores ambientales afectan a las interacciones ecológicas a través de la modificación de atributos funcionales relevantes para su desempeño.

ABSTRACT

Based on the centre-periphery hypothesis tenet that species encounter their ecological optimum at the center of their geographical distribution, we aim to test if suboptimal conditions at border of distribution that affects performance components as physiology and morphology, have a concomitant effect on species interactions, specifically we test whether leaf traits differ between centre and edge populations and its relation with herbivory damage. We perform this study in *Drimys winteri*, a wide distributed tree species that ranges in Chile from 30° to 50°S. We selected three populations from the centre and three from the north-edge of distribution and measure foliar traits related to plant performance that are responsive to abiotic factors, we also measured leaf damage frequency and identified herbivories feeding guilds. We found differences in foliar attributes, leaves were thicker and with lower nitrogen content in centre populations, these traits were significantly related to chewers damage frequency but not with herbivory guilds considered all together. We also found an increase of feeding guilds diversity at the centre of *D. winteri* distribution that can be attributed to herbivories pool. This study provides an insight on how environment affect ecological interactions through modulating species attributes along their geographic distribution.

INTRODUCTION

The understanding of what determines the boundaries of species geographic distribution is a fundamental topic in Ecology (Gaston 2009, Sexton *et al.* 2009, Hargreaves *et al.* 2013, Louthan 2016). The "centre-periphery hypothesis" is one of the most accepted explanations to the decrease of abundance towards the edges of distribution (Pironon *et al* 2015). Under the assumption that environmental conditions are autocorrelated and change in a monotonically fashion, the "centre-periphery hypothesis" postulate that species encounter an ecologic optimum at the center of their geographic distribution, while at the distribution borders, the abundance decreases in response to suboptimal environmental conditions (Brown 1984, Hoffmann and Blows, 1994).

In spite of recent reviews show that there is no sufficient empirical evidence supporting the "centre-periphery hypothesis", it is still is used as a conceptual reference to understand the ecological drivers that control species abundance and distribtution (Pironon *et al.* 2016, Pironon *et al.* 2015, Sexton et al 2009, Gaston 2006). One explanation on why the centre-periphery pattern is not always observed in species geographic range relays in that space is frequently presented as a complex mosaic of abiotic and biotic factors, where variables may not change continuously and or simultaneously. On the other hand, Pironon *et al.* (2016)

reviewed 248 studies that account for the differences in abundance, genetic diversity and performance along species distribution and found that there is no sufficient evidence that environmental suitability or that performance of populations systematically decline towards the distribution edges. Based on the idea that species follow their niche requirements, the authors propose that abundance will depend on the presence of sites that accomplish those abiotic requirements, thus, abundance decreases at borders of distributions because the frequency of suitable sites decreases as well (Holt and Keitt, 2000). Nevertheless, is a long standing prediction that individuals are limited or simply not adapted to the abiotic conditions beyond their range edge (Bridle and Vines 2007, Kawecki , Hargreaves *et al.* 2014).

Over time, the centre-periphery hypothesis has been extended to other components of species performance as genetics, physiology and morphology (Sexton *et al.*, 2009; Abeli *et al.*, 2014). These phenotype modifications in response to local conditions can have significant effects on species interactions (Schemske *et al.* 2009, Louthan *et al.* 2015). For example, in plants certain combinations of leaf traits arise primarily from strategies to treat abiotic stress, but these adaptations may also act as structural defenses against herbivore damage. Leaf mass per area (LMA) is identified as indicator of plant performance, which is strongly related to climatic conditions as precipitation and temperature (Levitt 1972, Witkowski and Lamont 1991, Smith *et al.* 1997, Lamont et al 2002, Gouveia and Freitas 2008, Sterck *et al.* 2006), and is also identified as *proxy* of mechanical resistance to herbivory (Hanley *et al.* 2007). Garibaldi and Kitzberger (2011) studied the relation between climate, insect density and foliar traits along the geographical distribution of a deciduous tree (*Nothofagus pumilio*) and found that climate modulates leaf damage by inducing change in traits than through affecting herbivore density. However, if a species along its geographical distribution tracks its niche, then it is expected that traits associated to performance (as LMA) remained constant or without significant changes along its distribution.

Plant species with wide distribution offer an opportunity to test whether phenotypic traits change across distribution and the implications of those changes on biotic interactions such as herbivory. Assuming niche conservatism, we expect no phenotypic changes because species occur in microclimate conditions similar to those observed at the center of their distribution. In the present work, we study how the frequency and type of folivory in *Drimys winteri* changes between populations at the center and the north-edge of its distribution and how it relates to foliar attributes. *Drimys* is one of the six genera of Winteraceae family which is considered as one of the most primitive families of angiosperms (Feild *et al.* 2005), principally because of the absence of xylem vessels. Consequently, the hydraulic constrain is pointed as the main factor in shaping Winteracea geographic distribution that has confined it to highly wet environments (Ehrendorfer *et al.* 1979; Feild *et al.* 2005). In Chile *D. winteri* is distributed from the 30°S to 56°S, where climatic conditions change from semi-arid to sub-antartic. In the north edge *D. winteri* is found strongly associated to water courses, which agrees with Pironon *et al.* (2016) idea of species tracking their ecologic optimum at the borders of distribution. However, it could also be expected that the notable seasonality and the summer droughts at the north-edge of *D. winteri* distribution will affect foliar attributes, and in consequence will affect foliar susceptibility to herbivory damage. Specifically, we attempt to answer the following questions: Are foliar attributes of *Drimys winteri* significantly different between north-edge and center populations? Is herbivory frequency and damage type different between north-edge and center populations? and is damage frequency related to foliar attributes?

MATERIALS AND METHODS

Drimys winteri present a discontinuous distribution from the north semi-arid region in Río Limarí (30° 30' S) to Cabo de Hornos (56°S) (Donoso 1974; Hernández 1992; Donoso, Navarro and Hernández 2004). Along this latitudinal range the mean annual precipitations varies from 138 mm. to 2000 mm. and mean annual temperature varies from 13°C to 5°C. The presence of *Drimys winteri* in the semi-arid region is rare and highly associated to streams, increasing its presence towards higher latitudes, reaching its maximum in the North Patagonian Rainforest (Donoso 1993).

Study sites

We selected six sites, three from the north-edge of distribution: La Campana National Park, Rio Clarillo National Reserve and Cajón del Maipo; and three populations from the center of distribution: Alerce Costero National Monument, Alerce Andino National Park and Estación Biológica Senda Darwin (Fig 1.). In the north-edge, sites present a Mediterranean climate, characterized by summer drought and cold and rainy winters, and schelerophyllous vegetation; Central populations occur in Nord-Patagonian forest, with a temperate climate with high oceanic influence. Information of mean annual temperature (MAT) and mean annual precipitation (MAP) are summarized in Table 1.

Foliar traits

Field observations and material collection were performed between October and November of 2016. In each site, we selected 6-10 adult individuals at least 10 meters apart. We collected 10 leaves fully expanded with no sign of damage from the first cohort, i.e. the group of leaves of a branch produced in the last growth season. We measure leaf thickness using a caliper gauge; leaf area was estimated using Image J software using digital images of flattened leaves, excluding petioles. Then, leaves where dried to 70°C for at least 48 hours in order to measure dry mass and calculate leaf mass per area (LMA g/m2). Leaves intended for use in the determination of nutrient concentration were stored at air temperature after harvest, dried at 70°C for 72 h and then milled prior to analyses. Leaf N content was determined by the semi-micro Kjeldahl method (Kjeldahl 1883). All chemical analyses were conducted in the Biogeochemistry Laboratory at the Pontificia Universidad Católica de Chile.

Leaf damage.

From each individual we selected three branches, and register the type of damage for the last leaves cohort, considering chewers, miners, pit feeders, leaf folders and surface feeders. Frequency damage, was determine as the number of leaves with damage over the total number of leave measured (Garibaldi and Kitzberger 2011).

Statistical analyses

We performed a Principal Component Analysis (PCA) in order to summarize foliar traits variation into main components, using log transformed data. We tested the differences of centre-and edge sites on leaf damage frequency and chewers damage frequency using One-way ANOVAs and a posteriori Tukey's test differences between sites, normality was checked using Shapiro-Wilcoxon normality test. Then, we used Pearson's product-moment correlations to test the relationship between leaf traits and leaf damage frequency of all types of damage and chewers damage. All analyses were conducted in R v. 2.7.1 (R Development Core Team, 2016).

RESULTS

We analyzed leaf traits and leaf herbivory damage of 53 trees, 26 from the north-edge and 27 from the centre of *D.winteri* distribution. Leaf traits mean values are summarized in Table 2. The PCA explained 82% of variation in leaf traits among individuals in the first two components (Fig. 2). The traits contributing most to the first component (explaining 50% of the variation) were leaf thickness and N_{mass}, which were negatively correlated, (component loadings > 0.6). Edge and centre populations were clearly separated along PC1, centre populations had greater Nmass and lamina thickness. PC2 was principally affected by LMA and leaf area (component loadings > 0.7), edge and centre populations showed no trend along PC2. Ellipses surrounding center and north-edge populations represent a normal confidence probability of 95%.

We identified 5 herbivores feeding guilds, the relative contribution on leaf damage frequency of each one in each sites are shown in Fig. 3. Considering all sites together, chewers were the most common feeding guild, representing the 84% of damage frequency. In the north-edge populations, damage is almost exclusively from chewers (94%), just one site in the north-edge (La Campana) presented miners damage. Diversity damage increased in the centre group, however, chewers was also the principal herbivory guild (71%). Alerce

Costero was the most diverse and the only site where we registered leaf surface feeders, and it was also the site with the lower relative damage by chewers (53%). Leaf folders damage was found in Alerce Andino and EBSD and represented 3% of damage of the centre group.

ANOVA analyses showed an effect of site on leaf damage frequency ($F_{5,47}$= 2.4, p< 0.001) and on chewer damage frequency ($F_{5,47}$= 2.4, p< 0.001). However, Tukey's test showed no clear differences between centre and north-edge populations in either leaf damage frequency considering all types of damage or in chewers leaf damage (Figure 4).

We test the relationship between leaf traits and herbivory by relating the PC1 of our leaf traits PCA and the leaf damage frequency data. First, when we consider all guilds together, we found no significant trends (r= -0.23, p= 0.1). Then, we analyze damage frequency for chewers separately because was the only guild founded in all sites. Chewers damage frequency showed a significant and negative relation with PC1 (r= -0.43, p< 0.01) (Figure 5).

DISCUSSION

In this study we investigated from a trait based approach whether *Drimys winteri*, a wide distributed species, is adapted to suboptimal conditions at the north-edge of its geographical range, which could be expressed by differences in foliar phenotype between centre and edge populations, or if in contrast, foliar traits remain constant suggesting niche tracking. Foliar traits showed contrasting patterns between populations. If *D. winteri* were affected to suboptimal conditions imposed by the Mediterranean climate in which in edge populations are located, LMA and thickness will be higher, while leaf area smaller. We found no significant differences in LMA, which is generally related to water availability. Because of the absence of xylem vessels and the poor stomatal control (Field *et al.* 1998), the presence of *D. winteri* in the north edge of distribution is restricted to highly water saturated soils, close to streams and brooks (Donoso 1993). The microclimatic conditions in the north edge may explain the similar values of LMA between center and edge populations. On the other hand, leaf thickness was significantly higher in center populations. Following the idea of the particularities of *D. winteri* presence in the north edge, we propose that *D. winteri* receives lower irradiance levels in north edge because is located at the bottom of creeks where the light is intercepted by mountains and forest canopy. In contrast, in centre populations the occupancy of *D. winteri* is not constrained to water courses, on contrary is found as a principal component of forest canopy and as a pioneer species, where receives higher irradiance. This could also explain why we found thicker leaves at the centre of distribution. Irradiance is positively related to LMA, specifically through increasing leaf thickness, moreover, irradiance relates more clearly with leaf morphology traits than MAT and rainfall (Niinemts 2001, Shipley and Cortez 2003). Furthermore, we found a weak but significant positive relation between LMA and thickness. However, when analyzed center and edge separately, the strength of the relation was greater for centre than for edge individuals, which indicates that LMA in center populations is more due to increase in lamina thickness (data non shown). We expected higher Nmass concentration in centre-populations because in Chile soil N increases towards higher latitudes (Gaxiola *et al.* manuscript in preparation). Nevertheless, lower N_{mass} concentration was found in centre populations, which is expected for thicker leaves (Reich *et al.* 1991, Reich 1999, Lamont *et al.* 2002), both traits were negatively related and explained most of the variance between edge and center populations, as showed in our PCA analysis.

Physically, tough leaves with high C/N ratios are related to higher leaf life span and confer damage protection to herbivory and wilting (Díaz et al 2001, Perez-Harguindeguy *et al.* 2003, Hanley *et al* 2007). We observed differences in the guild damage between center and edge. The damage in the north –edge is dominated by chewers. While in the center damage diversity increases, especially in Alerce Costero where only half of the damage is due to chewers. In order to clarify which factors influence leaf damage type and frequency in an environmental gradient it is necessary to analyze herbivories richness and density, because abiotic factors may also influence its presence and density (Bale *et al.* 2002). Our study sites are located within mid-latitudes (33-43°S) for where peaks of diversity in plants, mammals and butterflies are registered (Samaniego and Marquet 2009, Villagrán and Hinojosa 1997). Despite that, we did not measured folivories assemblages; these richness and diversity tendencies give us clues that there is not major differences in folivories between center and the north edge of distribution of *D. winteri*, as it could be in the south edge of distribution (56°S.), where diversity decreases. A previous herbivory study performed between north edge (30°S) and south edge (41°S) populations in saplings of a Chilean tree (*Aetoxicum punctatum*) founded no differences in damage by invertebrate herbivories (del-Val and Armesto 2010). However, feeding guilds were not analyzed and samplings were strongly affected by damage of small mammals, which can be masking the invertebrates effects and thus impeding comparisons with our study. We did not found additional studies testing herbivory or herbivories feeding guilds along species distribution in Chilean vegetation. However, because leaves are thinner and with lower LMA compared with Chilean Mediterranean vegetation (Read *et al.* 2016), this makes *D. winteri* more palatable not only compared with center populations but also in the context of Mediterranean plant composition.

We analyzed chewers damage separately because it was the predominant feeding guild and the only one found in all sites. Chewers damage relates with negatively with leaf thickness and positively with N mass (Figure 4b). Caldwell et al (2015) analyzed which foliar traits provided a mechanical resistance to herbivory among different feeding guilds in shrubs and trees of an Eucalypt forest in Australia and found that leaf thickness is negatively related with chewers damage ($r= -0.47$), consistent with our results ($r=- 0.43$). However, the mentioned study also found that LMA is also related to chewers damage, contrasting with our findings. Although we could not analyze the relation between foliar traits and the other feeding guilds, there is evidence that chewers are more strongly related to mechanical resistance than miners and pit feeders (Caldwell *et al.* 2015, Peeters *et al.* 2007)

Although our study delivers constrained information focused on a single species, this study gives preliminary idea on how abiotic conditions affect plants phenotype with concomitant consequences on ecological interactions. In the current climate change scenario an increasing research line has been developed principally focused on how climate directly affects species distribution ranges (Var den Putten et al 2010, Chen et al 2011, Halbritter et al 2015) but less attention has been paid on the climate influence on species interactions (Wisz et al 2013, Louthan 2015). This study provide valuable information on species variation along its geographic distribution populations and its relation with herbivory.

CONCLUSIONES

Este estudio se enmarca dentro de la hipótesis centro-periferia, la cual postula que en los bordes de distribución de las especies las poblaciones están bajo condiciones ambientales que restringen su expansión geográfica, lo que se puede evidenciar en el desempeño de los individuos y en los rasgos asociados a la performance, los que consecuentemente pueden afectar las relaciones interespecíficas. En este trabajo estudiamos rasgos foliares relacionados tanto con factores ambientales como con la susceptibilidad a la herbivoría entre poblaciones del borde norte y del centro de distribución del canelo. Entre estos sitios las condiciones climáticas son distintas, siendo más frías y húmedas en el centro que en el borde. En los rasgos foliares estudiados (LMA; área, grosor y contenido de nitrógeno), encontramos que las hojas del borde norte son más delgadas, con mayor concentración de nitrógeno y presentan mayor frecuencia de daño por masticadores en comparación con las poblaciones del centro de distribución, lo que es consistente con la preferencia de atributos foliares por parte de herbívoros según la literatura. Por otro lado, en todos los sitios estudiados el daño foliar estuvo representado principalmente por masticadores, en el centro de distribución registramos 5 gremios de herbívoros, mientras que en las poblaciones del borde norte de distribución sólo dos, donde el daño por masticadores concentró alrededor del 95% del daño total observado. De esta manera, concluimos que las condiciones ambientales locales afectan a las poblaciones de *Drimys winteri* en el borde de distribución haciéndolas más susceptibles al daño foliar por masticadores. Este trabajo entrega información apoyando la idea de que las poblaciones periféricas difieren en sus atributos morfológicos afectando en las interacciones ecológicas, en este caso la herbivoría es más intensa en el borde norte que en el centro de distribución. Si bien este estudio es un experimento natural basado en la observación de atributos foliares y la herbivoría en individuos adultos, aproximaciones experimentales futuras podría esclarecer la influencia de la comunidad de herbívoros en el desempeño de las poblaciones en los extremos de la distribución y en la regeneración de plántulas, la que potencialmente podría estar limitando la presencia y abundancia de la especie.

TABLES

Table 1. Study sites description of Mean anual temeprature (MAT) and Mean anual precipitation (MAP).

Site	MAT (°C)	MAP (mm/yr)
La Campana	13.8	398
Río Clarillo	9.1	714
Cajón del Maipo	9.1	644
Alerce Costero	7.5	2127
Alerce Andino	8.4	2376
EBSD	10.5	2240

Table 2. Leaf traits measures summarized by site.

	LMA (g m^{-2})		Thickness (mm)		Nmass		Area (m^2)	
ite	mean	s.d	Mean	s.d	mean	s.d	mean	s.d
,a Campana	54.2	± 5.3	0.3	± 0.01	1.25	± 0.06	80	± 7.7
Rio Clarillo	66.6	±4.7.	0.32	± 0.01	1.29	± 0.04	57.4	± 22.9
Cajón del Maipo	70.8	± 5.7	0.28	± 0.01	1.41	± 0.07	55.8	± 4.7
Alerce Costero	82	±3.1	0.52	± 0.01	0.76	± 0.03	90.9	± 10.2
Alerce Andino	64.1	± 2.6	0.47	±0.01	1	± 0.07	150.6	± 10.1
EBSD	66.8	± 3.4	0.49	± 0.01	1.08	± 0.04	79.2	± 2.9

FIGURES

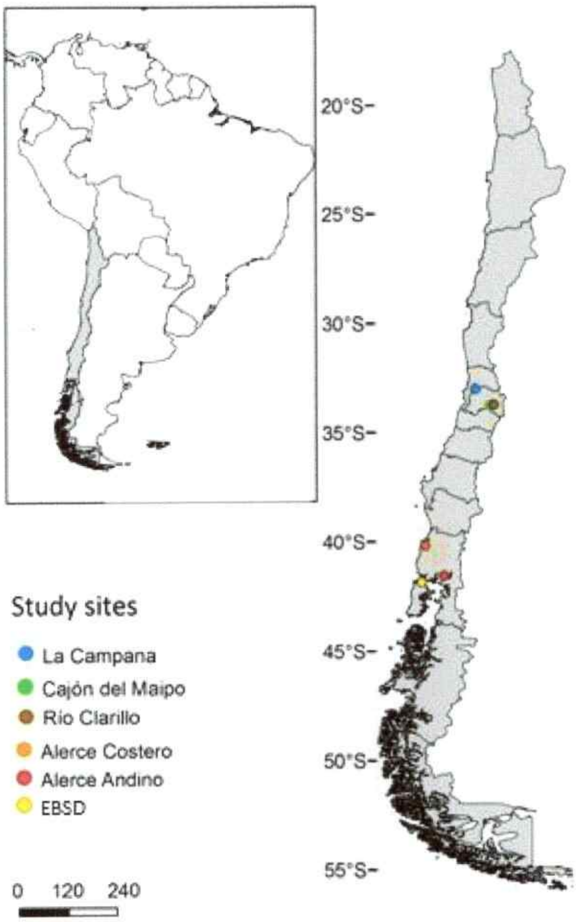

Figure 1. Study sites La Campana, Cajón del Maipo and Rio Clarillo represent north-edge populations; Alerece Costero, Alerce Andino and EBSD represent populations of the center of distribution of *Drimys winteri*.

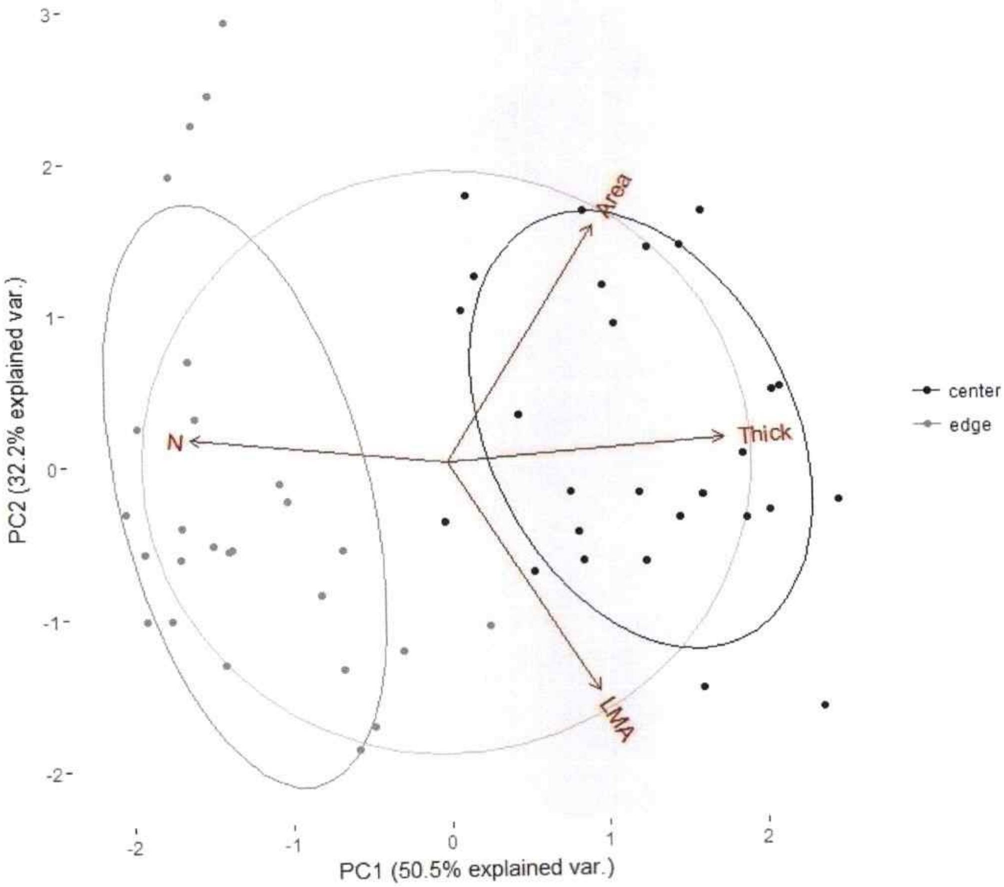

Figure 2. Principal component analysis of 4 foliar traits of 53 *Drimys winteri* trees. Loading plot for the first two components. Center in red indicates populations of *D. winteri* from center of its geographical distribution, Alerce Costero, Alerce Andino and EBSD. Edge in blue indicates populations from edge of distribution, La Campana, Río Clarillo and Cajón del Maipo. Elipse surrounding edge and center groups draws a normal data ellipse with a probability of 68%. Circle indicates a variables correlation with a 69% of probability.

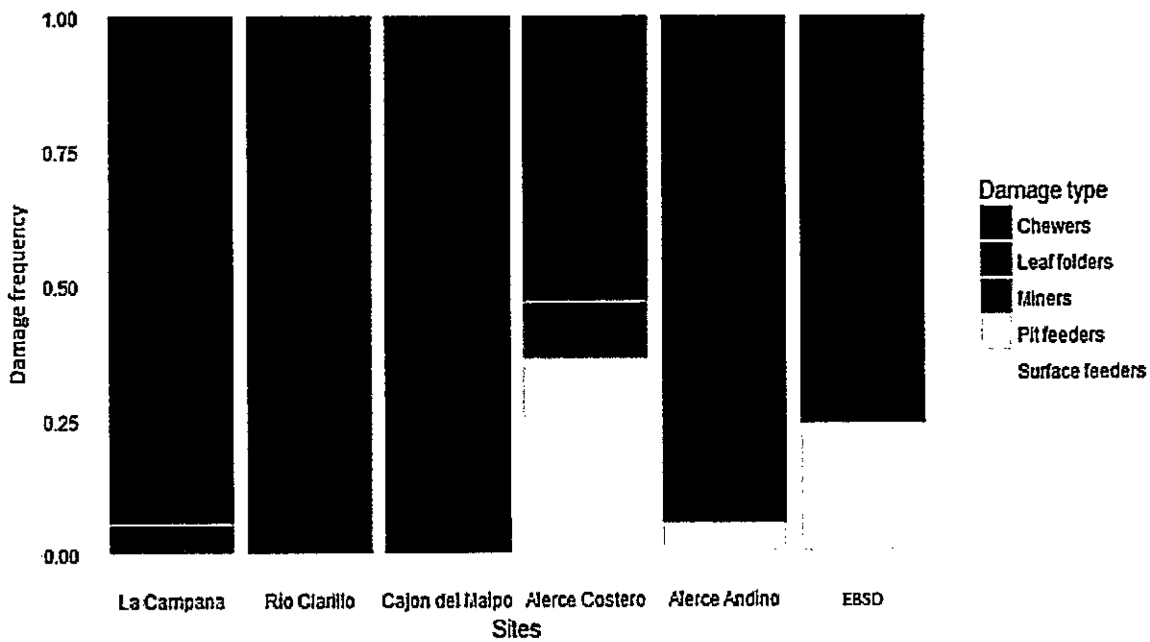

Figure 3. Decomposition of frequency of damage type between sites. La Camapana, Rio Clarillo and Cajon del Maipo belong to edge populations, while Alerece Costero, Alerce Andino and EBSD, to populations of the center of distrubtion.

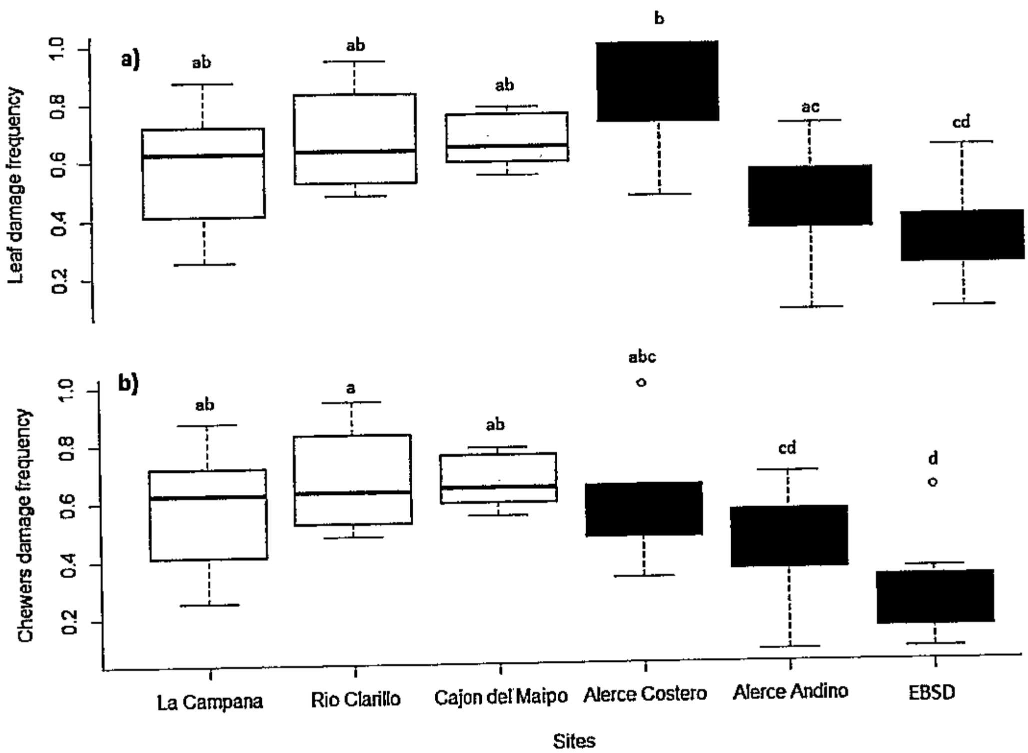

Figure 4. Leaf damage frequency considering all damage types described in figure 2 (a) and chewewrs frequency damage (b). Light grey boxes indicate north-edge populations, dark grey indicate centre populations. Letters above boxes describe differences between sites.

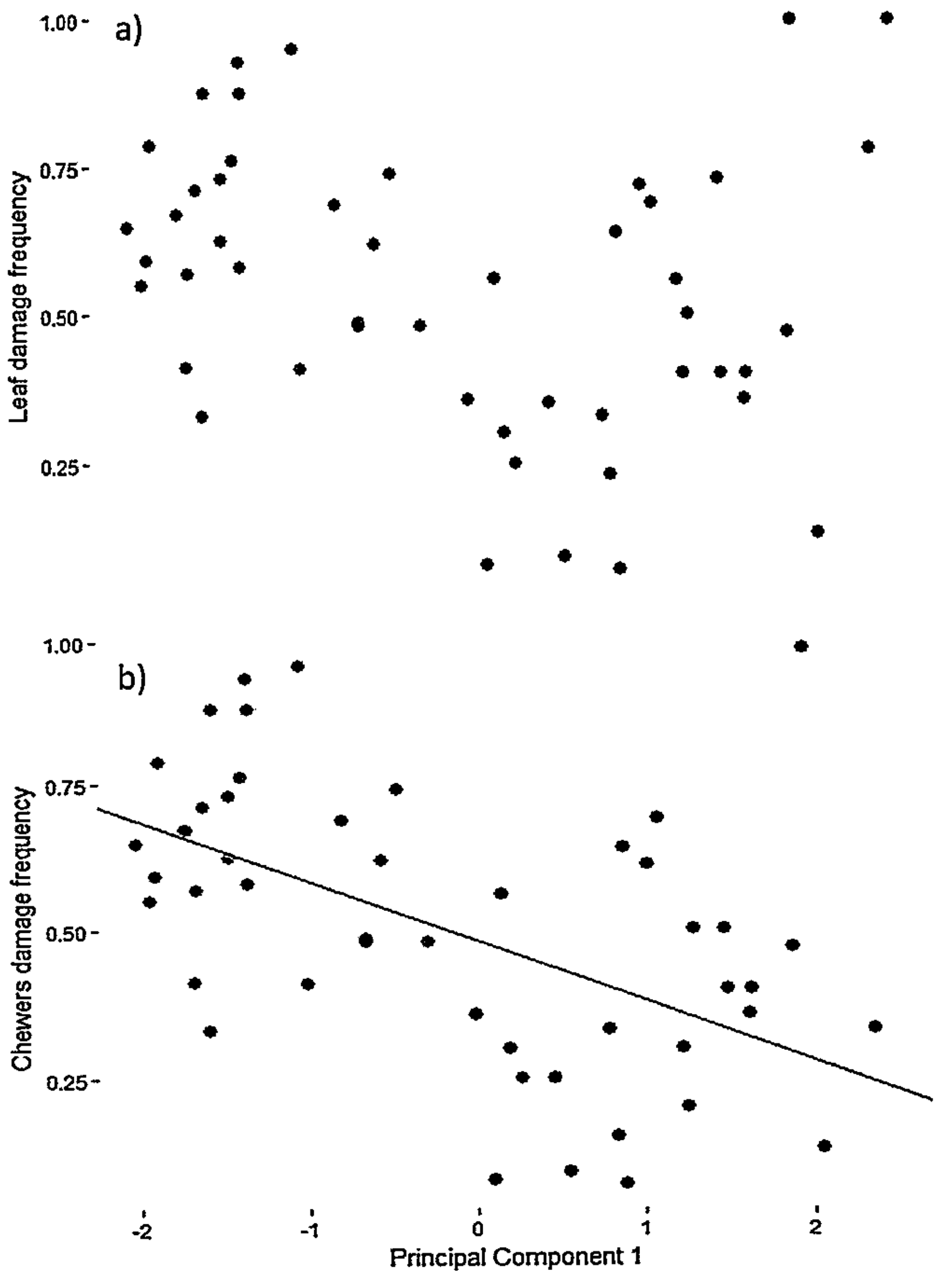

Fig. 5. Leaf damage frequency for all feeding guilds (a) and chewers exclusively (b) in relation with the fisrt component of the PCA of foliar traits. Light grey dots represent individuals of north-edge populations and black for those in the centre populations. Solid line indicates significant relationship.

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