PONTIFICIA UNIVERSIDAD CATÓLICA DE CHILE
FACULTAD DE CIENCIAS BIOLOGICAS
PROGRAMA DE DOCTORADO EN CIENCIAS BIOLÓGICAS
MENCION ECOLOGÍA

TESIS DOCTORAL

ESTRATEGIAS FUNCIONALES CONTRASTANTES EN EL GÉNERO *NOLANA* (SOLANACEAE) Y SU IMPORTANCIA EN LA RESPUESTA A LAS FLUCTUACIONES CLIMÁTICAS ACTUALES Y PASADAS

Tesis entregada a la Pontificia Universidad Católica de Chile en cumplimiento parcial de los requisitos para optar al Grado de Doctor en Ciencias Biológicas con mención en Ecología.

Por:
PAULINA GABRIELA OSSA ZAZZALI

Tutores:
Dra. Fernanda Pérez T.
Dr. Juan Armesto Z.

Septiembre, 2013

ESTRATEGIAS FUNCIONALES CONTRASTANTES EN EL GÉNERO *NOLANA* (SOLANACEAE) Y SU IMPORTANCIA EN LA RESPUESTA A LAS FLUCTUACIONES CLIMÁTICAS ACTUALES Y PASADAS

Tesis entregada a la Pontificia Universidad Católica de Chile en cumplimiento parcial de los requisitos para optar al Grado de Doctor en Ciencias Biológicas con mención en Ecología.

Por:

PAULINA GABRIELA OSSA ZAZZALI

Director de Tesis: Fernanda Pérez

Co-Director: Juan Armesto

Septiembre, 2013

A mi Sol y mis Estrellas.

AGRADECIMIENTOS

Esta Tesis fue financiada por CONICYT a través de la beca de estudios de doctorado nacional y beca de apoyo de realización de tesis doctoral nº 24091079.por proyecto FONDAP 1501-0001, CASEB, programa 3. Por IEB, proyectos ICM P05-002 y PFB-23 . por proyectos FONDECYT nº 1120215 y nº 1110929.

Quisiera agradecer a mis tutores, Fernanda Pérez y Juan Armesto por la confianza depositada, el apoyo constante, durante todo el proceso de desarrollo de esta Tesis. En especial a Fernanda por la amistad, conversaciones de tesis y de vida; y por darme el espacio para poder realizar esta tesis con toda la libertad posible.

A la comisión revisora de la Tesis, Francisco Bozinovic, Lohengrin Cavieres y Claudio Latorre, por sus comentarios que han ayudado a mejorar esta Tesis.

A M. O. Dillon por enseñarme sobre el sistema de Lomas y taxonomía de la flora del desierto costero y el género *Nolana*.

A Gioconda Peralta y al Laboratorio de Diversidad Molecular, por permitirme realizar gran parte de la tesis en su espacio físico y equipos, por el servicio de secuenciación molecular, pero por sobre todo por la paciencia, disposición de enseñar, ayuda constante y por hacer del laboratorio un grato espacio de trabajo.

A mis compañeros de laboratorio, en especial a Fabiola Orrego por su ayuda constante en el análisis de muestras, sin la cual esta Tesis no hubiese sido posible; a Patricio Spencer por su ayuda en el laboratorio; a Mauricio Bosshard en el análisis de datos; a Paz Montenegro en la confección de mapas; a Carmencha, Maureen, Ricardo, Isabel, Kester, Bea, por sus comentarios, discusiones a los resultados de la Tesis, apoyo, conversaciones de la vida, instantes esotéricos y más. A los que partieron en busca de nuevos horizontes; Thais, Rafa, Pato. Y a los que ya no están, Lore y Ale. A todos ustedes chiquillos del 5to piso y las que nos visitan a diario (Ale y Jessica), que le dan alma y vida propia a este laboratorio lleno de ideas, entusiasmo y amistad.

A Ariel Farias por su voluntad y ayuda en el análisis de resultados, a Andrés Parada en programas filogenéticos; a Sebastián Armesto y Nuria, por su ayuda en terreno; a mis compañeros Gabriela, Tamara, Leonor, Fernando, Fernanda por su apoyo y aportes durante el período de ramos y desarrollo del proyecto de Tesis.

Al Kongo por su ayuda constante en terrenos, análisis de datos y entregarme el apoyo espiritual y emocional para vivir este proceso. A mi familia y en especial a mis hermanas Valentina y Francisca. A mi hija Sol.

ÍNDICE DE MATERIAS

ÍNDICE DE FIGURAS

ÍNDICE DE TABLAS

LISTA DE ABREVIATURAS

SL:	Shrubby Lineage, linaje arbustivo
HL:	Herbaceous Lineage, linaje herbáceo
LGM:	Last Glacial Maximum, último máximo glacial
Ma:	Million years, millones de años atrás
ENSO:	El Niño-Southern oscillations
MANOVA:	Multivariate Analysis of Variance
PCA:	Principal component analysis
cpDNA:	Chloroplastidial DNA, ADN cloroplastidial
LMA:	Leaf mass per Area, Relación masa:área de la hoja
WUE:	Water use efficiency, eficiencia en el uso del agua
$\Delta^{13}C$:	Discriminación isotópica de carbono por la planta
SD:	stomatal density, densidad estomática
TD:	trichomes density, densidad de tricomas
SI:	stomatal index, índice estomático
PCI:	potential conductance index, índice de conductancia potencial

RESUMEN

La historia climática, la historia de vida y la ecología se conjugan para dar cuenta de la respuesta de las plantas a las variaciones del ambiente. La respuesta ecológica de las plantas apunta a optimizar el ajuste con el ambiente; así, en un gradiente ambiental, la interacción con el ambiente dará cuenta de un espectro de variación en rasgos funcionales correlacionados que explican distintas estrategias funcionales. Esta interacción con el ambiente a dejado una huella genética en las poblaciones de plantas y su filogeografía en respuesta a las fluctuaciones climáticas pasadas. En esta tesis se desarrolló una aproximación actual (ecológica) e histórica (filogegorafía) para entender el rol de los rasgos funcionales (ecofisiológicos y de historia de vida) en la respuesta de las plantas a las fluctuaciones del clima.

Se utilizó como modelo de estudio dos linajes del género *Nolana*, endémico del desierto de Atacama, co-distribuidos en la costa a lo largo del abrupto gradiente de precipitaciones desde el sur del Desierto de Atacama a la región mediterránea de Chile central. Los linajes estudiados fueron categorizados de acuerdo a sus rasgos funcionales de planta completa (historia de vida, forma de vida y forma de crecimiento) en: linaje herbáceo, comprendida por *N. rupicola, N. acuminata, N. reichei, N. paradoxa*; todas plantas herbáceas de ciclo de vida corto, hermanas en la filogenia y distribuidas secuencialmente en el gradiente ambiental. El segundo grupo, identificado como linaje arbustivo, comprendido por *N. incana y N. crassulifolia*, ambas arbustos siempreverdes de ciclo de vida largo, estrechamente emparentadas y distribuidas secuencialmente en el gradiente ambiental. Para indagar en las estrategias funcionales utilizadas y la capacidad de ajuste de estos grupos de plantas, se

estudió la variación de rasgos foliares (morfológicos y eco fisiológicos) a lo largo del gradiente de precipitaciones en el rango geográfico compartido. se estudiaron los patrones filogeográficos de ambos linajes a lo largo de todo el rango de distribución latitudinal a partir de la secuenciación de dos fragmentos de ADN cloroplastidiales.

Los resultados de esta Tesis muestran que estos dos linajes responden a estrategias contrastantes a lo largo de todo el gradiente, observándose una estrategia de conservación de agua por parte de las arbustivas y una estrategia de captura de recursos por parte de las herbáceas. Ambas estrategias fueron mantenidas a lo largo del gradiente, ajustando la variación en rasgos asociados a cada estrategia; mientras las arbustivas regulan principalmente el tamaño de los estomas y mantienen elevada relación masa por área, las herbáceas, regulan además la densidad estomática y mantienen elevado contenido de nitrógeno (asociado a mayor capacidad fotosintética en las hojas). En cuanto a la respuesta histórica, ambos grupos respondieron a las fluctuaciones climáticas durante el Cuaternario con signos de expansión de rango hacia el sur, pero con distintos patrones de estructuración filogeográfica y rango alcanzado.

En base a los resultados de esta tesis se puede concluir que estos dos grupos de *Nolana* corresponden a dos estrategias funcionales contrastantes lo que influye en su desempeño a lo largo de un gradiente ambiental y afectó su respuesta a las fluctuaciones climáticas pasadas. Se demuestra la importancia de los rasgos funcionales (historia de vida y morfo-ecofisiológicos) en la respuesta de las plantas a las fluctuaciones ambientales, haciendo hincapié en la influencia de la historia de vida (forma, ciclo de vida) en la capacidad de respuesta ecológica, ajuste al ambiente y en la respuesta histórica a las fluctuaciones climáticas pasadas.

ABSTRACT

One of the key questions in Ecology is to understand the factors that overlay the patterns of diversity and distribution in terrestrial plants. Climatic history, as well as life history and ecology account for the response of plants to climate fluctuations. Plant ecological response points to optimize the fit to the environment, the interaction with the environment determine spectra of correlated functional trait variation describing distinctive functional strategies. Likewise, plants had adjusted to past (Quaternary) climate fluctuations, imprinting a genetic mark in plant populations and phylogeography. In this Thesis, we study the current (ecological) and historic (phylogeographic) response to climatic fluctuations with emphasis in functional trait variations.

We used as a model of study of *Nolana*, endemic genus of the Atacama Desert; we select co-distributed plants along the abrupt precipitation gradient that develop in the coast, from southern Atacama Desert to Mediterranean central Chile. The studied species were categorized according to their whole plant functional traits (life history, life form, growth form) into: herbaceous lineage, comprising *N. rupicola, N. acuminata, N. reichei, N. paradoxa*; all herbaceous plants with short life-cycle, sisters in the phylogeny ad distributed sequentially in the environmental gradient. The second group was shrubby lineage, comprising *N. incana* and *N. crassulifolia;* both evergreen shrubs with long life-cycle, closely related and distributed sequentially in the gradient. To explore in the functional strategies used and fit capacity across

the gradient, we studied the variation in foliar traits (morphologic and ecophysiologic) along the precipitation gradient trough the shared geographical range between life form groups. To elucidate the response to past climatic fluctuations, we analysed the sequences of two chloroplastidial fragments to obtain the phylogeographic pattern across all the latitudinal distribution range.

The main results of this Thesis showed that this two group of plants behave as different plant strategies along all the gradient, shrubs displayed a water conservancy strategy, meanwhile herbs exhibited a resource capture strategy. Both strategies were maintained along the gradient, adjusting the variation in traits associated to each strategy; shrubs regulated stomatal size while keeping constant elevated leaf mas per area; herbs regulated stomatal density while keeping constant nitrogen content, commonly related with higher photosynthetic capacity. In terms of the historical response, both lineages responded to climatic fluctuations during the Quaternary, with southward range expansion, but with differences in the range reached and phylogeographic structuration across the pattern.

On the base of these results it's possible to conclude that these two lineages of *Nolana* belong to contrasting functional strategies which influences their performance along an environmental gradient, and impacted their response in the past to climatic fluctuations through the Quaternary. We demonstrate the importance of functional traits in the response of plants to environmental fluctuations, emphasizing the influence of life history (form, life cycle) in the ecological response, fit to the environment and the historical response to past climatic fluctuations.

INTRODUCCIÓN GENERAL

Uno de los objetivos claves de la biología (Darwin, 1859) ha sido entender los factores que explican los patrones de diversidad y distribución de la biota. Es evidente que la historia, en conjunto con los factores bióticos y abióticos, dan cuenta de la mayoría de los patrones observados; así, el conocimiento de las interacciones ecológicas, los rasgos de historia de vida y la historia climática son fundamentales para explicar los patrones biogeográficos (Cox & Moore, 2005; Alvarez *et al.,* 2009).

La respuesta ecológica de las plantas a las condiciones físicas de su hábitat puede ser predicha considerando los atributos morfológicos y fisiológicos que dan cuenta del ajuste dinámico entre las plantas y su entorno. El estudio de distintos grupos de plantas definidos en base a sus rasgos, en vez de taxa individuales, a lo largo de gradientes climáticos marcados ofrece una aproximación útil para entender la respuesta integrada de las plantas, como unidades ecológicas, a los cambios ambientales en el espacio y el tiempo (Reich *et al.,* 2003; Lavorel *et al*., 2007).

Durante el cuaternario los cambios climáticos y glaciaciones afectaron la distribución geográfica y diversidad de la biota (Hewitt, 2000; Jackson & Overpeck, 2000; Williams *et al*., 2004). Debido a la magnitud de los cambios en la distribución de las precipitaciones y descenso de temperaturas (5-7º C), los rangos geográficos de las plantas pudieron contraerse

y/o expandirse de acuerdo a sus caracteres ecofisiológicos, algunas poblaciones pudieron quedar aisladas en refugios o hábitats relictuales, o enfrentar la extinción local e incluso global (Taberlet *et al*., 1998, Dawson *et al*., 2011). Estas fluctuaciones en los patrones de distribución han dejado una huella genética en las poblaciones (Hewitt, 2000). Las expansiones post-glaciales por largas rutas de colonización y los sucesivos efectos fundadores se traducen en perdida de alelos y homocigocidad; la repetición de estos eventos va produciendo amplias áreas de homocigocidad con reducida variabilidad genómica. Cuando las poblaciones expanden dificultan el avance de poblaciones detrás de ellas, por lo que los genomas bloqueados tenderán a persistir en estas últimas (Hewitt, 2000).

El período de grandes cambios climáticos por el que ya atravesamos y que se espera se intensifique en las décadas y siglos venideros (IPCC, 2007), afectará los patrones actuales de diversidad con efectos dramáticos, gatillando grandes cambios desde los genes a ecosistemas en el corto plazo (Barnosky, 2008; Barnosky *et al*., 2011; Naeem *et al*., 2012). El estudio de la influencia de los rasgos ecofisiológicos de distintos grupos de plantas en las respuestas frente a tendencias climáticas pasadas y presentes representa una urgente necesidad para entender o modelar sus respuestas a futuras variantes del clima.

Es sabido que las zonas áridas y semiáridas son muy susceptibles a alteraciones en la disponibilidad de agua, por variaciones en la frecuencia y magnitud de los pulsos de lluvia, que pueden determinar en gran medida la composición florística de las comunidades de plantas (Armesto & Vidiella 1993; Armesto *et al*., 1993). Las tendencias climáticas pasadas y las proyectadas a futuro han afectado y afectarán tanto los patrones de distribución como la diversidad de la flora en los desiertos (Kelly & Goulden 2008; Ignace *et al*., 2007; Lenoir *et al*., 2008; Loarie *et al*., 2008). Sin embargo, en contraste con lo que ocurre con las zonas

templadas y tropicales, a la fecha es muy poco lo que sabemos sobre los impactos de las fluctuaciones climáticas del Pleistoceno sobre los patrones biogeográficos y diversificación de las plantas en ecosistemas áridos y semiáridos (Lessa *et al*, 2003). Los escasos antecedentes que existen se refieren a algunas floras de los desiertos de Norteamérica (Nason *et al*., 2002, Garrick *et al*., 2009; Rebernig *et al*., 2010), donde se han verificado expansiones post-glaciales de flora desértica. Sin embargo, para los extensos desiertos y zonas semiáridas de Sudamérica los antecedentes filogeográficos son extremadamente escasos y no permiten hacer predicciones sobre las distribuciones futuras. La transición árida-semiárida en la costa Pacífico de Sudamérica, es un área que dada la presencia imponente del macizo de los Andes en el este, puede servir como modelo para estudiar los cambios de distribución en dirección norte a sur, siguiendo las variaciones latitudinales en precipitación.

El estudio de especies de plantas relacionadas que habitan estos gradientes ambientales con un cambio gradual de los recursos hídricos con la latitud, proveen una oportunidad única de entender y predecir la respuesta de las plantas de las zonas áridas frente a cambios futuros de las condiciones climáticas en poblaciones naturales. El análisis de los factores que determinan los patrones filogeográficos a través de gradientes ambientales permitiría conocer las trayectorias evolutivas potenciales y reales de las poblaciones frente a la variación monotónica de los factores abióticos; y el conocimiento de sus caracteres, puede dar luces acerca de la selección sobre los rasgos que permiten el ajuste con el ambiente.

Clasificación funcional de las plantas: de formas de vida a espectros de rasgos funcionales

Se sabe bien que las plantas pueden ser clasificadas mas allá de su estatus taxonómico, sobre la base de sus afinidades morfológicas y ajustes con el clima (Du Rietz, 1931). Estas clasificaciones parten con la clasificación jerárquica fisionómica de Warming (1908, 1919) siguiendo con la clasificación de formas de vida de Raunkiaer (1935), que permiten visualizar la correspondencia entre clima y vegetación, según las adaptaciones para sobrevivir a la estación desfavorable. En tiempos modernos, las clasificaciones han integrado los rasgos morfológicos y ecofisiológicos en una clasificación funcional de las plantas de un ecosistema (Holdridge, 1947; Smith *et al*.,1996; Steffen *et al*., 1996; Woodward & Cranner, 1996; Lavorel *et al*., 1997). Rasgos funcionales, utilizados en la clasificación, son aquellos que tienen un impacto directo en el desempeño de la planta y reflejan adaptaciones a la variabilidad del ambiente local (McIntyre *et al*., 1999; Lavorel *et al*., 2007, Violle *et al*., 2007). Sobre la base de la variación de estos rasgos funcionales en gradientes ambientales, y correlaciones entre estos, es posible identificar un espectro de estrategias funcionales (Wright *et al*., 2001; Ackerly *et al*., 2002; Westoby *et al*., 2002; Ackerly, 2003, 2004; Wright *et al*., 2004; Reich *et al*., 2003, 2007).

Estas estrategias se asocian por lo general con diferencias en formas de crecimiento, ciclos de vida y rasgos funcionales, morfológicos y fisiológicos, que inciden fuertemente en el desempeño de las especies en su ambiente presente (Hooper *et al.,*2005). Así, plantas de ciclo de vida largo con rasgos que favorecen la economía de recursos (menor área foliar, menor tasa fotosintética máxima, mayor eficiencia en el uso del agua) constituyen una estrategia funcional de "conservación de recursos" que es ubica al otro extremo del espectro de variación

de rasgos que la de plantas de ciclo de vida corto que tienen menor eficiencia en el uso del agua, mayor área foliar, mayor tasa fotosintética y crecimiento rápido, cuya estarategia es de "captura de recursos".

Influencia del clima y estrategias funcionales en la filogeografía de las plantas

El patrón filogeográfico y la diversidad genética están fuertemente determinados por factores históricos y ecológicos (Hamrick & Godt 1989, 1996; Nybom & Bartish 2000; Nybom, 2004; Thiel-Egenter *et al*., 2009). Los rasgos de historia de vida, como el tiempo generacional, la capacidad de dispersión, y forma de reproducción; influyen en la tasa de divergencia genética y pueden determinar la respuesta de los taxa al cambio climático

(Willis & Niklas, 2004). Existe evidencia de que las plantas herbáceas de ciclo de vida corto, como las plantas anuales o efímeras, evolucionan más rápido que sus parientes leñosas más longevas (Smith & Donoghue, 2008), además de presentar mayores tasas de evolución fenotípica y cambio de nicho climático (Smith & Beaulieu, 2009). A modo de ejemplo, se ha demostrado que rasgos como el tiempo de floración pueden cambiar rápidamente en respuesta a las fluctuaciones climáticas (Franks *et al*., 2007). La comparación de los patrones filogeográficos de especies filogenéticamente cercanas, pero con estrategias funcionales contrastantes, en un mismo gradiente ambiental, es una poderosa herramienta para evaluar el papel del pasado climático común y los rasgos distintivos de cada grupo funcional en el patrón filogeográfico observado.

Modelo de estudio

En Chile, el Desierto de Atacama presenta una condición hiper-árida con precipitaciones escasas y variables (Houston & Hartley 2003). En la costa Pacífico del desierto existe un gradiente latitudinal de aridez marcada caracterizado por hiperaridez en el extremo norte, presencia de neblina bajo la capa de inversión térmica a los 800 m SNM (más común en invierno) y la influencia esporádica o nula de los frentes de precipitación sur-occidentales, los que comienzan a tomar mayor importancia hacia el sur de los 25º S (Lüebert & Pliscoff, 2004). El gradiente climático en el desierto costero es un experimento natural de aridización a través del Pleistoceno que permite indagar acerca del cambio espacial y temporal de rasgos funcionales relacionados al uso del agua.

Diversos estudios palinológicos (Maldonado & Villagrán, 2002, 2006; Maldonado *et al*., 2005), paleoecológicos (Latorre *et al*., 2005; Kaiser *et al*., 2008; Díaz *et al*., 2012) han indagado en la historia climática y biogeografía Cuaternaria del Desierto de Atacama, pero la respuesta filogeográfica de las plantas a las fluctuaciones Cuaternarias en el Desierto de Atacama aún permanece sin explorar (Viruel *et al*., 2012). Las formaciones de plantas del desierto costero de Chile y sur de Perú pueden ser dependientes de neblina, conocidas como formaciones de Lomas (Rundel *et al*.,1991), o encontrarse bajo la capa de neblina a muy pocos metros sobre el nivel del mar; y depender exclusivamente de la ocurrencia esporádica de lluvias frontales estacionales. Armesto & Vidiella (1993) y Armesto *et al*. (1993) muestran que la composición funcional de estas comunidades libres de neblina cambia de acuerdo a la estabilidad del régimen de precipitaciones, por ejemplo, hacia el norte son más comunes los arbustos, y la presencia y abundancia de plantas efímeras depende fuertemente de la frecuencia y magnitud de las lluvias.

Uno de los elementos florísticos más conspicuos del desierto costero es el género *Nolana* (Solanaceae) con una altísima diversidad de especies endémicas de esta región y especies clasificadas en varias estrategias funcionales. El género consta de 89 especies, 43 en Perú, 49 en Chile y una en islas Galápagos, corresponde a un clado monofilético y que tiene a Chile como su centro de origen (Dillon *et al*.,2007, Dillon *et al*.,2009). Se encuentra presente a todo lo largo del desierto costero, especialmente en formaciones de Lomas desde el Perú hasta Chile, y desde el nivel del mar hasta los faldeos andinos. La mayor diversidad de especies en Chile se encuentra en Antofagasta y Copiapó con 30 especies, hacia el sur la diversidad de *Nolana* disminuye progresivamente, en zonas con mayor precipitación, restringiéndose casi exclusivamente a hábitats costeros.

En la costa, entre los 24º S y 33º S coexisten en el gradiente especies del género *Nolana* que conforman dos linajes de especies con formas de vida contrastantes, herbáceas de ciclo de vida corto y arbustivas de ciclo de vida largo, que podrían representar dos estrategias funcionales igualmente exitosas para sobrevivir a las condiciones semiáridas. Las especies herbáceas *N. rupícola, N. reichei, N. acuminata* y *N. paradoxa*; poseen hojas de gran tamaño (>2 cm largo) suculentas de forma espatulada o lanceolada; grandes flores azuladas; crecen sobre sustratos de arena; son en su mayoría hierbas perennes (*N. paradoxa* se ha descrito como anual) cuya parte aérea es activa durante los períodos de primavera y muere en la época desfavorable (de verano a invierno). Las especies *N. incana, N. crassulifolia*, son arbustos siempreverdes, con hojas pequeñas (< 2cm largo), suculentas, de forma globosa y/o alargada, con flores pequeñas y blancas, que crecen sobre rocas o cerca de ellas, o en la arena muy cerca del mar.

Análisis filogenéticos basados en nDNA muestran que estas especies de *Nolana* se agrupan, junto con otras especies, según la estrategia de forma de vida, integrando dos clados muy bien sustentados pero con baja resolución entre las especies que lo conforman (Tu *et al*., 2008; Dillon *et al*., 2009). Esta baja resolución se corresponde con la similitud morfológica que existe entre las especies pertenecientes a un mismo grupo y con la dificultad visual para identificarlas, apoyando la distinción de estos clados como dos linajes o estrategias funcionales distintas. Se ha estimado la divergencia de este género de su ancestro mas cercano para los 4.02 Ma y la diversificación desde los 2.88 Ma Pleistoceno temprano (Dillon *et al*., 2009), diversificando en acorde con un clima árido en el desierto costero. Además, existe evidencia de que el límite de la transición latitudinal de clima árido-semiárido en Chile ha fluctuado numerosas veces durante el cuaternario (Lamy *et al*., 2000). Estos dos antecedentes nos permiten elegir a *Nolana*, y particularmente aquellas especies que habitan la transición árido-semiárido, como modelo para estudiar las adaptaciones a la aridez y su variación en el gradiente latitudinal, así como a través de su historia evolutiva, es decir entender como la variación del clima ha moldeado su expansión y adaptación.

Objetivos e Hipótesis

Hipótesis General

Los dos linajes de *Nolana* con rasgos de historia de vida contrastantes (arbustivas y herbáceas), constituyen estrategias funcionales opuestas de conservación y captura de recursos, y responden distinto a variaciones espaciales e históricas del régimen de precipitaciones.

Específicamente se espera que:

- los dos linajes de *Nolana* utilizen estrategias funcionales contrastantes a lo largo de un gradiente de precipitaciones. Las herbáceas, al perder la parte aérea en las estaciones desfavorables, presentarán rasgos asociados con una estrategia de captura de recursos y un menor ajuste (variación en los rasgos funcionales) a lo largo del gradiente; en contraste los arbustos son siempreverdes todo el año por lo que se espera que posean una estrategia de conservación y mayor ajuste a lo largo del gradiente de precipitaciones. (Capítulo I)
- Los arbustos fueron fuertemente afectados por las fluctuaciones climáticas durante el Cuaternario, presentando un claro patrón filogeográfico de contracción ante climas desfavorables y expansión post-glacial. Por el contrario, las herbáceas deben haber evadido los periodos desfavorables, por lo que su patrón filogeográfico presentará menos signos de expansión y contracción post-glacial. (Capítulo II, III).

Objetivo General

Evaluar la respuesta actual y pasada a las condiciones climáticas de aridez en dos formas de vida contrastantes del género *Nolana* comparando el patrón filogeográfico y la variación de rasgos funcionales relacionados con la captura y conservación de recursos a lo largo del gradiente de precipitaciones desde ambientes áridos a semiáridos en el margen sur del Desierto de Atacama.

Objetivos específicos:

1. Examinar la variación de rasgos funcionales foliares asociados a la adquisición de carbono y conservación de agua en dos formas de vida contrastantes a lo largo de un gradiente latitudinal (Capitulo I).
2. Reconstruir el patrón filogeográfico de plantas del género *Nolana* de forma de vida arbustiva-perenne (ciclo de vida largo) a lo largo de su rango geográfico (Capitulo II)
3. Reconstruir el patrón filogeográfico de plantas del género *Nolana* de forma de vida herbácea-anual (ciclo de vida corto) a lo largo de su rango geográfico en un gradiente (Capitulo III).

CAPITULO I

CHAPTER 1

Different life forms of *Nolana* (Solanaceae) exhibit contrasting functional strategies to drought in a pronounced aridity gradient at southern limit of Atacama Desert

Paulina G. Ossa[1,2], Fabiola Orrego[1], Jose M. Fariña[1], Juan J. Armesto[1,2], Fernanda Pérez[1,2]

[1]*Center for Advanced Studies in Ecology & Biodiversity (CASEB) and Departamento de Ecología, Pontificia Universidad Católica de Chile, Santiago, Chile,* [2]*Institute of Ecology & Biodiversity (IEB), Santiago, Chile*

1.1 ABSTRACT

In deserts, plants displays different strategies to resist extensive drought periods; the study of these strategies based on functional traits variation can provide insights on how they relates and respond to changes on their environment. The precipitation gradient in the latitudinal range of the Atacama Desert appear as the natural experiment to test if different plant forms perform as different strategies across an aridity gradient.

We studied a two lineages of the genus *Nolana* with contrasting life forms: short-lived herbaceous, and long-lived shrubs across a coastal latitudinal gradient (26º to 33º S). Leaf functional trait like: LMA; pubescence; stomatal size; stomatal density; nutrient (carbon and nitrogen) content and isotopes; were measured. We test between and within life forms differences with MANOVA, searched for relevant explanatory traits with PCA; compared correlations with precipitation of traits between lineages across the gradient.

We found significant differences between life form explained mostly by high pubescence and LMA in shrubs, and strong correlation with stomatal traits in herbs; $\Delta^{13}C$ didn't showed significant between lineages. Strategies across the gradient were differenced mostly by their ability to modify stomatal traits, with shrubs modifying their size and herb modifying their density.

In conclusion, both life forms perform as different strategies across the gradient, compensating the trade-off between carbon gain and water loss and maintaining equal average water use efficiency by employing strategies of water conservancy (shrubs) and resource capture (herbs).

1.2 INTRODUCTION

In arid and semiarid environments, three adaptive strategies to drought resistance have been classically identified (Orians & Solberg, 1977; Gibson, 1998; Lambers, 2008), this are: drought-escaping, based on the plant's ability to complete its life-cycle before the period of intense drought; drought-avoidance, based on the plant's ability to maintain its leaf water potential via adaptive traits that minimize water loss or improve water uptake; drought-tolerance, based on the plant's ability to maintain low water potentials trough osmotic regulation. Nevertheless, these strategies are not mutually exclusive and diverse morpho-functional traits can occur simultaneously in the same plant (Levitt, 1980; Ludlow, 1989; Chaves *et al.*, 2003), and most of the avoidance responses to drought are associated with the restriction of the growth cycle to the period when water supply is sufficiently abundant (Grene *et al*., 2011). Consequently, plant strategies in arid environments are closely associated with timing of phenophases and the presence of structural traits related to water storage and uptake, reduction of water loss, mechanical reinforcement (De Micco & Aronne, 2012).

In the recent years, most of the studies in plant ecology have focused in plant functional traits and their response to environmental variability (Ackerly *et al.*, 2002; Westoby *et al.*, 2002; Ackerly, 2004; Wright *et al.*, 2004; McGill *et al.*, 2006; Messier *et al.*, 2010). Plant functional traits can be defined as measurable characters of individuals that have an impact on fitness and reflect adaptations to the changing environments (McIntyre *et al*., 1999; Lavorel *et al.*, 2007, Violle *et al.*, 2007). The study of variation of functional traits across different ecological scales (Messier et al., 2010) and environmental gradients environments

(Reich *et al*. 1997 Ackerly, 2003; Reich *et al.*, 2004; Meng *et al.*, 2009) have revealed a spectrum of leaf functional traits correlations reflecting adaptive strategies and trade-offs in different environment (Wright *et al.*, 2001; Reich *et al.*, 2003; Wright *et al.*, 2004). At both ends of the spectrum the strategies are closely related to the ability of resource conservation and capture.

In desert, it's possible to find herbaceous plants with short life cycle and long-lived evergreen shrubs coexisting. The more relevant difference between them is the length of the period they are exposed to drought. Long-lived shrubs usually present various morphological and physiological adaptations that allow them to resist hot/dry periods and reduce transpiration losses, but that have a cost in resource capture (Reich et al., 2003). Short-lived herbs often complete their life cycles before the onset of the dry season; thus escaping aridity and reducing the costs associated with resource capture without incurring in energy-costly avoidance adaptations.

Across pronounced water supply gradients, plants can reach extreme arid conditions, where drought adaptations would be essential; and semiarid or humid conditions, where drought adaptations must be costly. Functional traits related to water conservancy and resource capture trade-off can vary across the gradient (Reich et al. 2003). The level and patterns of variation, however, might differ between life forms. Short-lived herbs could loss drought (and costly) leaf traits in humid condition to maximize resource uptake. Herbs exhibit higher rates of molecular evolution than their woody relatives (Smith & Donoghue, 2008), correlating with higher rates of phenotypic and climatic niche evolution (Smith and Beaulieu, 2009). This trend in evolution could influence the performance of annual herbs in an environmental gradient

favouring a better fit between the traits and the local climatic conditions. In contrast, perennial long-lived shrubs should maintain drought adaptations across gradient, as evolution is slower.

Several leaf traits are associated to the balance between water conservancy and resource capture. Stomatal density and stomatal size (Salisbury, 1927, Gibson, 1996; Larcher, 2006) are functional traits that favour carbon gain, but may also cause water loss. The distribution of stomas between upper and lower leaf surfaces is susceptible to change in response to aridity conditions. Stomata in both leaf surfaces allow for photosynthesis to occur at higher rates when water is available hence maximizing carbon gains. The stomatal density in the adaxial (upper) leaf surface is more sensitive to environmental cues and can be reduced under aridity (Mott & O'Leary, 1984; Tari, 2003; Driscoll, 2006). Pubescence (Ehleringer & Mooney, 1978) can reduce leaf temperature and water loss, but at the same time restrict the rate of photosynthesis through light reflection. Higher leaf mass per area (LMA) can provide structural reinforcement to increase resistance to wilting (Niinemets, 2001) and leaf lifespan (Reich *et al.,* 1997; Wright *et al.,* 2002), but at the same time cut down carbon gain (Wright *et al.,* 2001, 2004). In arid environments, water stress can make LMA even higher for a given leaf lifespan, changing substantially between plant functional types and biomes (Poorter *et al.,* 2009).

Intrinsic water-use efficiency (WUE) is defined as the unit of carbon gain via photosynthesis per unit of water loss from leaves via transpiration (Pérez-Harguindeguy *et al*., 2013); and can be estimated from carbon isotope discrimination (Farquhar *et al.*, 1984; Ehleringer & Cooper, 1988; Rundel *et al.*, 1988) as it allows time-integrated estimates of the proportion of CO2 in the substomatal cavity and the ambient (c_i:c_a). Foliar nutrient content (Schulze *et al.*, 1994) is also a functional trait related to photosynthetic capacity and water

availability. Leaf nitrogen content is generally proportional to the RUBP carboxylase concentration in the plant leaf. Nitrogen use efficiency can be measured as the carbon gain per unit of nitrogen (Livingston *et al*., 1999; Tsialtas & Veresoglou, 2007). Its expectable that long-lived shrub with drought-adaptations, seen restricted their adjustment capacity and present less trait variation across the gradient. A short lived herb will be able to respond to changing environmental conditions regulating traits that tend to maximize resource gain at the expense of water conservancy

The transition from the Atacama Desert to the Mediterranean-climate zone along the western coast of southern South America is characterized by a strongly marked latitudinal gradient in rainfall. Several plant life forms are present along this transition zone structuring a variety of coastal plant communities. *Nolana* (Solanaceae) is one of the most taxonomically and functionally diverse genera occurring in these transitional communities. Two lineages of different life form (herbs and shrubs) of *Nolana* expand through the coastal humidity gradient from coastal arid to semi-arid (26ºS to 33º S). These two co-distributed lineages of contrasting life forms serve as model for the study of functional trait variation in an extended climatic gradient.

In this work, we propose to assess the differences in leaf functional trait variation related to CO_2 uptake and water loss in two lineages of *Nolana* with contrasting life forms along a latitudinal rainfall gradient from hyperarid to Mediterranean climate. We tested two hypotheses. First, we expect that these two life form differ in the mean values of leaf functional traits, with shrubby linage showing functional traits that favour water conservancy and herbaceous lineage showing traits that favour resource capture. Second we expect that these lineages differ in the level and pattern of functional trait variation across

the latitudinal gradient of aridity. Specifically, we expect that herbaceous lineage loss leaf traits related with water conservation southward, as these traits are costly and reduce CO2 uptake. In contrast, shrubby lineage should maintain drought adaptations across the entire gradient, as they are exposed to drought period across all the year.

1.3 MATERIALS AND METHODS

Sampling and Study sites

We studied two lineages of congeneric species of *Nolana* classified into contrasting life forms (LFs), long-lived shrubs and short-lived herbs (Table 1). The widespread taxa in the herbaceous lineage is *Nolana paradoxa* Lindl., which grow in sandy to rocky beaches between 29 to 42°S. According to the nuclear marker GBSSI (Dillon *et al*., 2007b) and chloroplast marker (Ossa *et al*., 2013), this species is closely related to *N*. *acuminata* (Miers) Miers ex Dunal (25.33- 32°S), and *N*. *reichei* M.O. Dillon & Arancio (30.44°S). This relation is also supported by morphological similarities (Dillon et al. 2007a). The widespread taxa in the shrub lineage is *Nolana crassulifolia* Poepp., which grow in rocky seashore between 27.5-33.3° S, and is closely related with *N*. *incana* Phil. (25-26° S) (Dillon et al. 2007b, 2009; Tu et al. 2008; Ossa et al., 2013) that also inhabits rocky seashore. Species of these two lineages were sampled across the entire distributional range along the western coast of Chile, and treated each one as a taxonomic unit. The sampled geographic range encompasses two regions with different bioclimate: Arid (A) region (27-31ºS), characterized by less than 200 mm/yr of rainfall falling only in winter; semiarid (SA) region (31-34ºS), were winter-only precipitation can as high as 400 mm/yr.

Five populations of the herbaceous lineages and seven populations of the shrubby *Nolana* lineage were sampled between latitudes 26.4º S and 33.18 º S during the spring, growing period, October to December 2009. The herbaceous species sampled were *N. rupicola* (one population), *N. accuminata* (one population), *N. reichei* (one population) and *N. paradoxa* (two populations). The seven shrub populations belonged to two distinct species, *N. incana* (one population) and *N. crassulifolia* (six populations).

Leaf mass per area (LMA), nutrient content, and stable isotope.

Samples from 10 plants from each population were collected and subsamples were conserved fresh, dry with silica gel, and fixed in formalin aceto-alcohol solution (FAA) for further analyses. To calculate leaf mas per area (LMA), 10 fresh leaves per plant were collected, scanned and their surface calculated using ImageJ (Schneider *et al*., 2012); fresh leaves were weighed, dried at 60 ºC for 72 hours and weighed again to obtain dry mass. For isotopic and nutrient analysis (C & N), dry leaves from each plant were ground, mixed, and sent to Cornell Stable Isotope Laboratory for analysis, obtaining one average measure per plant. Carbon isotopic discrimination (Δ) was calculated with estimated ∂_a and ∂_p (air and plant) using Farquhar *et al*. (1982) equation as follows: $\Delta^{13}C = (\partial^{13}C_{air} - \partial^{13}C_{plant})/(1+ \partial^{13}C_{plant})$. We obtained mean values of ∂_a=8.19‰ for the period 2008-2010 from three stations located at different latitudes near the ocean (25º S, 27º S and 30º S) from the NOAA ESRL Carbon Cycle Cooperative Global Air Sampling Network database (White & Vaughn, 2011).

Stomata and trichomes on the leaf surface

To calculate stomatal density (SD), stomatal index (SI) (ratio of stomata to epidermal cell, see below), trichome density (TD), stomatal sizes (SS); two fixed leaf samples from five individuals from each population were dissociated in a 50% commercial chloride solution. Upper and lower leaf surfaces were mounted and stained with methylene blue for observation under an optical microscope OLYMPUS CX31 at 10×. For each preparation, one field of view (1.5 mm^2) in the central portion between the midrib and leaf edge of the leaves selected, and analysed with ImageJ to obtain the number of stomata, trichomes and epidermal cells and the length of ten stomata. Mean values for each trait by individual were used in statistical analyses

Stomatal index (SI; *ec.1*) was calculated as it normalizes for the effect of potential epidermal cell expansion, that is function of many environmental variables (light, temperature, water status, etc.), and could mask real amounts on stomatal frequency (Salisbury, 1927; Royer, 2001). Given that stomatal conductance depends on both stomatal density and pore size (area), we calculated the potential conductance index (PCI; *ec.2*) (Holland & Richardson, 2009).

$$\textbf{\textit{ec. 1}} \quad SI\ (\%) = \frac{stomatal\ density}{stomatal\ density + epidermal\ density} \times 100$$

$$\textbf{\textit{ec. 2}} \quad PCI = (guard\ cell\ length)^2 \times stomatal\ density \times 10^{-4}$$

Statistical Analyses

As a preliminary test, we evaluated differences in leaf traits (SS, SD, TD, SI, PCI) between leaf ‘surfaces and LFs using factorial two-way Analysis of Variance (ANOVA). When statistical differences among leaf surface traits and/or life forms were detected, these were

considered in the assessment of functional strategies (hypothesis 3). As we intended to look for general trends, variables were averaged for upper and lower leaf sides to evaluate hypotheses 1 and 2. Multivariate Analysis of Variance (MANOVA; Johnson, 1998) was run to test for differences between LFs (shrubs vs. herbs) and among populations within LFs independently. All variables were log transformed except for ∂^{15}N, % carbon, SS, LMA. One variable was square root transformed (TD+1). If differences proved significant, univariate ANOVAs were performed for each trait; Welch Anova test was used when the assumption of homogeneity of variances was not met. For the analysis of species within LFs, ANOVA and Tukey's post-hoc tests were performed for those traits that presented significant differences.

Principal component analysis (PCA) was conducted on those traits that showed significant differences between life forms to visualize the principal traits explaining life form differentiation and population arrangements on trait-related biplots. The first two principal component analyses were retained and rotated using VARIMAX to obtain factors that maximize variances of the squared loadings. PCAs within each life form were used to evaluate the relative contribution of traits to population differentiation. For all PCAs, only principal components with eigenvalues >1 were retained.

As precipitation changes with latitude on a log-normal scale ($r = 0.996$), we used the logarithm of rainfall as a surrogate of latitude to evaluate the strategies used by the two LFs. Pearson correlations between traits and latitude for species each LF were statistically compared with Fisher's z test (Fisher, 1929). All statistical analyses were done with the statistical packages JMP software (JMP10, SAS Institute, Cary, NC, USA) and Xlstat (Addinsoft, 2013).

1.4 RESULTS

Differences between lineages with contrasting life forms

Multivariate analysis (MANOVA) of the leaf trait measured between the two LFs (herbaceous vs. shrubby) was significantly different (Exact $F_{11, 102} = 74.4$; $P < 0.0001$). Partial ANOVAs were significant for 8 of the 11 traits tested (Supporting Information, table S2). The three traits that did not show significant differences between LFs, were $\partial 1^5N$ (Exact $F_{1,112} = 2.51$; P= 0.116), carbon content (Exact $F_{1,112} = 0.812$; P = 0.369) and $\Delta^{13}C$ (Exact $F_{1,112} = 2.35$; P = 0.127). The principal component analysis to display character differences between life form (PC_{LF}) considered all the significant leaf traits (SS, SD, TD, SI, PCI, LMA, C/N, %N). The two first PCA components explained the 80.6% of the variability in the set of traits examined and were the only components with eigenvalues > 1. The first component ($PC_{LF}1$) was correlated with high values for SI, SS, SD, and PCI, and with low values for LMA and TD (Table 1.3). $PC_{LF}2$ was correlated positively with leaf C/N ratio and negatively with %N.

$PC_{LF}1$ separated almost completely all the points in the PCA corresponding to each life form (Fig. 1.1a), which were split between the positive and negative portions of the first axis. Shrub populations were inversely correlated with $PC_{LF}1$, in accordance with their high LMA values and trichomes density. Herb populations in turn correlated positively the same axis, because of their high values for all stomatal traits (SI, SD, SS, PCI) and low values for LMA and TD. Population arrangement along both PCA axes was weak (Fig. 1b), but a slight north-to-south tendency was observed, which was explained by a reduction in TD for shrubs and an increase in stomatal traits for herb species.

The effect of leaf surface (adaxial vs abaxial) over the measured traits was significant for SD ($F_{1,1}$= 11.7, P< 0.05), SI ($F_{1,1}$= 6.67, P< 0.05), and for PCI ($F_{1,1}$= 4.52, P< 0.05), but a-posteriori tests only revealed significant differences for SI (abaxial > adaxial) within the herbaceous LF (Table S1.3).

Inter-population variation within LFs along aridity gradient

MANOVA was significant for shrubs (Pillai's Trace = 3.33; P < 0.0001), indicating significant differences among the studied populations for 9 of the 11 traits analysed (Table 1.2, Table S1.2). The leaf characters LMA (Exact $F_{6,60}$= 1.92; P= 0.09) and SI ($F_{6,60}$= 1.55; P= 0.18) did not show significant differences among shrub populations. Within those traits that showed differences among populations, only SS, TD, PCI and ∂^{15}N exhibited a weak latitudinal trend; with pronounced differences between the north and south extremes of the gradient. Other leaf characters, i.e., %N, C/N, %C, SD and Δ^{13}C, did not show a latitudinal trend among shrub populations.

For principal component analysis, we first removed the leaf traits that were less correlated with the first two components; the final PCA for shrubs considered six of these traits (SS, TD, PCI, ∂^{15}N, C/N, $\Delta 1^{3}$C). The two first components (PC_S1 and PC_S2) explained together 72.2% of the variability of the data. The PC_S1 was strongly and positively correlated with high values of leaf SS (r= 0.885), PCI (r= 0.831), and inversely with TD (r= -0.787) and ∂^{15}N (r= -0.773). The PC_S2 was positively correlated with leaf C/N ratio (0.894) and Δ^{13}C (r= 0.472). The first PCA axis displayed a north to south orientation of the shrub populations (Fig. 1.2). The northernmost population was placed in the negative side of PC_S axis1, thus correlating with high trichome density and high nitrogen isotope ratio, but with low stomatal

size and potential conductance index. The southernmost shrub populations (S6 & S7) were placed in the positive end of PC_S1, correlating with high values of SS and PCI. Central populations in the latitudinal gradient were positioned in the intersection of the axes.

In the case of herbs, a significant MANOVA was found for leaf traits (Pillai´s Trace= 2.76, $P< 0.0001$), with significant differences for 10 of the 11 measured traits. Only leaf nitrogen content was not significant (Exact $F_{4,41}= 1.77$; $P= 0.15$). Within those traits that showed significant differences among herbaceous populations, only TD, SI, PCI and $\Delta^{13}C$ exhibited a weak latitudinal trend; with marked differences between populations at the extremes of the latitudinal gradient. Other leaf characters, such as $\partial^{15}N$, C/N, %C, SD, SS and LMA, showed no clear trend across populations.

The principal component analysis (Fig 1.2.b) was computed initially with all the significant leaf traits, followed by removal of the less correlated ones. The final PCA_H was run with 5 leaf traits (SD, SI, PCI, $\Delta^{13}C$, TD). The two first components (PC_H1 and PC_H2) taken together explained the 81.9% of the variability. PC_H1 explained 62.2% with high correlations for SI (0.911), PCI (0.857), SD (0.811); and negative correlation for TD (-0.741). The PC_H2 explained the 19.7% of the variability with $\Delta^{13}C$ (-0.621) negatively correlated. In the case of herbs, there was no clear separation of populations along the PCA axes, but a trend from north to south was observed over the PC_H1. The northernmost populations were inversely correlated with PC_H1 along with high TD; while and southern populations were positively correlated with PC_H1, due to high SD, SI and PCI.

Pearson correlations of leaf traits with precipitation differed significantly for four of the traits compared between the two LFs; these traits were SS, SI and PCI and $\partial^{15}N$ (Fig. 1.3). Leaf traits SS, PCI and $\partial^{15}N$ correlated significantly with rainfall variation for both LFs, with

significantly higher correlations for shrubs than for herbs; SI was significantly and positively correlated with rainfall for herb species but not for shrubs. All these foliar traits (except for $\partial^{15}N$) are stomatal traits that influence water conductance. The rotated factor (Factor 1) obtained from PCA_{LF} was also significantly correlated for shrubs and statistically different from herbs. This factor was explained mostly by correlations with SS, TD and PCI (Fig. 1.4).

1.5 DISCUSSION

Differences between Life forms

Understanding the different response of life forms across a wide climatic gradient is an important step towards understanding plant diversity and distribution in a changing world. The study of a diverse plant genus with ample distribution across a pronounced aridity gradient can provide insights about the environmental constrains on the plasticity of traits related to water deficit. In this study, the two major LFs in the diverse desert genus *Nolana* were investigated to assess how contrasting life-cycle duration influences variation in morphological along the transition from arid to Mediterranean climate. These LFs showed remarkable differences in their mean values for key foliar traits, especially those that enhance or limit photosynthetic rates under restricted water supply. However, this pattern of variation was not observed for other ecophysiological (not morphological) traits examined, such as leaf carbon content, nitrogen isotopic ratio and carbon isotope discrimination. Only foliar C/N ratio presented significantly lower values and nitrogen content significantly higher values for herbs than for the shrubby species of *Nolana*.

Overall, the two main life forms characteristic of the genus *Nolana* are positioned in opposite ends of the foliar trait spectrum. Low LMA, high nitrogen content and high conductance capacity characterize short life-cycle herbaceous plants. At the opposite end, high LMA, low nitrogen content and low conductance capacity are the prevailing traits in long-lived shrubs. These findings are in agreement with the global trends of leaf trait variation (Reich et al., 2003; Wright *et al.*, 2004; Reich & Oleksyn, 2004), which are related primarily to contrasts in resource use and growth-phase duration, defining two main strategies, fast growth-high resource capture vs. slow growth-nutrient conservation. Herbs with short life-cycles are photosyntetically active only during short periods of the year, and because of their limited growing season, we expected that foliar traits tend to maximize carbon gain. In contrast, long-lived, perennial plants have to deal with periods of strong water stress during the year, and hence their traits tend to enhance water saving capacity at the cost of reducing photosynthesis. Similar trade-offs connected to WUE have been discussed for deciduous vs. evergreen woody plants (Eamus & Prichard, 1998; Givnish, 2002; Zhang *et al.*, 2012). Indeed we did not find significant differences between the two main LFs for intrinsic WUE ($\Delta\ ^{13}C$). For herb species as a group, PCA_{LF} revealed high correlation between stomatal traits (SD, SS, SI, PCI) affecting gas conductance and carbon fixation rates, whereas shrub species of *Nolana* showed stronger correlations among traits regulating water loss, such as LMA and pubescence. These findings support the prediction that the life-cycle duration is the major constraint on drought adaptations and leaf functional traits variation among life forms.

Differences across the latitudinal gradient

Trait variation in *Nolana* species across the aridity gradient studied was also significantly associated with plant LF, with some traits suggesting strong fixation of characters, while other traits reflecting plastic responses to aridity and still others responding to undetermined site-specific conditions. In both life forms, ecophysiological traits that varied in parallel with the latitudinal gradient in rainfall were those related to carbon fixation and water conservation. Among these traits, the notable exception was $\partial^{15}N$, which was strongly influenced by animal waste enrichment (by marine animals) of nitrogen source. This phenomenon has been reported as something of frequent occurrence in plants from coastal environments (Szpak *et al*., 2012). The marine isotopic signal was diluted southwards (toward areas of higher rainfall) in the case of shrubs, presumably due to the deeper root systems and availability of different nitrogen sources. In herbaceous *Nolana*, the dilution effect was not observed probably because of the shallower root system and their predominant use of more superficial and marine-influenced nitrogen sources.

The traits that did not show differences among *Nolana* populations along the latitudinal gradient were LMA, SI for shrubs and leaf nitrogen content for herbs, suggesting that these characters are strongly fixated to each life form. For LMA, trait invariance can be explained because the perennial habit imposes constrains on the potential variation across the aridity gradient. Shrubs that have to confront seasonality all-year round need to maintain their leaf mass per area investment. The stomatal index invariance suggests in turn that variation in stomatal density is regulated by epidermal cell expansion and does not imply higher investment in stomata initiation. The lack of variation in leaf nitrogen content in herbaceous *Nolana* across the latitudinal gradient may respond to the tendency observed in fast growing

plants with short life cycles to maintain high photosynthetic rates during short favourable periods and the need to allocate nitrogen rich compounds to photosynthetic tissues and thus enhance enzyme concentration (Reich *et al.,* 1997).

Plant strategies and trade-offs

Stomatal traits of *Nolana* species were the most relevant to differentiate among life forms and showed clear patterns of variation across the latitudinal aridity gradient, which strongly correlated with precipitation fitting a lognormal function.

When *Nolana* species of contrasting life forms are exposed to a pronounced soil moisture gradient, i.e., a latitudinal trend of declining aridity from arid to Mediterranean climate, they compensate for differences in water supply in different ways. Shrubby species of Nolana clearly display a water-conservative strategy regardless of their position in the aridity gradient. stomatal size, rotated PCA factor 1 (explained by variation in SS, PCI and TD) PCI correlated with precipitation; this together with the no significant trend in SI indicate a tendency across the gradient to regulate potential conductance by indirectly regulating stomatal size through constraints on epidermal cell expansion (i.e. less number of stomas and smaller pore size in arid sites and the opposite in wetter sites).

Herbaceous *Nolana* displayed a strategy driven by the enhancement of carbon capture, modifying those traits that maximize carbon fixation rather than those that improve water conservation. PCI and SI varied in a lognormal way with precipitation across the latitudinal gradient; further, $\Delta^{13}C$ was significantly correlated with latitude, indicating less water use efficiency at higher latitudes. The close adjustment of herbs trait variation to the rainfall gradient appear to be mostly due to significant changes in stomatal density and SI, reflecting

greater allocation of stomata to the abaxial leaf surface in southern (wetter) localities. This result documents a more plastic response of stomatal density than stomatal size (either guard cell length or stomata aperture length) (Ashton & Berlyn, 1994; Richardson *et al*., 2000; Richardson, *et al*., 2001). Accordingy, herbaceous *Nolana* appear to show more plasticity than shrubs in traits responding to changes in moisture along the latitudinal gradient.

This results strongly suggest the performance of two contrasting different strategies by this two types of life forms, and that this strategies are constrained by the life form, rather than the environment, across the entire latitudinal gradient; rejecting an scenario of strategy's shift across the gradient. As perspective of this work, remains to know whether this variations are given by a plastic response to the environment or are fixed to the populations.

Table 1.1 Geographic locations of life forms populations and man annual climatic conditions. Life forms and species within life forms, populations code, latitude as decimal degrees South, longitude as decimal degrees West, Annual precipitation (APP), minimal temperature of the coldest month (min T), maximal temperature of the warmest month (max T), potential evapotranspiration (PE; as in Thornthwaite, 1948). Values were taken from the published mean (at least 8 years of observations) records of the nearest weather stations to localities (Luebert & Pliscoff, 2006).

Life Form / species	Populations	Lat (ºS)	Lon (ºW)	APP (mm)	min T(ºC)	max T(ºC)	PE
Shrub							
N. incana	S1	26.41	70.7	25	13.4	21.8	804
N. crassulifolia	S2	27.25	70.96	29	12.9	19.9	745
N. crassulifolia	S3	28.13	71.17	39	12.3	20.9	774
N. crassulifolia	S4 (*)	29.62	71.29	78	11.7	18.6	710
N. crassulifolia	S5 (*)	30.49	71.69	108	12.0	17.9	702
N. crassulifolia	S6 (*)	31.76	71.51	209	11.1	17.0	681
N. crassulifolia	S7 (*)	33.18	71.69	351	11.4	17.8	701
Herbs							
N. rupicola	H1	26.87	70.81	29	12.9	19.9	745
N. acuminata	H2 (*)	29.62	71.29	78	11.7	18.6	710
N. reichei	H3 (*)	30.49	71.69	108	12.0	17.9	702
N. paradoxa	H4 (*)	31.76	71.51	209	11.1	17.0	681
N. paradoxa	H5 (*)	33.18	71.69	351	11.4	17.8	701

(*) indicate sympatric populations between life forms

Table 1.2 Variation of 11 functional traits across and within life forms. mean values ± standard errors of stomata size (SS), trichomes density (TD), stomatal density (SD),stomatal index (SI), potential conductance index (PCI), leaf mas per area (LMA), ^{15}N isotope ratio ($\partial^{15}N$), carbon isotopic discrimination ($\Delta^{13}C$), nitrogen content (%N), carbon content (%C) and C/N ratio. Data displayed between life forms (LF) and within LF; populations (Pops) within each life form ordered in north to south direction. *Mean* row are mean values across life forms, bold values indicate significant differences among life forms; asterisk (*) close to mean values refers to significant differences between populations within each LF. All statistical analysis were performed with transformed variables, originals mean values are shown. Bold and (*) are significant with $P<0.05$.

LF/ Pops	SS (μm2)	TD (no/mm2)	SD (no/mm2)	SI	PCI	LMA (g/mm2)	$\partial^{15}N$(‰)	$\Delta^{13}C$ (‰)	% N	% C	C/N ratio
Shrubs											
S1	22.10 ± 0.43^{C}	107.3 ± 10.0^{A}	25.82 ± 1.37^{AB}	11.19 ± 0.53	1.27 ± 0.13^{c}	113.08 ± 7.24	17.02 ± 0.60^{A}	15.79 ± 0.24^{D}	1.88 ± 0.20^{AB}	26.89 ± 1.21^{ABC}	15.21 ± 1.12^{BC}
S2	28.10 ± 0.42^{B}	70.80 ± 6.89^{AB}	20.25 ± 1.21^{B}	11.12 ± 0.41	1.55 ± 0.15^{BC}	120.81 ± 8.81	14.87 ± 0.48^{A}	17.07 ± 0.25^{BC}	1.26 ± 0.10^{B}	27.66 ± 0.82^{AB}	22.93 ± 1.32^{A}
S3	24.56 ± 0.54^{C}	55.16 ± 7.04^{BC}	27.18 ± 1.60^{AB}	11.76 ± 0.34	1.65 ± 0.16^{BC}	107.88 ± 6.49	16.54 ± 0.63^{A}	17.96 ± 0.32^{B}	1.55 ± 0.70^{AB}	23.99 ± 1.27^{BC}	18.58 ± 3.08^{ABC}
S4	29.07 ± 0.72^{B}	71.20 ± 5.49^{AB}	21.02 ± 1.13^{B}	10.76 ± 0.32	1.81 ± 0.25^{B}	138.85 ± 8.38	11.54 ± 0.60^{B}	17.70 ± 0.32^{BC}	1.22 ± 0.16^{B}	24.52 ± 0.76^{BC}	23.35 ± 2.96^{AB}
S5	31.30 ± 1.10^{AB}	49.59 ± 9.33^{BCD}	21.00 ± 1.96^{B}	10.39 ± 0.52	1.99 ± 0.26^{B}	124.4 ± 10.33	15.40 ± 0.55^{A}	16.71 ± 0.25^{CD}	2.28 ± 0.18^{A}	26.53 ± 1.01^{ABC}	12.28 ± 1.06^{C}
S6	34.15 ± 1.13^{A}	39.562 ± 4.95^{CD}	26.69 ± 2.52^{AB}	11.46 ± 0.49	3.06 ± 0.51^{A}	103.90 ± 6.67	11.03 ± 0.43^{B}	19.96 ± 0.38^{A}	1.31 ± 0.07^{B}	29.82 ± 0.58^{A}	23.23 ± 0.93^{A}
S7	32.20 ± 0.87^{A}	25.9 ± 3.99^{D}	31.60 ± 2.73^{A}	12.17 ± 0.38	3.40 ± 0.36^{A}	125.30 ± 9.44	6.39 ± 0.79^{C}	16.66 ± 0.25^{CD}	1.63 ± 0.27^{AB}	21.63 ±1.34^{C}	15.15 ± 1.39^{C}
Mean	**28.88 ± 0.56***	**59.70 ± 3.93***	**24.79 ± 0.83***	**11.27 ±0.17**	**2.10± 0.15***	**119.17 ± 3.28**	13.26 ± 0.48*	17.41 ± 0.18*	**1.59 ± 0.08***	25.86 ± 0.48*	**18.68 ± 0.85***
Herbs											
H1	39.30 ± 0.81^{BC}	24.01± 3.06^{A}	30.77 ± 4.12^{B}	16.00 ± 0.55^{B}	4.58 ± 0.62^{C}	79.37 ± 8.47^{AB}	14.44 ± 1.09^{A}	17.04 ± 0.51^{B}	2.17 ± 0.34	24.81 ± 0.45^{BC}	13.42 ± 1.53^{AB}
H2	35.16 ± 0.77^{D}	0.59 ± 0.33^{C}	44.61 ± 3.24^{A}	22.86 ±0.77^{A}	5.37 ± 1.1^{BC}	46.45 ± 2.57^{C}	13.06 ± 0.36^{A}	18.02 ± 0.38^{AB}	2.48 ± 0.20	31.06 ± 0.89^{A}	13.64 ± 1.66^{AB}
H3	37.53 ± 0.45^{CD}	5.41 ± 1.34^{B}	39.46 ± 4.17^{AB}	20.46 ± 0.58^{A}	5.55 ± 1.1^{BC}	77.72 ± 2.26^{AB}	14.37 ± 0.27^{A}	16.39 ± 0.42^{B}	2.63 ± 0.34	21.86 ± 1.26^{C}	9.24 ± 0.87^{B}
H4	43.02 ± 0.9^{A}	0.13 ± 0.08^{C}	44.96 ± 4.9^{AB}	23.18 ± 1.47^{A}	8.34 ± 0.62^{A}	89.37 ± 6.82^{A}	8.49 ± 0.96^{B}	19.21 ± 0.42^{A}	1.72 ± 0.14	30.12 ± 1.06^{AB}	14.41 ± 1.41^{A}
H5	42.89 ± 1.51^{AB}	0.08 ± 0.05^{C}	44.27 ± 5.1^{AB}	23.45 ± 1.20^{A}	8.20 ± 0.92^{AB}	64.99± 3.27^{BC}	12.07 ± 0.46^{A}	19.27 ± 0.52^{A}	2.26 ± 0.20	26.87 ± 1.25^{A}	15.21 ± 0.83^{A}
Mean	**39.33 ± 0.56***	**6.04 ± 1.46***	**43.61 ± 2.54***	**21.41 ± 0.58***	**6.75 ± 0.02***	**69.24 ± 3.16***	12.66 ± 0.42*	18.24 ± 0.27*	**2.24 ± 0.12**	27.52 ± 0.67*	**13.75 ± 0.67***

Table 1.3 Correlations between functional traits and location mean annual precipitation within each life form. Pearson correlations (*r*), number of individuals (n), lower 95% confidence interval (Lower CI), upper 95% confidence interval (Upper CI), significant probability (*P*), Fisher's z test, significant probability (*P*), bold *P* values indicate significant differences between correlations among LF with $P<0.05$. All correlations were made with variables transformed as explained in methods and precipitations log-transformed.

	Life Forms											
	Herb					**Shrub**					**F-test**	
	r	n	Lower CI	Upper CI	*P*	*r*	n	Lower CI	Upper CI	*P*	Z	*P*
SS	0.438	47	0.172	0.644	0.002*	0.777	67	0.660	0.857	<.001*	-2.91	**0.004**
SD	0.365	47	0.087	0.590	0.012*	0.235	67	-0.006	0.45	0.055	0.73	0.465
TD	-0.786	47	-0.878	-0.645	<.001*	-0.643	67	-0.765	-0.477	<.001*	-1.52	0.12
SI	0.629	47	0.417	0.776	<.001*	0.060	67	-0.183	0.296	0.629	3.471	**0.001**
PCI	0.593	47	0.369	0.752	<.001*	0.790	67	0.679	0.866	<.001*	-1.98	**0.047**
C/N	0.188	47	-0.105	0.451	0.207	-0.084	67	-0.318	0.159	0.498	—	—
LMA	0.103	47	-0.19	0.379	0.4922	-0.032	67	-0.27	0.21	0.798	—	—
$\Delta^{13}C$	0.458	47	0.196	0.658	0.001*	0.332	67	0.099	0.53	0.006*	0.76	0.447
$\partial^{15}N$	-0.412	47	-0.626	-0.142	0.004*	-0.744	67	-0.835	-0.614	<.001*	2.66	**0.007**
%C	0.26	47	-0.029	0.509	0.078	-0.019	67	-0.258	0.222	0.877	—	—
%N	-0.043	47	-0.326	0.247	0.774	0.069	67	-0.175	0.304	0.581	—	—
Factor1	0.281	47	-0.006	0.526	0.055	0.725	67	0.587	0.822	<.001	-3.2	**0.001**
Factor2	0.300	47	0.015	0.541	0.040*	0.04	67	-0.202	0.278	0.75	1.38	0.16

(*) Significant correlations within life forms, $P<0.05$.

Figure 1.1 Biplot of principal component analysis for selected traits between life forms. In (a) individuals are coloured by their life form and in (b) colours indicate populations. Percentages of variability explained are indicated in the axes. Arrows indicate loading values for leaf mass per area (LMA), stomatal size (SS), and the transformed response variables stomatal density (log(SD)), stomatal index (log(SI), potential conductance index (PCI), nitrogen content (log(%N)), trichomes density (sqrt(TD)) and C carbon to nitrogen ratio (log(C/N)).

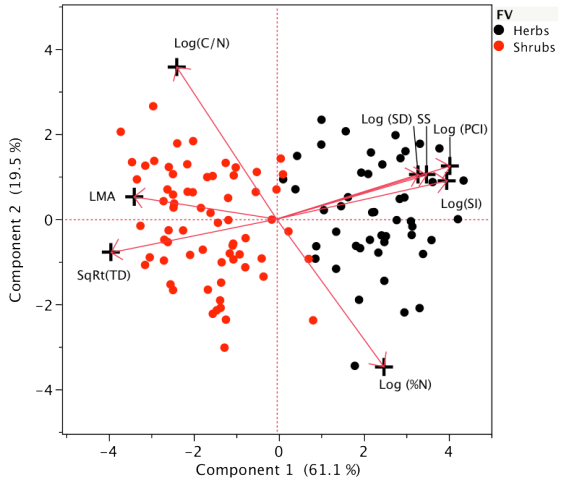
Figure 1.2 Biplot of principal component analysis for significant and most correlated traits within each life forms. Ellipses represents 50% probability ellipse for each population within life forms. (a) biplot illustrating the correlations within shrubs that mostly explain the latitudinal distribution of populations, rows indicate loadings for the different traits . (b) biplot illustrating the correlations within herbs that mostly explain the latitudinal distribution of populations.

Figure 1.3 Significant correlations between traits and precipitation gradient. (a) Stomatal size, (b) nitrogen isotope, (c) stomatal index, (d) potential conductance index. R2, equations and function of the fit are indicated in each plot. All correlations are graphed with non-transformed data.

Figure 1.4 Significant correlation between factor 1 (obtained from VARIMAX rotation of PCA_{LF}) and precipitation across the latitudinal gradient. (b) Loading factors of the traits explained by factor 1.

Figure 1.1

a.


FV
Herbs
Shrubs
Log(C/N)
Log (SD) SS
Log (PCI)
LMA
Log(SI)
SqRt(TD)
Log (%N)
Component 2 (19.5 %)
Component 1 (61.1 %)
4
2
0
−2
−4
−4
−2
0
2
4

b.

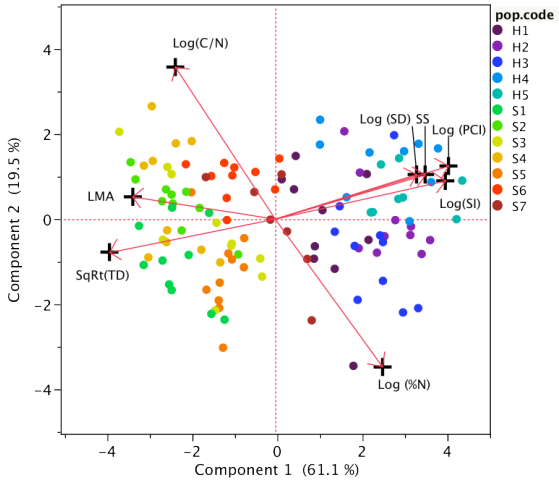
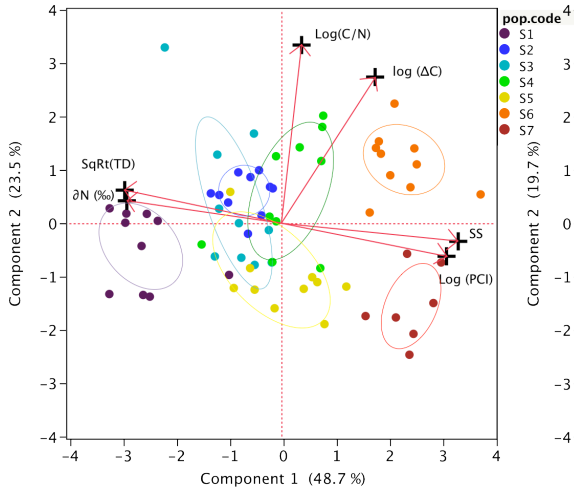

pop.code
H1
H2
H3
H4
H5
S1
S2
S3
S4
S5
S6
S7
Log(C/N)
Log (SD) SS
Log (PCI)
LMA
Log(SI)
SqRt(TD)
Log (%N)
Component 2 (19.5 %)
Component 1 (61.1 %)
4
2
0
−2
−4
−4
−2
0
2
4

Figure 1.2

a.


Log(C/N)
log (ΔC)
SqRt(TD)
∂N (‰)
SS
Log (PCI)
Component 2 (23.5 %)
Component 1 (48.7 %)
pop.code
S1
S2
S3
S4
S5
S6
S7

b.

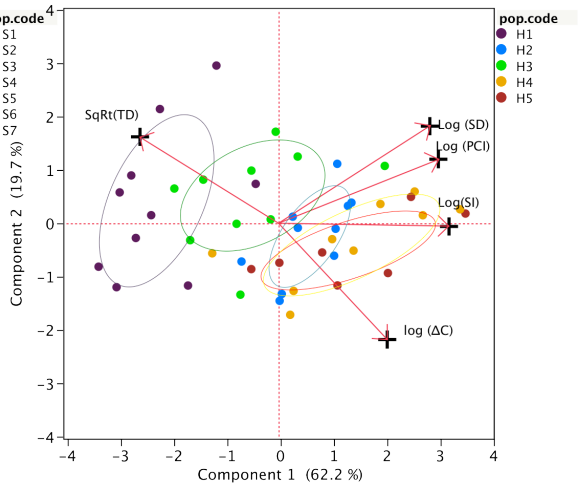

SqRt(TD)
Log (SD)
Log (PCI)
Log(SI)
log (ΔC)
Component 2 (19.7 %)
Component 1 (62.2 %)
pop.code
H1
H2
H3
H4
H5

Figure 1.3

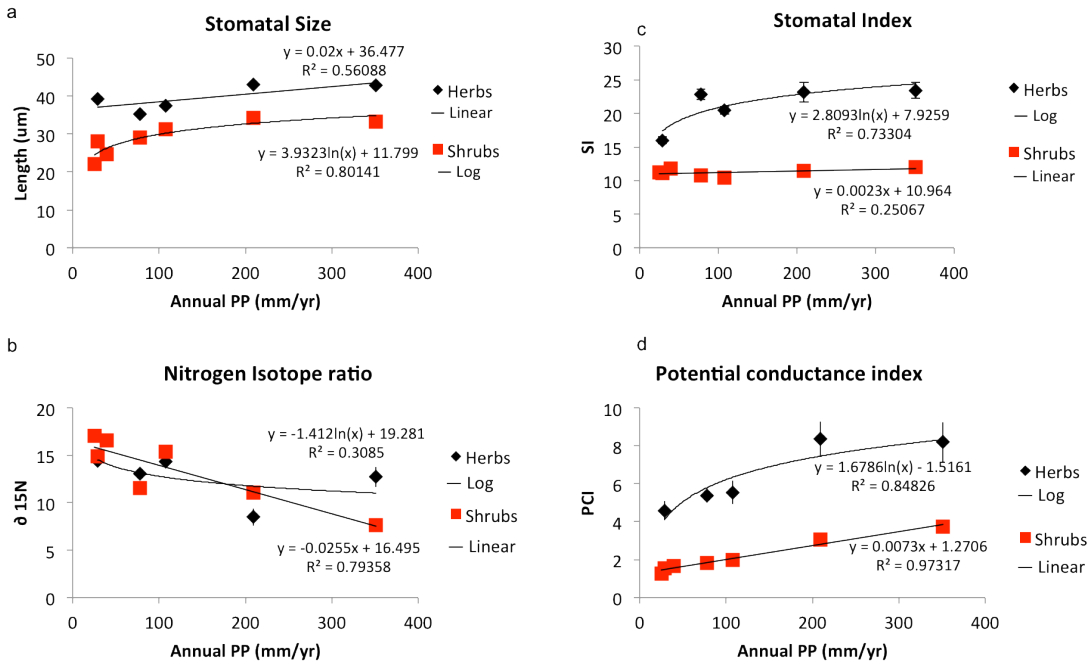
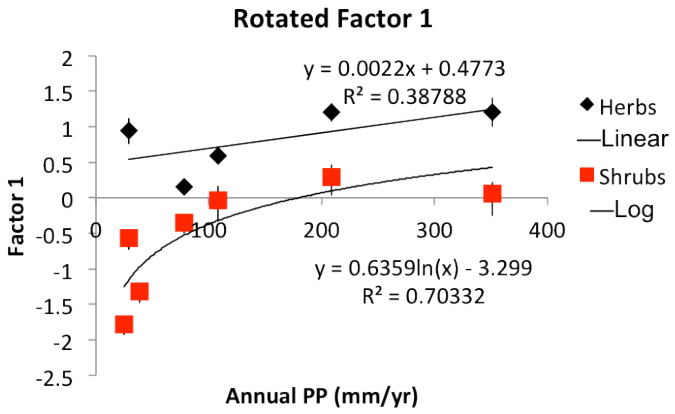

a
Stomatal Size
y = 0.02x + 36.477
R² = 0.56088
y = 3.9323ln(x) + 11.799
R² = 0.80141
Herbs
Linear
Shrubs
Log
Length (um)
Annual PP (mm/yr)
c
Stomatal Index
y = 2.8093ln(x) + 7.9259
R² = 0.73304
y = 0.0023x + 10.964
R² = 0.25067
Herbs
Log
Shrubs
Linear
SI
Annual PP (mm/yr)
b
Nitrogen Isotope ratio
y = -1.412ln(x) + 19.281
R² = 0.3085
y = -0.0255x + 16.495
R² = 0.79358
Herbs
Log
Shrubs
Linear
∂ 15N
Annual PP (mm/yr)
d
Potential conductance index
y = 1.6786ln(x) - 1.5161
R² = 0.84826
y = 0.0073x + 1.2706
R² = 0.97317
Herbs
Log
Shrubs
Linear
PCI
Annual PP (mm/yr)

Figure 1.4

a.



b.

Rotated factor loading

Variable	Factor 1
SS	0.965
LMA	-0.431
Log (SD)	0.213
SqRt(TD)	-0.730
Log(SI)	0.536
Log (PCI)	0.741
Log(C/N)	-0.213
Log (%N)	0.242

CHAPTER 1

SUPPLEMENTARY MATERIAL

Table S1.1 Between leaf 'surfaces differences among traits in Shrubs and Herbs. Mean and standard error (SE) for abaxial and adaxial leaves surface of the measured traits. SS, stomata size; SD, stomatal density; TD, trichomes density; SI stomatal index; PCI potential conductance index. Life form (LF) and leaf surface (LS) crossed effects were evaluated with ANOVA; F value, *P* value.

	Leaf surface	SS	SD	TD	SI	PCI
Shrubs	Abaxial	29.03 ± 0.82^{A}	23.59 ± 1.13^{A}	61.35 ± 5.50^{A}	10.96 ± 0.25^{A}	1.99 ± 0.14^{A}
	Adaxial	28.43 ± 0.82^{A}	25.98 ± 1.30^{A}	51.57 ± 5.80^{A}	11.46 ± 0.24^{A}	2.17 ± 0.18^{A}
Herbs	Abaxial	39.31 ± 0.89^{B}	46.92 ± 2.98^{B}	6.40 ± 2.22^{B}	22.25 ± 0.87^{B}	7.34 ± 0.68^{B}
	Adaxial	39.41 ± 0.80^{B}	35.0 ± 2.19^{C}	6.45 ± 2.18^{B}	20.00 ± 0.73^{B}	5.37 ± 0.27^{B}
Effect Test LF x LS	F ratio	0.1706	11.7116	0.0001	6.6700	4.5248
	P	0.6804	0.0009*	0.9933	0.0111*	0.0356*

* Indicate significant values with $P<0.05$

Table S1.2 MANOVA between LF and partial ANOVA by traits. (*) Indicate significant values at $P<0.05$. no significant values are in bold case. Rows are: all traits were tested transformed as explained in materials and methods section.

Trait	NumDF /DenDF	Exact F	Prob>F
Stomatal Size	1/112	156.1413	<.0001*
Stomatal Density	1/112	64.9095	<.0001*
Stomatal Index	1/112	421.5330	<.0001*
P. conductance index	1/112	221.4158	<.0001*
LMA	1/112	96.5218	<.0001*
Leaf carbon content	1/112	0.8123	**0.3694**
∂^{15}N	1/112	2.5145	**0.1156**
Leaf nitrogen content	1/112	21.0357	<.0001*
Leaf C/N ratio	1/112	21.0357	<.0001*
Trichome density	1/112	228.2793	<.0001*
Δ^{13}C	1/112	2.3584	**0.1274**
Between LF	10/102	74.4019	<.0001*

Table S1.3 Univariate ANOVA test for each trait by LF. (*) indicate significant values at $P<0.05$. no significant values are in bold case. rows are: all traits were tested transformed as expliened in materials and methods section.

	Shrubs			Herbs		
Trait	**NumDF /DenDF**	**Exact F**	**Prob>F**	**NumDF /DenDF**	**Exact F**	**Prob>F**
Stomatal Size	6/60	30.8705	<.0001*	4/41	21.21	<.0001*
Stomatal Density	6/60	5.4213	0.0002*	4/41	2.71	0.0433*
Stomatal Index	6/60	1.5465	**1.5465**	4/41	12.49	<.0001*
PCI	6/60	20.4968	<.0001*	4/41	6.74	<.0001*
LMA	6/60	1.9293	0.0002*	4/41	8.71	<.0001*
Leaf C content	6/60	5.3184	0.0002*	4/41	14.78	<.0001*
∂^{15}N	6/60	33.2190	**0.1156**	4/41	10.98	<.0001*
Leaf N content	6/60	4.9377	<.0004*	4/41	1.7678	**0.1539**
Leaf C/N ratio	6/60	7.7345	<.0001*	4/41	5.08	0.0020*
Trichome density	6/60	12.2079	<.0001*	4/41	66.12	<.0001*
Δ^{13}C	6/60	20.2991	<.0001*	4/41	8.23	<.0001*
Between populations	66/330	Pillai's Trace 3.33	<.0001*	44/136	Pillai's Trace 2.767	<.0001*

Capitulo II

Journal of Biogeography (J. Biogeogr.) (2013)

ORIGINAL ARTICLE

Phylogeography of two closely related species of *Nolana* from the coastal Atacama Desert of Chile: post-glacial population expansions in response to climate fluctuations

Paulina G. Ossa[1,2]*, Fernanda Pérez[1,2] and Juan J. Armesto[1,2]

[1]*Center for Advanced Studies in Ecology & Biodiversity (CASEB) and Departamento de Ecología, Pontificia Universidad Católica de Chile, Santiago, Chile,* [2]*Institute of Ecology & Biodiversity (IEB), Santiago, Chile*

*Correspondence: Paulina G. Ossa, Departamento de Ecología, Pontificia Universidad Católica de Chile, Alameda 340, Santiago, Chile.
E-mail: pgossa@bio.puc.cl

ABSTRACT

Aim To investigate the impact of Quaternary climate cycles on the coastal Atacama Desert flora by assessing phylogeographical patterns of the desert shrub *Nolana crassulifolia* (Solanaceae) and its congener *Nolana incana*.

Location The latitudinal aridity gradient from the southern margin of the coastal Atacama Desert to the mediterranean semi-arid region of Chile (25–33° S).

Methods Two cpDNA regions were sequenced for 130 individuals in 15 populations of these two closely related species, covering their entire distribution range (1000 km) along the arid to semi-arid Chilean coast. We explored haplotype relationships in a statistical parsimony network, and assessed population genetic diversity, population differentiation and phylogeographical structure. In addition, we conducted demographic analyses and spatial analysis of genetic variation, identified barriers to gene flow with Monmonier's algorithm, and used Bayesian phylogenetic reconstruction to estimate divergence dates between lineages.

Results We found a total of 14 haplotypes – four of them shared by both species – and high levels of genetic differentiation among populations, but no past distribution breaks that could account for major vicariant events. Genetic diversity decreased continuously from north to south, with loss of haplotypes and a greater number of monomorphic populations in the southern range. Landscape analysis revealed greater genetic differentiation in the northernmost populations of both species.

Main conclusions The documented north–south gradient of declining genetic diversity, the origin and location of ancestral haplotypes in northern sites, and the loss of haplotypes from southern populations all support the hypothesis of post-glacial range expansion of *Nolana* southwards during arid/warmer cycles in the Atacama. The higher genetic diversity and greater differentiation of northern populations of both *Nolana* species support the hypothesis that populations survived in northern arid sites during wetter/colder episodes of the glacial cycles. We suggest that Quaternary arid phases in the Atacama promoted southward expansion of the coastal desert vegetation.

Keywords
Coastal Atacama Desert, cpDNA, *Nolana*, perennial shrub, plant phylogeography, post-glacial expansion, Quaternary, semi-arid region.

INTRODUCTION

The Pleistocene was characterized by extensive perturbations of the global climate that strongly affected the distributions of organisms and communities (Hewitt, 2000; Jackson & Overpeck, 2000; Williams *et al.*, 2004). Its effects on biogeographical patterns have been well documented for temperate, alpine and high-latitude regions (Taberlet *et al.*, 1998). At

Journal of Biogeography

arid subtropical latitudes, however, where the impact of glacial cycles was less evident, the results are not so conclusive or well documented (Lessa *et al.*, 2003). For the desert biome, the phylogeographical patterns associated with Quaternary climate cycles have been poorly explored, and research has focused primarily on animals (Riddle *et al.*, 2000; Jezkova *et al.*, 2009). The limited genetic evidence suggests that the distribution ranges of drought-resistant desert plants were strongly affected by glacial climate changes in the Northern Hemisphere (Nason *et al.*, 2002; Garrick *et al.*, 2009; Rebernig *et al.*, 2010) often supporting post-glacial range expansions. In contrast, we lack information on the glacial dynamics of distribution ranges for plants from the hyper-arid Atacama Desert of western South America, where intermittent episodes of high aridity interrupted by intervals of increased rainfall have been reported in the late glacial period (Latorre *et al.*, 2005).

The Atacama Desert of northern Chile, considered one of the driest places on Earth, extends along the western margin of South America from 17° to 30° S. The hyper-arid condition prevailing over much of the region dates back to the late Miocene (Dunai *et al.*, 2005), coeval with the final phase of central Andean uplift and intensification of the cold Humboldt Current (Placzek *et al.*, 2009). Coastal fog and winter rains caused by westerly storm fronts, modulated by the South Pacific Anticyclone (SPA), supply moisture to the southern margin of the Atacama, whereas summer rains transported by easterly winds are the principal source of rainfall in the Andean highlands. The Quaternary history of the Atacama Desert remains unresolved, although researchers agree that several wet episodes affected the Atacama during the last glacial–interglacial cycle (Betancourt *et al.*, 2000; Latorre *et al.*, 2005; Díaz *et al.*, 2012). At the time of the Last Glacial Maximum (LGM), easterly winds became weaker and westerly winds were displaced towards the equator (Lamy *et al.*, 1999), thus reducing summer rainfall in the northern Atacama, but intensifying winter rains at the southern margin of the desert (Maldonado *et al.*, 2005; Maldonado & Villagrán, 2006; Kaiser *et al.*, 2008). Moreover, a hypothetical scenario of weaker SPA during the LGM (Rojas *et al.*, 2009) probably led to a reduction of coastal fog in the Atacama, and the decline in the global ice accumulation in polar regions may have exposed a greater extent of the seafloor, thus creating new habitats for the coastal desert flora.

The coastal Atacama Desert in Chile exhibits an outstanding floristic diversity for a region of extreme aridity, with nearly 550 vascular plant species, 40% of them endemic to the region (Dillon & Hoffmann, 1997). Plant diversity in the hyper-arid core of the Atacama is largely restricted to fog-dependent ecosystems known as *lomas* formations, found on coastal mountain-tops above 600 m (Rundel *et al.*, 1991). At lower elevations, fog-free plant communities are also present, and are maintained by sporadic seasonal precipitation, which becomes more regular south of 26° S along the transition from arid to semi-arid (Armesto *et al.*, 1993).

Range contraction and isolation in multiple refugia during wet/cold episodes during glacial–interglacial cycles might have played a major role in promoting population divergence and vicariant speciation in the Atacama Desert flora. However, genetic evidence of population fragmentation is only available for *Dioscorea biloba* (Viruel *et al.*, 2012), a desert plant that currently has a disjunct distribution close to the margins of the Atacama Desert. Very little is known about the response to Pleistocene climatic oscillations of desert plants with currently continuous distributions across the southern margin of the Atacama. It can be postulated that these plant species should have been less affected by climate changes during glacial–interglacial cycles, or alternatively, that they could have tracked semi-arid conditions, contracting their ranges northwards during wet/cold episodes and expanding southwards during warmer and drier periods.

One of the most representative and diverse plant genera of the coastal Atacama Desert is *Nolana* (Solanaceae), with about 85 species and subspecies of annuals, perennial herbs and shrubs distributed in the western margin of the southern Peruvian and northern Chilean deserts, extending southwards into the semi-arid mediterranean region of Chile (Dillon, 2005). The shrub *Nolana crassulifolia* Poepp. is among the most widespread species in the genus, extending across the arid to semi-arid transition from 27° to 33° S, along the Pacific shoreline. Individuals of this species closely resemble the related *Nolana incana* (Phil.) Johnst. (Johnston, 1936), which occupies the same habitats; the latter species is smaller in size and grows at the northern end of the range (25°–27° S), in hyper-arid conditions. Recent studies of phylogenetic relationships within the genus *Nolana*, using four chloroplast DNA regions (Tu *et al.*, 2008) and the nuclear regions *GBSSII* (Dillon *et al.*, 2007) and *LEAFY* (Tu *et al.*, 2008) have not completely resolved the relationship between *N. crassulifolia* and *N. incana*. However, the combination of molecular and morphological data suggests that *N. crassulifolia* and *N. incana* are sister species, constituting a monophyletic clade (see Materials and Methods and Appendices S1–S2 in Supporting Information). The crown age established for the entire genus *Nolana*, 4.02 Ma, dates back to the Pliocene, followed by diversification in the early Pleistocene, from 2.88 to 1.25 Ma (Dillon *et al.*, 2009). Thus, the history at population level of these two closely related species with a broad distribution in the southern margin of the Atacama Desert might provide evidence for plant responses to glacial–interglacial climatic fluctuations in the Atacama.

In the present study, we used DNA sequence data from two chloroplast DNA (cpDNA) regions to infer the population responses of these two closely related *Nolana* species to glacial–post-glacial climatic fluctuations in the southern Atacama Desert. Based on the palaeoclimatic scenario described above for the southern Atacama Desert, we expected to find evidence for contraction of the species' ranges to northern latitudes during wetter/colder episodes of the last glacial cycle, and document a post-glacial population expansion to occupy the southern part of the range, concurrent with the

increased aridity during the Holocene. Recent population expansions are predicted to cause a north-to-south trend of decreasing genetic diversity for both closely related species, with large areas of the present distribution fixed for a single haplotype near the southern margin of the geographical range. This genetic pattern could result from founder effects and successive population bottlenecks (Hewitt, 1996). In addition, we tested for evidence of population fragmentation and isolation that should be reflected in geographically structured haplotypes, with high levels of genetic differentiation between populations and isolation by distance. We treated all the populations of the two closely related species, *N. incana* and *N. crassulifolia*, distributed at the southern margin of the Atacama and in the Chilean semi-arid region, as components of a hypothetical single evolutionary unit that responded similarly to climate fluctuations.

MATERIALS AND METHODS

Species, study sites and plant collections

The study was focused on *N. crassulifolia* and *N. incana*. Both species inhabit the rocky sea shores of the Pacific coastal desert, are prostrate subshrubs with succulent leaves and a small white funneliform (bell-shaped) corolla (Fig. 1). Their seeds are located in 5–9 small rounded mericarps that remain inside the fruit until germination. Mericarp dispersal is basically passive, mostly persisting in the accrescent calyx, but some mericarps may be dispersed by wind or water (ocean). Although no reports of animal dispersal are known, fossil mericarps of *Nolana* spp. have been collected from ancient rodent middens (Betancourt *et al.*, 2000; Díaz *et al.*, 2012).

The phylogenetic relationship between the two species is still unresolved (Dillon *et al.*, 2007; Tu *et al.*, 2008). Chloroplast data placed both in an unresolved clade that also includes other Chilean endemic shrub species (clade FH in Tu *et al.*, 2008). Nuclear data (Dillon *et al.*, 2007; Tu *et al.*, 2008) placed *N. crassulifolia* and *N. incana* in the same clade as *N. divaricata*, *N. peruviana* and *N. werdermannii*. In order to clarify the phylogenetic relationships within the clade including the two species, we reconstructed a total-evidence tree using 11 morphological traits (Appendix S1) and concatenated DNA sequences for the Chilean shrub species of *Nolana* (clade FH in Tu *et al.*, 2008). We only included individuals with data for the six DNA markers (named according to collector's vouchers). For morphological data, we examined three individuals per species from samples deposited at the Museo Nacional de Historia Natural in Santiago, Chile (MNHN; herbarium code SGO), and descriptions in the literature (Johnston, 1936; Dillon *et al.*, 2007). Results supported with 91% confidence (bootstrap value) the close sister relationship between *N. crassulifolia* and *N. incana* (Appendix S2).

The sampled range in our study extended along the littoral zone of the mediterranean region, covering 1000 km between 25° S and 33° S, and including the complete geographical range of the focal taxa. Plant material was collected from 14 localities, belonging to three populations of *N. incana*, 10 of *N. crassulifolia*, and one sympatric site. Sample size ranged from two to 22 individuals (130 plants in total), according to population density; in every locality, we defined transects of 200 m along the coast from where samples were taken. All populations from which samples were taken were georeferenced and relevant vouchers from localities were deposited at MNHN.

DNA extraction and sequencing of non-coding regions of cpDNA

Dried leaf tissue was ground in a TissueLyser (Qiagen, Valencia, CA, USA); total genomic DNA was extracted using DNeasy Plant Mini Kit (Qiagen) and verified on 2% agarose gels. We tested nine individuals for amplification and polymorphism in 12 non-coding cpDNA regions. The intergenic spacers *rpl*32–*trn*L and *rpl*16 intron showed the highest variability and were amplified for the full sample. Amplifications were carried out following Shaw *et al.* (2005, 2007), using 12.5 μL GoTaq Colorless Master Mix (Promega, Madison, WI, USA), 0.5 μM each primer, 20 ng of DNA template, and nuclease-free water (Promega) to reach a final volume of 25 μL. PCR products were verified on 1% agarose gels and sent to Macrogen (Seoul, South Korea) for purification and sequencing with forward and reverse primers. Difficult PCR products were purified using QIA-quick purification kit (Qiagen) and sequenced in the Molecular Diversity Laboratory of Pontificia Universidad Católica (Chile). Sequences were aligned and edited with CLC SEQUENCE VIEWER 6.6 (CLC Bio A/S, 2008); indels due to poly-N were ignored. All sequences were deposited in GenBank, with accession numbers KC434177–KC434307 (*rpl*32–*trn*L) and KC434308–KC434438 (*rpl*16).

Data analysis

Genetic diversity

The number of haplotypes (K), haplotype (gene) diversity (Hd), nucleotide diversity (π) and the average number of pairwise nucleotide differences per site (Π; Nei, 1987) were estimated using DNASP 5.1 (Librado & Rozas, 2009). To assess possible founder effects and find evidence of the latitudinal migration southwards from a past distribution further north, we analysed the trends of within-population genetic diversity with latitude. We subdivided the distribution range into three approximately equal geographical regions, based on mean climatic conditions (di Castri & Hajek, 1976; Luebert & Pliscoff, 2006): North (25–27° S; mostly hyper-arid; mean annual precipitation: < 25 mm yr^{-1}), Central (27–30° S; mostly arid; mean annual precipitation: 29–108 mm yr^{-1}) and South (30–33° S, semi-arid; mean annual precipitation: 120–350 mm yr^{-1}). To account for

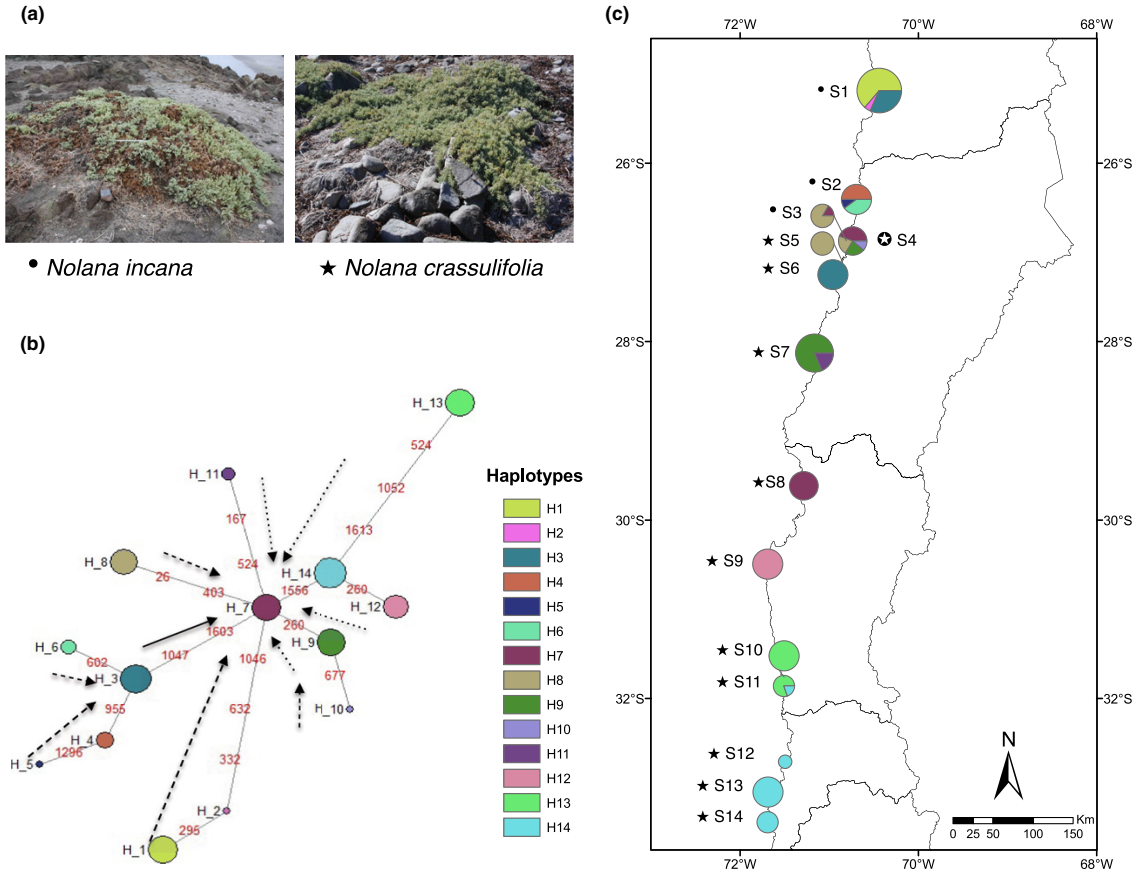


Figure 1 Geographical distribution and genealogical relationships of the 14 cpDNA haplotypes recovered from 14 populations of *Nolana incana* and *N. crassulifolia* in the Atacama Desert, Chile. (a) Habitus of *N. incana* and *N. crassulifolia*. (b) Statistical parsimony network; circle sizes and branch lengths are proportional to haplotype frequencies and the number of mutational steps between haplotypes, respectively. Dashed, dotted and solid arrows represent the three different contraction events according to the star-contraction algorithm. (c) Geographical map of the sampling locations, with pie charts displaying the frequency of occurrence of each haplotype in each locality; pie chart size is proportional to population size, and haplotype colours correspond to those shown in panel (b). The symbols beside localities denote populations of *N. incana* (black circle), *N. crassulifolia* (star) or both (star inside a circle).

differences in sample size among populations, we performed Monte Carlo simulations to standardize population sizes to $n = 9$ and recalculated the genetic diversity estimators mentioned above. These procedures were performed with an R script and the R package PEGAS 0.4-1 (Paradis, 2010).

Network representation

Relatedness among haplotypes was represented by a statistical maximum parsimony network (MP) using the software TCS (Clement *et al.*, 2000), and a median-joining (MJ) network with the software NETWORK 4.6.1.0 (Bandelt *et al.*, 1999). This software was also used to run the star contraction algorithm that simplifies networks estimating historical demographic expansion events (Forster *et al.*, 2001). A minimum spanning tree (MST) was calculated using Prim's algorithm (Prim, 1957) with HAPSTAR (Teacher & Griffiths, 2011).

Populations differentiation and geographical structure analyses

Genetic differentiation among populations was evaluated using the G_{ST} and N_{ST} coefficients (Pons & Petit, 1996). Both coefficients estimate the ratio between the mean within-population genetic diversity and total genetic diversity, but while the G_{ST} index makes use only of the allelic frequencies, N_{ST} also takes into account the genetic distances among haplotypes. These indices were statistically compared using 1000 permutations in PERMUTCPSSR 2.0 (Pons & Petit, 1996). A greater N_{ST} means that more closely related haplotypes occur in the same population, indicating phylogeographical structure. Only populations with a sample size larger than three individuals were considered. A spatial autocorrelation analysis (SAA) and a Mantel test were performed with GENALEX 6.1 (Peakall & Smouse, 2006) to identify

patterns of isolation by distance. SAA was run with distance intervals of 82 km, equal to the average distance between sampling locations, and a 95% confidence interval around the null hypothesis of no spatial structure was calculated using 1000 permutations. Affinities between the populations were assessed considering genetic and geographical distances using spatial analysis of molecular variance (SAMOVA) with SAMOVA 1.0 (Dupanloup *et al.*, 2002). Populations with fewer than two individuals were excluded from the analysis. To test for neutral evolution, we calculated the mismatch distribution, Tajima's D and Fu's F_S using ARLEQUIN 3.5.1.3 (Excoffier & Lischer, 2010). The significance of these tests was evaluated as the proportion of random statistics less than or equal to the observed value. For positive values of Tajima's test, we used the published critical values (Tajima, 1989).

A genetic landscape shape analysis was performed using ALLELES IN SPACE (Miller, 2005). This analysis identifies genetic discontinuities among populations in a landscape shape and produces a three-dimensional surface plot where the x and y-axes correspond to the geographical coordinates of populations, and the z-axis corresponds to the interpolated genetic distances. We investigated the genetic barriers associated with each geographical location and populations, using Monmonier's maximum-difference algorithm, implemented in BARRIER 2.2 (Manni *et al.*, 2004). Significance of barriers was estimated using 10,000 bootstrapped pairwise distance matrices generated in R (R Development Core Team, 2012).

Bayesian divergence time estimations

To estimate divergence times between haplotypes, we performed a Bayesian analysis using BEAST 1.7.4 (Drummond *et al.*, 2012) with a log-normal relaxed clock, extended Bayesian skyline tree prior and TVM+I substitution model, which was selected using the Akaike information criterion (AIC) as implemented in JMODELTEST 2.1.1 (Darriba *et al.*, 2012). Given the lack of fossil records for *Nolana*, we calibrated the clock using a strong prior on the substitution rate (Drummond *et al.*, 2006). Using the available data published in a previous independent study (Dillon *et al.*, 2009), we estimated a mean of 2.23×10^{-9} substitutions per site per year (s s^{-1} yr^{-1}) and a standard deviation (SD) of 0.6×10^{-9} s s^{-1} yr^{-1}; this values lies within the mutation rate interval described for most angiosperms ($1.0–3.0 \times 10^{-9}$ s s^{-1} yr^{-1}, see Wolfe *et al.*, 1987). We ran three independent MCMC analyses for 40,000,000 generations sampling every 4000. Logs and tree files were combined in LOGCOMBINER 1.7.4 (Drummond *et al.*, 2012).

We verified the convergence of the estimated parameters and that the effective sample sizes were at least 200 in TRACER 1.5 (Rambaut & Drummond, 2007); trees were summarized in TREEANOTATOR 1.7.4 (Drummond *et al.*, 2012) with 10% burn-in, and edited in FIGTREE 1.4 (Rambaut, 2012).

RESULTS

Haplotypes and network analysis

The alignments of the *rpl*32–*trn*L and *rpl*16 data sets of *Nolana* were 720 and 929 bp in length, respectively, including two variable mononucleotide repeats. The 1649-bp combined data set contained 18 nucleotide substitutions. A total of 14 haplotypes were identified among the 130 individuals sampled from 10 populations of *N. crassulifolia* and three populations of *N. incana* at the southern margin of the Atacama Desert. The two species occurred in sympatry at only one site. Four haplotypes (H3, H7–H9) were shared between populations of the two closely related *Nolana* species.

Six of the 14 haplotypes we identified occurred in more than two *Nolana* populations, with frequencies of 10–14% (Fig. 1). Three haplotypes were very rare, with frequencies below 1%. The most widely distributed haplotypes in the entire sample were H14, H7 and H3. Haplotype H14 was present in four *Nolana* populations (S11–S14) within a range of 170 km and was restricted to the South (semi-arid) region; haplotypes H7 and H3 were found in three (S3, S4 and S8) and two (S1 and S6) populations, respectively, occurring in both the North and Central portions of the species' range and encompassing a geographical range of 300 km and 218 km, respectively.

Network constructions of haplotype structure with the MJ and MP algorithms showed the same results (Fig. 1). The network presented a loop involving four haplotypes (H7, H14, H12, H9), which was broken between H12 and H9 in both trees. The central and most connected haplotype was the widespread haplotype H7, which was inferred to be the most likely ancestral haplotype. A maximum of four mutational steps separated H7 from the remaining haplotypes, with the longest branches formed by haplotypes H13–H14 (from the southernmost populations) and haplotypes H1–H2 (from the northernmost populations). The star contraction algorithm suggested stepped demographic expansion events that converged in H7. The first contraction shrank haplotypes H6, H4 and H5 into H3; H1, H2 and H8 into H7; and H10 into H9. The second contraction shrank all remaining haplotypes into H7, except for H3.

Population differentiation and phylogeographical structure

The coefficient of differentiation N_{ST} (0.81) was high, and significantly higher than G_{ST} (0.76) (permutation test: $P = 0.04$), indicating significant population differentiation and suggesting the existence of phylogeographical structure. The Mantel test ($r = 0.38$; $P = 0.001$) and the spatial autocorrelation analysis (SAA) (omega = 165.786; $P = 0.001$) were both significant, thus supporting the hypotheses of isolation by distance. The correlogram showed a general trend of decreasing autocorrelation (r) with increasing geographical distance (Appendix S3); despite this, a smaller increment in r at intermediate distances (about 300 km) was detected,

which is probably a consequence of low genetic differentiation among populations in the southern portion of the distribution range.

Based on the cpDNA haplotypes and matrix of geographical distances, SAMOVA identified five phylogeographical groups with an F_{CT} = 0.63 (Fig. 2). These five groups, however, did not represent clearly separated geographical units. The northern populations (S1–S5) were disaggregated into four groups: Group 1, containing only the northernmost site (S1); Group 2, including both S2 and the central S6; Group 3, connecting S3 and S5; and Group 4, connecting S4 with all central and populations located south of 28° S, except S10 and S11, which together comprise the final Group 5. Monmonier's algorithm also revealed strong differentiation among northern populations. The three major genetic barriers were located in the north between 25° and 27° S (Fig. 2), with bootstrap values that sum to 92.4%. These barriers isolate S1 from S2 (26.4%), S2 from S3–S5 (58.2%), and S6 from the northernmost populations (7.8%). Genetic landscape analysis was congruent with these results and showed an overall reduction in genetic distance among *Nolana* populations from north to south, delimiting two zones of pronounced differentiation (Fig. 3). The first was found at the extreme north and was composed of three peaks (25.8°, 26.7° and 27.1° S), and the second was smaller and located at the southern margin of the range, at around 32° S.

The Bayesian chronogram of haplotypes showed a geographical pattern of divergence closely congruent with SAMOVA groupings (Fig. 2). The ages of divergence of haplotype lineages were estimated at between 0.7 and 0.15 Ma in all cases, corresponding to the mid-Pleistocene period.

Latitudinal patterns of genetic diversity

Genetic diversity varied markedly among the populations (Table 1), but was higher in the northernmost cases. The

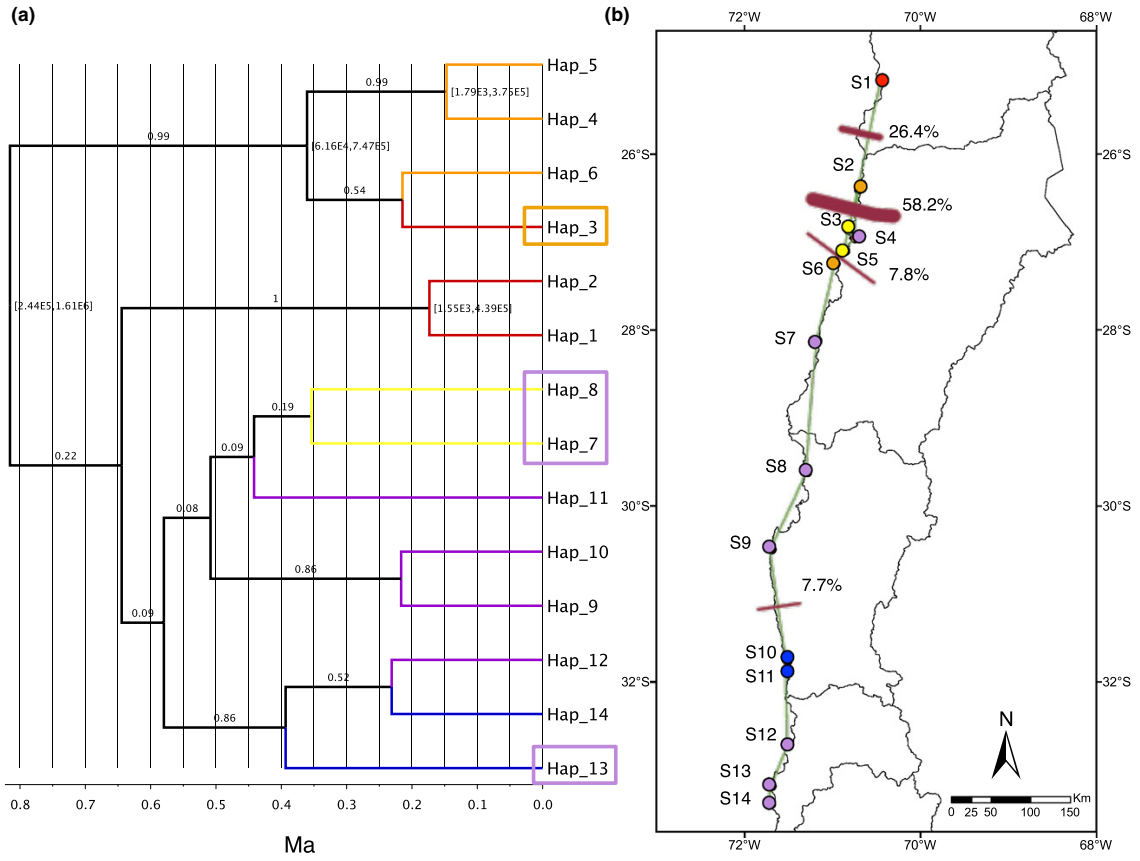


Figure 2 Geographical relationships among haplotypes of *Nolana* in Chile. (a) Bayesian chronogram of haplotypes; axis scale represents million years ago (Ma); numbers above branches represent posterior probabilities; only 95% intervals of nodes with posterior probabilities > 0.9 are shown; branch colours correspond to those shown in panel (b) and represent the SAMOVA groups in which these haplotypes were found; highlighted haplotypes are those shared between populations that belong to different groups. (b) Barriers obtained with Monmonier's algorithm and SAMOVA groups are shown on the map; the thickness of each barrier is proportional to the number of times it was included in one of the 10,000 computed barriers from bootstrap matrices and expressed as percentages; SAMOVA groups are represented by the colours red (Group 1), orange (Group 2), yellow (Group 3), purple (Group 4) and blue (Group 5).

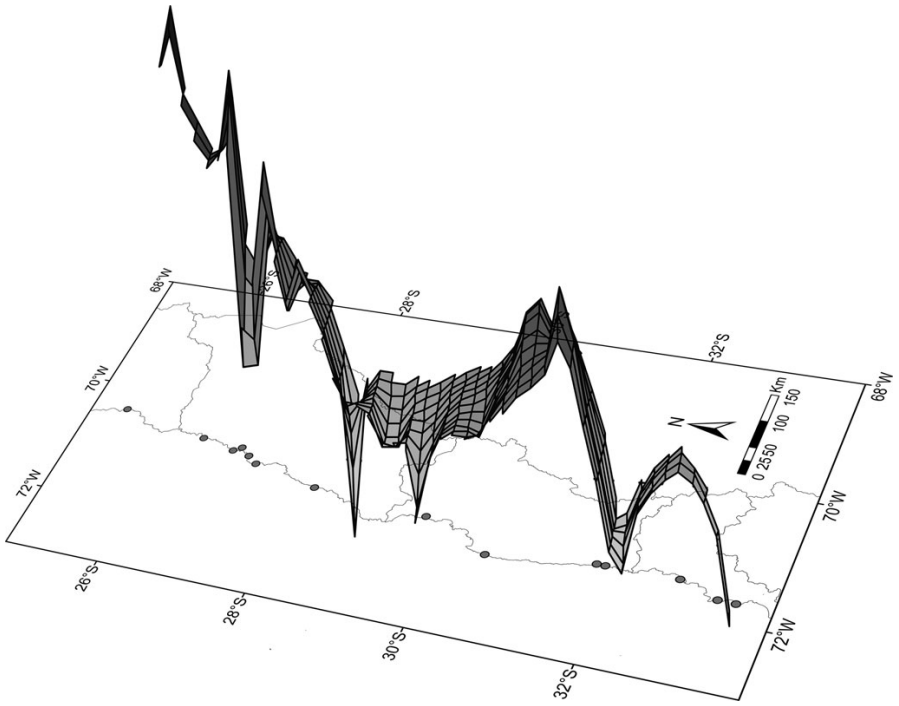


Figure 3 Genetic landscape analysis showing the latitudinal trend of differentiation between *Nolana* populations. The landscape is standardized with a grid of 50 × 30 cells comprising the entire geographical range of the close relatives *Nolana crassulifolia* and *N. incana* in all localities, superimposed on a map of the southern Atacama Desert. Peaks and depressions of the surface are proportional to genetic distances.

highest haplotypic diversity (*Hd*) was found in S2 and S6, situated at latitudes 26.4° and 26.9° S, whereas mean pairwise differences (Π) among individual plants were highest in the northernmost site situated at latitude 25.1° S. Eight populations were fixed for a single haplotype, and five of them occurred south of 30° S, in the semi-arid region. When differences in sample size among populations were considered (Fig. 4), excluding the smaller populations, and the genetic diversity index was standardized for $n = 9$, we detected a significant reduction of within-population genetic diversity with increasing latitude (*Hd*: $r = -0.73$, $P = 0.012$, $n = 9$; Π: $r = -0.70$, $P = 0.017$, $n = 9$). The same tendency was observed when populations were pooled into three a priori geographical groups: North (north to 27° S), Central (27°–31° S) and South (> 31° S) (Table 2).

Demographic analyses

The pairwise mismatch distribution for the entire sample was unimodal, with a maximum at 4 bp, and did not differ significantly from the null population expansion model (Table 3). However, the more conservative estimate of population expansion, Tajima's *D*, was non-significant, and therefore, did not indicate a departure from population equilibrium ($D = 0.111$, P = n.s.). When the analysis was performed for the SAMOVA groupings, Groups 2–5 showed unimodal mismatch distributions, but only Group 5 showed a significant negative value for Tajima's *D*, suggesting population expansion (Group 2: $D = 0.11$, P = n.s.; Group 3: $D = -1.45$, $P = 0.08$; Group 4: $D = -0.26$, P = n.s.; Group 5: $D = -1.68$, $P = 0.01$). Group 1 – containing only the northernmost population S1 – had a bimodal mismatch distribution with peaks at 0 and 6 bp, as well as a significantly positive Tajima's *D* value ($D = 2.09$, $P < 0.05$), suggesting population contraction.

DISCUSSION

Chloroplast DNA markers did not recover the distinction between *N. crassulifolia* and *N. incana*. The finding of four haplotypes shared by both species supports the hypothesis of a recent divergence between these two closely related taxa and incomplete lineage sorting. Therefore, it seems legitimate to analyse the phylogeography of *N. crassulifolia* and *N. incana* together, treating them as a single evolutionary complex using cpDNA (see for example Grivet & Petit, 2002).

We detected high levels of genetic differentiation among *Nolana* populations, and significant phylogeographical structure (high $G_{ST} = 0.76$ and $N_{ST} > G_{ST}$), but SAMOVA did not produce an arrangement of sites in discrete geographical

Table 1 Genetic diversity of *Nolana* populations found at the southern margin of the Atacama Desert. Location, latitude, longitude, voucher number, climate, sample size (*n*) and measurements of genetic diversity for three populations of *N. incana*, 10 of *N. crassulifolia,* and one sympatric site (S4).

Code	Species	Locality	Climatic region	Lat. (°S)	Long. (°W)	Collector voucher	*n*	*K*	*Hd*	Π	π
S1	*N. incana*	Bandurrias	North	25.18	70.44	Ossa 267	22	3	0.51 (0.08)	2.76 (1.52)	0.0017 (0.001)
S2	*N. incana*	Playa Hippie	North	26.41	70.70	Ossa 251	10	3	0.64 (0.10)	1.27 (0.87)	0.0008 (0.0006)
S3	*N. incana*	Zenteno	North	26.87	70.81	Ossa 107	6	2	0.33 (0.21)	0.67 (0.59)	0.0004 (0.0004)
S4	Sympatric	Q. El León	North	26.96	70.74	Ossa 315, 321	9	4	0.78 (0.11)	1.50 (0.99)	0.0009 (0.0007)
S5	*N. crassulifolia*	Bahía Inglesa	Central	27.11	70.85	Ossa 114	6	1	0	0	0
S6	*N. crassulifolia*	Bahía Cisne	Central	27.25	70.96	Ossa 237	10	1	0	0	0
S7	*N. crassulifolia*	Hualtata	Central	28.13	71.17	Ossa 256	16	2	0.33 (0.13)	0.98 (0.70)	0.0006 (0.0005)
S8	*N. crassulifolia*	Hornos	Central	29.62	71.29	Ossa 171	9	1	0	0	0
S9	*N. crassulifolia*	Talcaruca	South	30.49	71.69	Ossa 201	10	1	0	0	0
S10	*N. crassulifolia*	Chigualoco	South	31.76	71.51	Ossa 157	10	1	0	0	0
S11	*N. crassulifolia*	Playa Amarilla	South	31.86	71.51	Ossa 286	5	2	0.40 (0.24)	1.20 (0.91)	0.0007 (0.0006)
S12	*N. crassulifolia*	Caucau	South	32.71	71.50	Ossa 302	2	1	0	0	0
S13	*N. crassulifolia*	Quintay	South	33.18	71.69	Ossa 210	10	1	0	0	0
S14	*N. crassulifolia*	El Quisco	South	33.39	71.69	Ossa 086	5	1	0	0	0
						Total	130	14	0.90 (0.01)	3.44 (1.77)	0.0021 (0.0012)

K, number of haplotypes; *Hd*, haplotypic diversity; Π, mean pairwise differences; π, nucleotide diversity. Standard deviations are given in parentheses.

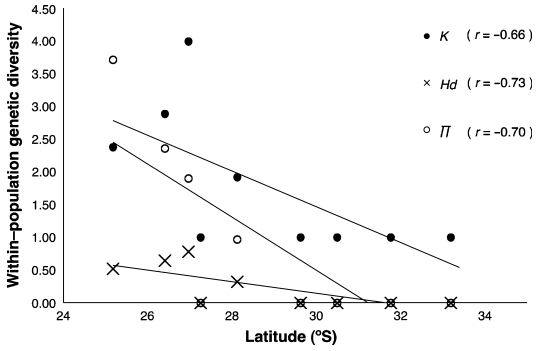


Figure 4 Latitudinal variation of within-population genetic diversity in *Nolana* at the southern margin of the Atacama Desert and the transition to mediterranean climate. *K*, number of haplotypes; *Hd*, haplotypic diversity; Π, mean pairwise differences. Sample sizes were standardized to $n = 9$ for the populations shown.

units as expected under a hypothesis of biogeographical breaks related to vicariance. The present geographical distribution of the *Nolana* complex could be the result of several population contractions/expansions rather than fragmentation of an extended ancient population, as proposed in the only phylogeographical study available for an Atacama Desert plant (Viruel *et al.*, 2012). Both *Nolana* species are restricted to the rocky coastline, showing a nearly continuous distribution over 1000 km, and the current level of genetic differentiation among the populations studied is most probably the result of population isolation derived from habitat discontinuities along the coast. A geographical pattern similar to *Nolana* has been documented for other rock-dwelling shoreline taxa, such as macroalgae (Fraser *et al.*, 2010), rock-pool copepods (Burton & Feldman, 1981) and also terrestrial plants (Cain *et al.*, 2000). These taxa often exhibit significant levels of genetic differentiation despite usually being able to disperse over long distances. Accordingly, a clear isolation-by-distance signal was found in SAA for short geographical distances (0–250 km), strongly biased by the high differentiation shown by the northern populations.

The conspicuous latitudinal gradient in both genetic differentiation and genetic diversity of *Nolana* – along the transition from hyper-arid to semi-arid – is also supported by genetic landscape analysis and barriers detected by Monmonier's algorithm, pointing to marked population differentiation at 25–27° S. Northern populations within this species complex also showed the highest number of haplotypes and greater haplotypic and nucleotide diversity, suggesting that they have been able to persist for long time. These data support the hypothesis that populations within this species complex remained in northern areas during the glaciations and wet Holocene episodes, when increased run-off or groundwater upwelling could have allowed the expansion of local populations of *Nolana* along creeks and mountain slopes, as suggested for lomas vegetation by Díaz *et al.* (2012). Exposed seashore as a consequence of lower sea level also favoured population expansion in lowland areas (Faure *et al.*, 2002; Sakaguchi *et al.*, 2010). In fact, Monmonier analyses grouped coastal sites S3, S5 and the inland S4 – located in the head of a dry stream bed – together, reflecting past and present interconnections by alluvial fans in coastal Atacama. During Holocene and interglacial arid episodes, populations in the hyper-arid Atacama probably contracted, as a consequence of an overall trend of declining rainfall and groundwater discharge, as well as rising sea

Table 2 Within-population mean and total genetic diversity for *Nolana* populations occurring in three climatic regions at the southern margin of the Atacama Desert.

Climatic regions	Mean or total	No. of individuals	No. of populations	*K*	*S*	*Hd*	Π	π
North (25–27° S)	Mean within-population	27	3	3.1	4.33	0.65 (0.12)	2.65 (2.67)	0.0016 (0.0011)
	Total	47	4	10	13	0.85 (0.03)	3.82 (1.95)	0.0023 (0.0001)
Central (27–30° S)	Mean within-population	27	3	1.3	2	0.11 (0.05)	0.32 (0.40)	0.0002 (0.0002)
	Total	41	4	5	7	0.78 (0.03)	1.99 (1.15)	0.0012 (0.0001)
South (30–33° S)	Mean within-population	27	3	0	0	0	0	0
	Total	42	6	3	4	0.66 (0.002)	1.74 (1.03)	0.0011 (0.0001)

K, number of haplotypes; *S*, number of polymorphic sites; *Hd*, haplotypic diversity; Π, mean pairwise differences; π, nucleotide diversity. Standard deviations are given in parentheses. For mean within-population diversity in each climatic region, sample sizes were standardized to $n = 9$.

Table 3 Patterns of demographic expansion for five groups of Chilean *Nolana* defined by SAMOVA. Standard deviations are given in parentheses. Mismatch distributions were evaluated under a model of demographic expansion and spatial expansion using the sum of square differences (SSD) between the observed and the expected mismatch as a test statistic.

								Demographic expansion model		Spatial expansion model	
	n	*K*	Π	Tajima's *D*	*P*-value	Fu's F_S	*P*-value	SSD	*P*-value	SSD	*P*-value
Group 1	22	3	3.21 (1.72)	2.093	< 0.05†	5.473	0.978	0.450	0.000	0.129	0.062
Group 2	20	4	1.61 (1.00)	0.105	0.607	1.170	0.746	0.205	0.049*	0.179	0.013*
Group 3	12	2	0.50 (0.46)	−1.454	0.084	1.054	0.597	0.040	0.063	0.018	0.244
Group 4	61	7	1.42 (0.88)	−0.247	0.452	−0.569	0.402	0.008	0.101	0.008	0.069
Group 5	15	2	0.53 (0.47)	−1.685	0.013*	1.318	0.714	0.026	0.060	0.012	0.228
Total	130	14	3.44 (1.77)	0.111	0.595	−0.267	0.469	0.004	0.492	0.005	0.604

*Significant by coalescent simulations.
†Significant following published critical values (Tajima, 1989).

levels. Populations could have been restricted to rocky beaches and the foothills of coastal mountains where fog and marine spray can be trapped. Accordingly, Tajima's *D* is significantly high in the northernmost population S1, suggesting recent population contraction. Expansion/contraction cycles could have occurred several times over the Quaternary, producing remixing of lineages. Some lineages could have expanded southwards into the semi-arid region when aridity became widespread at the southern margin of the Atacama.

In contrast to populations from the extremely arid north, southern populations showed lower genetic variation, with most populations fixed for a single haplotype, as could be expected for colonizing populations. Reduced genetic diversity within southern populations of *Nolana* is consistent with the prediction derived from founder effects, as successive population bottlenecks during range expansion tend to reduce diversity (Hewitt, 1996). Furthermore, Group 5, composed of populations S10 and S11, revealed higher genetic diversity than their closest neighbours, with the presence of an ancestral and a derivative haplotype, suggesting long-term persistence of taxa during adverse periods in small habitat refugia followed by population mixing. This finding is supported by values of Tajima's *D* that suggest recent population expansion.

Long-distance dispersal into mediterranean climate areas can probably be assisted by seabirds (see Gillham, 1956), as plants of *Nolana* are commonly found close to seabird nesting sites (P.G.O., pers. obs.; see also Bernal *et al.*, 2006), or by oceanic dispersal, given the characteristics of plants and fruits (vegetative growth and mericarp buoyancy). One type of barrier for the continuous southward expansion of the distribution range of *Nolana* could be a river estuary interrupting terrestrial connectivity. The Copiapó River, the first major east–west river crossing the latitudinal gradient occupied by *Nolana*, could be a barrier for seed dispersal during humid periods. During arid phases of the glacial and post-glacial, dispersed seeds of *Nolana* could presumably cross these barriers, but river flooding during humid periods could promote isolation. Such barriers would explain the allele fixations that occur in the southernmost populations studied. In fact, the outlet of the Maipo River – one of the largest rivers in central Chile – represents the southern end of the current distribution range of *N. crassulifolia*.

The hypothesis of north-to-south migration raised here is also supported by the gradual divergence pattern observed in the Bayesian chronogram of haplotypes (Fig. 2). This result suggests that several migratory events occurred from north to south along the coast, followed by local northward

recolonization. The calculated divergence dates position the split of haplotypes before the last glacial period, in the mid-Pleistocene, indicating that the current distribution could be the product of climatic fluctuations in the last glacial period and the Holocene.

CONCLUSIONS

Overall, we provide genetically and phylogeographically based evidence that populations of two closely related species in a *Nolana* species complex persisted at northern locations of their geographical range during glacial events, accumulating much genetic diversity, which is still evident today. During drier periods associated with the interglacial phases, the northernmost populations became restricted to moist/foggy coastal refugia, while the southern range expanded following higher temperatures and greater aridity. This sequence of events may have recurred during glacial–interglacial cycles, promoting lineage mixing. Some populations remained isolated in southern habitats, showing signs of expansion today. These patterns are in agreement with the classic scenario of post-glacial range expansion documented for the North American desert floras (Nason *et al.*, 2002; Clark-Tapia & Molina-Freaner, 2003; Rebernig *et al.*, 2010), where wetter and colder glacial conditions triggered the contraction of drought-resistant desert plant species to lower, more arid latitudes. To our knowledge, this is the first study to offer genetic evidence of post-glacial range expansion of a desert plant in the southern Atacama Desert. Although no fossil records are available to document contraction/expansion events for *Nolana*, palaeoclimatic reconstructions for the last glacial–interglacial cycle (Maldonado & Villagrán, 2002, 2006; Kaiser *et al.*, 2008; Díaz *et al.*, 2012) tend to support our conclusions.

ACKNOWLEDGEMENTS

We thank: M.O. Dillon for his comments that helped to improve the manuscript, help in plant identification, and especially for his dedication to the taxonomy of *Nolana* and the natural history of the Atacama Desert flora, which inspires P.G.O.'s line of research; J. M. Fariña for help in the field; G. Rojas from MNHN Santiago, Chile, for showing us voucher specimens; P. Spencer and G. Peralta for help in lab procedures; P. Montenegro for help in drafting maps; and A. Farias and M. Boshard for help with statistical analyses. Funding was provided by CONICYT #24091079 (to P.G.O.), FONDAP 1501-2001 to CASEB, PFB-23 and ICM P05-002 to the IEB, and FONDECYT #1090339 (to F.P.).

REFERENCES

Armesto, J.J., Vidiella, P.E. & Gutiérrez, J.R. (1993) Plant communities of the fog-free coastal desert of Chile: plant strategies in a fluctuating environment. *Revista Chilena de Historia Natural*, **66**, 271–282.

Bandelt, H.-J., Forster, P. & Röhl, A. (1999) Median-joining networks for inferring intraspecific phylogenies. *Molecular Biology and Evolution*, **16**, 37–48.

Bernal, M., Simeone, A. & Flores, M. (2006) Breeding of wedge-rumped storm-petrels (*Oceanodroma tethys*) in northern Chile. *Ornitologia Neotropical*, **17**, 283–287.

Betancourt, J.L., Latorre, C., Rech, J.A., Quade, J. & Rylander, K.A. (2000) A 22,000-year record of monsoonal precipitation from northern Chile's Atacama Desert. *Science*, **289**, 1542–1546.

Burton, R.S. & Feldman, M.W. (1981) Population genetics of *Tigriopus californicus*. II. Differentiation among neighboring populations. *Evolution*, **35**, 1192–1205.

Cain, M.L., Milligan, B.G. & Strand, A.E. (2000) Long-distance seed dispersal in plant populations. *American Journal of Botany*, **87**, 1217–1227.

di Castri, F. & Hajek, E.R. (1976) *Bioclimatología de Chile*. Ediciones Universidad Católica de Chile, Santiago, Chile.

Clark-Tapia, R. & Molina-Freaner, F. (2003) The genetic structure of a columnar cactus with a disjunct distribution: *Stenocereus gummosus* in the Sonoran desert. *Heredity*, **90**, 443–450.

CLC Bio A/S (2008) *CLC Sequence Viewer*. Version 6.6 (and previous). CLC Bio, Aarhus, Denmark. Available at: http://www.clcbio.com/products/clc-sequence-viewer/ (accessed 1 September 2012).

Clement, M., Posada, D. & Crandall, K.A. (2000) TCS: a computer program to estimate gene genealogies. *Molecular Ecology*, **9**, 1657–1659.

Darriba, D., Taboada, G.L., Doallo, R. & Posada, D. (2012) jModelTest 2: more models, new heuristics and parallel computing. *Nature Methods*, **9**, 772.

Díaz, F.P., Latorre, C., Maldonado, A., Quade, J. & Betancourt, J.L. (2012) Rodent middens reveal episodic, long-distance plant colonizations across the hyperarid Atacama Desert over the last 34,000 years. *Journal of Biogeography*, **39**, 510–525.

Dillon, M.O. (2005) The Solanaceae of the lomas formations of Coastal Peru and Chile. *Festschrift for William G. D'Arcy: the legacy of a taxonomist* (ed. by R.C. Keating, V.C. Hollowell and T.B. Croat), pp. 131–155. Monographs in Systematic Botany, Vol. 104. Missouri Botanical Garden, St Louis, MO.

Dillon, M.O. & Hoffman J., A.E. (1997) Lomas formations of the Atacama Desert, northern Chile. *Centres of plant diversity, a guide and strategy for their conservation* (ed. by S.D. Davis, V.H. Heywood, O. Herrera-McBryde, J. Villa-Lobos and A.C. Hamilton), pp. 528–535. World Wildlife Fund, Information Press, Oxford.

Dillon, M.O., Tu, T., Soejima, A., Yi, T., Nie, Z., Tye, A. & Wen, J. (2007) Phylogeny of *Nolana* (Nolaneae, Solanoideae, Solanaceae) as inferred from granule-bound starch synthase I (GBSSI) sequences. *Taxon*, **56**, 1000–1011.

Dillon, M.O., Tu, T., Xie, L., Quipuscoa Silvestre, V. & Wen, J. (2009) Biogeographic diversification in *Nolana* (Solanaceae), a ubiquitous member of the Atacama and Peruvian Deserts along the western coast of South America. *Journal of Systematics and Evolution*, **47**, 457–476.

Drummond, A.J., Ho, S.Y.W., Phillips, M.J. & Rambaut, A. (2006) Relaxed phylogenetics and dating with confidence. *PLoS Biology*, **4**, e88.

Drummond, A.J., Suchard, M.A., Xie, D. & Rambaut, A. (2012) Bayesian phylogenetics with BEAUti and the BEAST 1.7. *Molecular Biology and Evolution*, **29**, 1969–1973.

Dunai, T.J., González López, G.A. & Juez-Larré, J. (2005) Oligocene–Miocene age of aridity in the Atacama Desert revealed by exposure dating of erosion-sensitive landforms. *Geology*, **33**, 321–324.

Dupanloup, I., Schneider, S. & Excoffier, L. (2002) A simulated annealing approach to define the genetic structure of populations. *Molecular Ecology*, **11**, 2571–2581.

Excoffier, L. & Lischer, H.E.L. (2010) Arlequin suite ver 3.5: a new series of programs to perform population genetics analyses under Linux and Windows. *Molecular Ecology Resources*, **10**, 564–567.

Faure, H., Walter, R.C. & Grant, D.R. (2002) The coastal oasis: ice age springs on emerged continental shelves. *Global and Planetary Change*, **33**, 47–56.

Forster, P., Torroni, A., Renfrew, C. & Röhl, A. (2001) Phylogenetic star contraction applied to Asian and Papuan mtDNA evolution. *Molecular Biology and Evolution*, **18**, 1864–1881.

Fraser, C.I., Thiel, M., Spencer, H.G. & Waters, J.M. (2010) Contemporary habitat discontinuity and historic glacial ice drive genetic divergence in Chilean kelp. *BMC Evolutionary Biology*, **10**, 203.

Garrick, R.C., Nason, J.D., Meadows, C.A. & Dyer, R.J. (2009) Not just vicariance: phylogeography of a Sonoran Desert euphorb indicates a major role of range expansion along the Baja peninsula. *Molecular Ecology*, **18**, 1916–1931.

Gillham, M.E. (1956) Ecology of the Pembrokeshire islands. V. Manuring by the colonial seabirds and mammals, with a note on seed distribution by gulls. *Journal of Ecology*, **44**, 429–454.

Grivet, D. & Petit, R.J. (2002) Phylogeography of the common ivy (*Hedera* sp.) in Europe: genetic differentiation through space and time. *Molecular Ecology*, **11**, 1351–1362.

Hewitt, G.M. (1996) Some genetic consequences of ice ages, and their role in divergence and speciation. *Biological Journal of the Linnean Society*, **58**, 247–276.

Hewitt, G.M. (2000) The genetic legacy of the Quaternary ice ages. *Nature*, **405**, 907–913.

Jackson, S.T. & Overpeck, J.T. (2000) Responses of plant populations and communities to environmental changes of the late Quaternary. *Paleobiology*, **26**, 194–220.

Jezkova, T., Jaeger, J.R., Marshall, Z.L. & Riddle, B.R. (2009) Pleistocene impacts on the phylogeography of the desert pocket mouse (*Chaetodipus penicillatus*). *Journal of Mammalogy*, **90**, 306–320.

Johnston, J.S. (1936) A study of the Nolanaceae. *Contributions from the Gray Herbarium of Harvard University*, **112**, 1–87.

Kaiser, J., Schefuß, E., Lamy, F., Mohtadi, M. & Hebbeln, D. (2008) Glacial to Holocene changes in sea surface temperature and coastal vegetation in north central Chile: high *versus* low latitude forcing. *Quaternary Science Reviews*, **27**, 21–22.

Lamy, F., Hebbeln, D. & Wefer, G. (1999) High-resolution marine record of climatic change in mid-latitude Chile during the last 28,000 years based on terrigenous sediment parameters. *Quaternary Research*, **51**, 83–93.

Latorre, C., Betancourt, J.L., Rech, J.A., Quade, J., Holmgren, C., Placzek, C., Maldonado, A.J.C., Vuille, M. & Rylander, K.A. (2005) Late Quaternary history of the Atacama Desert. *23°S: archaeology and environmental history of the southern deserts* (ed. by M. Smith and P. Hesse), pp. 73–90. National Museum of Australia Press, Canberra.

Lessa, E.P., Cook, J.A. & Patton, J.L. (2003) Genetic footprints of demographic expansion in North America, but not Amazonia, during the Late Quaternary. *Proceedings of the National Academy of Sciences USA*, **100**, 10331–10334.

Librado, P. & Rozas, J. (2009) DnaSP v5: a software for comprehensive analysis of DNA polymorphism data. *Bioinformatics*, **25**, 1451–1452.

Luebert, F. & Pliscoff, P. (2006) *Sinopsis bioclimática y vegetacional de Chile*. Editorial Universitaria, Santiago, Chile.

Maldonado, A. & Villagrán, C. (2002) Paleoenvironmental changes in the semiarid coast of Chile (~32 S) during the last 6200 cal years inferred from a swamp-forest pollen record. *Quaternary Research*, **58**, 130–138.

Maldonado, A. & Villagrán, C. (2006) Climate variability over the last 9900 cal yr BP from a swamp forest pollen record along the semiarid coast of Chile. *Quaternary Research*, **66**, 246–258.

Maldonado, A., Betancourt, J.L., Latorre, C. & Villagrán, C. (2005) Pollen analyses from a 50 000-yr rodent midden series in the southern Atacama Desert (25° 30′ S). *Journal of Quaternary Science*, **20**, 493–507.

Manni, F., Guerard, E. & Heyer, E. (2004) Geographic patterns of (genetic, morphologic, linguistic) variation: how barriers can be detected by using Monmonier's algorithm. *Human Biology*, **76**, 173–190.

Miller, M.P. (2005) Alleles in Space: computer software for the joint analysis of interindividual spatial and genetic information. *Journal of Heredity*, **96**, 722–724.

Nason, J.D., Hamrick, J.L. & Fleming, T.H. (2002) Historical vicariance and postglacial colonization effects on the evolution of genetic structure in *Lophocereus*, a Sonoran Desert columnar cactus. *Evolution*, **56**, 2214–2226.

Nei, M. (1987) *Molecular evolutionary genetics*. Columbia University Press, New York, NY.

Paradis, E. (2010) pegas: an R package for population genetics with an integrated–modular approach. *Bioinformatics*, **26**, 419–420.

Peakall, R. & Smouse, P.E. (2006) GENALEX 6: genetic analysis in Excel. Population genetic software for teaching and research. *Molecular Ecology Notes*, **6**, 288–295.

Placzek, C., Quade, J., Betancourt, J.L., Patchett, P.J., Rech, J.A., Latorre, C., Matmon, A., Holmgren, C. & English, N.B. (2009) Climate in the dry central Andes over geologic, millennial, and interannual timescales. *Annals of the Missouri Botanical Garden*, **96**, 386–397.

Pons, O. & Petit, R.J. (1996) Measuring and testing genetic differentiation with ordered *versus* unordered alleles. *Genetics*, **144**, 1237–1245.

Prim, R.C. (1957) Shortest connection networks and some generalizations. *Bell System Technical Journal*, **36**, 1389–1401.

R Development Core Team (2012) *R: a language and environment for statistical computing*. R Foundation for Statistical Computing, Vienna, Austria. Available at: http://www.r-project.org/.

Rambaut, A. (2012) *FigTree v1.4*. University of Edinburgh, Edinburgh, UK. Available at: http://tree.bio.ed.ac.uk/software/figtree/.

Rambaut, A. & Drummond, A.J. (2007) *Tracer v1.4*. University of Edinburgh, Edinburgh, UK. Available at: http://beast.bio.ed.ac.uk/Tracer/ (accessed 1 December 2012).

Rebernig, C.A., Schneeweiss, G.M., Bardy, K.E., Schönswetter, P., Villaseñor, J.L., Obermayer, R., Stuessy, T.F. & Weiss-Schneeweiss, H. (2010) Multiple Pleistocene refugia and Holocene range expansion of an abundant southwestern American desert plant species (*Melampodium leucanthum*, Asteraceae). *Molecular Ecology*, **19**, 3421–3443.

Riddle, B.R., Hafner, D.J. & Alexander, L.F. (2000) Phylogeography and systematics of the *Peromyscus eremicus* species group and the historical biogeography of North American warm regional deserts. *Molecular Phylogenetics and Evolution*, **17**, 145–160.

Rojas, M., Moreno, P., Kageyama, M., Crucifix, M., Hewitt, C., Abe-Ouchi, A., Ohgaito, R., Brady, E.C. & Hope, P. (2009) The Southern Westerlies during the last glacial maximum in PMIP2 simulations. *Climate Dynamics*, **32**, 525–548.

Rundel, P.W., Dillon, M.O., Palma, B., Mooney, H.A., Gulmon, S.L. & Ehleringer, J.R. (1991) The phytogeography and ecology of the coastal Atacama and Peruvian deserts. *Aliso*, **13**, 1–49.

Sakaguchi, S., Sakurai, S., Yamasaki, M. & Isagi, Y. (2010) How did the exposed seafloor function in postglacial northward range expansion of *Kalopanax septemlobus*? Evidence from ecological niche modelling. *Ecological Research*, **25**, 1183–1195.

Shaw, J., Lickey, E.B., Beck, J.T., Farmer, S.B., Liu, W., Miller, J., Siripun, K.C., Winder, C.T., Schilling, E.E. & Small, R.L. (2005) The tortoise and the hare II: relative utility of 21 noncoding chloroplast DNA sequences. *American Journal of Botany*, **92**, 142–166.

Shaw, J., Lickey, E.B., Schilling, E.E. & Small, R.L. (2007) Comparison of whole chloroplast genome sequences to choose noncoding regions for phylogenetic studies in angiosperms: the tortoise and the hare III. *American Journal of Botany*, **94**, 275–288.

Taberlet, P., Fumagalli, L., Wust-Saucy, A.-G. & Cosson, J.-F. (1998) Comparative phylogeography and postglacial colonization routes in Europe. *Molecular Ecology*, **7**, 453–464.

Tajima, F. (1989) Statistical method for testing the neutral mutation hypothesis by DNA polymorphism. *Genetics*, **123**, 585–595.

Teacher, A.G.F. & Griffiths, D.J. (2011) HapStar: automated haplotype network layout and visualization. *Molecular Ecology Resources*, **11**, 151–153.

Tu, T., Dillon, M.O., Sun, H. & Wen, J. (2008) Phylogeny of *Nolana* (Solanaceae) of the Atacama and Peruvian deserts inferred from sequences of four chloroplast markers and the nuclear *LEAFY* second intron. *Molecular Phylogenetics and Evolution*, **49**, 561–573.

Viruel, J., Catalán, P. & Segarra-Moragues, J.G. (2012) Disrupted phylogeographical microsatellite and chloroplast DNA patterns indicate a vicariance rather than long-distance dispersal origin for the disjunct distribution of the Chilean endemic *Dioscorea biloba* (Dioscoreaceae) around the Atacama Desert. *Journal of Biogeography*, **39**, 1073–1085.

Williams, J.W., Shuman, B.N., Webb, T., III, Bartlein, P.J. & Leduc, P.L. (2004) Late-Quaternary vegetation dynamics in North America: scaling from taxa to biomes. *Ecological Monographs*, **74**, 309–334.

Wolfe, K.H., Li, W.H. & Sharp, P.M. (1987) Rates of nucleotide substitution vary greatly among plant mitochondrial, chloroplast, and nuclear DNA. *Proceedings of the National Academy of Sciences USA*, **84**, 9054–9058.

SUPPORTING INFORMATION

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

Appendix S1 States of morphological characters used in phylogenetic analyses of 12 Chilean shrub *Nolana* species.

Appendix S2 Majority-rule consensus tree of 12 Chilean shrub *Nolana* species based on Bayesian analysis of morphological characters and combined molecular data.

Appendix S3 Spatial correlogram illustrating the isolation-by-distance pattern.

BIOSKETCHES

Paulina G. Ossa is a PhD student in the Departamento de Ecología at Pontificia Universidad Católica de Chile in Santiago, and is affiliated to the Instituto de Ecología y Biodiversidad. Her research interest is the ecology and evolution of desert plants; she initiated the present work when she started the PhD program. She is currently working on the effects of ecophysiological and life-history traits on the evolutionary history of desert plants.

Fernanda Pérez is an assistant professor at the Pontificia Universidad Católica de Chile, and adjunct scientist at the Instituto de Ecología y Biodiversidad. She works on phylogeography and plant evolutionary ecology.

Juan J. Armesto is a professor at the Pontificia Universidad Católica de Chile and Director at the Instituto de Ecología y Biodiversidad. He works on ecosystem ecology and long-term ecology.

Author contributions: P.G.O. had the original idea and collected the data. F.P. and P.G.O. analysed the data. P.G.O., F.P. and J.J.A. designed the study and wrote the manuscript.

Editor: Liliana Katinas

Journal of Biogeography

SUPPORTING INFORMATION

Phylogeography of two closely related species of *Nolana* from the coastal Atacama Desert of Chile: post-glacial population expansions in response to climate fluctuations

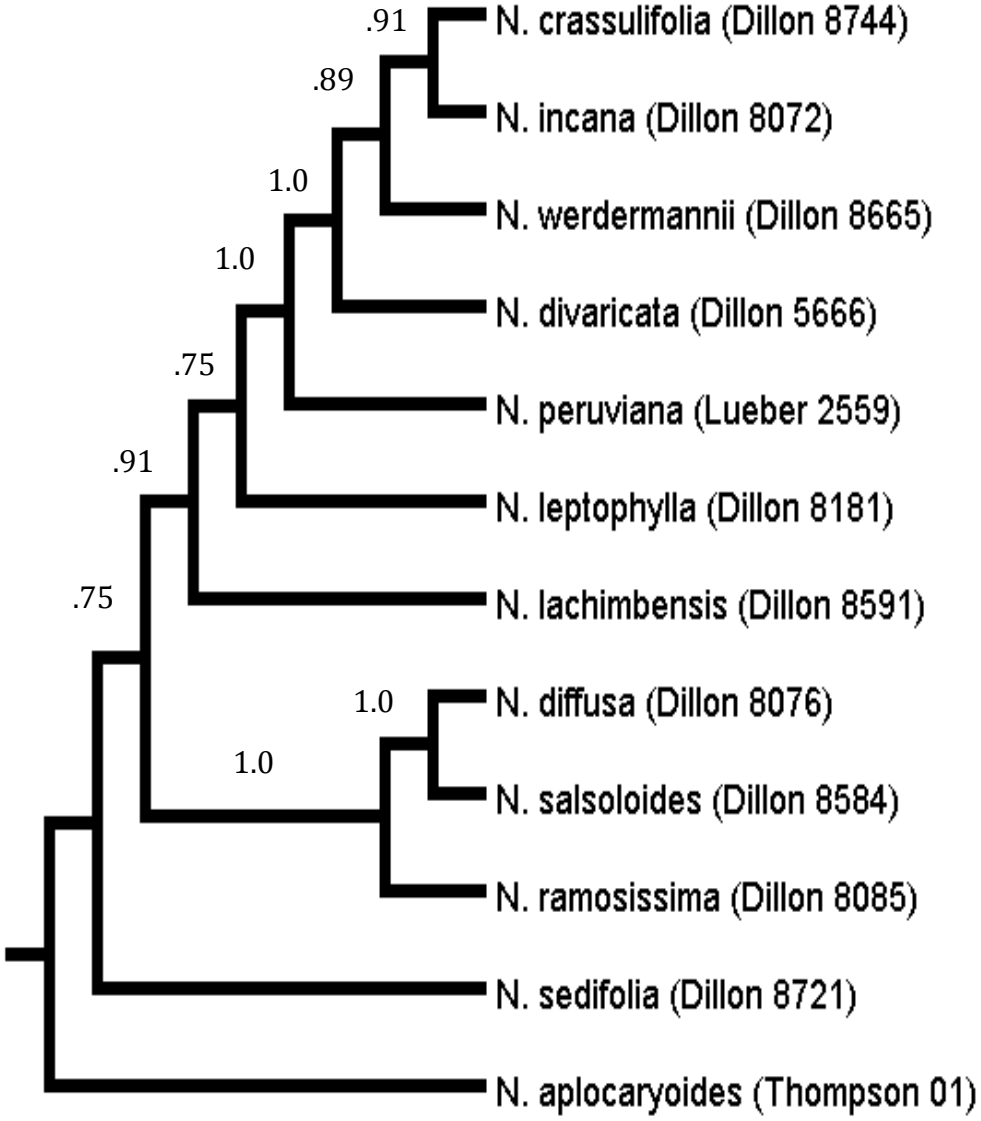
Paulina G. Ossa, Fernanda Pérez and Juan J. Armesto

Appendix S1 States of morphological characters used in phylogenetic analyses of Chilean shrub *Nolana* species.

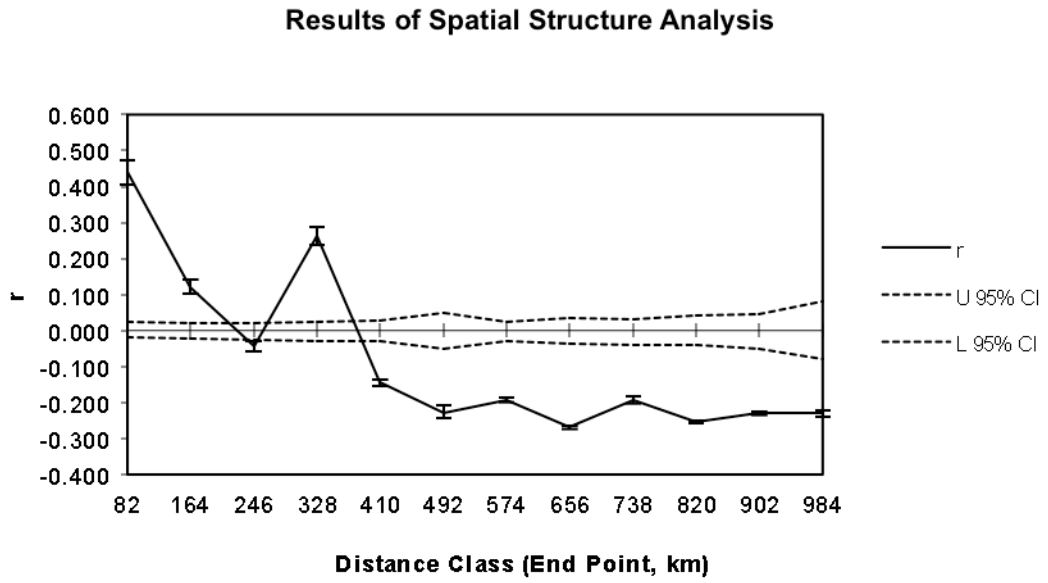
Species	LL	RW	LS	LM	LC	TR	GH	CP	FS	PL	LF
N. aplocaryoides	0	0	0	0	0	0	0	0	0	0	0
N. crassulifolia	0	1	0	0	1	1	1	1	0	1	1
N. diffusa	1	0	0	0	0	0	1	0	0	0	1
N. divaricata	1	0	1	1	0	0	0	0	0	0	1
N. incana	0	1	0	0	1	1	1	1	0	0	1
N. lachimbensis	1	0	1	0	1	0	0	0	0	0	1
N. leptophylla	1	0	0	0	0	0	0	0	1	1	1
N. peruviana	1	0	1	1	0	1	0	1	0	0	1
N. ramosissima	1	0	0	0	0	0	0	0	0	0	1
N. salsoloides	1	0	0	1	0	0	0	0	0	1	1
N. sedifolia	1	0	1	1	0	0	0	1	0	0	1
N. werdermannii	0	1	2	0	1	1	0	1	0	1	1

LL, leaf length (0, > 8 cm; 1, < 8 cm); RW, relative leaf width (0, length/width ratio < 6; 1, length/width ratio > 6); LS, leaf shape (0, spathulate; 1, globose; 2, linear); LM, leaf margin (0, revolute; 1, non-revolute); LC, leaf cleft (0, absent; 1, present); TR, star-like trichomes (0, absent; 1, present); FS, flower size (0, < 15 cm; 1, > 15 cm); GH, growth habit (0, erect; 1, postrate); CP, corolla pigmentation (0, blue to lavender; 1, white); PL, pedicel length (0, < 3 mm; 1, > 3 mm); LF, life form (0, annual; 1, perennial).

Appendix S2 Majority-rule consensus tree of Chilean shrub *Nolana* species (Clade FH in Tu *et al.*, 2008) based on Bayesian analysis of 11 morphological characters (Appendix S1) and combined sequences of *GBSII* nDNA (taken from Dillon *et al.*, 2007), *LEAFY* nDNA (taken from Tu *et al.*, 2007) and four cpDNA markers (taken from Tu *et al.*, 2008). Values above branches are Bayesian posterior probabilities. Bayesian inference of phylogeny with Markov chain Monte Carlo sampling was conducted for the concatenated data using MRBAYES 3.04 (Huelsenbeck & Ronquist, 2001). A general time-reversible model of DNA substitution and shape parameter of the gamma distribution (GTR+G) model was used with parameters partitioned across the six DNA regions. *Nolana aplocaryoides* was used as outgroup. One cold chain and three heated chains were run simultaneously for one million generations, and one tree per 100 generations was sampled. The first 500 trees were discarded as burn-in, and Bayesian posterior probabilities were estimated on the 50% majority rule consensus of the remaining trees.



Appendix S3 Spatial correlogram illustrating the isolation by distance pattern. Dashed lines represent upper and lower bounds of the 95% confidence intervals for *r*, estimated using bootstrapping.



REFERENCES

Dillon, M.O., Tu, T., Soejima, A., Yi, T., Nie, Z., Tye, A. & Wen, J. (2007) Phylogeny of *Nolana* (Nolaneae, Solanoideae, Solanaceae) as inferred from granule-bound starch synthase I (GBSSI) sequences. *Taxon*, **56**, 1000–1011.

Huelsenbeck, J.P. & Ronquist, F. (2001) MRBAYES: Bayesian inference of phylogenetic trees. *Bioinformatics*, **17**, 754–755.

Tu, T., Dillon, M.O., Sun, H. & Wen, J. (2008) Phylogeny of *Nolana* (Solanaceae) of the Atacama and Peruvian deserts inferred from sequences of four chloroplast markers and the nuclear *LEAFY* second intron. *Molecular Phylogenetics and Evolution*, **49**, 561–573.

Capitulo III

CHAPTER 3

On the role of life-form in shaping phylogeographic patterns: a comparative study in two *Nolana* (Solanaceae) congener lineages with contrasting life history traits

Authors: Paulina G. Ossa[1,2], Juan J. Armesto[1,2], Fernanda Pérez[1,2]

[1]Center for Advanced Studies in Ecology & Biodiversity (CASEB) and Departamento de Ecología, Pontificia Universidad Católica de Chile, Santiago, Chile, [2]Institute of Ecology & Biodiversity (IEB), Santiago, Chile

3.1 ABSTRACT

Plant diversity and distribution is intimately linked to the climatic history and life historic traits. To assess the influences of life form and life cycle in the patterns of diversity and distribution, we explored the phylogeographical pattern of an herbaceous set of *Nolana* species that grow from Atacama desert to Chiloé island, and compared with those published results for co-distributed congeneric shrubs.

Thirteen localities (89 individuals, 3 species) from 29º S to 43ºS were sampled, covering the entire distributional range. Two cpDNA regions were sequenced; we analysed the haplotype network, assessed population genetic diversity, differentiation and phylogeographical structure. We conducted demographic analyses, spatial analysis of genetic variation and identified barriers to gene flow. Finally we compare this finding with those from a shrubby *Nolana* lineage.

We found 14 haplotypes, three of them shared between species; significant genetic population differentiation but no phylogeographic structure. The ancestral haplotype was located in northernmost population, southernmost population consists of haplotypes genetically distant. Genetic diversity was higher north and southern populations, with haplotype fixation in central populations. In contrast, we found higher population structure in shrubs, with a linear pattern of decreasing diversity.

This results support a decreasing latitudinal pattern of diversity in both lineages between arid to semiarid transition, but with increment in diversity in herbs southern populations explained by glaciations and population refugia. The herbaceous habitus allowed plants to scape from colder year periods and colonize colder environments, reaching a wide distribution.

3.2 INTRODUCTION

The population genetic structure of species and their geographical distribution is the result of the interaction of historical, ecological and life-history factors (Hamrick & Godt, 1989, 1996; Nybom & Bartish, 2000; Nybom, 2004; Alvarez et al., 2009; Thiel-Egenter *et al*., 2009). The effect of historical events have been deeply studied (Taberlet *et al*., 1998; Hewitt, 2000), standing the Quaternary and its climatic oscillations as the most likely responsible of species modern distribution and genetic variation patterns (Hewitt, 1996, 2000). By contrast, the role of life-history traits on the plant species ability to track climate changes, and subsequently, their contribution to observed phylogeographical patterns remains poorly explored (Meirmans *et al*., 2011).

Life form, as well as generation time and dispersal ability, influence genetic divergence rates and therefore, might determinate the individualistic response of taxa to climate changes (Willis & Niklas, 2004). Herbaceous plants with short generation times exhibit higher rates of molecular evolution than their woody relatives (Smith & Donoghue, 2008), which are also correlated with higher rates of phenotypic and climatic niche evolution (Smith & Beaulieu, 2009). In addition, their rapid growth facilitates the colonization of new habitats (Baker & Stebbins, 1965), distinct patterns of fragmentation and postglacial expansions would be expected. Comparing phylogeographical patterns of co-distributed

species with contrasting life forms arise as a powerful tool to evaluate the past geographical and the relevance life traits in shaping those distributions.

The Chilean coastline from southern Coastal Atacama Desert to Mediterranean Central Chile offers a wide aridity latitudinal gradient (25°-33°S) to compare phylogeographic patterns of distinct plant life forms. During Quaternary, this area was affected by rainfall intensity fluctuations characterized by wet-cold phases and warm-dry phases (Kaiser *et al*., 2008; Diaz *et al*., 2012), that probably resulted from northward and southward displacement of westerly winds belt during glacial and interglacial respectively, in late Pleistocene (Heusser *et al*., 1999; Lamy *et al*., 1999; Shulmeister *et al*., 2004). Also, El Niño-Southern oscillations (ENSO) settlement (Moy *et al*., 2002; Rein *et al*., 2005; Koutavas *et al*., 2006) triggered wet/dry phases along this latitudinal gradient during Holocene. This fluctuations favoured vegetation shifts, as has been documented for swamp forest and xerophytic taxa at 32°S (Maldonado & Villagrán, 2002; Maldonado *et al*., 2005; Maldonado & Villagran, 2006) and northward expansion of temperate forests (Heusser, 1990; Valero-Garcés *et al*., 2005). Increased rainfalls and river floods during glacial or wet periods must have promoted local extinctions of southernmost arid coastal plant formations and persistence and divergence for the northern ones.

The coastal plant communities in the southern Atacama are strongly linked to the north-to-south increasing humidity gradient established along the coast (Armesto & Vidiella, 1993; Armesto *et al*., 1993). With a great functional diversity, some floristic elements extend their distribution from arid coastal plant communities to more humid, southern coastal plant formations. One of the most representative floristic elements of coastal Atacama Desert is the genus *Nolana* (Solanaceae), remarked by great species and life form diversity, going from

annual-herbs to perennial-shrubs (Dillon, 2005). Two lineages of different life form (herbs and shrubs) of *Nolana* expand through the coastal humidity gradient from coastal arid to semi-arid (until 33º S Lat.) and even temperate oceanic (42° for herbs). The widespread taxa in the herbaceous lineage is *Nolana paradoxa* Lindl., which grow in sandy to rocky beaches between 29 to 42°S. According to the nuclear marker GBSSI (Dillon et al. 2007b), this species is closely related to *N. acuminata* (Miers) Miers ex Dunal (25.33- 32°S), and *N. reichei* M.O. Dillon & Arancio (30.44°S). This relation is also supported by morphological similarities (Dillon et al. 2007a). The widespread taxa in the shrub lineage is *Nolana crassulifolia* Poepp., which grow in rocky seashore between 27.5-33.3° S, and is closely related with *N. incana* Phil. (25-26° S) (Dillon et al. 2007b, 2009; Tu et al. 2008) that also inhabits rocky seashore. Results from a previous study with the shrub lineage comprised by *N. crassulifolia* and *N. incana* show evidences of northward contraction during wetter glacial periods and southward expansion when aridity increase, a north-to-south gradient of decreasing genetic diversity, presence of ancestral haplotype in northern sites and loss of haplotypes to south (Ossa et al., 2013).

The aim of this work was to study the phylogeographical pattern of an herbaceous lineage of coastal desert plant conformed by *Nolana paradoxa* and its sister species *N. acuminata* and *N. reichei* along a wide range of the coast of Chile. To asses for the effect of distinct life form strategies in the phylogeography, we compared these findings with that obtained in a previous study for a shrubby lineage comprised by *N. crassulifolia* and *N. incana* (Ossa et al., 2013). We expect that given that these two lineages had a common quaternary history, their phylogeographic pattern should share general trends like loss of diversity to south according to a southern postglacial expansion pattern. But, as they differ on their life

strategies, we expect to find differences attributable to differences in life form and life cycle duration, as greater number of haplotypes with shorter branches and lesser population and phylogeographic structure as evidence of rapid evolutionary rate in herbaceous lineage.

3.3 MATERIALS AND METHODS

Species Lineages and study sites

We sampled 13 localities of herbaceous *Nolana* between 29.6ºS and 43ºS (1800 km) during the flowering period between October to December of 2009 and March/2009 for the southern limit of the distributional rank. The species sampled were *N. accuminata* (3 populations, 19 individuals), *N. reichei* (2 populations, 14 individuals) and *N. paradoxa* (9 populations, 56 individuals) (Table 3.1, Fig. 3.1). These herbaceous lineage (HL) goes from perennial with reduce vegetative growth during winter-cold period in the northern limit of the distribution to ephemeral or even annual in the southern limit; the flowering extend from spring (September-October) to Summer/Autumn limit in March. All are herbs with basal rosettes of leaves, thick taproot, stems prostrate to decumbent, relative large flowers, campanulate calyx; corollas blue to lavender with white to yellow centre; in *N. reichei* stands the absence of white but the presence of black. 10 to 20 mericarps, rounded (*N. paradoxa*) to angular (*N. acuminata*). *N. paradoxa* are restricted to coastal enviornments, whereas *N. acuminata* and *N. reichei* can grow further from the sea. The scarce studies in reproductive biology available suggest that this *Nolana* species are outcrossing (Freyre et al., 2005; Freyre & Douglas 2008). Mericarps dispersal occurs principally by gravity or ocean currents.

The sampled range was divided *a priori* in three coastal regions with different bioclimate based on Leubert & Pliscoff (2006): North region (27-31ºS), with desertic-oceanic Mediterranean bioclimate characterized by less than 200 mm/yr of precipitation; Central region (31-34ºS), with xeric-oceanic Mediterranean bioclimate were precipitation can reach 400mm/yr; and South region (up to 43ºS), characterized with Temperate-hiperoceanic bioclimate were precipitations easily exceed the 1000 mm/yr.

DNA extraction and molecular analyses

Leaf samples were stored in hermetic plastic bags and dried with silica gel. All samples were geo-referenced. Dried foliar tissues were ground in a QIAGEN TissueLyser and total genomic DNA was extracted using QIAGEN DNeasy Plant Mini Kit (Qiagen, Hilden, Germany). All extraction products were verified in 2.0% agarose gels. We tested 9 individuals (3 individuals from 1 population from north, central and south distribution) for amplification and polymorphism in 12 non coding cpDNA regions, including the regions: *trnS-trnR* (Doyle *et al*., 1992), *trnD-trnT* (Demesure *et al*., 1995), chloroplastidial microsatellites *ccmp4, ccmp6, ccmp7* (Wising & Gardner, 1999); *rpl16* intron*, rps16* intron (Shaw *et al*., 2005); and *trnV-ndL, trnS-trnG, trnQ-rps16, rpl32-trnL, ndhF-rpl32* (Shaw *et al*., 2007). The intergenic spacers *rpl32-trnL* and *rpl16* intron showed sufficient variability and were amplified for the totality of individuals. Amplifications were carried out following Shaw *et al*. (2005) and Shaw *et al*. (2007), using 12.5 uL GoTaq® Colorless Master Mix (Promega, Madison, USA), 0.5uM of each primer, 20 ng of DNA template, and nuclease free water (Promega) until reach a final volume of 25 uL. PCR products were verified in 1% agarose gels and sent to Macrogen Inc.

(Seoul, South Korea) for purification and sequentiation (forward and reverse primers). Some sequences that showed difficulties during PCR were purified using QIA-quick purification kit (Qiagen) and sequenced in the Molecular Diversity Laboratory of P. Universidad Católica (Chile). Sequence alignment and edition were performed using CLC SEQUENCE VIEWER 6.6 software (CLC Bio A/S, 2012). Indels due to Poly-N were ignored.

Data Analysis

The degree of genetic differentiation between species and population was examined with the Analysis of Molecular Variance (AMOVA) implemented in ARLEQUIN software package (version 3.5.1.3; Excoffier & Lischer, 2010). Estimations of number of haplotypes (K), haplotype (gene) diversity (Hd), nucleotide diversity (π) and the average number of nucleotide differences per site between two sequences ($\prod$) (Nei, 1987), were carried out using DNASP version 5.1 (Librado & Rozas, 2009).

Relatedness among haplotypes was represented by a statistical parsimony network, generated by the program TCS (Clement *et al*., 2000) and a network obtained by median joining with NETWORK 4.6.1.0 software (Bandelt *et al*., 1999). In order to simplify networks and estimate historic demographic expansion events, we also run the star contraction algorithm implemented in the NETWORK software (Forster *et al*., 2001). Minimum spanning tree (MST) was calculated using Prim's Algorithm (Prim, 1957) with HAPSTAR program (Teacher & Griffiths, 2010).

Genetic differentiation among populations was evaluated using the G_{ST} and N_{ST} coefficients (Pons & Petit, 1996). Both coefficients estimate the ratio between the mean

within-population genetic diversity and total genetic diversity, but while G_{ST} index makes use only of the allelic frequencies, N_{ST} also takes into account the genetic distances between haplotypes. These indices were statistically compared using 1000 permutations in PERMUTCPSSR (version 2.0, Pons & Petit, 1996). A greater N_{ST} means that more closely related haplotypes occur in the same population, indicating phylogeographic structure (Pons & Petit, 1996). A spatial autocorrelation analysis (SAA) with equal distance classes and a Mantel test were perfomred with GENALEX (Peakall & Smouse, 2006) to identify patterns of isolation by distance, for SAA a 95% confidence interval about the null hypothesis of no spatial structure was determined by 1000 permutations. Affinities between the populations were studied considering genetic and geographic distances with the spatial analysis of molecular variance (SAMOVA) with SAMOVA 1.0 (Dupanloup *et al*., 2002). The genetic barriers associated with each geographical location and population were investigated using Monmonier´s maximum-difference algorithm (Monmonier, 1973) in BARRIER v. 2.2 (Manni *et al*., 2004), this software allow to test for significance estimating bootstrap values when bootstrap matrixes are provided. 1000 bootstrap genetic distance matrix between populations were calculated with a script in R using functions provided in packages Pegas (Paradis, 2010) and Adegenet (Jombart, 2008) that allow to deal with sequence data and calculate Nei´s genetic distance between populations.

To examine possible founder effects and explore the latitudinal migration southward from a potential refugium in the north, we analyzed the variation of within-population genetic diversity with latitude. Given that the sampled sizes differ between sites, we performed Monte Carlo simulations and standardized population size to 10. These procedures were performed with a script made in R CRAN using function nuc.div from Pegas v.0.4-1 R package (Paradis,

2010) to calculate π. Mismatch distribution, Tajima´s Neutrality test, Fu´s neutrality test and fixation index pairwise comparison table were calculated using ARLEQUIN 3.5.

Finally, we compared this results with the one obtained for two shrubby *Nolana*: N. *crassulifolia* and *N. incana* from a previous study (Ossa *et al*., 2013). In that work those species were considered as species lineages, named in this work *Shrubby Lineage* (SL).

3.4 RESULTS

Phylogeography of Herbaceous Lineage

The alignment lengths of the *rpl32-trnL* and *rpl16* dataset were 834 and 915 bp, including one variable mononucleotide repeat. The 1749 bp combined data set contained 17 nucleotide substitutions where 11 were parsimony informative (Appendix S3.1). 14 haplotypes were identified within 89 sampled individuals from *N. acuminata* (8 haplotypes), *N. reichei* (6 haplotypes) and *N. paradoxa* (3 haplotypes). *Nolana acuminata* shared haplotypes with *N. paradoxa* (H1) and *N. reichei* (H9 and H11), and AMOVA (Table 3.2.a) showed no significant (n.s.) differences between species (FCT= 0.183, p>0.05). Therefore, we found no inconvenience to study the phylogeography of *N. paradoxa, N. acuminata* and *N. reichei* as a species lineage.

Network and Haplotypes diversity for the herbaceous lineage. Total haplotype (gene) diversity (Hd), total nucleotide diversity (π) and mean pairwise differences (∏) were 0.808, 0.0013 and 2.231 respectively (Table 1). Two haplotypes account for the 60% of the individuals with

frequencies of 33% (H2) and 27% (H1). These two haplotypes also reached the widest distribution, H1 with 374 Km. and H2 with 1150 Km., covering all the *N. paradoxa* type distributional. The remaining haplotypes have frequencies lower than 10% (Appendix 3.2), and nine of them were unique to one population. Eleven of these haplotypes (H4- H14) were distributed exclusively between 29-31ºS (A region).

The MJ and MP analysis resulted in the same network construction. A loop involving H5, H1, H3 and H7 was initially formed; the MST and MP tree broke the link between H3 and H7 (fig. 3.2(b)). The network was centred in H5, the ancestral haplotype according to out-group weight calculated by TCS program; with 9 links around connecting with closer descendant haplotypes by one (5 links) or two mutational (4 links) steps. The two longest branches involved 2 haplotypes and three mutational steps, and the four shortest linked to terminal haplotypes by one step. A secondary ramification was located around H9, with 2 one-step links to terminal haplotypes and one by two-steps; this node involve haplotypes from *N. acuminata* and *N. reichei* types from three geographically closer populations. The star contraction algorithm identified at first contraction a star-like formation around H4 and H9, contracting all the links around.

Population differentiation and phylogeographic structure. We found significant population differentiation using the coefficients for both unordered ($G_{st} = 0.561$, $p<0.0001$) and ordered alleles ($N_{st} = 0.532$, $p<0.0001$). However, they were not significantly different from each other, suggesting lack of phylogeographic structure.

The Monmonier algorithm (fig. 3.3) identified a strong genetic barrier between S4 and S5 (30-31ºS) with a bootstrap value (BV) of 82%, and also several small genetic barriers with

values lesser than 10% between the remaining southern populations. In order to explore whether this small barriers constitute real barriers to gene flux, we allow the algorithm to identify a second barrier; obtaining one between S10-S11 with a BV of 78.5%. A similar arrangement was detected by SAMOVA (Fig. 3.3) when the number of groups (k) was set to three. Group 1 comprised populations northern to 31ºS (S1-S4), group 2 included the central populations fixed for haplotype H1 (S5, S7, S9 and S10); group 3 comprised the remaining central populations (S6, S8) with the three southernmost populations (S11-S13) and is characterized by the presence of haplotypes H2 and H3. The proportion of variance explained by differences among groups (F_{CT}) was 53.1%; increasing F_{CT} values were obtained with increasing number of groups but failing to reach a plateau.

Latitudinal pattern in genetic diversity. The diversity indices K, Hd, and π varied strongly between populations (Table 3.1), reaching the highest values at both extremes of the distributional range. The highest levels of haplotype diversity were found in three populations located northern 31°S (S2, S3 and S4), whereas the highest level of nucleotide diversity were found in the southernmost populations (S13, S14). By contrast, the majority of central populations were fixed for a single haplotype. Accordingly, significant quadratic relations with latitude were detected with haplotype diversity (fig. 3.4.a) and nucleotide diversity (fig. 3.4.b). When differences in sample size among populations were considered, and genetic diversity indices were standardized for n=5, this quadratic relationship persisted (R^2= 0.39 for π and R^2=0.44 for Hd) without statistical significance.

Demographic analyses. The pairwise mismatch distribution for the entire sample was unimodal, with a maximum at 3 bp, and differ significantly from the null population expansion model (Table 3.4). Also, the more conservative estimate of population expansion, Tajima D,

was not significant, and therefore, did not indicate a departure from population equilibrium (D=-0.964, p=n.s.). The mismatch distributions of the three SAMOVA groups differed in shape, suggesting that these groups experienced independent demographic histories (Table 3.3). The mismatch distribution of the northern group was unimodal, with a low and insignificant SSD value. Tajima D and Fu´s FS were significant and negatives, suggesting that it had undergone demographic expansion. By contrast the southern group had a bimodal and significant mismatch distribution, and positive and non-significant values for Tajima D and Fu´s FS (Table 3.3), which is congruent with old stationary populations; while, central group was completely fixed for one haplotype, indicating neutral evolution.

Comparison between life form species lineages

The dataset for the SL consist of 130 sequences from 14 populations, with 1649 base pairs aligned and 18 polymorphic sites. Although the haplotype number were the same, SL had higher genetic diversity (Table 3.4) overall. AMOVA analyses (Table 3.2) reveal low molecular variance explained by the species level (11.4%, p=n.s. for SC; and 18.4%, p=n.s. for HL), and a strong bias in SL to among population variance explained (F_{ST}=0.774) rather than in HL (F_{ST}=0.599). Although both lineages were highly structured as expected for coastal species with a linear, fragmented distribution (Franks *et al.,* 2004); genetic population structure was stronger in SC, with significant signs of phylogeographic structure (Nst>Gst; p<0.05). For both lineages Fu´s FS and Tajima´s D didn't showed departures of neutrality, in HL, only mismatch analysis detect departures of the null expansion model. We found

differences in the trend of the latitudinal pattern of variation in genetic diversity, with a linear decreasing trend for SL (fig. 3.4(c) and (d)) and a quadratic and significant trend in HL.

3.5 DISCUSSION

The herbaceous lineage phylogeographic pattern responded to a southward migration tendency with high levels of genetic diversity and genetic differentiation in northern populations of the HL. These results, together with the presence of the ancestral haplotype in the northernmost population, suggest that these taxa contracted toward low latitudes during last glacial maxima (LGM). The absence of DNA haplotype variation within and among central-south populations (SAMOVA group 2), suggest recent population expansion from north to central-south, probably during increased-aridity phases of Holocene. One or more population(s) carrying haplotype H1 probably colonized the coast and extended to southern Chile. Founder effects during such a rapid expansion would be responsible for genetic homogeneity in these populations. Long-distance dispersal probably occurs by passive animal dispersion (*Nolana* individuals are commonly found close to sea-birds nidification areas, personal observation) or by oceanic currents, given the characteristics of the plant and fruits (vegetative growth and mericarp floatability).

Geographic distribution of genetic variation is in agree with the model of postglacial colonization with high genetic variation in northerly distributed areas and loss of diversity to south in the recolonized areas, but an increase on diversity was observed in the southernmost populations. These results suggest that these taxa was able to persist and survive since Quaternary to present in the north, but also that at higher latitudes they survived in refugia in

the limit of ice-sheet (Veit & Garleff, 1995) that would have covered the entire western slope of the Andes to 42°S. The fact of being a short-lived herb could have allowed *N. paradoxa* to reach distant geographic locations during favourable conditions and survive to wetter and colder periods maybe trough dormance or shortening the life cycle. Evidence of multiple refugia have been documented for cold tolerant arboreal species (Sersic et al, 2012; Premoli et al, 2010) from southern south America, but, to our knowledge, this is the first study that documents isolation in multiple refugia for a herbaceous species with an evolutionary origin linked to the Atacama Desert.

A rapid growth short-lived herb will reach a higher geographic range, and their ability to scape from the colder season might favoured their survivance in latitudes were the mean weather condition is colder. Once this plants reach higher latitudes, they can survive a major climate disruptance like glaciations, trough seed dormance or shorter life cycle when conditions allowed it. For the case of this Nolana herbs, wich center of origin is central-north Chile, the adaptations to drough they carry might help on their survivance in colder latitudes; like hard and dry mericarps covering seeds, seed dormance ability very common in deserts), succulent leaves with high concentration of solutes can confer frost resistance.

The phylogeographic pattern of HL differed from that found in the SC, conformed by *N. crassulifolia* and *N. incana,* which showed a monotonic decrease in diversity with latitude (fig. 3.4.c, d); concordant with a model of northern contraction of the flora during glacial and wetter periods, and subsequent southward expansion during postglacial and periods of increasing aridity. Also, the genetic diversity and differentiation were lower than those documented for the SL (Table 3.4). These results contrast with the idea that annual plants with short generation times should exhibit higher rates of molecular evolution (Smith & Donoghue,

2008), and high levels of population differentiation than their woody relatives (Nybom & Bartish, 2000). These results could be the reflection of prolonged aridity periods that have affected these herbaceous populations. The ephemeral and annual flora of the Atacama Desert usually germinate only during rainy years, specially those with positive ENSO anomalies giving account of the phenomena known as "flowering desert" (Vidiella *et al*., 1999.). On that way they have a smaller number of generation per unit of time than expected.

Conclusions

In summary, we find that differences in life form and other life-history traits (life-cycle, growth rate) can affect in a significant manner the phylogeography of plant taxa. The shorter life cycle in these *Nolana* herbs, together with the outcrossing condition, promote higher intrapopulation differentation, with genetically closer haplotypes. in contraposition, in shrubs with long life cycle, more mutations accumulates between generations, resulting in higher differentation between populations, with less haplotypes within populations but more distant. This differences account for different phylogeographic structures.

To our knowledge this is the first study comparing phylogeographic patterns of codistributes plant strategies that try to disentangle the role of functional traits in shaping the geographic distribution of genetic diversity.

Table 3.1. Genetic diversity indexes by populations. Location, latitude, longitude, geographic region, sample size (n) and measurements of genetic diversity of two populations of *N. acuminata* one of *N. reichei*, one sympatric site between *N. acuminata* and *N. reichei* (S04); and 9 of *N. paradoxa*. K, number of haplotypes; S, number of polymorphic sites; H, haplotype diversity; ∏, mean pairwise differences; π, nucleotide diversity. Standard deviations are indicated in parenthesis. Numbers after localities indicate administrative regions of Chile. Geographic regions based on bioclimatic classification as described in text.

Code	Species	Locality	Lat (ºS)	Lon (ºW)	Region	n	K	S	Hd	∏	π
S01	*N. acuminata*	Hornos	29.61	71.29	North	11	4	5	0.6 (0.154)	1.055 (0.754)	0.0006 (0.0005)
S02	*N. acuminata*	Tongoy	30.26	71.49	North	6	4	6	0.800 (0.172)	2.200 (1.407)	0.0013 (0.0009)
S03	*N. reichei*	Talcaruca	30.49	71.69	North	10	4	5	0.733 (0.101)	2.022 (1.239)	0.0012 (0.0008)
S04	*Sympatric N.ac & N.re*	Limarí	30.73	71.70	North	6	3	4	0.733 (0.155)	2.333 (1.476)	0.0013 (0.0009)
S05	*N. paradoxa*	Chigualoco	31.76	71.51	Central	10	1	0	0	0	0
S06	*N. paradoxa*	P. Amarilla	31.86	71.51	Central	10	1	0	0	0	0
S07	*N. paradoxa*	Caucau	32.71	71.49	Central	3	1	0	0	0	0
S08	*N. paradoxa*	Quintay	33.18	71.69	Central	6	2	3	0.533 (0.172)	1.600 (1.095)	0.0009 (0.0007)
S09	*N. paradoxa*	El Quisco	33.38	71.69	Central	5	1	0	0	0	0
S10	*N. paradoxa*	S. Domingo	33.67	71.64	Central	3	1	0	0	0	0
S11	*N. paradoxa*	Mehuin	39.44	73.22	South	10	1	0	0	0	0
S12	*N. paradoxa*	Hueicocolla	40.16	73.66	South	5	2	4	0.6 (0.175)	2.4 (1.557)	0.0014 (0.0010)
S13	*N. paradoxa*	Chiloé	42.9	73.48	South	4	2	4	0.667 (0.204)	2.667 (1.778)	0.0015 (0.0012)
					Total	**89**	**14**	**17**	**0.808 (0.201)**	**2.231 (1.241)**	**0.0013 (0.0008)**

Table 3.2 AMOVA results for the (a) herbaceous lineage of species and the (b) shrubby lineage. For both species lineages the analyses was performed considering the species identities. differentiation indexes: FCT, among groups; FSC, among populations within groups; FST, among populations.

Source	df	SS	Est. Var.	%	index	score	*P*
(a) Herbaceous Lineage							
Species level							
Among Species	2	20.598	0.232	18.4%	**FCT**	0.183	0.06
Among Pops	11	39.386	0.522	41.3%	**FSC**	0.506	<0.0001
Within Pops	85	38.173	0.509	40.3%	**FST**	0.597	<0.0001
Total	88	98.157	1.263	100%			
(b) Shrubby Lineage							
Species level							
Among Species	1	30.281	0.223	11.4%	**FCT**	0.114	0.085
Among Pops	13	141.128	1.286	66.0%	**FSC**	0.745	<0.001
Within Pops	115	50.584	0.440	22.6%	**FST**	0.774	<0.001
Total	129	221.992	1.948	100%			

Table 3.3 Patterns of demographic expansion of 3 groups (SAMOVA) for Herbaceous Lineage. Standard deviations are given between parentheses. * Significant P-value of the statistic as the proportion of random statistics less or equal to the observation following coalescent simulations.

	Group 1	Group 2	Group 3	Total
n	33	21	35	89
K	12	1	3	14
∏	2.136	0	1.086	2.23
Tajima´s D	-1.527	0	0.291	-0.964
D *P*-value	0.044*	0	0.657	0.176
Fu´s FS	-4.706	0	1.996	-3.336
FS *P*-value	0.011*	0	0.847	0.098
Mismatch Distribution				
Model: Demographic				
SSD	0.006	0	0.079	0.05
SSD *P*-value	0.251	0	0.048*	0.037*
Model: Spatial				
SSD	0.006	0	0.021	0.042
SSD *P*-value	0.287	0	0.372	0.095

Table 3.4 Comparison of genetic diversity between Shrubby and Herbaceous lineage. Number of individuals (N); number of populations; number of ncleotides (bp); haplotypes; number of haplotypes (K); number of polymorphic sites (S); haplotypes diversity (Hd); nucleotide diversity (π); mean pairwaise differences ($\prod$); Genetic population differentiation indexes (Gst, Nst) and P-value for Nst > Gst test; Tajima neutrality test (D), Fu neutrality test (FS); mismatch distribution analysis (SSD), significance between parentheses.

	Shrubby lineage	**Herbaceous lineage**
N	130	89
nº pops	14	13
bp	1649	1749
K	14	14
S	18	17
Hd	0.904	0.808
π	0.0021	0.0013
$\prod$	3.442	2.231
Gst	0.76	0.561
Nst	0.81	0.532
***P* (Nst>Gst)**	0.04*	n.s.
D	0.111	0.176
FS	-0.267	-3.336
SSD	0.004 (n.s)	0.05 (*)

Figures legend

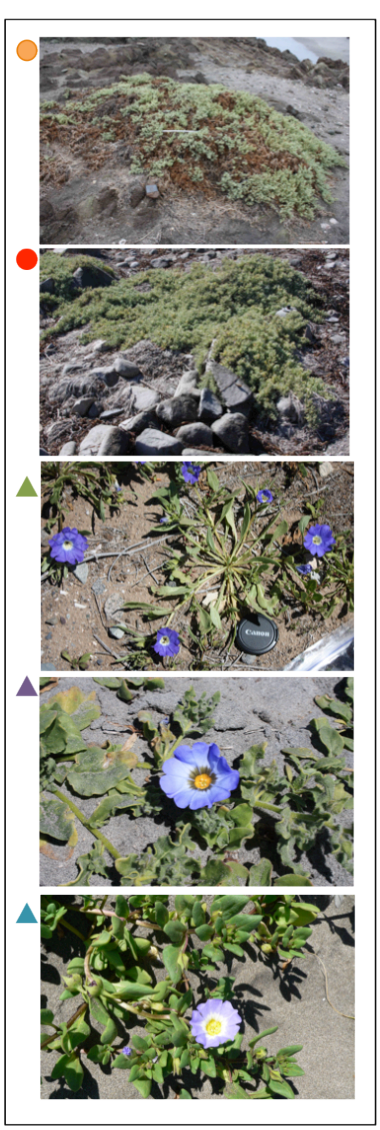
Figure 3.1 Illustration of species sampled (a) in this study; symbol in the left corner indicate life form: shrubs (circle), herbs (triangle); in the Map (b) the sampling localities are identified by species (colour) and life form (symbol). The species are: *N. incana* (orange), *N. crassulifolia* (red) *N. acuminata* (green), *N. reichei* (purple) and *N. paradoxa* (turquese); sympatric localities within life form are indicated by bi-colour symbols.

Figure 3.2 Geographic distribution and genealogical relationships of the 14 cpDNA haplotypes found in the herbaceous lineage. (a) Pie charts proportional to population size illustrating the frequency of each haplotype by site, colours correspond to those shown in the network. (b) Statistical maximum parsimony network linking the fourteen haplotypes, Circle sizes represents haplotype frequencies and branch lengths the number of mutational steps.

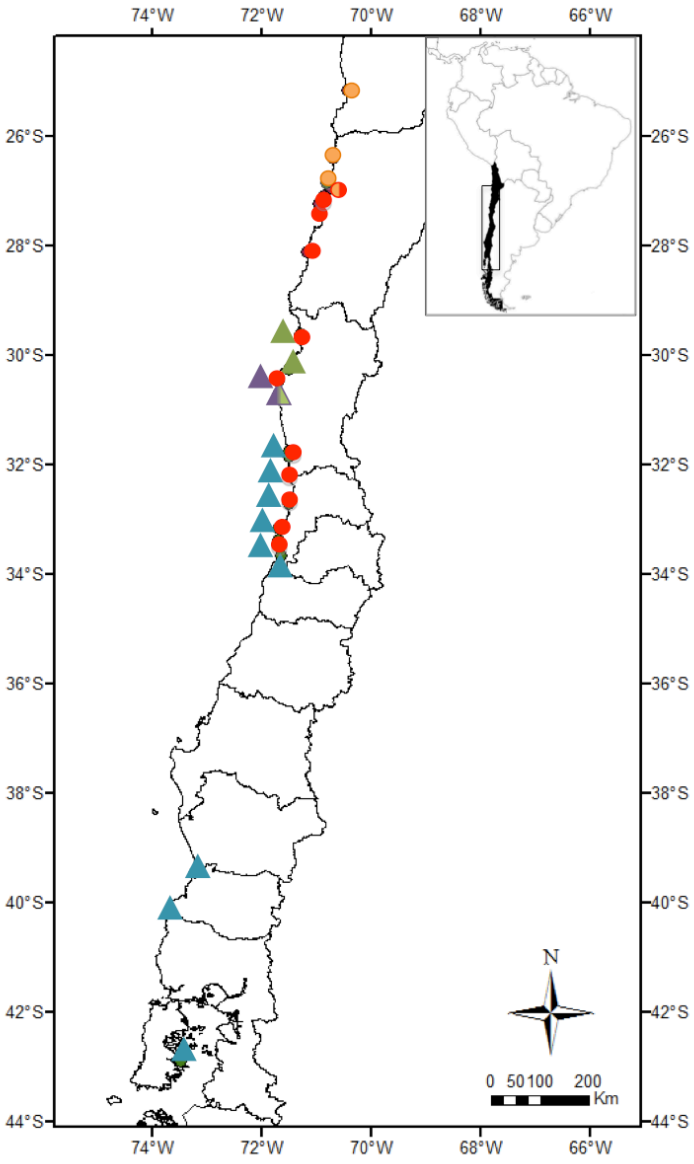
Figure 3.3 Barriers to gene flow calculated according the Monmonier algorithm; two most supported separated three phylogeographic regions. The barriers were calculated allowing the search of two barriers per matrix for a total of 1000 bootstrapped matrixes; barriers were considered as additives, values indicate the % of times a barrier was constructed in both surveys; only barriers with bootstrap values higher than 0.5% are shown. Symbols near populations indicate the SAMOVA aggrupation's: group 1 (star), group 2 (triangle), group 3 (circle).

Figure 3.4 Latitudinal gradient on genetic diversity measured with raw values and standardized at n=5 . (a) and (b) quadratic regressions for haplotype and nucleotide diversity for the herbaceous lineage.(c) and (d) linear regressions for haplotype and nucleotide diversity for the shrubby lineage. * statistically significant, $p<0.05$.

Figure 3.1



(a)


74°W
72°W
70°W
68°W
66°W
26°S
28°S
30°S
32°S
34°S
36°S
38°S
40°S
42°S
44°S
N
0 50 100 200
Km

(b)

Figure 3.2

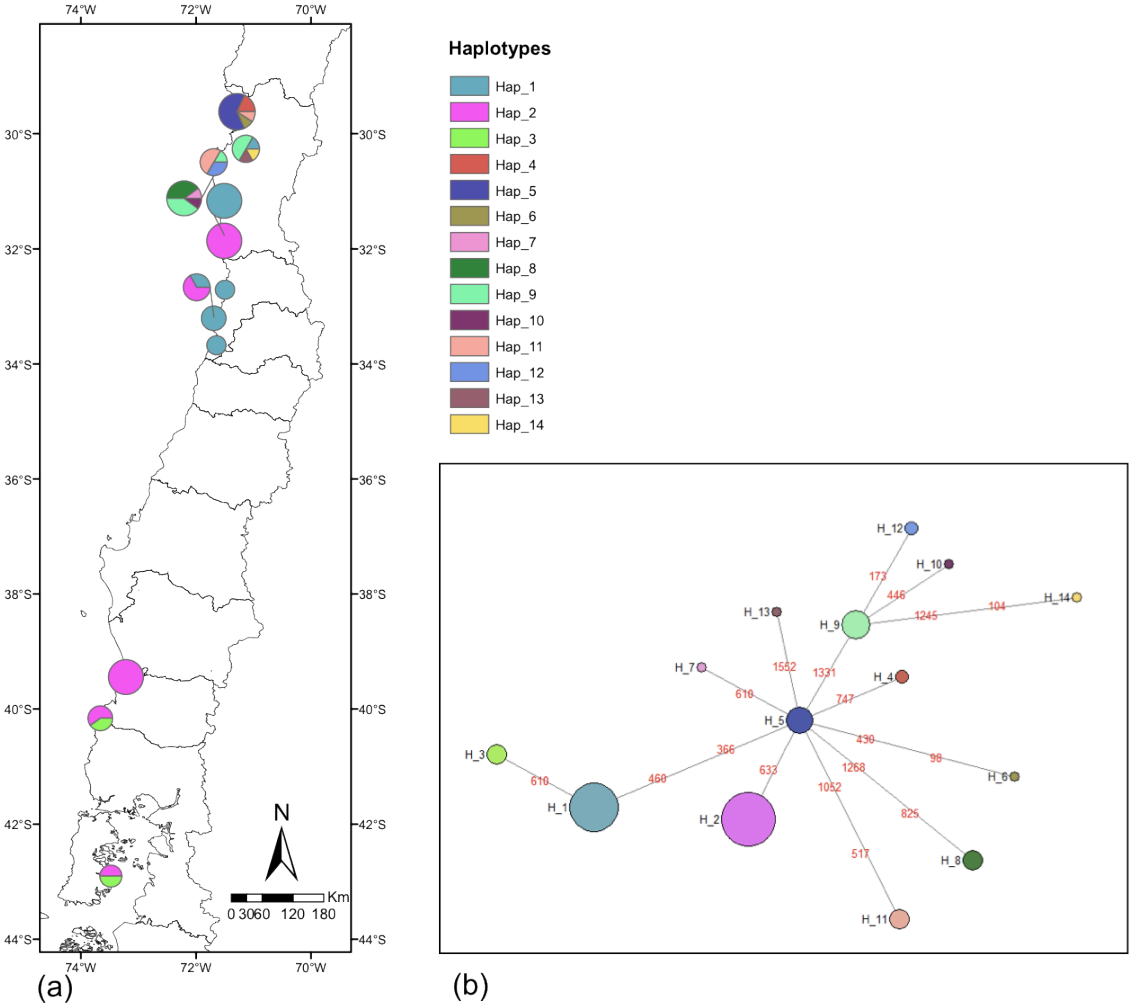

74°W
72°W
70°W
30°S
32°S
34°S
36°S
38°S
40°S
42°S
44°S
N
Km
0 3060 120 180
(a)
Haplotypes
Hap_1
Hap_2
Hap_3
Hap_4
Hap_5
Hap_6
Hap_7
Hap_8
Hap_9
Hap_10
Hap_11
Hap_12
Hap_13
Hap_14
H_12
H_10
H_14
H_13
H_9
H_7
H_4
H_5
H_3
H_6
H_1
H_2
H_8
H_11
173
446
1245
104
1552
1331
610
747
366
439
610
460
633
1268
98
1052
825
517
(b)

Figure 3.3

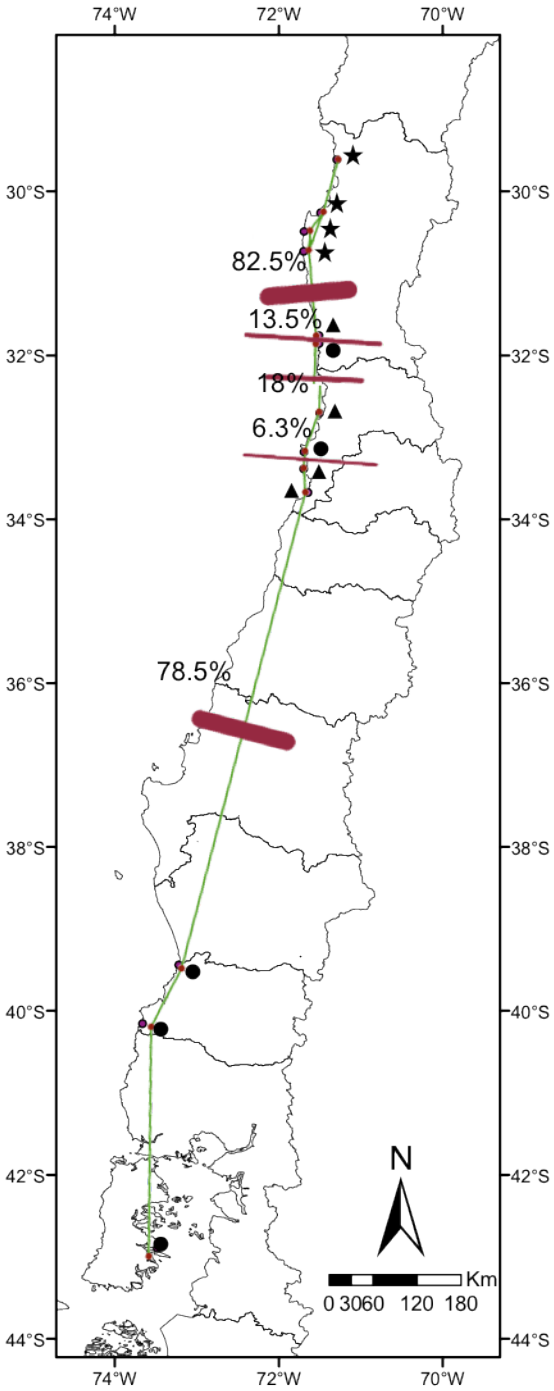
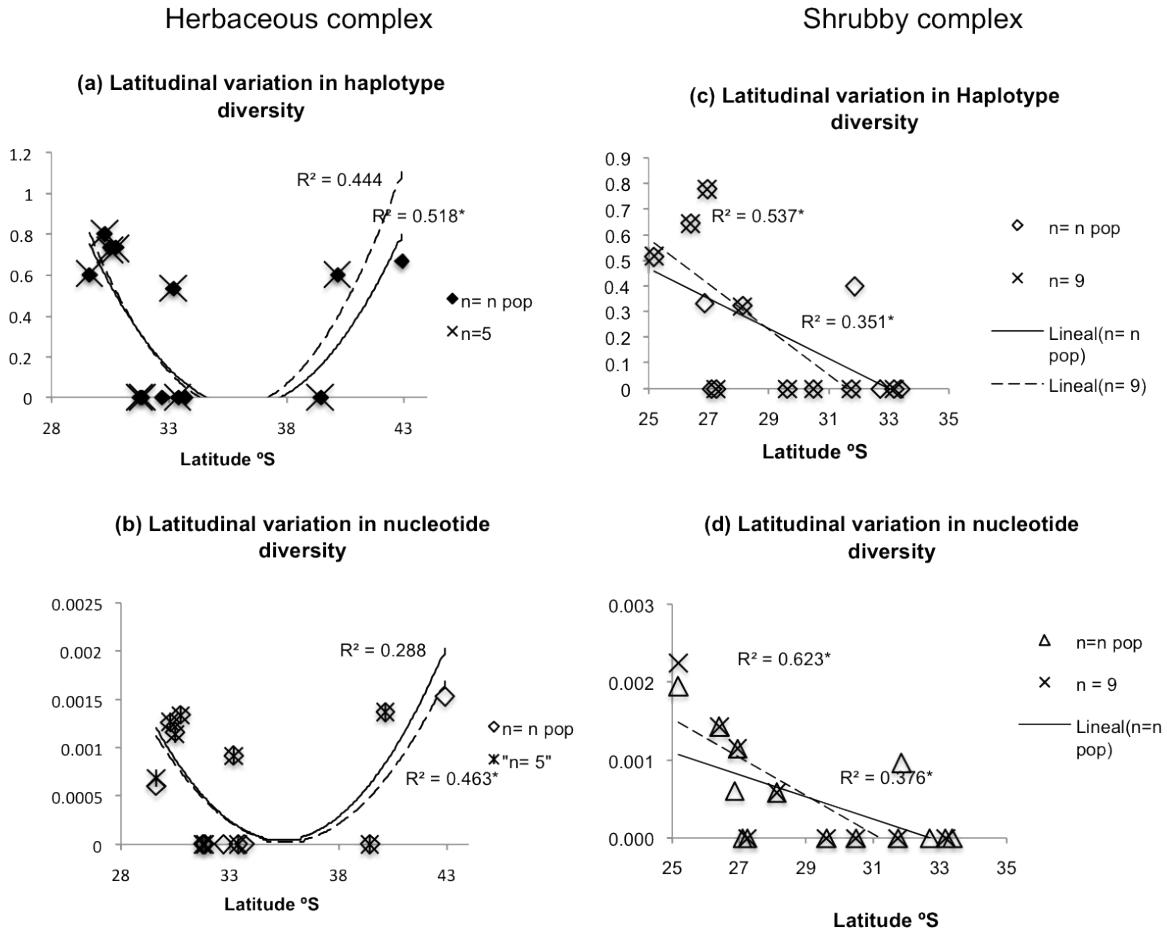

74°W
72°W
70°W
30°S
32°S
34°S
36°S
38°S
40°S
42°S
44°S
82.5%
13.5%
18%
6.3%
78.5%
N
Km
0 3060 120 180

Figure 3.4


Herbaceous complex
Shrubby complex
(a) Latitudinal variation in haplotype diversity
R² = 0.444
R² = 0.518*
n= n pop
n=5
Latitude ºS
(c) Latitudinal variation in Haplotype diversity
R² = 0.537*
R² = 0.351*
n= n pop
n= 9
Lineal(n= n pop)
Lineal(n= 9)
Latitude ºS
(b) Latitudinal variation in nucleotide diversity
R² = 0.288
R² = 0.463*
n= n pop
"n= 5"
Latitude ºS
(d) Latitudinal variation in nucleotide diversity
R² = 0.623*
R² = 0.376*
n=n pop
n = 9
Lineal(n=n pop)
Latitude ºS

CHAPTER 3

SUPLEMENTARY MATERIAL

Table S3.1 Sequences of haplotypes identified in the herbaceous lineage.

	Sequence Position																
	9	1	1	3	4	4	4	5	6	6	7	8	1	1	1	1	1
	8	0	7	6	3	4	6	1	1	3	4	2	0	2	2	3	5
		4	3	6	0	6	0	7	0	3	7	5	5	4	6	3	5
													2	5	8	1	2
Haplotype	***rpl32-TrnL***												***rpl16 intron***				
Hap_1	C	G	C	A	C	G	T	C	G	G	C	C	G	C	A	A	G
Hap_2	.	.	.	C	.	.	G	.	.	T	.	.	.	.	.	.	.
Hap_3	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.
Hap_4	.	.	.	C	.	.	G	.	.	.	A	.	.	.	.	.	.
Hap_5	.	.	.	C	.	.	G	.	.	.	.	.	.	.	.	.	.
Hap_6	A	.	.	C	A	.	G	.	.	.	.	.	.	.	.	.	.
Hap_7	.	.	.	C	.	.	G	.	T	.	.	.	.	.	.	.	.
Hap_8	.	.	.	C	.	.	G	.	.	.	.	T	.	.	G	.	.
Hap_9	.	.	.	C	.	.	G	.	.	.	.	.	.	.	.	C	.
Hap_10	.	.	.	C	.	T	G	.	.	.	.	.	.	.	.	C	.
Hap_11	.	.	.	C	.	.	G	A	.	.	.	.	T	.	.	.	.
Hap_12	.	.	A	C	.	.	G	.	.	.	.	.	.	.	.	C	.
Hap_13	.	.	.	C	.	.	G	.	.	.	.	.	.	.	.	.	T
Hap_14	.	T	.	C	.	.	G	.	.	.	.	.	.	A	.	C	.

Table S3.2 Frequencies of haplotypes in populations of the herbaceous lineage.

	Haplotypes														
Localities	**H1**	**H2**	**H3**	**H4**	**H5**	**H6**	**H7**	**H8**	**H9**	**H10**	**H11**	**H12**	**H13**	**H14**	**total**
S01				2	7	1					1				11
S02	1								3				1	1	6
S03							1	4	4	1					10
S04									1		3	2			6
S05	10														10
S06		10													10
S07	3														3
S08	2	4													6
S09	5														5
S10	3														3
S11		10													10
S12		3	2												5
S13		2	2												4
Total	**24**	**29**	**4**	**2**	**7**	**1**	**1**	**4**	**8**	**1**	**4**	**2**	**1**	**1**	**89**
Freq.	**27%**	**33%**	**4%**	**2%**	**8%**	**1%**	**1%**	**4%**	**9%**	**1%**	**4%**	**2%**	**1%**	**1%**	**100%**

DISCUSIÓN Y CONCLUSIÓN GENERAL

DISCUSIÓN Y CONCLUSIÓN GENERAL

Los resultados analizados en esta tesis permiten concluir que la duración del ciclo de vida y la forma de vida son rasgos funcionales de la planta completa que a su vez se correlacionan con otros rasgos foliares (morfológicos y ecofisiológicos) y afectan el desempeño de las plantas en ambientes fluctuantes. Las restricciones impuestas por rasgos funcionales y su interacción con el clima influyen en la historia evolutiva y biogeográfica, lo que puede ser observado en el desierto costero, donde el efecto de las fluctuaciones climáticas Cuaternarias no fue tan devastador como en la zona austral de Chile.

Los rasgos funcionales dan una mejor apreciación de las restricciones y oportunidades desafiadas por la planta en los distintos hábitats, que la identidad taxonómica por si sola. La novedad de este trabajo yace en analizar dentro de un mismo género, *Nolana*, grupos de plantas con ciclo y formas de vida contrastante (herbáceas de ciclo corto versus arbustivas de ciclo largo) que coexisten en un abrupto gradiente de precipitaciones y determinar hasta que punto estos rasgos de historia de vida restringen o condicionan la variación de otros rasgos funcionales relacionados más directamente con el uso y conservación de recursos, como eficiencia en el uso de agua, LMA, densidad de estomas, tamaño de estomas, contenido de nutrientes.

Los resultados muestran que las formas de vida analizadas (herbáceas y arbustivas) corresponden a estrategias de uso de recursos distintas: la forma de vida arbustiva se

correlacionan con rasgos que favorecen la conservación de agua, mientras que la forma de vida herbácea con rasgos que favorecen la captura de CO2. Ambas formas de vida mantuvieron sus estrategias a lo largo del gradiente de aridez, optimizando distintos rasgos: las arbustivas modifican el tamaño de estomas y mantienen elevado LMA, mientras que herbáceas modifican la densidad de estomas y mantienen bajo LMA.

Las diferencias en estrategias de forma de vida se vieron también reflejadas en la respuesta histórica a las fluctuaciones climáticas del Cuaternario. Los patrones filogeográficos estudiados sugieren fuertemente que estas plantas han experimentado procesos de expansión y contracción de rango en el pasado, lo que es apoyado por la evidencia paleoclimática, paleo ecológica y palinológica. Sin embargo, estos patrones también presentan fuertes diferencias, que dan cuenta de la importancia de la historia de vida en la repuesta a las variaciones climáticas del Cuaternario. En el linaje de *Nolana* formado por especies arbustivas encontramos mayor diferenciación interpoblacional, menor diversidad intrapoblacional, disminución de la diversidad genética hacia el sur y signos de contracción poblacional en el norte (mezcla de haplotipos de linajes distantes) y de expansión reciente de rango en el sur. En contraste, en el linaje de especies herbáceas encontramos menor diferenciación interpoblacional y mayor diversidad intrapoblacional; siguiendo una tendencia cuadrática con la latitud, con gran diversidad en los extremos del gradiente, signos de expansión poblacional en la zona norte (gran diversidad de haplotipos cercanamente emparentados), expansión de rango hacia el centro y contracción poblacional en el sur, con características que sugieren persistencia en el tiempo, y que dan cuenta de poblaciones antiguas que probablemente sobrevivieron a las fases glaciales.

La gran expansión de rango alcanzada por las herbáceas y su capacidad de sobrevivirlas fases glaciales a latitudes altas estaría asociada principalmente a la duración del ciclo de vida. En comparación con las especies arbustivas, las herbáceas tienen rasgos funcionales asociados con la maximización de la captura de CO_2 y no de conservación de agua, por lo tanto una estrategia de evasión de los periodos fríos, y no una mayor tolerancia al congelamiento (que se correlaciona con tolerancia al estrés hídrico), podría explicar su persistencia a latitudes altas. La duración del ciclo de vida, también podría explicar la mayor diversidad y patrón de expansión poblacional encontrado en el norte en el linaje herbáceo respecto al arbustivo, pues se ha documentado que ciclos mas cortos y exposición a condiciones severas de aridez promueven mayor tasa de mutación y mayor diversidad. Para las arbustivas, el ciclo de vida largo podría haberles permitido aprovechar las distintas fuentes de humedad que se presentan en el año (lluvias de invierno y de verano, y neblina), y su sobrevida a los periodos secos es consistente con su estrategia de conservación de agua, así como con la persistencia en "refugios de aridez" aislados y recolonización. Otros factores que pueden estar influyendo y que no fueron abordados en esta tesis son tasas de crecimiento, germinación, viabilidad de semillas. Los arbustos frecuentemente tienen tasas de germinación mas bajas, lo que provocaría que ante condiciones extremas, las probabilidades de extinción sean más alta, explicando la baja diversidad poblacional y alto promedio de diferencias pareadas (mayor diferenciación de haplotipos).

Los resultados de esta tesis enfatizan fuertemente en la importancia de los rasgos y su interacción con el ambiente como promovedor del cambio evolutivo y modelador de la distribución de las plantas. Es importante considerar y conocer la capacidad de respuesta de los rasgos y sus restricciones a la hora de hacer predicciones de cambio ante futuros escenarios

de cambio climático. El considerar las plantas como entidades con tendencias en correlación de rasgos, mas que entidades taxonómicas con atributos particulares, permite visualizar de mejor manera la respuesta al cambio, además de una mejor interpretación de los patrones filogeográficos y su historia climática pasada.

A la luz de esta tesis, surgen al menos dos líneas de investigación a abordar:

1. Comprender la relación entre diversidad genética y diversidad funcional

2. Entender de manera mas acabada el patrón de persistencia de poblaciones en el extremo hiperárido del desierto costero, en función de los gradientes altitudinales de humedad que se desarrollan, y la variación en los rasgos funcionales que dan cuenta del ajuste planta-ambiente.

Referencias

Ackerly, D.D. (2003) Community assembly, niche conservation, and adaptive evolution in changing environments. *International Journal of Plant Sciences*, **164**, S165–S184.

Ackerly, D.D. (2004) Functional traits of chaparral shrubs in relation to seasonal water deficit and disturbance. *Ecological Monographs,* **74**, 25–44.

Ackerly, D.D., Knight, C.A., Weiss, S.B., Barton, K. & Starmer, K.P. (2002) Leaf size, specific leaf area and microhabitat distribution of chaparral woody plants: contrasting patterns in species level and community level analyses. *Oecologia* **130**, 449-457.

Alvarez, N., Thiel-Egenter, C., Tribsch, A., Holderegger, R., Manel, S., *et al*.(2009) History or ecology? Substrate type as a major driver of spatial genetic structure in Alpine plants. Ecology Letters, **12**, 632-640.

Armesto, J.J. & Vidiella, P.E. (1993) Plant life forms and biogeographic relations of the flora of lagunillas (30°S) in the fog-free pacific coastal desert. *Annals of the Missouri Botanical Garden* **80**, 499-511.

Armesto J.J., Vidiella, P.E. & Gutierrez, J.R. (1993) Plant communties of the fog-free coastal desert of Chile: plant strategies in a fluctuating environment. Revista Chilena de Historia Natural **66**, 271-282.

Ashton, P.M.S., & Berlyn, G.P. (1994). A comparison of leaf physiology and anatomy of Quercus (section Erythrobalanus–Fagaceae) species in different light environments. American Journal of Botany**, 81**, 589–597.

Baker, H.G. & Stebbins, G.L. (1965) *The genetics of colonizing species*. Academic Press, New York.

Bandelt, H.-J., Forster, P. & Röhl, A. (1999) Median-joining networks for inferring intraspecific phylogenies. *Molecular Biology and Evolution*, **16**, 37–48.

Barnosky, A.D. (2008) Climatic change, refugia, and biodiversity: Where do we go from here? An editorial comment.*Climatic Change* **86**, 29-3.

Barnosky, A.D., Matzke, N., Susumu Tomiya, S., Guin Wogan, G., Swartz, B., Quental, T., Marshall, C., McGuire, J.L., Lindsey, E.L., Maguire, K.C., Mersey, B. & Ferrer, E.A (2011) Has the Earth's sixth mass extinction already arrived? *Nature,* **471**, 51-57.

Chaves, M.M., Maroco, J.P. & Pereira, J.S. (2003) Understanding plant responses to drought: from genes to the whole plant. *Functional Plant Biology* **30**, 239–26.

Clement, M., Posada, D. & Crandall, K.A. (2000) TCS: a computer program to estimate gene genealogies. *Molecular Ecology*, **9**, 1657–1659.

Condon A.G., Richards, R.A., Rebetzke G.J. & Farquhar G.D. (2004) Breeding for high water-use efficiency. *Journal of Experimental Botany*, **55**, 2447-2460.

Cox, C.B. & Moore, P.D. (2005). *Biogeography: An Ecological and Evolutionary Approach*, 7th edn. Blackwell, Malden

Darwin, C. (1859) On the Origin of Species by Means of Natural Selection, or the Preservation of Favoured Races in the Struggle for Life. London.

Dawson, T.P., Jackson, S.T., House, J.I., Prentice, I.C. & Mace, M.G. (2011) Beyonds predictions: biodiversity conservation in a changing climate. *Science* **332**: 53-58

De Micco, V. & Aronne, G. (2012) Morpho-anatomical traits for plant adaptation to drought. *Plant responses to drought stress* (Ed. Aroca R.) Springer-Verlag, Berlin, Heidelberg. 37- 61

Díaz, F.P., Latorre, C., Maldonado, A., Quade, J. & Betancourt, J.L. (2012) Rodent middens reveal episodic, long-distance plant colonizations across the hyperarid Atacama Desert over the last 34,000 years. *Journal of Biogeography*, **39**, 510–525.

Dillon, M.O. (2005) The Solanaceae of the Lomas Formations of Coastal Peru and Chile. *Festschrift for William G. D'Arcy: the legacy of a taxonomist* (ed. by R.C. Keating, V.C. Hollowell and T.B. Croat), pp. 131–155. Monographs in Systematic Botany, Vol. 104. Missouri Botanical Garden, St Louis, MO.

Dillon M.O., Arancio, G. & Luebert, F. (2007a) Five new species of *Nolana* (Solanaceae-Nolaneae) from Chile. *Arnaldoa,* **14,** 191-212.

Dillon, M.O, Tu, T., Soejima, A., Yi T, Nie, Z., Tye, A. & Wen,J. (2007b) Phylogeny of Nolana (Solanoideae-Solanaceae) inferred from granule-bound starch synthase I (GBSSI or Waxy) marker. *Taxon,* **56**, 1000-1011.

Dillon, M.O., Tu, T., Xie, L., Quipuscoa Silvestre, V. & Wen, J. (2009) Biogeographic diversification in *Nolana* (Solanaceae), a ubiquitous member of the Atacama and Peruvian Deserts along the western coast of South America. *Journal of Systematics and Evolution*, **47**, 457–476.

Driscoll SP, Prins A, Olmos E, Kunert KJ, & Foyer CH (2006) Specification of adaxial and abaxial stomata, epidermal structure and photosynthesis to CO2 enrichment in maize leaves. Journal of Experimental Botany **57**, 381-390.

Du Rietz, G. E. (1931) Life-forms of terrestrial flowering plants. *I. Acta Phytogeographica Suecica* **3** (1): 95 pp.

Dupanloup, I., Schneider, S. & Excoffier, L. (2002) A simulated annealing approach to define the genetic structure of populations. *Molecular Ecology*, **11**, 2571–2581.

Eamus, D. & Prichard, H. (1998) A cost-benefit analysis of leaves of four Australian savanna species. *Tree Physiol* **18,** 537–545.

Ehleringer, J.R. & Mooney H.A. (1978) Leaf hairs: effect on physiological activity and adaptive value to a desert shrub. *Oecologia*, **37**, 183-200.

Ehleringer, J. R. & Cooper, T. A.(1988) Correlations between carbon isotope ratio and microhabitat in desert plants. *Oecologia*, **76**, 562-566.

Excoffier, L. & Lischer, H.E.L. (2010) Arlequin suite ver 3.5: a new series of programs to perform population genetics analyses under Linux and Windows. *Molecular Ecology Resources*, **10**, 564–567.

Farquhar G.D., Ehleringer, J.R. & Hubick, K.T. (1989) Carbon Isotope Discrimination and Photosynthesis. *Annual Review of Plant Physiology and Plant Molecular Biology,* **40**, 503–537

Farquhar G. & Richards, R. (1984) Isotopic Composition of Plant Carbon Correlates With Water-Use Efficiency of Wheat Genotypes. *Australian Journal of Plant Physiology,* **11**, 539–552.

Forster, P., Torroni, A., Renfrew, C. & Röhl, A. (2001) Phylogenetic star contraction applied to Asian and Papuan mtDNA evolution. *Molecular Biology and Evolution*, **18**, 1864–1881.

Franks, S.J., Sim, S. & Weis, E.A. *(2007) Rapid evolution of flowering time by an annual plant in response to a climate fluctuation. Proceedings of the National Academy of Science USA* **104**, 1278–1282

Freyre, R. & Douglas, A.C. (2008) Studies on sexual compatibility between eight *Nolana* (Solanaceae) species. *Hortscience,* **43**, 1094–1094.

Freyre, R., Douglas, A.C., Dillon, M.O., 2005. Artificial hybridizations in five species of Chilean Nolana (Solanaceae). *Hortscience* **40**, 532–536.

Garrick, R.C., Nason, J.D., Meadows, C.A. & Dyer, R.J. (2009) Not just vicariance: phylogeography of a Sonoran Desert euphorb indicates a major role of range expansion along the Baja peninsula. *Molecular Ecology*, **18**, 1916–1931

Gibson AC (1996) Structure-function relations of warm desert plants. Springer-Verlag Berlin Heidelberg. 215pp.

Gibson A.C. (1998) Photosynthetic organs of desert plants. *BioScience*. **48**, 911-920.

Givnish ,T.J. (2002) Adaptive significance of evergreen vs. deciduous leaves: solving the triple paradox. *Silva Fennica,* **36**, 703–743.

Grene, R., Vasquez-Robinet, C. & Bohnert, H.J. (2011) Molecular biology and physiological genomics of dehydration stress. *Plant desiccation tolerance* (Eds. Lüttge U, Beck E, Bartels D), Springer, Heidelberg, 255–288.

Grime, J.P. (1979) Competition and struggle for existence. In: *Population dynamics*. (Eds R.M., Anderson, B.D., Turner, L.R. Taylor) pp. 123-139, Blackwell Scientific Publications, Oxford, UK.

Grivet, D. & Petit, R.J. (2002) Phylogeography of the common ivy (*Hedera* sp.) in Europe: genetic differentiation through space and time. *Molecular Ecology*, **11**, 1351–1362.

Hamrick, J. & Godt, M. (1989). Allozyme diversity in plant species. In: *Plant Population Genetics, Breeding and Genetic Resources* (eds Brown AHD, Clegg MT, Kahler AL, Weir BS), pp. 43– 63. Sinauer, Sunderland, MA.

Hamrick, J.L. & Godt, M.J.W. (1996) Effects of life history traits on genetic diversity in plant species. Philosophical Transactions of the Royal Society of London Series B, Biological Sciences, 351, 1291–1298.

He JS, Fang JY, Wang ZH, Guo DL, Flynn DFB, Geng Z. 2006. Stoichiometry and large-scale patterns of leaf carbon and nitrogen in the grassland biomes of China. Oecologia **149**, 115–122.

Heusser, C.J. (1990) Ice vegetation and climate of subtropical Chile. *Paleogeography, Paleoclimatology, Paleoecology* 80:107-127.

Heusser, L., Heusser, C.J., Kleczkowski, A. & Crowhurst, S. (1999). A 50,000-yr pollen record from Chile of South American millennial-scale climate instability duringthe last glaciation. *Quaternary Research* **52**, 154–158.

Hewitt, G.M. (1996) Some genetic consequences of ice ages, and their role in divergence and speciation. *Biological Journal of the Linnean Society*, **58**, 247–276.

Hewitt, G.M. (2000). The genetic legacy of the Quaternary ice ages. *Science*, 405, 907–913.

Holland, N. & Richardson, A.D. (2009) Stomatal length correlates in elevation of growth in four temperate species. *Journal of Susteinable Forestry*, **28**, 63-73.

Hooper DU, Chapin FSIII, Ewel JJ, Hector A, Inchausti P, Lavorel S *et al*. (2005) Effects of biodiversity on ecosystem functioning: a consensus of current knowledge. Ecological Monograph 75:3-35.

Ignace DD, Huxman TE, Weltzin JF & Williams DG (2007) Leaf gas exchange and water status responses of a native and non-native grass to precipitation across contrasting soil surfaces in the Sonoran Desert. Oecologia 152(3): 401-413

Intergovernmental Panel on Climate Change (IPCC) (2007) Climate change 2007: mitigation. Contribution of working group III to the fourth assessment report of the intergovernmental panel on climate change. Cambridge University Press, Cambridge

Jackson, S.T. & Overpeck, J.T. (2000) Responses of plant populations and communities to environmental changes of the late Quaternary. *Paleobiology*, **26**, 194–220.

Jombart T. (2008) adegenet: a R package for the multivariate analysis of genetic markers. *Bioinformatics* **24**: 1403-1405. doi: 10.1093/bioinformatics/btn129

Kaiser, J., Schefuß, E., Lamy, F., Mohtadi, M. & Hebbeln, D. (2008) Glacial to Holocene changes in sea surface temperature and coastal vegetation in north central Chile: high *versus* low latitude forcing. *Quaternary Science Reviews*, **27**, 21–22.

Kelly A & Goulden M (2008) Rapid shifts in plant distribution with recent climate change. Proceedings of the National Academy of Science **105**, 11823–11826.

Koutavas, A., deMenocal, P.B., Olive, G.C. & Lynch-Stieglitz, J. (2006) Mid-Holocene El Nino-Southern Oscillation (ENSO) attenuation revealed by individual forami- nifera in eastern tropical Pacific sediments. *Geology*, **34**, 993–996.

Lambers H, Chapin FS & Pons TL (2008) Plant physiological ecology. Second edition, Springer. 604pp.

Lamy F, Klump J, Hebbeln D & Wefer G (2000) late quaternary rapid climate change in northern Chile. *Terra Nova* **12**, 8-13.

Larcher, W. (2003) Physiological plant ecology. Fourth edition. Springer-Verlag, Berlin. 513pp.

Latorre, C., Betancourt, J.L., Rech, J.A., Quade, J., Holmgren, C., Placzek, C., Maldonado, A.J.C., Vuille, M. & Rylander, K.A. (2005) Late Quaternary history of the Atacama Desert. *23°S: archaeology and environmental history of the southern deserts* (ed. by M. Smith and P. Hesse), pp. 73–90. National Museum of Australia Press, Canberra

Lavorel, S., McIntyre, S., Landsberg, J. & Forbes, D. (1997) Plant functional classifications: from general groups to specific groups based on response to disturbance. *Trends in Ecology and Evolution* **12**, 474–478.

Lavorel S., Díaz S., Cornelissen, J.H.C., Garnier, E., Harrison, P.S., *et al*. (2007) Plant functional types: are we getting any closer to the Holy Grail?. *Terrestrial ecosystems in a changing world* (eds Canadell J, Pitelka LF, Pataki D) Berlin: Springer-Verlag, 149–164.

Lenoir, J., Gégout, J.C., Marquet, P.A., de Ruffray, P. & Brisse, H. (2008) A significant upward shift in plant species optimum elevation during the 20th century. *Science* **320**, 1768-177.

Lessa, E.P., Cook, J.A. & Patton, J.L. (2003) Genetic footprints of demographic expansion in North America, but not Amazonia, during the Late Quaternary. *Proceedings of the National Academy of Sciences USA*, **100**, 10331–10334.

Levitt J (1980) Response of plants to environmental stresses. Chilling, freezing, and high temperature stresses. Academic, New York.

Librado, P. & Rozas, J. (2009) DnaSP v5: a software for comprehensive analysis of DNA polymorphism data. *Bioinformatics*, **25**, 1451-1452.

Livingston, N.J., Guy, R.D., & Ethier, G.J. (1999) The effects of nitrogen stress on the stable carbon isotope composition, productivity and water use efficiency of white spruce (*Picea glauca* (Moench) Voss) seedlings. *Plant Cell and Environment*, **22**: 281-289.

Loarie SR, Carter BE, Hayhoe K, McMahon, S., Moe, R., *et al.,* (2008) Climate change and the future of California´s endemic flora. *PloS ONE* **3**, e2505.

Luebert, F. & Pliscoff, P. (2006) *Sinopsis bioclimática y vegetacional de Chile*. Editorial Universitaria, Santiago, Chile.

Ludlow, M.M. (1989) Strategies of response to water stress. *Structural and functional responses to environmental stresses: water shortage* (Eds. Kreeb KH, Richter H, Hinckley TM) SPB Academic Publishing, The Hague, 269–281.

Maldonado, A. & Villagrán, C. (2002) Paleoenvironmental changes in the semiarid coast of Chile (~32ºS) during the last 6200 cal years inferred from a swamp-forest pollen record. *Quaternary Research,* **58**, 130–138.

Maldonado, A. & Villagrán, C. (2006) Climate variability over the last 9900 cal yr BP from a

swamp forest pollen record along the semiarid coast of Chile. *Quaternary Research*, **66**, 246–258.

Maldonado, A., Betancourt, J.L., Latorre, C. & Villagrán, C. (2005) Pollen analyses from a 50 000-yr rodent midden series in the southern Atacama Desert (25° 30′ S). *Journal of Quaternary Science*, **20**, 493–507.

Manni, F., Guerard, E. & Heyer, E. (2004) Geographic patterns of (genetic, morphologic, linguistic) variation: how barriers can be detected by using Monmonier's algorithm. *Human Biology,* **76**, 173–190.

McIntyre, S., Lavorel, S., Landsberg, J. & Forbes, T.D.A. (1999). Disturbance response in vegetation – towards a global perspective on functional traits. *Journal of Vegetation Science*, **10**, 621-630.

McGill, B.J., Enquist, B.J., Weiher, E. & Westoby, M. (2006) Rebuilding community ecology from functional traits. *Trends in Ecology and Evolution,* **21**, 178–185.

Meirmans, P.G., Goudet, J., IntraBioDiv Consortium & Gaggiotti, O.E. (2011) Ecology and life-history affect different aspects of the population structure of 27 high-alpine plants. *Molecular Ecology,* **20**, 3144-3155.

Meng TT, Ni J. & Harrison S.P. (2009) Plant Morphometric traits and climate gradients in northern China: a meta-analysis using quadrat and flora data. *Annals of Botany*, **104**, 1217-1229.

Messier, J., McGill, B.J. & Lechowicz, M.J. (2010) How do traits vary across ecological scales? A case for trait-based ecology. *Ecology Letters*, **13**, 838-848.

Monmonier M (1973) Maximum-difference barriers: an alternative numerical regionalization method. Geogr Anal 3: 245–61.

Mott, K.A. & O'Leary, J.W. (1984) Stomatal behavior and CO2 exchange characteristics in amphistomatous leaves. *Plant Physiology* **74**, 47-51.

Naeem, S., Duffy, J.E. & Zavaleta, E. (2012) The Functions of Biological Diversity in an Age of Extinction, *Science,* **336**, 1401-1406

Nason, J.D., Hamrick, J.L. & Fleming, T.H. (2002) Historical vicariance and postglacial colonization effects on the evolution of genetic structure in *Lophocereus*, a Sonoran Desert columnar cactus. *Evolution*, **56**, 2214–2226.

Nei, M. (1987) *Molecular evolutionary genetics*. Columbia University Press, New York, NY.

Niinemets, U. (2001) Global-scale climatic controls of leaf dry mass per area, density, and thickness in trees and shrubs. *Ecology*, **82**, 453–469.

Nybom H (2004) Comparison of different nuclear DNA markers for estimating intraspecific genetic diversity in plants. *Molecular Ecology*, **13**, 1143–1155.

Nybom H, Bartish I (2000) Effects of life history traits and sampling strategies on genetic diversity estimates obtained with RAPD markers in plants. *Perspectives in Plant Ecology, Evolution and Systematics*, **3**, 93–114.

Orians, G. H., & Solbrig, O. T. (1977). A cost-income model of leaves and roots with special reference to arid and semiarid areas. *American Naturalist*, **111**, 677-690.

Ossa P.G., Pérez, F., Armesto J.J. (2013) Phylogeography of two closely related species of Nolana from the coastal Atacama Desert of Chile: post-glacial population expansions in response to climate fluctuations. *Journal of Biogeography*. DOI: 10.1111/jbi.12152

Paradis, E. (2010) pegas: an R package for population genetics with an integrated–modular approach. *Bioinformatics*, **26**, 419–420.

Peakall, R. & Smouse, P.E. (2006) GENALEX 6: genetic analysis in Excel. Population genetic software for teaching and research. *Molecular Ecology Notes*, **6**, 288–295.

Pérez-Harguindeguy N., Diaz, S., Garnier, E., Lavorel, S., Poorter, H., et al. (2013) New handbook for standardized measurement of plant functional traits worldwide. *Australian Journal of Botany*, **61**, 167-234.

Pons, O. & Petit, R.J. (1996) Measuring and testing genetic differentiation with ordered *versus* unordered alleles. *Genetics*, **144**, 1237–1245.

Poorter H., Niinemets U., Poorter L., Wright I.J. & Villar R. (2009) Causes and consequences of variation in leaf mass per área (LMA) a meta-analysis. *New Phytologist*, **182**, 565–588.

Premoli, A.C., Del Castillo, R.F., Newton, A.C., Bekessy, S., Caldiz, M., Martínez-Araneda, C. Et Al. (2007) Patterns of Genetic Variation in Tree Species and their Implications for Conservation. In: AC Newton (ed.) *Biodiversity Loss and Conservation in Fragmented Forest Landscapes: The Forests of Montane Mexico and Temperate South America* . CAB International.

Prim, R.C. (1957) Shortest connection networks and some generalizations. *Bell System Technical Journal*, **36**, 1389–1401.

Raunkiær, C. (1934) *The Life Forms of Plants and Statistical Plant Geography, being the collected papers of C. Raunkiær*. Oxford University Press, Oxford.

Rebernig, C.A., Schneeweiss, G.M., Bardy, K.E., Schönswetter, P., Villaseñor, J.L., Obermayer, R., Stuessy, T.F. & Weiss-Schneeweiss, H. (2010) Multiple Pleistocene refugia and Holocene range expansion of an abundant southwestern American desert plant species (*Melampodium leucanthum*, Asteraceae). *Molecular Ecology*, **19**, 3421–3443.

Reich P.B. & Oleksyn J. (2004) Global patterns of plant leaf N and P in relation to temperature and latitude. *Proceedings of the National Academy of Sciences*, **101,** 11001-11006.

Reich, P.B., Walters, M.B. & Ellsworth, D.S. (1997) From tropics to tundra: global convergence in plant functioning. *Proceedings of the National Academy of Sciences*, **94**, 13730 –13734.

Reich, P.B., Wright, I.J., Cavender-Bares, J., Craine, J.M., Oleksyn, J. Westoby, M. & Walters, M.B. (2003) The evolution of plant functional traits, scpectra, and strategies. *International Journal of Plant Sciences*, **164**, S143-S164

Reich, P.B., Wright, I.A. & Lusk, C.H. (2007) Predicting leaf physiology from simple plant and climate attributes: a global Glopnet analysis. *Ecological Applications*, **17**, 1982–1988.

Rein, B., Lückge, A., Reinhardt, L., Sirocko, F., Wolf, A. & Dullo, W.C. (2005) El Niño variability off Peru during the last 20,000 years. *Paleoceanography*, **20**, PA4003. doi:10.1029/2004PA001099

Richardson, A.D., Ashton, P.M.S., Berlyn, G.P., Mcgroddy, M.E. & Cameron,.I.R. (2001) Within-crown foliar plasticity of western hemlock, Tsuga heterophylla, in relation to stand age. *Annals of Botany***, 88**, 1007–1015.

Richardson, A.D., Berlyn, G.P., Ashton, P.M.S., Thadani, R. & Cameron, I.R. (2000) Foliar plasticity of hybrid spruce in relation to crown position and stand age. *Canadian Journal of Botany*, **78**, 305–317.

Royer D.L. (2001) Stomatal density and stomatal index as indicators of paleoatmospheric CO_2 concentration.*Review of Palaeobotany and Palynology*, **114**, 1-28.

Rundel, P. W., Ehleringer, J. R., & Nagy, K. A. (1988). Stable Isotopes in Ecological Research. Springer-Verlag 544 pp.

Rundel PW, Dillon MO, Palma B, Mooney HA, Gulmon SL & Ehleringer JR (1991) The phytogeography and ecology of the coastal Atacama and Peruvian deserts. *Aliso* **13**, 1-50.

Salisbury, E.J. (1927) On the causes andecological significance of stomatal frequencywith special reference to thewoodland floor. *Philosophical Transactions of the Royal Society,* **216**, 1-65.

Schneider, C.A., Rasband, W.S. & Eliceiri, K.W. (2012) NIH Image to ImageJ: 25 years of image analysis. *Nature Methods,* **9,** 671-675.

Schulze, E. D., Kelliher, F. M., Korner, C., Lloyd, J., &Leuning, R. (1994). Relationships among maximum stomatal conductance, ecosystem surface conductance, carbon assimilation rate, and plant nitrogen nutrition: a global ecology scaling exercise. *Annual Review of Ecology and Systematics*, **25**, 629-660.

Sersic, A., Cosacov, A, Cocucci, A.A,. Johnson, L.A., Pozner, R., Avila, L.J., Sites, J.W. & Morando, M. (2011). Emerging phylogeographical patterns of plants and terrestrial vertebrates from Patagonia. Biological Journal of the Linnean Society 103:475-494.

Shaw, J., Lickey, E.B., Beck, J.T., Farmer, S.B., Liu, W., Miller, J., Siripun, K.C., Winder, C.T., Schilling, E.E. & Small, R.L. (2005) The tortoise and the hare II: relative utility of 21 noncoding chloroplast DNA sequences. *American Journal of Botany*, **92**, 142–166.

Shaw, J., Lickey, E.B., Schilling, E.E. & Small, R.L. (2007) Comparison of whole chloroplast genome sequences to choose noncoding regions for phylogenetic studies in angiosperms: the tortoise and the hare III. *American Journal of Botany*, **94**, 275–288.

Shulmeister, J., Goodwin, I., Renwick, J., Harle, K., Armand, L., McGlone, M.S., Cook, E., Dodson, J., Hesse, P.P., Mayewski, P. & Curran, M. (2004) The Southern hemisphere westerlies in the Australasian sector over the last glacial cycle: a synthesis. *Quaternary International,* **118–119**, 23–53.Smith, S. A., & Donoghue, M. J. (2008). Rates of molecular evolution are linked to life history in flowering plants. *Science*, **322**, 86-89.

Smith, S. A., & Donoghue, M. J. (2008). Rates of molecular evolution are linked to life history in flowering plants. *Science*, **322**, 86-89.

Smith, S. A., & Beaulieu, J. M. (2009). Life history influences rates of climatic niche evolution in flowering plants. *Proceedings of the Royal Society B: Biological Sciences*, **276**, 4345-4352.

Smith, T.M., Shugart, H.H. & Woodward, F.I. eds (1996) *Plant Functional Types: Their relevance to ecosystem properties and global change*. Cambridge University Press.

Southwood, T.R.E. (1977) Habitat. The themplet for ecologival sttrategies?. *Journal of Animal Ecology*, **43**, 337-365.

Steffen, W.L, Cramer W., Plöchl, M. & Bugmann, H. (1996) Global vegetation models: incorporating transient changes to structure and composition. *Journal of Vegetation Science,* **7**, 321-328.

Szpak P, Longstaffe FJ, Millaire J-F, White CD (2012) Stable Isotope Biogeochemistry of Seabird Guano Fertilization: Results from Growth Chamber Studies with Maize (*Zea mays*). PLoS One 7: e33741.

Szpak P, White CD, Longstaffe FJ, Millaire J-F, Vásquez Sánchez VF (2013) Carbon and Nitrogen Isotopic Survey of Northern Peruvian Plants: Baselines for Paleodietary and Paleoecological Studies. PLoS ONE 8(1): e53763. doi:10.1371/journal.pone.0053763

Taberlet, P., Fumagalli, L., Wust-Saucy, A.-G. & Cosson, J.F. (1998). Comparative phylogeography and postglacial colonization routes in Europe. *Molecular Ecology*, **7**, 453–464.

Tari, I. (2003) Abaxial and adaxial stomatal density, stomatal conductances and water status of bean primary leaves as affected by poclobutrazol. *Biologia Plantarum***,** 47, 215-220

Teacher, A.G.F. & Griffiths, D.J. (2011) HapStar: automated haplotype network layout and visualization. *Molecular Ecology Resources*, **11**,151–153.

Thiel-Egenter, C., Gugerli, F., Alvarez, N., Brodbeck, S., Cieslak, E., Colli, L., Englisch, T., Gaudeul, M., Gielly, L., Korbecka, G., Negrini, R., Paun, O., Pellecchia, M., Rioux, D., Ronikier, M., Schonswetter, P., Schupfer, F., Taberlet, P., Tribsch, A., van Loo, M., Winkler, M. & Holderegger, R. (2009) Effects of species traits on the genetic diversity of high-mountain plants: a multi-species study across the Alps and the Carpathians, *Global Ecology and Biogeography*, **18**, 78-87.

Tsialtas, I.T. & Veresoglou, D.S. (2007) Nitrogen use efficiency in a semi-arid comunity and its relationship with water use efficiency and soil water and nitrogen. *World Journal of Agricultural Sciences*, **3**, 485-488.

Tu, T., Dillon, M.O., Sun, H. & Wen, J. (2008) Phylogeny of *Nolana* (Solanaceae) of the Atacama and Peruvian deserts inferred from sequences of four chloroplast markers and the nuclear *LEAFY* second intron. *Molecular Phylogenetics and Evolution*, **49**, 561–573.

Valero-Garcés, B.L., Jenny, B., Rondanelli, M., Delgado-Huertas, A., Burns, S.J., Veit, H. & Moreno, A. (2005) Palaeohydrology of Laguna de Tagua Tagua (34º30' S) and moisture fluctiations in Central Chile for the last 46 000yr. *Journal of Quaternary Science*, **20**, 625-641.

Veit, H. & K. Garleff (1995) Evolución del paisaje cuaternario y los suelos en Chile central-sur. Páginas 29-49. In: J. Armesto, C. Villagrán y M. Kalin Arroyo (eds.). *Ecología de los Bosques Nativos de Chile*. Editorial Universitaria. Santiago, Chile.

Vidiella, P.E., J.J., Armesto & Gutiérrez, J.R. (1999) Vegetation changes and sequential flowering after rain in the southern Atacama Desert. *Journal of Arid Environments* **43**, 449-458.

Violle, C., Navas, M.-L., Vile, D., Kazakou, E., Fortunel, C., Hummel, I. and Garnier, E. (2007), Let the concept of trait be functional!. *Oikos*, **116**, 882–892.

Viruel, J., Catalán, P. & Segarra-Moragues, J.G. (2012) Disrupted phylogeographical microsatellite and chloroplast DNA patterns indicate a vicariance rather than long-distance dispersal origin for the disjunct distribution of the Chilean endemic *Dioscorea biloba* (Dioscoreaceae) around the Atacama Desert. *Journal of Biogeography*, **39**, 1073–1085.

Warming, E. (1908) Om planterigets livsformer. G.E.C. Gad, København.

Warming, E. (1919) Dansk Plantevrekst. Botanisk Tidsskrift, **35**, 6.

Westoby, M., Falster, D.S., Moles, A.T., Vesk, P.A. & Wright, I.J. (2002) Plant ecological strategies: some leading dimensions of variation between species. *Annual Review of Ecology and Systematic*s, **33**, 125–159.

White, J.W.C. and B.H. Vaughn (2011), University of Colorado, Institute of Arctic and Alpine Research (INSTAAR), Stable Isotopic Composition of Atmospheric Carbon Dioxide (13C and 18O) from the NOAA ESRL Carbon Cycle Cooperative Global Air Sampling Network, 1990-2012, Version: 2013-04-05, Path: ftp://ftp.cmdl.noaa.gov/ccg/co2c13/flask/event/.

Williams, J.W., Shuman, B.N., Webb, T., III., Bartlein, P.J. & Leduc, P.L. (2004) Late-Quaternary vegetation dynamics in North America: scaling from taxa to biomes. *Ecological Monographs*, **74**, 309–334.

Willis, K.J. and Niklas, K.E. The role of Quaternary environmental change in plant macroevolution – the exception or the rule? *Philosophical Transactions of the Royal Society, Series B*, **359**, 159-172.

Woodward, F.I. & Cramer, W. (1996) Plant functional types and climatic changes - Introduction. *Journal of Vegetation Science,* **7**, 306-308.

Wolfe, K.H., Li, W.H. & Sharp, P.M. (1987) Rates of nucleotide substitution vary greatly among plant mitocondrial, chloroplast, and nuclear DNA. *Proceedings of the National Academy of Sciences USA,* **84,** 9054-9058.

Wright, I.J., Reich, P.B. & Westoby, M. (2001) Strategy-shifts in leaf physiology, structure and nutrient content between species of high and low rainfall, and high and low nutrient habitats. *Functional Ecology*, **15,** 423–434

Wright I.J., Westoby M., & Reich P. B. (2002) Convergence towards higher leaf mass per area in dry and nutrient –poor habitats has different consequences for leaf life span. *Journal of Ecology*, **90**, 534-543.

Wright, I.J., Reich, P.B., Westoby M., Ackerly, D.D., Baruch, Z., *et al*. (2004) The worldwide leaf economics spectrum. *Nature*, **428**, 821-827.

Zhang J-L., Poorter L., & Cao, K-F. (2012) Productive leaf functional traits of Chinese savanna species. *Plant Ecology*. **213**, 1449-1460.