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INSTITUTO TECNOLÓGICO DE CD. VICTORIA

Programa

DOCTORADO EN CIENCIAS EN BIOLOGÍA

Tesis

VARIACIÓN GENÉTICO-MORFOLÓGICA DE *Agave lechugilla* TORR. Y *Agave gentryi* ULLRICH., EN UN GRADIENTE ELEVACIONAL

Presenta

ALFREDO SÁNCHEZ GONZÁLEZ

Como requisito para obtener el grado de
DOCTOR EN CIENCIAS EN BIOLOGÍA

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Ciudad Victoria, Tamaulipas DICIEMBRE, 2021

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e-mail: dir_cdvictoria@tecnm.mx
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Conforme a los numerales 2.15.4 y 2.15.5 de los Lineamientos para la Operación de Estudios de Posgrado en el TecNM (ver. 2018) y por recomendación del H. Comité Tutorial, ésta división le **AUTORIZA** imprimir y reproducir digitalmente la Tesis: **"Variación genético-morfológica de *Agave lechuguilla* Torr. y *Agave gentryi* Ullrich. en un gradiente elevacional"**, que tuvo a bien desarrollar en la División de Estudios de Posgrado e Investigación de éste instituto.

Ruego a Usted dar puntual seguimiento al formato en vigor que, para tal caso, indica las características de diseño que deberá contener tan importante documento.

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A mi hermosa Familia:

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Esta contribución es por y para ellos, LOS AMO.

“Por siempre Narnianos”

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ABREVIATURAS

°C	Grados Centígrados
µl	Microlítros
µM	Micromolar
A	promedio de alelos por locus/localidad
AM	Altiplano Mexicano
AMOVA	Análisis de Varianza Molecular
cm	Centímetros
CTAB	*Cetyl Trimethyl Ammonium Bromide* (Bromuro de hexadeciltrimetilamonio)
DE	Desviación Estándar
DH	Diferencia de Heterocigosis
dH2O	Agua Destilada
DNA	*Desoxyribonucleic Acid* (Ácido desoxiribonucléico)
dNTP	Deoxinucleósidotrifosfato
EDTA	*Ethylene Diamine Tetra Acetic Acid* (Ácido etildiaminotetraacético)
F	Índice de Fijación
F_{IT}	Coeficiente de Endogamia Entre Individuos y la Población Total
F_{IS}	Coeficiente de Endogamia Entre Individuos y su Subpoblación
F_{ST}	Coeficiente de Diferenciación Poblacional
G	Gramos
HCL	Ácido Clorhídrico
H_E	Heterocigosis Esperada
H_{EQ}	Heterocigosis en el equilibrio
H_O	Heterocigosis Observada
HW	Hardy-Weinberg
I	Índice informativo para cada iniciador
km^2	Kilómetros Cuadrados
m	Metro
M	Molar
mg	Miligramo
$MgCl_2$	Cloruro de Magnesio
min	Minutos
ml	Mililitro
mm	Milímetros
mM	Milimolar
msnm	Metros Sobre el Nivel del Mar
N	Promedio de muestras amplificadas por locus/localidad
NaCl	Cloruro de Sodio
Ne	número efectivo de alelos por locus/localidad
ng	Nanogramos
Nm	Número de migrantes por generación
PCR	*Polimerase Chain Reaction* (Reacción en Cadena de la Polimerasa)
PVP	Polivinilpirrolidona
rpm	Revoluciones por Minuto
S.M.M.	*Step Mutation Model* (Modelo de Mutación por Pasos)

seg	Segundos
SMO	Sierra Madre Oriental
Taq	Polimerasa de *Thermus aquaticus*
TFPGA	*Tools For Population Genetic Analyses* (Herramientas para Análisis de Genética de Poblaciones)
U	Unidades Enzimáticas
UPGMA	*Unweighted Pair Group Method with Arithmetic Mean* (Método de Agrupamiento Pareado No Balanceado con Medias Aritméticas)
WSW	*West South West* (Orientación Oeste Sur Oeste)
Φ_{IS}	PhyIS Coeficiente Hipervariable de Endogamia Entre de las Poblaciones
Φ_{IT}	PhyIT Coeficiente Hipervariable de Endogamia dentro de las Poblaciones
Φ_{ST}	PhyST Coeficiente Hipervariable de Diferenciación Poblacional
χ^2	Chi cuadrada

RESUMEN

El presente trabajo es un estudio sobre la variación morfológica y genética de dos especies del género *Agave* con diferente tolerancia ambiental, en un gradiente elevacional.

El estudio se realizó en un intervalo de elevación de 500 a 3500 msnm, con la cuantificación de variables climáticas, de asociaciones vegetales y suelos a lo largo del gradiente, se definieron seis estratos elevacionales cada 500 metros. Partiendo de esto, se realizaron mediciones de variables morfológicas de *Agave lechuguilla* y *Agave gentryi* para evaluar la tolerancia ambiental de las especies y su respuesta fenotípica frente a las condiciones cambiantes del ambiente. De esta forma, se logró identificar su amplitud de nicho y su capacidad de respuesta.

Del mismo modo, se tomaron muestras de tejido foliar de cada uno de los individuos incluidos en la evaluación morfológica, para realizar extracciones de DNA y, utilizando marcadores moleculares microsatélites, se generó un perfil genético de las poblaciones. Con lo cual, se logró reportar la diversidad y la estructura genética de las especies. Esto permitió evaluar cómo se relaciona la amplitud de nicho (generalista o especialista) con el grado de diversidad genética; así como identificar si hay una asociación entre grupos genéticos y los estratos elevacionales.

Este trabajo tuvo como objetivo contribuir con el tema de la capacidad adaptativa de las plantas frente a las oscilaciones ambientales a partir de la plasticidad morfológica y genética, considerando el generalismo y especialismo de las especies.

ABSTRACT

The present work is a study on the morphological and genetic variation of two species of the *Agave* genus with different environmental tolerance, in an elevation gradient.

The study was carried out in an elevation interval between 500 to 3,500 m asl, with the quantification of climatic variables, plant associations, and soil along the gradient, six elevation strata were defined every 500 m asl. Starting from this, measurements of morphological variables of *Agave lechuguilla* and *Agave gentryi* were carried out to evaluate the environmental tolerance of the species and their phenotypic response to the changing conditions of the environment. In this way, it was possible to identify its breadth of niche and its responsiveness.

In the same way, leaf tissue samples were taken from each of the individuals included in the morphological evaluation, to carry out DNA extractions and, using microsatellite molecular markers, a genetic profile of the populations was generated. This made it possible to report the genetic diversity and structure of the species. It also allowed corroborating whether generalist and specialist are defined by the degree of genetic diversity; as well as to identify if there is an association between genetic groups and the elevational strata.

This work to shed light into the adaptive capacity of plants in the face of environmental oscillations based on morphological and genetic plasticity, considering the generalism vs specialism of the species

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INTRODUCCIÓN GENERAL

El funcionamiento adecuado de los ecosistemas depende del rol ecológico de las especies, mediado por su capacidad adaptativa frente a las condiciones ambientales (Lande, 1988; Medail y Diadema, 2009); por ello, identificar los factores que influyen en la adecuación de la biota debe ser un tema fundamental en el diseño de estrategias sustentables. Aunado a esto, el cambio climático trabaja de manera acelerada; las proyecciones muestran que el incremento en la temperatura es su principal efecto, se estima que para el año 2100 es muy probable que el incremento oscile entre 1.5°C y 4.0°C (Harvell *et al*., 2002; Williams *et al.*, 2003; Araujo *et al*. 2005; Kosanic *et al.,* 2018). Por lo tanto, el cambio climático constituye una condición importante para aumentar los esfuerzos de evaluar la respuesta ecológica de las especies y su riesgo potencial de extinción. En este sentido, los gradientes elevacionales, considerados experimentos naturales, permiten evaluar la capacidad de respuesta de las especies frente a cambios súbitos en el ambiente ya que generan variaciones en los diferentes estratos de elevación que pueden estimular la respuesta morfológica y genética de las poblaciones (Körner, 2007; Premoli y Mathiasen, 2011).

Es importante tomar en cuenta que las especies han desarrollado diferentes grados de variación a lo largo de un proceso evolutivo para enfrentar las oscilaciones ambientales; estas estrategias adaptativas pueden ser morfológicas, de comportamiento y/o fisiológicas, sustentadas en la expresión diferencial de su variabilidad genética acumulada (Lande, 2009; Hedrick, 2009; Arteaga *et al.,* 2015). Por esta razón, es relevante que las poblaciones mantengan el mayor número de variantes (morfológicas y alélicas) ya que cada una constituye un reservorio

adaptativo asociado a ambientes particulares (Leisca y Allendorf, 1995; Frankham, 2005; Galván-Hernández *et al.*, 2015); lo cual puede ocurrir con algunas especies del género *Agave.*

El género *Agave* L. es uno de los taxa más representativos de México por su amplia distribución en el país y por la estrecha relación con pueblos indígenas y mestizos desde la época prehispánica hasta el presente, incluso se reconoce que no hay otra planta que tenga tantas modalidades de uso como los agaves (Gómez-Pompa, 1963; García-Mendoza, 2007; Eguiarte *et al.*, 2017). Cabe mencionar que este grupo de plantas tiene representantes en ambientes heterogéneos como *Agave lecheguilla* Torr, y otros en sitios con requerimientos más específicos como *A. gentryi* Ullrich (Gentry, 1982; García-Mendoza, 2007). Por lo cual, pueden funcionar como especies modelo para evaluar y generar un referente sobre la capacidad adaptativa de las plantas frente a los cambios ambientales.

En este trabajo se genera información sobre la capacidad adaptativa que tienen dos especies de *Agave* con diferente nicho ecológico; *A. lechuguilla,* una especie generalista con una importante distribución a lo largo del territorio mexicano, y *A. gentryi,* una especie especialista asociada a zonas montañosas en el noreste del país. A partir de las diferencias adaptativas de estas dos especies, se describe la respuesta morfológica y genética en un gradiente elevacional, el cual puede simular los cambios repentinos que genera el cambio climático. Este estudio se divide en tres capítulos.

El primer capítulo contextualiza el enfoque del trabajo a partir de una revisión sobre lo que actualmente se conoce del efecto del gradiente ambiental sobre la variación morfológica y genética en plantas. Se analizan las respuestas puntuales

mediante el modelado de las condiciones futuras esperadas y un consumo general de variabilidad genética y morfológica, identificando las principales amenazas sobre especies vegetales.

En el segundo capítulo se explica el efecto de la variación ambiental generada por el gradiente elevacional sobre *A. lechuguilla* (generalista) y *A. gentryi* (especialista); el objetivo de este capítulo fue evaluar la respuesta morfológica de cada especie bajo el contexto de la teoría de nicho ecológico. Primeramente, se definieron los estratos de elevación identificando a qué nivel de elevación la variación es significativa, posteriormente se aplicó un análisis de marginalidad media para conocer la amplitud de nicho ecológico de las especies y finalmente se determinó si existe una respuesta morfológica asociada con la variación ambiental del gradiente elevacional.

Finalmente, en el capítulo tres se evalúan la diversidad y estructura genética de *A. lechuguilla* y *A. gentryi* en el gradiente elevacional, a partir de marcadores moleculares se obtuvo un sondeo del perfil genético de cada especie y se analizó si la diferencia de tolerancia ambiental entre ambas especies se relaciona con su variación y diferenciación genética.

Este trabajo es una breve contribución al tema de la capacidad adaptativa de las plantas frente a las oscilaciones ambientales a partir de la plasticidad morfológica y genética.

Hipótesis

“Si la elevación provoca cambios graduales en el ambiente, además de estimular a que las poblaciones formen fenotipos y genotipos asociados con el ambiente, y si las poblaciones de *A. lecheguilla* y *A. gentryi* se distribuyen de manera generalista y especialista, respectivamente, en el gradiente elevacional; entonces se espera que la especie generalista presente mayor diversidad genética con genotipos y morfotipos asociados a cada estrato elevacional en comparación con la especie especialista, la cual contará con menor diversidad genética y poca respuesta morfológica asociada a las variables ambientales”.

Objetivos

Objetivo general

Determinar la variación morfológica-genética de *A. lechuguilla* Torr. y *A. gentryi* Ullrrich., al interior y entre poblaciones ubicadas a lo largo de un gradiente elevacional, mediante correlaciones morfométricas y análisis moleculares, para establecer los atributos ambientales asociados con la variación morfológica-genética.

Objetivos específicos

1) Evaluar la variación ambiental que ocurre en la distribución de *A. lechuguilla* y *A. gentryi* a lo largo de un gradiente elevacional mediante georreferencias y asociación con base de datos; para determinar los atributos más importantes que segregan el ambiente de los sitios.
2) Caracterizar las poblaciones morfológica y genéticamente, mediante análisis morfométricos lineales de caracteres vegetativos y el uso de marcadores moleculares microsatélites; para establecer los niveles de diversidad genética dentro y entre poblaciones.
3) Evaluar las fuerzas evolutivas, mediante análisis bayesianos y correlaciones, que predominan a lo largo del gradiente; para correlacionar las diferencias morfo-genéticas con los atributos ambientales en cada gradiente elevacional.

I. CAPÍTULO 1: Environment gradient effects on morphological and genetic variation of plants: an accelerated response to climate change.

Alfredo Sánchez-González[1], Ludivina Barrientos-Lozano[1], Pablo Octavio-Aguilar[2]

1. Tecnológico Nacional de México-I. T. Ciudad Victoria, Departamento de Posgrado e Investigación. Blvd. Emilio Portes Gil No. 1301. C.P. 87010. Ciudad Victoria Tamaulipas.

2. Universidad Autónoma del Estado de Hidalgo-Centro De Investigaciones Biológicas, Instituto de Ciencias Básicas e Ingeniería, Laboratorio de Genética. Pachuca, Hidalgo, México.

ABSTRACT

The variability is an appreciated background that allows species to adapt and its geographic distribution depends on environmental gradients. Some general patterns across altitudinal and latitudinal gradients predict a clinal demised of variation in the opposite sense between alpine and tropical vegetation. Both gradients could condition the capacity of the species to successful radiation and colonization events, with better adaptability of generalist versus specialist species. In a global warming panorama, the clinal distribution of two principal sources of variation, morphologic and genetic pools, could suffer an accelerated process of variation loss, more severe to alpine and subalpine vegetation than tropical plants. Moreover, tropical and subtropical vegetation would express a limited radiation capacity from low to high altitudinal and latitudinal strata because a subsequent population reduction consumes genetic variability in serial founded events. These putative responses are analyzed in this work by modeling the future expected conditions and general consumption of genetic variability identifying the main threats on vegetal species.

KEY WORDS: clinal loss, global warming, environmental gradients, evolutionary genetics, vegetal response.

INTRODUCTION

Global climate change constitutes an important condition to increase the efforts for evaluating ecological responses of the biotic resources and its potential extinction risk (Harvell et al., 2002; Williams et al., 2003; Araujo et al., 2005). For example, several studies have shown that some areas, in particular alpine and subalpine lands, would act as a refuge of the 5.5 - 14% of the globe plants and animal species after a climate change panorama (Noce et al., 2017; Mattingly and Leopold, 2018; Panetta et al., 2018; Smith et al., 2018). In this sense, the persistence of the species is related to the interaction of environmental stochasticity and its genetic pool, strategically distributed among the populations (Medail and Diadema, 2009). Therefore, the elevational gradients are considered natural experiments for the evaluation of the particular response of species. Since the gradient generates changes in the abiotic parameters as the altitude increases, temperature and atmospheric pressure decrease, and O2 - CO2 partial pressure diminishes (Körner, 2007, Othman et al., 2015), which can modify the allelic frequencies in the populations associating adaptable morphotypes to each stratum (Premoli and Mathiasen, 2011; Galván-Hernández et al., 2015b; Qian, 2017).

For a better understanding of the environmental variation effect over the organisms, it is convenient to consider that each biological species is a dynamic evolutionary unit in time (Griesemer, 2000; Frankham et al, 2009) and from each genetic combination, results in a different hereditable variation set, that responds as an adaptive strategy to the environment, expressed in morphology, behavior and/or physiology particularities (Hedrick, 2009). Inside each population, a greater variation represents a non-depurated genetic pool, a general pattern of healthy populations.

In contrast, a deplete genetic pool is typical of populations subject to natural selection which normally occurs in the geographic, physical or altitudinal limits of the species distribution (Lesica and Allendorf, 1995; Frankham, 2005; Parmesan, 2006).

In fact, when evaluating morphological or genetic responses in environmental gradients it must be taken into account that, despite analyzing individuals of the same group (family, genus, or species), some have a higher environment tolerance range than others, because they are able to take advantages of a greater amount of resources (generalist), while others have exchanged this ability for efficient use of a particular set of resources in specific environmental conditions (specialists) (Boulangeat et al., 2011). Therefore, range-restricted species or specialists, particularly those that are adapted to cold environments, show severe range contractions and have been the first groups in which the entire species have gone extinct due to recent climate change (Parmesan, 2006). Plants suffer more than other organisms this change, because they require time to perform an adaptive process, limited by their motility. Particularly plants that survive in alpine landscapes are constantly challenged by the harsh and often unpredictable environmental conditions, stochasticity linked to global warming. Steep environmental gradients and patchy distribution of habitats lead to a small size and spatial isolation of populations, restricting its gene flow (Stöcklin et al., 2009).

In this work, we review plants' adaptive responses considering morphological and genetic changes, related to an environmental variation on elevational and latitudinal gradients in a global warming panorama. The main causes of extinction that currently threaten alpine and subalpine plant species are identified, and the importance of variation as a potential source of adaptation is highlighted.

Elevational-latitudinal effects on environment traits

An elevational gradient is a gradual or acute change in altitude of the surface with respect to the sea level. This change causes scenarios with an important variation in environment traits that influence the distribution and diversity of biota (Furtado and Neto, 2018). Nevertheless, authors consider that altitudinal gradients are less important with respect to latitudinal gradients as a source of plant variation, because a greater total environment and energetic variability, also a distance restriction of a mixture of vegetal communities, have been shown among latitudinal distances (Brown et al., 1996; Hawkins et al., 2003). However, the limited radiation capacity of some plant species only allows a geographically close establishment that is why the altitudinal gradient becomes more important. In particular, atmospheric factors that act on smaller scales could be associated with sea level and the mentioned limited radiation (atmospheric pressure, temperature, solar radiation), potentiated by latitudinal position (precipitation, wind speed, hours of sunshine, seasonality, etc.) (Körner, 2007), both factor sources interact in the adaptive potential of tropic, subtropic, temperate and boreal-arctic vegetation. The interaction between gradients makes a synergistic effect over the biota, increasing the environmental changes associated with the elevation on Ecuador and homogenizing the environment among elevational strata on latitudinal extremes, that is why on the tropics we might find all life zones of the globe packed into a 5 km altitudinal range (Körner, 2000). However, considering a global temperature increase (2.1°C) on a high CO_2 emissions model (Matthews et al., 2009) at 2050 and 2070, a standard decrease of 6.5°C on each km of altitude (Konovalov and Nagornov, 2017) and a decrease of 5°C by each 10° of latitude (Haltiner and Williams, 1980), using a distance-weighted least squares

model of fitting [Loss = (Obs-Pred)2 * (1/x2)] (Neter et al., 1985), we can predict the loss of boreal-arctic clime in all the elevational strata from Ecuador to the Tropics, and localize tropical areas on sea level since 40 degrees of latitude by the 2070 year (Figure 1), causing warming in the circumpolar zone, more or less above the border between Canada and the United States, reducing the seasonal temperature over time because cooler season are warming faster than summer (Wu et al., 2018).

These temperature changes imply that mountain islands of vegetation could act as refuges but only over 40° latitude because on equatorial zones these types of clime will be extinct below five thousand meters. In America, only five mountains in the tropical zone could conserve refuges of boreal-arctic climes: Huascaran Volcano, Peru (6768 masl), Chimborazo Volcano, Ecuador (6268 masl), Cotopaxi, Ecuador (5890 masl), Cristobal Colon Peak, Colombia (5776 masl) and Citlaltepetl Mountain, Mexico (5747 masl). In Africa, seven mountains are putative clime refuges in the tropics: Kibo Volcano, Tanzania (5895 masl), Batian Peak, Kenya (5199 masl), Nelion Peak, Kenya (5188 masl), Mawenzi Volcano, Tanzania (5149 masl), Mount Stanley, Congo (5109 masl), Alezandra Peak, Congo (092 masl) and Albert Peak, Congo (5088 masl). Even when Asia has the highest mountains in the world, none of them is in the tropics.

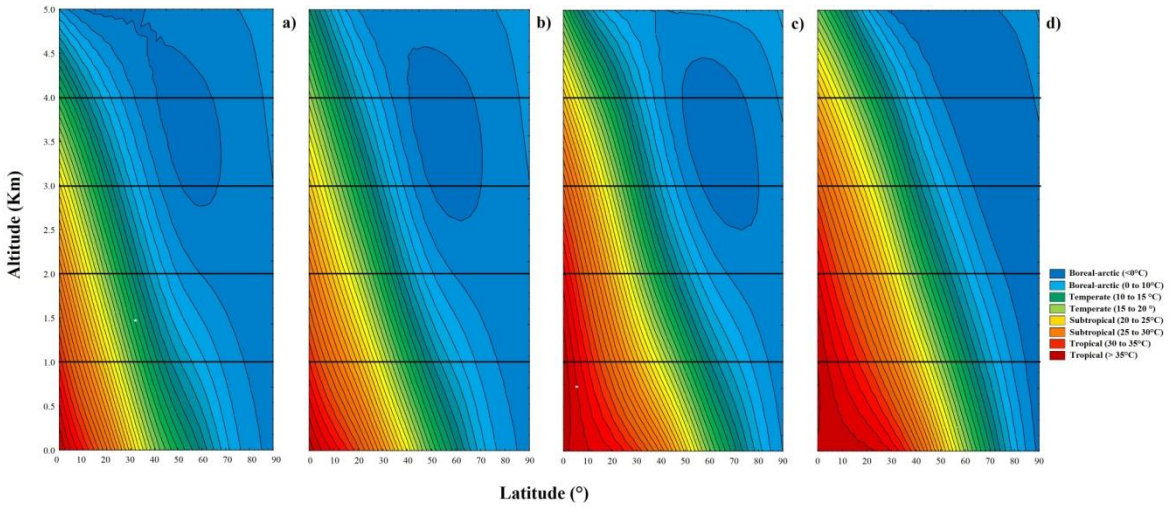

Figure 1. Clime relation among temperature, altitude, and latitude across the time. a) 1950 model, b) actual model, c) 2050 model (high CO_2 emissions), d) 2070 model (high CO_2 emissions).

Morpho-physiological plant responses to the global warming in elevational gradients

As mentioned above, one of the most notable effects of climate change on the ecosystems, mainly in plants, is the redistribution of species and/or morphological changes by have the best performance against environmental oscillations (King et al. 2018). It is known that, in the plant communities, the climatic factors are those that influence more in their performance and the morphological feature that responds more to these factors, is the leaf (Ackerly et al, 2002; Galván-Hernández et al, 2015b); for this reason, climate change stimulates the morphological response of plants and their redistribution in the elevational gradient. In this sense, it is known that the atmospheric pressure decreases 11% by each kilometer of elevation. In particular, O2 and CO2 partial pressure decrease until to 21% at 2000 masl (Körner and Diemer, 1987, Allen et al., 1998); the exchange of these gases is important to the physiology process of plants; for example, on high

temperatures, the diffusion between leaf surface and gas molecules through stomata is increased at constant pressure, in response tropical plants required fewer stomata by foliar surface. Contrastingly, alpine plants increased their stomata density to facilitate the diffusion process by the decrease of gas pressure, but the clime change exposes non-adapted plants to high temperatures and low atmospheric pressures; this scenario promotes an accelerated humidity loss and desiccation (Beerling and Rundgren, 2000, Franks et al., 2013). In consequence, plants of arid or semi-arid climate with crassulacean acid metabolism (CAM) (González-Salvatierra et al, 2013), could occupy new elevational strata; while the most common response of the alpine plants is a morphological variation of the leaves in size, thickness, exposure, number, and/or persistence (Abrams, 1990; Poorter et al., 2012; Liu et al., 2019).

According to this, leaf variation is also promoted by solar incidence, which is less intense on northern than southern latitudes as a response to the earth tilt and make more resistant photosynthetic organisms in southern than northern hemispheres (Agusti et al., 2015). In general, trees of equatorial populations have wider, thinner, and more exposed leaves to direct light than trees of the highest latitudes (Escribano-Rocafort et al., 2017). Consequently, leaf variation is a response to a complex interaction among environmental variables as carbon and water exchange, temperature, precipitation, and the mentioned solar incidence, particularly intense on low latitudes (McGuire et al., 2002). Nevertheless, the elevational gradients act in synergy with latitude because on the top strata more intense radiation is caused by a clean atmosphere in absence of clouds by less humidity. Both gradients increase the physiological stress on alpine plants (or an inverse pattern in tropical plants) and increase the extinction risk and/or the

morphological response of these species including biomass increase with disease infestations or losses in productivity (Sullivan et al., 1992; Ziska, 1996, Cybulske and Peterjohn, 1999; Sullivan, 2005). It shall be mentioned that, unfortunately, the increase of plant canopy size and density in tropics resulting in greater biomass of high nutritional quality on new strata, combined with more environment humidity, promote certain diseases such as rusts, powdery mildews, leaf spots, and blights (Manning and Vontiedemann, 1995). This response could be a consequence of disequilibrium generated by the morphological response accentuated to the climatic change.

The morphological response is a plastic trait defined by the species, related to its phylogenetic history and the mentioned strategy to use of the environment resources (specialist-generalist) (Turner et al., 2015). Generally, good environments result in high-performance trait plasticity, and the specialization for bad environments results in low-performance trait plasticity (Fazlioglu and Bonser, 2016). In a global warming scenario, the alpine plants do not have a phylogenetic history associated with the adaptive process, in consequence, a limited plastic response is expected and low performance of morphological traits (Meng, et al., 2016; Schmid et al., 2017). This homogeneity implies a limited environment response even on the highest elevational strata. In consequence, only generalist plant species could be adapted to a temperature increase scenario in top elevational strata, generating monospecific forest in tropical mountains, susceptible to sickness, because it is expected an inverse association between the abundance of life forms with respect to altitude and a more restricted functional attributes response of specialist species (Pavon et al., 2000, Octavio-Aguilar et al., 2019). In addition, an

accelerated invasion of tropical plants of the highest latitudes but with a limited morphological variation consumed by colonization and genetic bottlenecks, it is expected too.

Genetic response of plants to environmental gradients

The morphological plasticity has a limited capacity to respond to stochastic environments because some attributes have a wide range of variation (polygenic traits) but others are a unique product of genetic expression (Mendelian traits), generating an inconsistent non depurated morphological variability that could be associated with a pioneer-non pioneer plants type and a trade-off interchange between vegetative-reproductive growth (Colautti et al., 2009; Cheesman and Winter, 2013; Louw, et al., 2015).

In contrast, real adaptation results of the change in the frequency of allelic combinations that manifest a specific array of physiological, morphological, and behavior attributes, which in conjunction, increase the propagation, reproduction, and survival success of the carrier plants in a specific environment, but with a loss of other allelic combinations less successful. For this reason, plant genetic resources are related with variety and possible combinations of alleles that allow plant species to adapt (Octavio-Aguilar et al., 2018); but its sessile condition imposes a pattern dependent on the underlying environmental conditions and an elevational gradient generates a heterogeneous environment pushing the phytogenetic variability to the limit (Premoli, 2003), even more intense in a global warming scenario.

High and continuous genetic variation among elevational strata is a rare phytogenetic response associated with a wide possibility to use resources and different strategies to confront evolutionary problems (generalist species). This

response implies gene variation independent of natural selection, a wide ecological niche, geographic proximity to a source of variability (island-continent theory), and an optimal genetic flow (Gaudel et al 2007; Maciel-Mata et al., 2015). The niche center proximity is a theory associated with less intense selection and conditioning the genetic homogeneity to a successive radiation process in both elevational and latitudinal gradients. In this sense, genetic variation of boreal-arctic vegetation could be high and continual in elevational gradients but only at major latitudes, and tropical-subtropical vegetation would be genetically diverse and continual along equatorial elevations (Conover et al., 2009).

More common phytogenetic responses to the elevational gradients are consumption of genetic variability in a monotonically clinal pattern (same distance-weighted least squares model of fitting) from low selection pressures localities to higher stressing strata, in inverse sense between boreal (high to low altitudes, Figure 2a) and tropical (low to high altitudes, Figure 2b) species (Jonas and Geber, 1999, Etterson, 2004; Alberto et al., 2013). In the first example, the boreal-arctic species Fagus crenata showed higher heterozygosity at the top altitudinal and latitudinal levels in Japan (Tomaru et al., 1996), in contrast, tropical species Cedrela fissilis showed an inverse pattern with higher heterozygosity levels at a lower and intermediate south latitude in Brazil (Mangaravite et al., 2016). The dynamic history of the Pleistocene generated periodic glacial cycles with more than 10 major glaciations over the past million years and climatic fluctuation over 103 to 104 years, with a hypothetical expansion of alpine vegetation from some restricted refuges (Knowles, 2000, Gong et al., 2016). This contraction-expansion dynamic in a reticular historical pattern consumes the genetic variation by a successive pattern of

colonization-extinction along both gradients (founder effect) (Coyne, 1992; Dlugosch and Parker, 2008). In consequence, a depleted gene pool is the only actual base to the future adaption to global climatic change, particularly stronger conditions on sky islands with boreal-arctic vegetation in tropic and subtropical latitudes, and opposite conditions in low-lands with temperate and tropical vegetation on high latitudes. These last tropical populations in boreal latitudes could be a source of expansion in a global warming scenario but from limited genetic resources, that will not permit a clinal genetic structure increasing the extinction risk of tropical vegetation at higher strata in major latitudes (Arruda et al., 2018; Hohmann, et al., 2018).

The capacity to resist the mentioned genetic variability reduction implies an accelerated population growth rate, short life cycle, massive production of offspring, and invasive tendencies (Collevatti et al., 2013; Evans et al., 2015). Herbal and shrub life forms could be more resistant to global warming because a rapid evolutionary response permit a demographic expansion and a consequent wide distribution range (Davidson et al., 2011; Gong et al., 2016), but trees, native species, and long-life plants could be more menaced by its intrinsic ecological characteristics (Long et al., 2004) restringing its distribution to patched and isolated sky islands, more and more reduced as they are closer to the equator. In this sense, the prospection of protection of 5.5-14% of the globe vegetal and animal species in alpine and subalpine refuges after a climate change (Noce et al., 2017) may not include native, long life, menaced, or susceptible groups of plants.

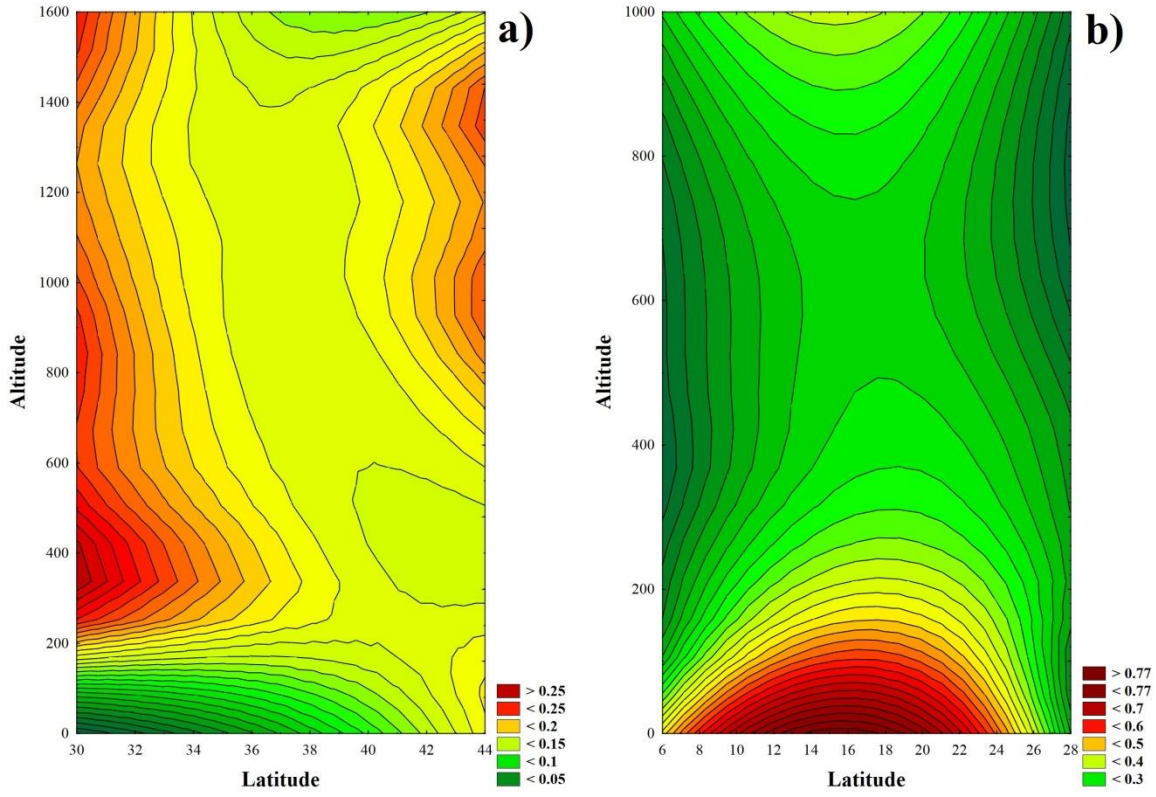

Figure 2. Observed heterozygosity relation among altitude and latitude gradients. a) boreal-arctic species *Fagus crenata* (Tomaru et al., 1996), b) tropical species *Cedrela fissilis* (Mangaravite et al., 2016).

In conclusion, the main threats of alpine and subalpine plants it is the reduction of distribution to a patchy and unconnected fragment with limited capacity of adaptation, associated with a reduction of morphological and genetic variability. These sky islands only could be found at high altitude-latitude in synergic gradients effect, and always over 40° latitudes. In opossum sense, tropical vegetation climb in an altitudinal range and extend its distribution to the north, but could suffer a restricted capacity dependent to its life-form and strategy, showing a colonization.

REFERENCES

Abrams, M. D. 1990. Adaptations and responses to drought in Quercus species of North-America. Tree Physiology, 7(1-4): 227-238. DOI: 10.1093/treephys/7.1-2-3-4.227

Ackerly, D. D., C. A. Knight, S. B. Weiss, K. Barton and K. P. Starmer. 2002. Leaf size, specific leaf area and microhabitat distribution of chaparral woody plants: contrasting patterns in species level and community level analyses. Oecología. 130: 449-457. DOI: 10.1007/s004420100805.

Agusti, S., Llabres, M., Carreja, B., Fernández, M. and Duarte, C. M. 2015. Contrasting sensitivity of marine biota to UV-B radiation between southern and northern hemispheres. Estuaries and Coasts, 38(4): 1126-1133. DOI: 10.1007/s12237-014-9790-9

Alberto, F. J., Aitken, S. N., Alia, R., González-Martínez, S. C., Hanninen, H., Kremer, A., Lefevre, F., Lenormand, T., Yeaman, S., Whetten, R. and Savolainen, O. 20123. Potential for evolutionary responses to climate change evidence from tree populations. Global Change Biology, 19(6): 1645-1661. DOI: 10.1111/gcb.12181

Allen, R. G., Pereira, L., Raes, D. and Smith, M. 1998. Crop evapotranspiration - Guidelines for computing crop water requirements. FAO Irrigation and Drainage, 56: 1-15.

Araujo, M. B., Pearson, R. G., Thuiller, W. and Erhard, M. 2005. Validation of species-climate impact models under climate change. Global Change Biology, 11(9):1504-1513. DOI: 10.1111/j.1365-2486.2005.001000.x

Arruda, D. M., Schaefer, C. E. G. R., Fonseca, R. S., Solar, R. R. C. and Fernandes, E. L. 2018. Vegetation cover of Brazil in the last 21 ka: New insights into the Amazonia refugia and Peistocenic arc hypotheses. Global Ecology and Biogeography, 27(1): 47-56. DOI: 10.1111/geb.12646

Beerling, D. J. and Rundgren, M. 2000. Leaf metabolic and morphological responses of dwarf willow (*Saliz herbacea*) in the sub-arctic to the past 9000 years of global environmental change. New Physiologist, 145(2): 257-269. DOI: 10.1046/j.1469-8137.2000.00582.x

Boulangeat, I., Lavergne, S., Van Es, J., Garraud, L. and Thuiller, W. 2011. Niche breadth, rarity and ecological characteristics within a regional flora spanning large environmental gradients. Journal of Biogeography, 39(1): 204-204. DOI: 10.1111/j.1365-2699.2011.02581.x

Brown, J. H., Stevens, G. C. and Kaufman, D. M. 1996. The geographic range: size, boundaries, and internal structure. Annual Review of Ecology and Systematics, 27: 597-623. DOI: 10.1146/annurev.ecolsys.27.1.597

Cheesman, A. W. and Winter, K. 2013. Growth response and acclimation of CO2 exchange characteristics to elevated temperatures in tropical tree seedlings. Journal of Experimental Botany, 64(12): 3817-3828. DOI: 10.1093/jxb/ert211

Colautti, R. I., Maron, J. L. and Barrett, S. C. H. 2009. Evolutionary Applications, 2(2): 187-199. DOI: 10.1111/j.1752-4571.2008.00053.x

Collevatti, R. G.Telles, M. P. C., Nabout, J. C., Chaves, L. J. and Soares, T. N. 2013. Demographic history and the low genetic diversity in *Dipteryx alata* (Fabaceae) from Brazilian neotropical savannas. Heredity, 111(2): 97-105. DOI: 10.1038/hdy.2013.23

Conover, D. O., Duffy, T. A. and Hice, L. A. 2009. The covariance between genetic and environmental influences across ecological gradients reassessing the evolutionary significance of countergradient and cogradient variation. Year in Evolutionary Biology, Annals of the New Yorj Academy of Sciences, 1168: 100-129. DOI: 10.1111/j.1749-6632.2009.04575.x

Coyne, J. A. 1992. Genetics and Speciation. Nature, 355(6360): 511-515. DOI: 10.1038/355511a0

Cybulski, W. J. and Peterjohn, W. T. 1999. Effects of ambient UV-B radiation on the above-ground biomass of seven temperate-zone plant species. Plant Ecology, 145(1): 175-181. DOI: 10.1023/A:1009820320031

Davidson, A. M., Jennions, M. and Nicotra, A. B. 2011. Do invasive species show higher phenotypic plasticity than native species and, if so, is it adaptive? A meta-analysis. Ecology Letters, 14(4): 419-431. DOI: 10.1111/j.1461-0248.2011.01596.x

Dlugosch, K. M. and Parker, I. M. 2008. Founding events in species invasions: genetic variation, adaptive evolution, and the role of multiple introductions. Molecular Ecology, 17(1): 431-449. DOI: 10.1111/j.1365-294X.2007.03538.x

Escribano-Rocafort, A. G., Ventre-Lespiaucq, A. B., Granado-Yela, C., de Casas, R. R., Delgado, J. A., Escudero, A. and Balaguer, L. 2017. Intraindividual variation in light-related functional traits: magnitude and structure of leaf trait variability across global scales in *Olea europea* trees. Trees-Structure and Funcion, 31(5): 1505-1517. DOI: 10.1007/s00468-017-1565-z

Etterson, J. R. 2004. Evolutionary potential of *Chamaecrista fasciculata* in relation to climate change. 1. Clinal patterns of selection along an environmental gradient in the great plains. Evolution, 58(7): 1146-1458.

Evans, L. M., Allan, G. J., DiFazio, S. P., Slavov, G. T:, Wilder, J. A., Floate, K. D:, Rood, S. B. and Whitham, T. G. 2015. Gographical barriers and climate influence demographic history in narrowleaf cottonwoods. Heredity, 114(4): 387-396. DOI: 10.1038/hdy.2014.115

Fazlioglu, F. and Bonser, S. P. Phenotypic plasticity and specialization in clonal versus non-clonal plants: A data synthesis. Acta Oecologica-International Journal of Ecology, 77: 193-200. DOI: 10.1016/j.actao.2016.10.012

Frankham, R. 2005. Genetics and extinction. Biological Conservation, 126: 131-140. DOI: 10.1016/j.biocon.2005.05.002

Frankham, R., Ballou J.D. and Briscoe DA. 2009. Introudction to Conservation Genetic. Cambridge, UK: University Press, Cambridge.

Franks, P. J., Adams, M. A., Amthor, J. S., Barbour, M. M., Berry, J. A., Ellworth, D. S., Farquhar, G. D., Ghannoum, O., Lloyd, J., McDowell, N., Norby, R. J., Tissue, D. T. and von Caemmerer, S. 2013. Sensitivity of plants to changing atmospheric CO2 concentration: from the geological past to the next century. New Phytologist, 197(4): 1077-1094. DOI: 10.1111/nph.12104

Furtado, S. G. and Neto, L. M. 2018. Elevational and phytophysiognomic gradients influence the epiphytic community in a cloud forest of the Atlantic phytogeographic domain. Plant Ecology, 219(6): 677-690. DOI: 10.1007/s11258-018-0826-2

Galván-Hernández, D., Lozada-García, J. A., Flores-Estévez, N., Galindo-González, J. and Vázquez-Torres, S. M. 2015. Variation and genetic structure in *Platanus mexicana* (Platanaceae) along riparian altitudinal gradient. International Journal of Molecular Sciences, 16(1): 2066-2077. DOI: 10.3390/ijms16012066

Galván-Hernández, D., J. A. Lozada-García, N. Flores-Estévez, J. Galindo-González and S. M. Vázquez-Torres. 2015b. Altitudinal gradient effect on morphometric variation and leaf symmetry of *Platanus Mexicana* Moric. Revista Chapingo Serie Ciencias Forestales y del Ambiente. 21: 171-183. DOI: 10.5154/r.rchscfa.2014.08.034.

Gong, W., Liu, W. Z., Gu, L., Kaneko, S., Koch, M. A. and Zhang, D: X. 2016. From glacial refugia to wide distribution range: demographic expansion of *Loropetalum chinese* (Hamamelidaceae) in Chinese subtropical evergreen broadleaved forest. Organisms Diversity & Evolution, 16(1): 23-38. DOI: 10.1007/s13127-015-0252-4

González-Salvatierra, C., J. L. Andrade, R. Orellana, L. M. Peña-Rodríguez and C. Reyes-García. 2013. Microambiente lumínico y morfología y fisiología foliar de *Bromelia karatas* (Bromeliaceae) en una selva baja caducifolia de Yucatán, México. Botanical Sciences. 91: 75-84.

Griesemer, J. 2000. The units of evolutionary transition. Selection 1(3): 67-80. DOI: 10.1556/Select.1.2000.1-3.7

Gaudel, M., H. K. Stenoien and J. Agren. 2007. Landscape structure, clonal propagation, and genetic diversity in Scandinavian populations of

Arabidopsis lyrata (Brassicaceae). American Journal of Botany, 94: 1146-1155.

Haltiner, G. J. and Williams R. T. 1980. Numerical prediction and dynamic meteorology. Wiley, New York. 477 pp.

Harvell, C. D., Mitchell, C. E., Ward, J. R., Altizer, S., Dobson, A. P., Ostfeld, R. S. and Samuel, M. D. 2002. Ecology-Climate warming and disease risks for terrestrial and marine biota. Science, 296(5576): 2158-2162. DOI: 10.1126/science.1063699

Hawkins, B. A., Field, R., Cornell, H. V., Currie, D. J., Guegan, J. F., Kaufman, D. M., Kerr, J. T., Mittelbach, G. G., Oberdorff, T., O'Brien, E. M., Porter, E. E. and Turner, J. R. G. 2003. Energy, water, and broad-scale geographic patterns of species richness. Ecology, 84(12): 3105-3117. DOI: 10.1890/03-8006.

Hedrick, P. 2009. Population Genetics in Ecology. Pp 109-116. In: P. Hedrick (Ed.) Genetics of Populations. Jones y Bartlett Publisher. Sudbury, Massachusetts.

Hohmann, N., Wolf, E. M., Rigault, P., Zhou, W. B.m Kiefer, M., Zhao, Y. P., Fu, C. X. and Koch, M. A. 2018. *Ginkgo biloba´*s footprint of dynamic Pleistocene history dates back only 3900,000 years ago. BMC Genomics, 19: 299. DOI: 0.1186/s12864-018-4673-2

Jonas, C. S. and Geber, M. A. 1999. Variation among populations of *Clarkia ungiculata* (Onagraceae) along altitudinal and latitudinal gradients. American Journal of Botany, 86(3): 333-343. DOI: 10.2307/2656755

King, N.G., N. J. McKewon, D. A. Sale and J Moore. 2018. The importance of phenotypic plasticity and local adaptation in driving intraspecific variability in thermal niches of marine macrophytes. Echography. 41: 1469-1489. DOI: 10.1111/ecog.03186.

Knowles, L. L. 2000. Test of Pleistocene speciation in montane grasshoppers (genus Melanoplus), from the sky islands of western North America. Evolution, 54(4): 1337-1348. DOI: 10.1111/j.0014-3820.2000.tb00566.x

Konovalov, Y. V. and Nagornov, O. V. 2017. Two-dimensional pronostic experiments for fast-flowing ice streams from the Academy of Sciences Ice Cap. Earth Systems Dynamics, 8(2): 283-294. DOI: 10.5194/esd-8-283-2017

Körner, C. and Diemer, M. 1987. In situ photosynthetic responses to light, temperature and carbon dioxide in herbaceous plants from low and high altitude. Functional Ecology, 1(3): 179-194. DOI: 10.2307/2389420

Körner, C. 2000. Why are there global gradients in species richness? Mountains might hold the answer. Trends in Ecology & Evolution, 15: 513-514. DOI: 10.1016/S0169-5347(00)02004-8

Körner, C. 2007. The use of altitude in ecological research. Trends in Ecology and Evolution, 22: 569-574. DOI: 10.1016/j.tree.2007.09.006

Lesica, P. and Allendorf, F. W. 1995. When are peripheral populations valuable for conservation? Conservation Biology, 9(4): 753-760.

Long, S. P., Ainsworth, E. A., Rogers, A. and Ort, D. R. 2004. Rising atmospheric carbon dioxide: Plants face the future. Annual Review of Plant Biology, 55: 591-328. DOI: 10.1146/annurev.arplant.55.031903.141610

Liu, L. L., Ji, H. T., An, J. P., Shi, K. J., Ma, J. F., Liu, B., Tang, L., Cao, W. X. and Zhu, Y. 2019. Response of biomass accumulation in wheat to low-temperature stress at jointing and booting stages. Environmental and Experimental Botany, 157: 46-57. DOI: 10.1016/j.envexpbot.2018.09.026

Louw, E. L., Hoffman, E. W., Theron, K. I. and Midgley, S. J. E. 2015. Physiological and phenological responses of Protea 'Pink Ice' to elevated temperatures. South African Journal of Botany, 99: 93-102. DOI: 10.1016/j.sajb.2015.04.002

Maciel-Mata, C. A., Manríquez-Morán, N., Octavio-Aguilar, P. and Sánchez-Rojas, G. 2015. Geographical distribution of the species: a concept review. Acta Universitaria, 25(2): 3-19. DOI: 10.15174/au.2015.690

Mangaravite, E., Vinson, C. C., Rody, H. V. S., García, M. G., Carniello, M. A., Silva, R. S. and Olveira, L. O. 2016. Contemporary patterns of genetic diversity of *Cedrela fissilis* offer insight into the shaping of seasonal forest in eastern South America. American Journal of Botany, 103(2): 307-316. DOI: 10.3732/ajb.1500370

Manning, W. J. and Vontiedemann, A. 1995. Climate-change - potential effects of increased atmospheric carbon-dioxide (CO2), ozone (O-3), and ultraviolet-B (UV-B) radiation in plant -diseases. Environmental Pollution, 88(2): 219-245. DOI: 10.1016/0269-7491(95)91446-R

Matthews, H. D., Gillett, N. P., Stott, P. A. and Zickfeld, K. 2009. The proportionality of global warming to cumulative carbon emissions. Nature, 459(7248): 829-U3. DOI: 10.1038/nature08047

Mattingly, K. Z. and Leopold, D. J. 2018. Habitat indicators of a federally listed glacial relict plant species restricted to cliffs in the northern United States. Natural Areas Journal, 38(1): 54-67.

McGuire, A. D., Wirth, C., Apps, M., Beriger, J., Clein, J., Epstein, H., Kicklighter, D. W., Bhatti, J., Chapin, F. S., de Groot, B., Efremov, D., Eugster, W., Fukuda, M., Gower, T., Hinzman, L., Huntley, B., Jia, G. J., Kasischke, E., Melillo, J., Romanovsky, V., Shvidenko, A., Vaganov, E. and Walker, D. 2002. Environmental variation, vegetation distribution, carbon dynamics and water/energy exchange at high latitudes. Journal of Vegetation Science, 13(3): 301-314. DOI: 10.1111/j.1654-1103.2002.tb02055.x

Medail, F. and Diadema, K. 2009. Glacial refugee influence plant diversity patterns in the Mediterranean basin. Journal of Biogeography, 36(7): 1333-1345. DOI: 10.1111/j.1365-2699.2008.02051.x

Meng, F. D., Zhou, Y., Wang, S. P., Duan, J. C:, Zhang, Z. H., Niu, H. S., Jiang, L. L., Cui, S. J., Li, X. E., Lou, C. Y., Zhang, L. R., Wang, Q., Bao, X. Y., Dorji, T., Li, Y. N., Du, M. Y., Zhao, X. Q., Zhao, L., Wang, G. J. and Inouye, D. W. 2016. Temperature sensitivity threshold to warming and cooling in phenophases of alpine plants. Climatic Change, 139(3-4): 579-590. DOI: 10.1007/s10584-016-1802-2

Neter, J., Wasserman, W. and Kutner, M. H. 1985. Applied linear statistical models: regression, analysis if variance, and experimental designs. Volume 469. Second Edition. 1127 pp. Michigan, US.

Noce, S., Collalti, A. and Santini, M. 2017. Likelihood of changes in forest species suitability, distribution, and diversity under future climate: The case of

southern Europe. Ecology and Evolution, 7(22): 9358-9375. DOI: 10.1002/ece3.3427

Octavio-Aguilar, P., García-Montes, M. A., Galván-Hernández, D. M., Macedo-Villarreal, M. A. and Sánchez-González, A. 2018. Conservation of phytogenetic resources, smart use of biodiversity. Revista Biológico Agropecuaria Tuxpan, 6(2): 2064-2073.

Octavio-Aguilar, P., Martínez-Falcón, A. P., Sánchez-González, A., Rojas-Martínez, A., Meerow, W. A., Ramírez-Bautista, A., Ortiz-Pulido, R., Caballero-Cruz, P., Hernández-Rico, G. N. and Berriozabal-Islas, C. S. 2019. Influence of microhabitat on functional attributes of two columnar cacti with different distribution ranges. Journal of Arid Environments, 162: 18-25. DOI: 10.1016/j.jaridenv.2018.12.003

Othman, R., Latiff, N. H. M., Tukiman, I. and Hashim, K. S. H. Y. 2015. Effects of altitude and microclimate on the distribution ferns in an urban areas. Journal Technology, 77: 30.

Panetta, A. M., Stanton, M. L. and Harte, J. 2018. Climate warming drives local extinction: Evidence from observation and experimentation. Science Advances, 4(2): eaaq1819. DOI: 10.1126/sciadv.aaq1819

Parmesan, C. 2006. Ecological and Evolutionary responses to recent climate change. Annual Review of Ecology, Evolution, and Systematics, 37:637–69. DOI: 10.1146/annurev.ecolsys.37.091305.110100

Pavon, N. P., Hernández-Trejo, H. and Rico-Gray, V. 2000. Distribution of plant life forms along an altitudinal gradient in the semi-arid valley of Zapotitlán, Mexico. Journal of Vegetation Science, 11(1): 39-42. DOI: 10.2307/3236773

Poorter, H., Niklas, K. J., Reich, P. B., Oleksyn, J., Poot, P. and Mommer, L. 2012. Biomass allocation to leaves, stems and roots: meta-analyses of interspecific variation and environmental control. New Phytologist, 193(1): 30-50. DOI: 10.1111/j.1469-8137.2011.03952.x

Premoli, A. C. 2003. Isozyme polymorphisms provide evidence of clinal variation with elevation in *Nothofagus pumilio*. Journal of Heredity, 94: 218-226. DOI: 10.1093/jhered/esg052

Premoli, A. C. and Mathiasen, P. 2011. Adaptive vs. plastic ecophysiological responses in dry montane habitats: *Nothofagus pumilio*, the tree that took over the Southern Andes. Ecología Austral, 21(3): 251-269.

Qian, H. 2017. Climatic correlates of phylogenetic relatedness of woody angiosperms in forest communities along a tropical elevational gradient in South America. Journal of Plant Ecology, 11(3): 394-400. DOI: 10.1093/jpe/rtx006

Schmid, S. F., Stocklin, J., Hamann, E. and Kesserling, H. 2017. High-elevation plants have a reduced plasticity in flowering time in response to warming compared to low-elevation congeners. Basic and Applied Ecology, 21: 1-12. DOI: 10.1016/j.baae.2017.05.003

Smith, P., Price, J. Molotoks, A., Warren, R. and Malhi, Y. 2018. Impact on terrestrial biodiversity of moving from a 2 degrees C to a 1.5 degrees C target. Philosophical transactions of the Royal Society A-Mathematical Physical and Engineering Sciences, 376(2119): 20160456. DOI: 10.1098/rsta.2016.0456

Stöcklin, J., Kuss, P. and Pluess, A. R. 2009. Genetic diversity, phenotypic variation and local adaptation in the alpine landscape: case studies with

alpine plant species. Botanica Helvetica, 119: 125-133. DOI: 10.1007/s00035-009-0065-1

Sullivan, J. H., Teramura, A. H. and Ziska, L. H. 1992. Variation in UV-B sensitivity in plants from a 3,000-m elevational gradient in Hawaii. American Journal of Botany, 79(7): 737-743. DOI: 10.2307/2444938

Sullivan, J. H. 2005. Possible impacts of changes in UV-B radiation on North American trees and forests. Environmental Pollution, 137(3): 380-389. DOI: 10.1016/j.envpol.2005.01.029

Tomaru, N., Mitsutsuji, T., Takahashi, M., Tsumur, Y., Uchida, K. and Ohba, K. 1996. Genetic diversity in *Fagus crenata* (Japanese beech): influence of the distributional shify during the late-Quaternary. Heredity. 78: 241-251.

Turber, K. G., Freville, H. and Rieseberg, L. H. 2015. Adaptive plasticity and niche expansion in an invasive thistle. Ecology and Evolution, 5(15): 3183-3197. DOI: 10.1002/ece3.1599

Williams, S. E., Bolitho, E. E. and Fox, S. 2003. Climate change in Australian tropical rainforests: an impending environmental catastrophe. Proceedings of the Royal Society B-Biological Sciences, 270(1527): 1887-1892. DOI: 10.1098/rspb.2003.2464

Wu, C. Y., Wang, X. Y., Wang, H. J., Ciais, P., Penuelas, J., Myneni, R. B., Desai, A. R., Gough, C. M., Gonzamo, A., Black, A. T., Jassal, R. S., Ju, W. M., Yuan, W. P., Fu, Y. S., Shen, M. G., Li, S. H., Liu, R. G., Chen, J. M. and Ge, Q. S. 2018. Nature Climate Change, 8(12): 1092+. DOI: 10.1038/s41558-018-0346-z

Ziska, L. H. 1996. The potential sensitivity of tropical plants to increased ultraviolet-B radiation. Journal of Plant Physiology, 148(1-2): 35-41. DOI: 10.1016/S0176-1617(96)80291-4

II. CAPÍTULO 2: Effect of elevation strata on morphological variation of two *Agave* species with different niche amplitude

Sánchez-González, Alfredo[1]; Octavio-Aguilar, Pablo[2], Barrientos-Lozano, Ludivina[1], Meerow W. Alan[3]*

[1]Tecnológico Nacional de México-Instituto Tecnológico de Ciudad Victoria. División de Estudios de Posgrado e Investigación. Blvd. Emilio Portes Gil No. 1301. Ciudad Victoria Tamaulipas. México. C.P. 87010.

[2]Universidad Autónoma del Estado de Hidalgo-Centro de Investigaciones Biológicas. Carretera Pachuca-Tulancingo Km. 4.5. Col. Carboneras, Mineral de la Reforma, Hidalgo. México. C.P. 42184.

[3]USDA-ARS-SHRS, National Germplasm Repository, 13601 Old Cutler Road, Coral Gables, Florida 33158, USA.

* Corresponding author: ludivinab@yahoo.com

Tecnológico Nacional de México-Instituto Tecnológico de Ciudad Victoria. División de Estudios de Posgrado e Investigación. Blvd. Emilio Portes Gil No. 1301. Ciudad Victoria Tamaulipas. México. C.P. 87010. ludivinab@yahoo.com Teleph: 52 + 834 153 2000 Ext. 364

Abstract

Species modify their morphological attributes in response to environmental variation as an adaptive strategy, a situation aggravated by current conditions of rapid alteration of global ecosystems. Altitudinal gradients represent an ideal source of environmental variation to evaluate such adaptations. Along these gradients, generalist species are the most adaptable, which increases their radiation potential. In the present study, the plastic response of two species with different requirements was evaluated: *Agave lechuguilla* (generalist) and *A. gentryi* (specialist) along an elevational gradient (500-3500 masl). Our hypothesis was to expect differences in environmental tolerance between *A. lechuguilla* (with a wide distribution) and *A. gentryi* (with a restricted distribution), and greater phenotypic plasticity in the generalist species with respect to the specialist with a grade of morphological variability associated with the different elevation strata. The objective of the present study was to evaluate the plastic response of each species in the context of ecological niche amplitude. An ecological niche analysis of average marginality index (Outlying Mean Index OMI) was used to measure the range amplitude of each species. To quantify the morphological response of each species along the elevational strata, a generalized discriminant function analysis (AFDG) was calculated, and a spatial interpolation of morphological attributes related to environmental variation was generated. The results indicate that *Agave lechuguilla* has a wider niche than *A. gentryi*, explained by higher morphological variation, in particular leaf size (length and width) and length of the terminal spine, attributes that decrease at higher elevation. *A. gentryi* has a restricted distribution limited to three elevation strata and does not exhibit any morphological differences between

gradients. In other words, elevation gradient modified the plastic capacity of the more widely distributed species.

Keywords: elevational gradients, morphometry, outlying mean index, species-environment relationships, and multivariate methods.

Introduction

Accelerated alteration of ecosystems may stress the adaptive processes of species, especially those with less environmental tolerance (Foley *et al*, 2005; Gerstner *et al*, 2014; Völler *et al*, 2017). This tolerance limitation is accentuated in wild populations when there is selective harvest of individuals by homogenizing the morphological and genetic variation and reducing the adaptive potential of the species (Lande, 1988). Therefore, it is important to evaluate both ecologic tolerance and morphological variation to identify the adaptive capacity of species to respond to environmental variation, and subsequently design better management and conservation measures.

Studies that evaluate a species' response to environmental conditions are important to identify morphological variation related to plastic and adaptive responses (Lande, 2009). Altitudinal gradients allow us to test this response, because they generate significant environmental variations as altitude increases or decreases; they are considered "natural experiments" (Fabro and Körner, 2004; Körner, 2007; Premoli and Mathiasen, 2011). The pressure exerted by the environmental variation produced by elevation leads to: *i)* variation patterns

correlated with altitudinal strata (Nicotra *et al,* 2010), and *ii)* spatial distribution ranges related to ecological niche (Tsiftsis *et al*, 2008).

Variation as a response to the environment is the capacity of organisms to modify their behavior, anatomy, physiology, or gene pool, conferring adaptability to tolerate changes in the environment (Hedrick, 2009). Therefore, species with greater interspecific variation are more likely to adapt in changing environments; morphological variation provides a greater advantage in biotic and abiotic interactions (Lande, 2009, Nicotra *et al*, 2010, Turrcote and Levine, 2016). For this reason, populations exhibit adaptive peaks that allow them to optimally meet their requirements. In plants, the organs with the greatest variation are leaf and flower, which is why they are commonly used for taxonomic questions and environmental correlations (Andrade *et al*, 2008, Premoli and Mathiasen, 2011, Arteaga *et al*, 2015, Galván-Hernández *et al*, 2015). Plants that grow in arid and semi-arid zones often evolve succulent leaves with deposits of wax and thick cuticles, rosette phyllotaxy, low stomatal density and generally have a CAM system, all of which reduces the consequences of high insolation (García-Mendoza, 2007; Liu *et al*, 2018).

Environmental variation can influence the ecological niche of a species if spatial distribution is considered an underlying effect of resource availability. This fits the concept of ecological niche proposed by Hutchinson (1957), defined as the hypervolume of *n*-dimensions required by a species to persist, each dimension representing a variable. Within this niche approach is an extension: the fundamental niche corresponds to the total range of environmental conditions that are favorable for the existence of a species without the influence of interspecific competition, predation, limitation of dispersal and natural or human disturbances (Pulliam, 2000).

The realized niche refers to the part of the fundamental niche actually occupied by the species under these restrictions and defines the spatial distribution of the species in a community and or study area (Austin and Smith, 1989). In order to generate a much more accurate niche model, we propose to formulate a relationship between the data on the dynamics of the resources present in the space occupied by the species and the population dynamics of the species (population density). In this way the requirement and impact niche is assembled, although obtaining and integrating this information is extremely complicated as is implementing it in a mathematical model (Leibold and Geddes, 2005; Peterson *et al*, 2011). However, defining the niche based on the requirements (environmental or Grinnellian niche) is correct, especially considering that the present study is aimed at determining the niche breadth as a plastic response perhaps associated with adaptive changes (Soberón, 2010; Peterson *et al*, 2011; Blonder, 2017).

This allows the clarification of which type of environmental variables can contribute favorably to niche adaptation and the morphological response of the species. There are environmental factors that, by exerting pressure on organisms, change their usefulness (bionomic). Therefore, it is important to establish variables that are not modified when used by organisms (scenopoetics) (Hutchinson, 1978, Soberón, 2010, Peterson *et al*, 2011). For plant in particular, it has been shown that the niche is defined by the capacity to develop under climatic, physiographic and edaphic conditions (Malanson *et al*, 1992, Thuiller *et al*, 2004, Tsiftsis *et al*, 2008).

The genus *Agave* L. (Asparagaceae) includes species with both broad and restricted distribution. They are among the most conspicuous and representative taxa in Mexico due to their ecological, economic, and cultural value (Delgado-Lemus

et al, 2014). Several human communities exploit agave wild populations without management strategies or regular evaluation of the resource (García-Mendoza, 2007, Casas *et al*, 1999, Treviño-Carreón *et al*, 2011; Félix-Valdez *et al*, 2015). *Agave lechuguilla* Torr, is a widely distributed species in Mexico, mainly in the Altiplano Mexican and in the mountainous areas of northeastern Mexico, in the states of Chihuahua, Coahuila, Nuevo León, Tamaulipas, Durango, Zacatecas, San Luis Potosí, Querétaro, Hidalgo and Mexico, as well as in the United States in Texas and southern New Mexico (García-Mendoza, 1995; Gentry, 1982; Hochztätter, 2015), with an elevational amplitude of 500 to 3,000 masl. This plant has been used for bioremediation of chromium and cadmium leachates (Mohan and Pittman, 2006; Sawalha *et al*, 2006), has potential to generate biofuels (Davis *et al*, 2011), and is used to produce cellulose fibers and derivatives (Vieira *et al*, 2002). The latter is its principal use in the northern region of Mexico. According to the IUCN *A. lechuguilla* is in a low risk category (Least Concern), since it has a continuous and abundant distribution (IUCN, 2017).

By contrast, the distribution of *A. gentryi* Ullrich (green agave) is restricted mainly to mountainous areas of northeastern Mexico, in the states of Coahuila, Nuevo León, Tamaulipas, Durango, Zacatecas, San Luis Potosí, Hidalgo, Mexico and Puebla. It is associated with limestone in temperate forests and scrub. Very little is known about its distribution and abundance. Based on records obtained from herbarium specimens, it appears that its elevational distribution is between 1,500-3,000 masl (IBUNAM:MEXU:8267). Hernández-Castillo and Treviño-Carreón (2009) evaluated the reproductive biology of the species, which is nocturnal flowering and associated with a chiropterophilous pollination syndrome, like other *Agave* species

with restricted distribution such as *A. difformis* and *A. striata* (Trejo-Salazar *et al*, 2015). There is little basic information on *A. gentryi*, such as demographic characterization, risk status and genetic variation.

The evolutionary history of a species is an essential factor in its accumulation of variation. Paleontological and molecular evidence suggests that climatic and glacial cycles that occurred in the last 2.5 million years affected the distribution, diversity and genetic structure of plant and animal populations in North America (Castellanos-Morales *et al*, 2016; Sosa *et al*, 2018). *A. lechuguilla* was exposed to different diversification events associated with the formation of the Altiplano Mexicano and the Sierra Madre Oriental, generating several lineages associated with geographic isolation. *A. gentryi* has a much more recent origin (Scheinvar *et al*, 2015; Castellanos-Morales *et al*, 2016). This could also influence the reproductive biology of the species, as agaves have coevolved with bats of the genus *Leptonicteris* (Gentry, 1982; Rocha *et al*, 2006). Floral morphological variation corresponds to a latitudinal gradient associated with the distribution of chiropteran (Silva-Montellano and Eguiarte, 2003). However, *A. lechuguilla*, unlike *A. gentryi*, can be pollinated by birds and insects as well; this could influence the amplitude of its distribution. (Rocha *et al,* 2006). Consequently, some species have developed the capacity to take advantage of a wide range of resources and establish in diverse environmental conditions (generalist), while others have evolutionarily exchanged that ability to be more efficient in taking advantage of a set of resources and require specific environmental conditions (specialists) (Gaston, 1997, Boulangeat *et al*, 2011).

Considering the contrast between *A. gentryi* and *A. lechuguilla*, it is possible to evaluate the differential effect of the environment on the adaptive and plastic capacity of both agaves. We hypothesize differences in the environmental tolerance between *A. lechuguilla* (wide distribution) and *A. gentryi* (restricted distribution), with greater morphological variation in the generalist species with respect to the specialist, with a grade of morphological variability associated with elevation gradient. The objective of the present study is to evaluate the plastic response of these species through the measure of range amplitude of ecological niche and of morphological variation to elevational gradient.

Material and Methods

Study area

The study area is located on the southwestern region of Tamaulipas State between 23° 47’ and 23° 24’north latitude and 99°58’ and 99°35’ west longitude (Figure 1). It includes two biogeographical provinces (Rzedowski, 1978): i) Sierra Madre Oriental (SMOr) and ii) Mexican Altiplano (MA) dominated by pine and oak forest and rosetophyll thicket, respectively, two vegetation types associated with agaves (Rzedowski, 1978; García-Mendoza, 2007). The study area has an elevation gradient of 500 to 3,500 masl, implying an important source of environmental variation.

Elevation strata of 500 masl were defined based on an AFD; this multivariable technique identifies the global differences between groups (grouping variable) with respect to a set of elements (independent variables), generates linear combinations

of the variables used to maximize the difference between groups and indicates the variable or variables that best discriminate each group (Jongman *et al*, 1995, Gotelli and Ellison 2004). In this case, the grouping variable corresponds to each elevational floor of 500 m. Each stratum was assigned an environmental identity from a set of climatic and phenological variables and from which environmental differences between the gradients were delimited. Eleven environmental variables were taken (Table 1); these were obtained from World Clime (Hijmans *et al,* 2005) and Soil Grids v 0.5.5 databases (Shangguan *et al*, 2017; Hengl *et al*, 2017).

Once the elevational gradients were defined, a stratified-type systematic sampling was applied to *Agave gentryi* and *A. lechuguilla*, using the same stratification criterion and applying independent radii of 30 m around each localized individual to avoid a coancestry effect (Flores-Abreu, 2007). Everyone was measured across seven morphological attributes related to vegetative growth, including the dimensions of the rosette, the length and width of the leaf, mucron and the number of teeth in the margin of the leaf in a range of 10 cm (Table 2). Based on other studies, it was established that the characters related to the dimensions of the leaf are those that respond significantly to environmental variation independently of the species (Premoli and Mathiasen, 2011, Galván-Hernández, 2015).

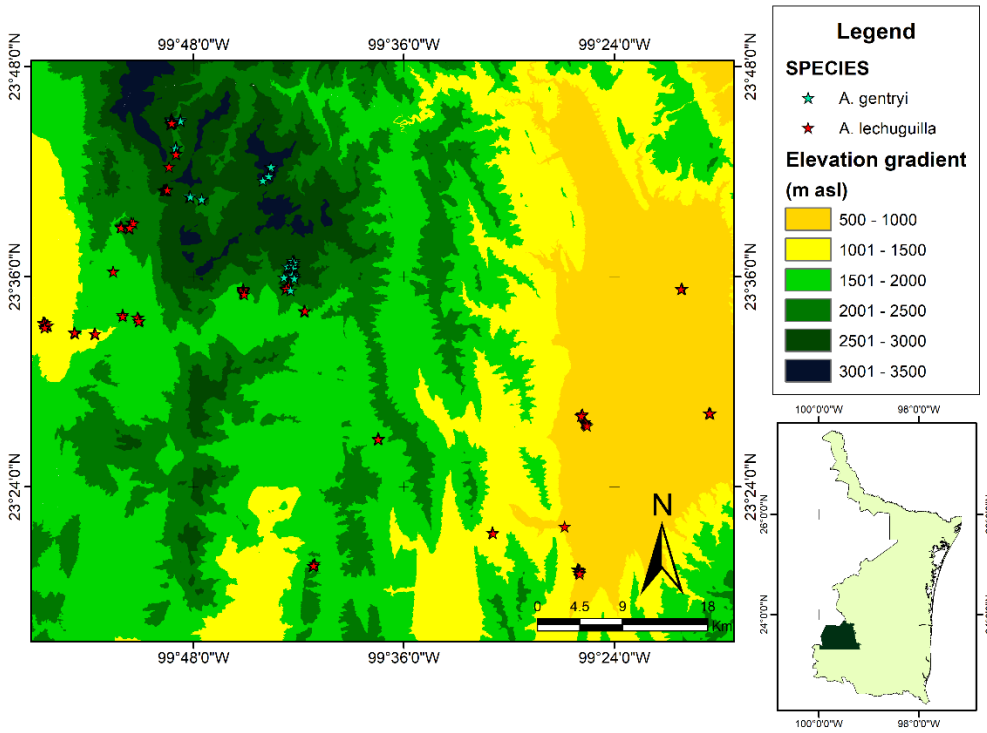

Figure 1. Study area. Red stars indicate *Agave lechuguilla*; green stars, *A. gentryi*.

Table 1. Average and standard deviation of 11 environmental variables at six elevation strata. Note. Contribution of each attribute to the AFD is shown. Coef: Correlation coefficient. G1 gradient 1; G2 gradient 2; G3 gradient 3; G4 gradient 4 and G5 gradient 5

VARIABLE	G1	G2	G3	G4	G5	G6	Coef.
Average temperature (°C)	21.8 ± 0.5	19.8 ± 0.2	18 ± 0.6	16 ± 1	15 ± 0.2	13.4 ± 0.5	-0.7468
Bulk density (kg/m3)	1389.7 ± 21	1378 ± 12	1330.6 ± 23	1309.6 ± 20	1278 ± 14	1223.5 ± 36	-0.362
Soil pH X 10 in H2O	76.3 ± 2	78.8 ± 2	72.6 ± 2	68.3 ± 3	66 ± 3	65.8 ± 1.5	-0.253
Soil pH X 10 in KCl	62 ± 2	67.3 ± 3	58.8 ± 4.5	55.4 ± 3	53 ± 3	50 ± 1	-0.196
Average solar radiation (kJ m-2 day-1)	17817 ± 77	17778 ± 68	17764 ± 59	17735 ± 46	17658 ± 32	17634 ± 31	-0.157
Precipitation of the wettest quarter (mm)	91.7 ± 4	71.4 ± 4.5	63 ± 8	63.5 ± 7.7	73.6 ± 5	94 ± 8	-0.12
Sand content (50-2000 µm) mass fraction in %	38.5 ± 2	33.4 ± 1	37.2 ± 1.4	36.7 ± 1.4	34.9 ± 1.5	33.2 ± 1.5	-0.08
Stilt content (2-50 µm) mass fraction in %	26 ± 2	30 ± 1.4	26.7 ± 2.6	27.6 ± 2	29.2 ± 1.7	30.8 ± 2.6	0.06
Precipitation of the driest quarter (mm)	13.8 ± 0.2	14 ± 0.3	13.5 ± 0.2	13.7 ± 0.8	14.9 ± 0.5	17.7 ± 1	0.125
Cation exchange capacity of soil (cmolc/kg)	21 ± 1.4	20.7 ±2	24 ± 3.7	25.6 ± 2	25.3 ± 1.7	29.8 ± 1.5	0.128
Soil organic carbon stock per ha	20.7 ± 2	19.4 ± 2	23.4 ± 2.7	27.2 ± 2	31.4 ± 3.2	33.8 ± 1.5	0.257

Analysis

Niche analysis was conducted using the average marginality index (Outlying Mean Index OMI) from 11 variables; seven are related to geological factors and four are related to climatic factors (Table 4). The climatic variables were obtained from World Clim (Hijmans *et al,* 2005) and the geological variables from Soil Grids v0.5.5 databases (Shangguan *et al*, 2017; Hengl *et al*, 2017). With the tool "Extract Multi Values to Points" of ArcGis ver. 10.3 (ESRI, 2012) we extracted the values of the environmental variables to determinate the ecological tolerance of both *Agave* species, measuring the range amplitude of their ecological niche.

The OMI identifies the group of ecological variables that exert greater pressure on the structure and composition of the community. As a first step, measure of the marginality of the species that corresponds to the distance among the average habitat conditions used (centroid of the species) and the average of the sample area conditions (origin of the niche hyperspace) illuminates the difference between the species that occurs in atypical habitats of the region (marginal), and species that occurs in typical habitats of the region (nonmarginal) (Maciel-Mata *et al,* 2015). The posterior from permutation (1,000 random simulations) tests if the deviation among the centroid of the species is statistically different from the environmental average conditions. Finally, the analysis generates values for the ecological tolerance of the species expressed as the niche range and the residual tolerance that quantifies the degree of association among the environmental variables and the presence or absence of the species (Dolédec *et al*, 2000; Tsiftsis *et al*, 2008). ADE-4 (Thioulouse *et al*, 1997) was used for the analysis of ecological niche. The analysis combines the linear response of the principal components (PCA) and the unimodal response

of the canonical correspondence analysis (CCA). The first step of the analysis is to order the sites according to the environmental variables by applying a PCA, for which the "correlation matrix PCA" function was used; later, the generated ordering is used to associate the presence-absence of the species with a CCA using the option "Niche".

Table 2. Morphological attributes evaluated in individuals of Agave lechuguilla and A. gentryi across six elevation strata.

Strata	Elevation	*A. lechuguilla*	*A. gentryi*	Morphological attributes
1	500 -1000	30	0	Rosette dimension (height and width in cm)
2	1001-1500	30	0	Leaf number
3	1501-2000	30	0	Leaf length (cm)
4	2001-2500	16	11	Leaf width (cm)
5	2501-3000	19	42	Average mucrons in 10 cm
6	3001-3500	0	4	Length of terminal spine (cm)

Finally, an analysis of discriminant functions generalized (AFDG) among elevational strata with the Statistica v.10 software (StatSoft, 2011) was employed to evaluate the morphological variation of *A. gentryi* and *A. lechuguilla* within the elevation gradient. From the linear combinations of the independent variables (morphological variables) taken from each individual, it was determined whether there are significant differences between them, generating comparisons taking as reference the grouping variable (elevational gradient). The morphometric analyses were completed separately for each species. The variables that were most important in the model obtained by AFDG were used to develop a model of altitudinal regression with the interpolation function in ArcGis 10.3 (ESRI, 2014).

Results

ADF shows that under that parameter the combinations of 11 environmental variables present significant differences (Wilks' Lambda = 0.0002, $F_{(65, 901)} = 64.342$, $p < 0.05$). Six elevation strata were obtained (Table 1). The most important variables are evapo-transpiration of the driest trimester, evapo-transpiration of the wettest trimester and average temperature. The first two roots explain 92% the variation of the data among six elevation strata.

A total of 47 individuals of *Agave gentryi* were heterogeneously distributed among three elevation strata (11 at 2,000-2,500, 32 at 2,500-3,000 and 4 at 3,000-3,500), the majority at middle elevation. One hundred thirty-nine *A. lechuguilla* individuals were widely distributed among five elevation strata (30 at 500-1,000, 33 at 1,000-1,500, 40 at 1,500-2,000, 16 at 2,000-2,500 and 20 at 2,500-3,000) (Table 2). The niche analysis range shows that both species have a significant deviation between their expected distribution centroid and the average environmental conditions of the sample area ($p < 0.05$); it also shows a different tolerance pattern in *A. lechuguilla* with respect to *A. gentryi* (Figure 2). Both species exhibited significant association between environmental variation and presence-absence of individuals, but *A. lechuguilla* has a wider tolerance and stronger environment relationship (Table 3). The niche amplitude variation for both species depends mainly on the soil organic carbon concentration, pH of soil, and soil density (Table 4, Figure 3). The first two components of the analysis explain 72% of the total variation.

The AFDG showed that there are no significant differences of morphological attributes of the individuals of *A. gentryi* along the altitudinal gradient ($F_{2,44} = 2.34$,

$p > 0.05$). In contrast, *A. lechuguilla* showed significant morphological variation on most of the elevation strata (Wilks' $\lambda = 0.390$, $F_{(28,462)} = 4.918$, $p < 0.05$). The morphological variables that contributed the most to separate the individuals among the altitudinal strata were in root one (R1 eigenvalue = 1.0178, $\chi^2 = 123.957$, $p = 0.000$): leaf length ($r = -0.8621$, $p < 0.05$) and length of terminal spine (cm)($r = 0.4322$, $p < 0.05$). For root two (R2 eigenvalue = 0.1753, $\chi^2 = 31.2898$, $p = 0.026$), length of terminal spine (cm) and leaf width were relevant. The cumulated variance among the two roots was 93% (Table 5, Figure 4). The individuals distributed in the first gradient differ from the rest, the individuals distributed on two and three gradients are morphologically similar ($F_{7,128} = 0.7111$, $p = 0.6626$) but different in the rest of the group, while the individuals of gradient 4 differ from those of the other gradients and the individuals of gradient 5 are like individuals of gradient 2 (Table 6).

Discussion

The results of the present work indicate that *Agave lechuguilla* has a wider niche than *A. gentryi*, which corresponds to higher morphological variation particularly in leaf size (length and width) and length of the terminal spine, attributes that decrease at higher elevation. *A. gentryi* has a restricted distribution limited to three elevation strata and does not exhibit any morphological difference between gradients. The elevation gradient indirectly generates a variation in environmental conditions, which stimulates morphological variation in species with a greater adaptive reserve (Andrade *et al*, 2009, Premoli and Mathiasen, 2011, Arteaga *et al*, 2015; Galván-Hernández *et al*, 2015). This can be associated with a much more basal evolutionary origin and exposure to greater environmental pressure due to

geological events, compared with species with a relatively more recent origin (Nicotra *et al*, 2010; Castellanos-Morales *et al*, 2016; Turrcote and Levine, 2016; Sosa *et al*, 2018). Similarly, it leads to greater distribution capacity and a greater niche.

Adaptive capacity for ecological niche amplitude

The niche analysis showed a significant marginality in both agaves; this means that average environmentally suitable conditions for the species' development are different from the conditions exhibited within the elevation strata. Comparatively, the niche amplitude of *Agave lechuguilla* was 50% higher than *A. gentryi* and the residual tolerance was lowest in the widely distributed species. In this sense, stress-tolerators, habitat specialists and species of infertile habitats have higher values of residual tolerance, particular niche requirements, and generally poor performance (Dolédec *et al*, 2000; Thuiller *et al*, 2004; Pywell *et al*, 2003), much like we see in *A. gentryi*. Presence-absence of individuals for *A. gentryi* was associated with average environmental requirements for the species, which is restricted to middle elevations, while presence-absence of *A. lechuguilla* individuals was related to environmental variation among elevation strata.

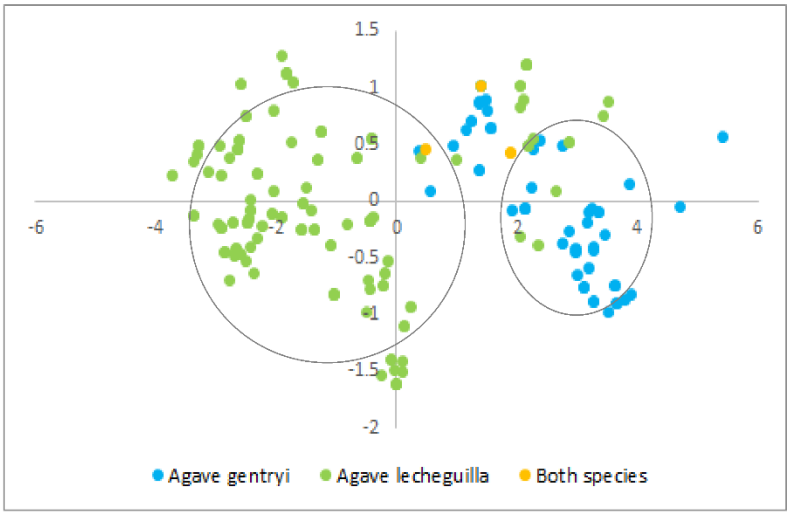

Figure 2. Graph representing the range of the ecological niche of the species *Agave gentryi* and *A. lechuguilla*. Ellipses include 90% of the observations for each species.

Table 3. Amplitude niche analysis comparing two *Agave* species on an altitudinal gradient. Note: *P* values are shown in italics. OMI p marginality index; Tol p tolerance; TolR p relation between the environmental variables and the presence or absence of the individuals

Species	OMI	Tol	TolR	*p*-value
A. gentryi	6.23	1.178	3.11	0.00*
A. lechuguilla	1.02	3.18	6.85	0.00*

Studies on patterns of distribution and ecological niche of species establish that the climatic and geological factors are those that have the greatest influence. Thuiller and collaborators (2004) analyzed the amplitude and niche separation in *Leucadendron* species, reporting ecoregions derived from the gradient of aridity and precipitation. Tsiftsis *et al*. (2008) calculated the ecological niche of orchids and determined that the temperature and the availability of organic matter in the soil are the variables that most influence the model.

Table 4. Contribution of the 11 climatic and edaphic attributes that explain the differential niche amplitude of two *Agave* species.

VARIABLE	PC1	CP2
Soil organic carbon stock per ha	0.689	0.000
Cation exchange capacity of soil (cmolc/kg)	0.389	-0.003
Stilt content (2-50 µm) mass fraction in %	0.243	0.006
Precipitation of the driest quarter (mm)	0.241	0.000
Precipitation of the wettest quarter (mm)	-0.075	0.003
Sand content (50-2000 µm) mass fraction in %	-0.301	0.006
Average solar radiation (kJ m-2 day-1)	-0.398	-0.003
Soil pH X 10 in KCl	-0.549	0.000
Average temperature (°C)	-0.564	0.003
Bulk density (kg/m3)	-0.671	-0.001
Soil pH X 10 in H2O	-0.707	-0.002
Eigenvalue	5.4195	2.561
Cumulative variance	49.26%	72.5%

Aguilar-Romero *et al*. (2016) analyzed the distribution pattern of nine *Quercus* species, reporting that temperature and precipitation are the most important attributes. The species of the genus *Agave* are plants well adapted to arid and semi-arid regions (García-Mendoza, 2007; Scheinvar *et al,* 2016); the variation of attributes related to humidity, light incidence and temperature do not significantly influence the structure of its ecological niche. This is supported by the niche analysis, which included both climatic and edaphic variables, and showed that soil attributes (soil organic carbon stock per ha, soil pH and bulk density at km/m^3) were the most important factors in the establishment of individuals (Table 4). AFD shows differences between elevation strata related to pH decrease as elevation increases, and that the soil organic carbon stock (COS) has a positive correlation with elevation (Table 1). Studies on physical, chemical and biological quality of soil report that community richness and availability of nutrients is conditioned by pH and COS; when the pH of the soil is close to neutral, the COS assists in the solubility of nutrients

(Bartelheimer and Poschlod, 2014; Glassman *et al*, 2017; Zhou *et al*, 2017). The sites where *A. gentryi* is distributed have a pH close to neutral (7-7.5) and higher concentration of COS (27-33 ton/ha/year). This allows a better assimilation of nutrients for this species, but it restricts its niche amplitude to a single elevation stratum.

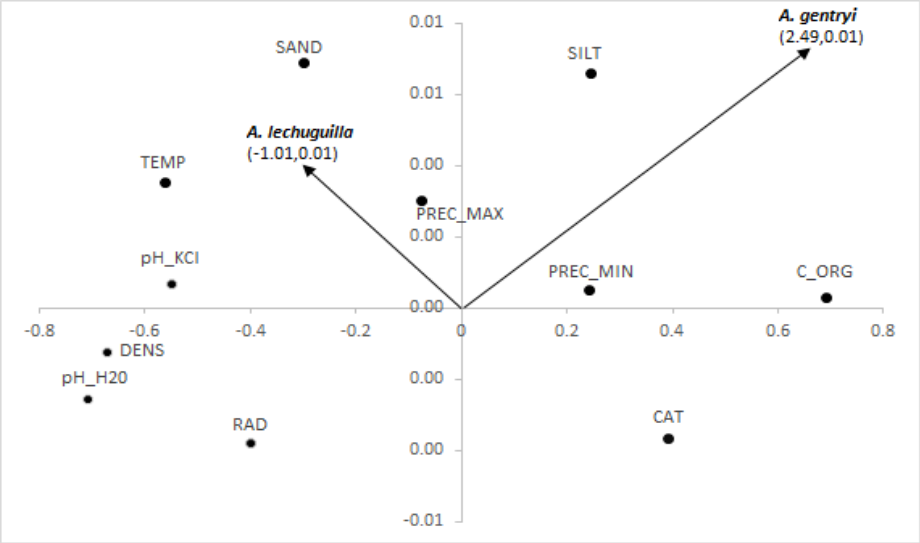

Figure 3. Graph representing the distance between the centroid of Agave species and the origin of the hyperspace of the niche (average of the environmental variables).

Agave lechuguilla, in contrast, presented marginality preference towards sites with arid conditions, has high tolerance values (3.18), and was observed in most strata of the elevational gradient, except for the highest (3,000-3,500 m asl). It was noted that from the third gradient to higher (1,500 to 3,000 m asl), its distribution was restricted to exposed spaces. This suggests that climatic conditions did not affect its distribution, but the availability of spaces with particular soil conditions did, as supported by the niche analysis. In this context, morphological analysis showed

variation in response to altitudinal gradients, with the largest plants at lower elevation. Vegetative traits of several species (e.g. leaf dry weight) for example, *Festuca christianii-bernardii* and *Helianthemum canum*; and abundance of life-history stages (e.g. seedling, sapling and tree) as it happens with *Abies alba*, *Acer campestre*, *Betula pendula*, *Carpinus betulus*, *Fagus sylvatica*, amongst others, diverged on shallow soils, reflecting the coexistence of distinct nutrient use strategies in these constrained habitats, and converged with increasing soil resource availability, particularly necessary to early life-history stages (Bertrand *et al*, 2011; Bernard-Verdier *et al*, 2012).

Table 5. AFDG for morphological attributes across six elevation strata for *Agave lechuguilla.*

Variable	ROOT 1	ROOT 2	*F*-value	*p-value*
Leaf length (cm)	-0.862103	-0.352928	26.122890	0.00
Length of terminal spine (cm)	0.432256	-0.476036	14.014280	0.00
Leaf width (cm)	0.280405	-0.410881	4.918934	0.00
Average mucrons in 10 cm	-0.165741	0.259571	9.487672	0.00
Rosette dimension (Height in cm)	-0.307984	-0.300861	7.251884	0.00
Leaf number	0.213329	-0.409941	5.976438	0.00
Rosette dimension (Width in cm)	-0.231748	-0.202617	5.338907	0.00

Agave lechuguilla originated in the Altiplano Mexicano; genetic analysis with chloroplast microsatellites concluded that this specie was exposed to a process of diversification with the formation of the Sierra Madre Oriental (Scheinvar *et al*, 2016). This could explain the affinity to arid sites and their gradual adaptation to elevation strata. It is necessary to carry out genetic studies for *A. gentryi* in order to contrast the origin and radiation of this more narrowly distributed species with *A. lechuguilla*.

Adaptive capacity for phenotypic plasticity

The AFDG showed a differential morphological response of *A. lechuguilla* stimulated by the elevation strata, but morphological homogeneity for *A. gentryi*. It is reported that environmental gradients more suitable for a species are associated with radiation centers and optimal developmental conditions, exhibiting a healthier population structure depending on its adaptive strategy (Lesica and Allendorf, 1995; Guisan and Zimmermman, 2000; Cabrera, 2002). In this regard; generalist species display varying adaptive strategies in response to the environment, while specialist species do not show the same phenotypic plasticity. Lower and middle elevations are conducive to development of *A. lechuguilla*, whereas a higher but more limited elevation range is optimal for *A. gentry.*

Schlichting (1986) and Xu *et al* (2008) note that some species, in this case *Agave gentryi* and *A. lechuguilla,* have the ability to modify their morphological or physiological attributes in response to environmental changes, which is particularly important for plants as sessile organisms. This phenotypic plasticity is reflected in color variation of leaves, heterophylly and cleistogamy (Galván-Hernández *et al,* 2015). The environmental adaptations at the extreme of a gradient requires improving physiological efficiency, i.e., avoiding the loss of water or increasing the nutrient uptake (Galván-Hernández *et al*, 2012). The morphological heterogeneity of *A. lechuguilla* reflected a case of heterophylly in which leaf length decreases with elevation. This could be an adaptation for better use of nutrients in the preferred sites of this species, while a longer leaf may be more photosynthetically efficient.

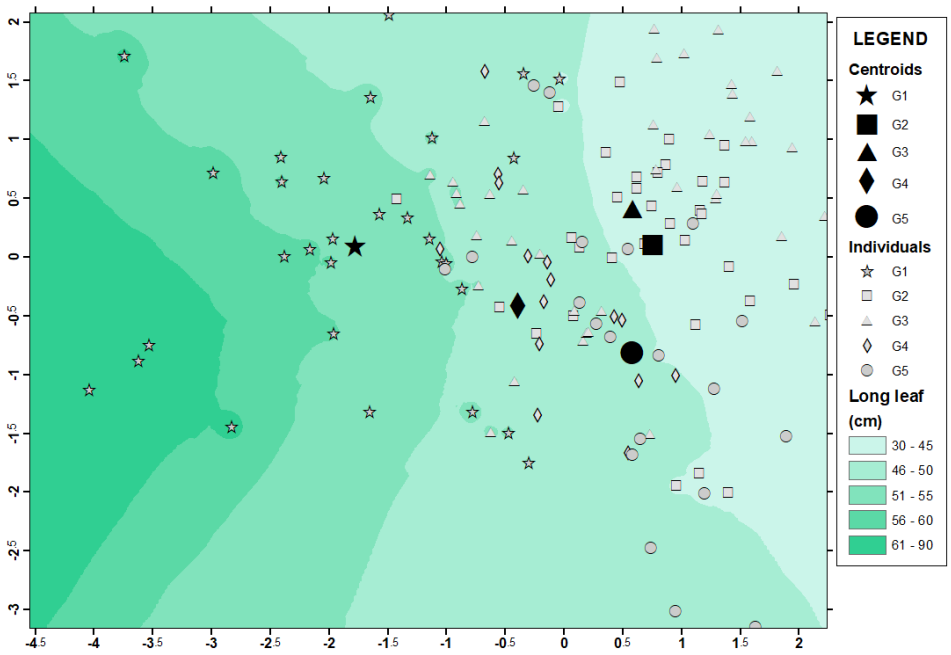

Figure 4. Graph of the generalized function discriminant analysis representing the two roots and the interpolation of the variable value "leaf length" (more intense color represents areas with longest leaves; clear value represents shorter leaves). The gray symbols represent the individuals of each gradient; the black symbols represent the centroid of the Agave lechuguilla individuals at each elevation strata (G1–G5 p gradients 1–5). A color version of this figure is available online.

Table 6. Probability values and *F* value of the distances between the groups of *Agave lechuguilla* defined by elevation strata. Note: upper diagonal corresponds to the values of p; lower diagonal corresponds to the values of F; G1 gradient 1; G2 gradient 2; G3 gradient 3; G4 gradient 4 and G5 gradient 5.

Elevation strata	G1	G2	G3	G4	G5
G1	------	0.000	0.000	0.001	0.000
G2	13.871	-------	0.662$^{(NS)}$	0.005	0.110 $^{(NS)}$
G3	13.395	0.711	--------	0.006	0.008
G4	3.870	3.059	3.005	--------	0.042
G5	10.537	1.720	2.892	2.162	---------

Conclusion

Agave gentryi shows narrower elevation gradient amplitude and is restricted to higher elevational strata, while *A. lechuguilla* can colonize a wider elevation range. *A. gentryi* shows a putative low adaptive capacity as a specialist species; it does not respond to environmental variations and its phenotypic plasticity is insufficient to adapt to heterogeneous conditions. *A. lechuguilla* is a species with greater adaptive capacity, higher environmental tolerance, and the ability to adapt to different environmental conditions. This suggests different radiation patterns for both species, with less adaptive capacity for *A. gentryi* and a better survival strategy in *A. lechuguilla* in response to environmental variation across lower and middle elevation strata.

References

Aguilar-Romero R, F García-Oliva, F Pineda-García, I Torres, E Peña-Vega, A Ghilardi, K Oyama 2016 Patterns of distribution on nine Quercus species along an environmental gradient in a fragmented landscape in central Mexico. Botanical Sciences 94:471-482.

Andrade IM, SJ Mayo, D Kirkup D, C Van den Berg 2008 Comparative morphology of populations of *Monstera adans*. (Araceae) from natural forest fragments in Northeast Brazil using elliptic Fourier analysis of leaf outlines. Kew Bulletin 63: 193–211. doi:10.1007/s12225-008-9032-z.

Arteaga MC, R Bello-Bedoy, JL León de la Luz, J Delgadillo, R Domínguez 2015 Phenotypic variation of flowering and vegetative morphological traits along the distribution for the endemic species *Yucca capensis* (Agavaceae). Botanical Sciences 93: 765-770. DOI: 10.17129/botsci.214

Austin MP and TM. Smith 1989 A new model for the continuum concept. Vegetatio 83:35–47.

Bartelheimer M, P Poschlod 2014 The response of grassland species to nitrate versus ammonium coincides with their pH optima. Journal of Vegetation Science 25(3):760-770.

Bernard-Verdier M, M L Navas, M Vellend, C Violle, A Fayolle, E Garnier 2012 Community assembly along soil depth gradient: contrasting patterns of plant traits convergence and divergence in a Mediterranean range land. Journal of Ecology 100(6):1422-1433.

Bertrand R, J C Gegout, J D Bontemps 2011 Niches of temperate tree species converge towards nutrient-richer conditions over ontogeny. OIKOS 120(19):1479-1488.

Blonder B 2017 Hypervolume concepts in niche- and trait-based ecology. Ecography 40: 001-013.

Boulangeat I, S Lavergne, J Van, L Garraud, W Thuiller 2011 Niche breadth, rarity and ecological characteristics within a regional floras panning large environmental gradients. Journal of Biogeography 39:204-214.

Cabrera M 2002 Respuestas ecofisiológicas de plantas en ecosistemas de zonas con clima mediterráneo y ambientes de alta montaña. Revista Chilena de Historia Natural 75:625-637.

Casas A, J Caballero, A Valiente-Banuet, JA Soriano, P Dávila 1999 Morphological variation and the process of domestication of *Stenocereus stellatus* (Cactaceae) in Central Mexico. American Journal of Botany 86:522-533.

Davis SC, FG Dohleman, SP Long 2011 The global potential for Agave as a biofuel feedstock. Global Change Biology Bioenergy 3:68-78.

Delgado-Lemus A, A Casas, O Téllez 2014 Distribution, abundance and traditional management of *Agave potatorum* in the Tehuacán Valley, Mexico: bases for sustainable use of non-timber forest products. Journal of Ethnobiology and Ethnomedicine 10:2-12.

Dolédec S, D Chessel, C Gimaret-Carpentier 2000 Niche separation in community analysis: a new method. Ecology 81:2914-2927.

ESRI 2014 ArcMap 10. Environmental Systems Research Institute, Redlands, California.

Fabbro T, CKörner 2004 Altitudinal differences in flower traits and reproductive allocation. Flora 199:70-81.

Félix-Valdez LI, O Vargas-Ponce, D Cabrera-Toledo, A Casas, A Cibrián-Jaramillo, L De la Cruz-Larios 2015 Effects of traditional management for mescal production on the diversity and genetic structure of *Agave potatorum* (Asparagaceae) in central Mexico. Genetic Resources and Crop Evolution 63:1255-1271.

Flores-Abreu IN 2007 Coevolución entre género *Agave sensu lato* y sus murciélagos Polinizadores (Phyllostomidae). Tesis para obtener el grado en Maestría en Ciencias Biológicas. Instituto de Ecología, Universidad Nacional Autónoma de México, México.

Foley J, T DeFries, G Asner, C Barford, GBonan, S Carpenter, S Chapin, M Coe, G Daily, H Gibbs, J Helkowski, T Holloway, E Howard, CKucharik, C Monfreda, J Patz, I Prentice, N Ramankutty, P Snyder 2005 Global Consequences of Land Use. Science 309:560-574.

Galván-Hernández D, JA Lozada-García, N Flores-Estevez, J Galindo-González, SM Vázquez-Torres 2015 Altitudinal gradient effect on morphometric variation and leaf symetry of *Platanus mexicana* Moric. Revista Chapingo Serie Ciencias Forestales y del Ambiente 21:171-183.

García-Mendoza A, R Galván 1995 Riquezas de la familia Agavaceae y Nolinaceae en México. Boletín de la Sociedad Botánica de México 56: 7-24. DOI: 10.17129/botsci.1461

García-Mendoza A 2007 Los Agaves de México. Ciencias 87:4-23.

Gaston KJ 1997 What is rarity? The biology of rarity: causes and consequences of rare–common differences. Pages 30-47 *in* WE Kunin, KJ Gaston, eds. Chapman and Hall, London.

Gentry, S 1982 Agaves of continental North America University of Arizona Press, Tucson.

Gerstner K, CF Dormann, A Stein, AM Manceur, R Seppelt, 2014 Effects of land use on plant diversity–A global meta-analysis. Journal of Applied Ecology 51:1690-1700.

Glassman SI, IJ Wang, TD Bruns 2017 Environmental filtering by pH and soil nutrients drives community assembly in fungi at fine spatial scales. Molecular Ecology on-line. DOI: 10.1111/mec.14414.

Gotelli NJ and AM Ellison 2004 A Primer of Ecological Statistics, Sunderland.

Guisan A, NE Zimmermman 2014 Predictive habitat distribution model in ecology Ecological Modelling 135:147-186.

Hedrick P 2009 Population genetics and ecology. Pages 109-116 *in* P Hedrick ed Genetics of Populations. Jones and Bartlett Publisher. Sudbury, Massachusetts.

Hengl T, J Mendes, GBM Heuvelink, MG Ruiperez, M Kilibarda, A Blagotic, W Shangguan, MN Wright, X Geng, B Bauer-Marschallinger, MA Guevara, R Vargas, RA MacMillan, NH Batjes, JGB Leenaars, E Ribeiro, I Wheeler, S Mantel, B Kempen 2017 Soil Grids 250 m: Global gridded soil information based on machine learning. Plos One 12: e0169748.

Hernández-Castillo H, J Treviño-Carreón 2009 Biología floral de *Agave gentryi* Ullrich (Agavaceae) en la localidad de La Marcela, Miquihuana, Tamaulipas. Ciencia UAT 4:62-66.

Hijmans R J, S E Cameron, J L Parra, P G Jones, A Jarvis 2005 Very high-resolution interpolated climate surfaces for global land areas. International Journal of Climatology 25:1965-1978.

Hochstätter F 2015 *Agave* Linné (Agavaceae). Publicado por el autor, Mannheim.

Huston M 1994 Biological Diversity: The coexistence of species on changing landscapes. Cambridge University. United Kingdom.

IUCN 2017 Red list of endangered species. http://www.iucnredlist.org/search. Recovered on August 30, 2017.

Jongman RHG, CJF Ter Braak, OFR Van Tongeren 1995 Data analysis in community and landscape ecology. Cambridge University Press, Cambridge.

Körner C 2007 The use of altitude in ecological research. Trends in Ecology and Evolution 22:569-574.

Lande R 2009 Adaptation to an extraordinary environment by evolutionof phenotypic plasticity and genetic assimilation. Journal of Evolutionary Biology 22:1435-1446.

Lande R 1988 Genetics and demography in biological conservation. Science 241:1455-1460.

Leibold MA and P Gedes 2005 El concepto de nicho en las meta-comunidades. Ecología Austral 15:117-129.

Lesica P, FW Allendorf 1995 When are peripheral populations valuable for conservation? Conservation Biology 9:753-760.

Liu RX, MA Yingemi, Z Xinghua, Ch Shichao, M Yanjie, W Tiange 2018 Anatomical structures of *Reaumurias oongorica* in Alashan desert Pakistan Journal of Botany 50:2291-2299.

Malanson G P, W E Westman, YL Yan 1992 Realized versus fundamental niche functions in a model of chaparral response to climatic change. Ecological Modelling 64:261–277.

Maciel-Mata C, N Manríquez-Morán, P Octavio-Aguilar, G Sánchez-Rojas 2015 Geographical distribution of the species: a concept review. Acta Universitaria: Multidisciplinary Scientific Journal 25(2):3-19.

Mohan D,CU Pittman 2006 Activated carbons and low cost adsorbents for remediation of tri-and hexavalent chromium from water. Journal of Hazardous Materials 137:762-811.

Nicotra AB, OK Atkin, SP Bonser, AM Davidson, EJ Finnegan, U Mathesius, P Poot, MD Purugganan, CL Richards, F Valladares, M Van Kleunen 2010 Plan phenotypic plasticity in a changing climate. Trends in Plant Science 15:684-692.

Peterson AT, J Soberón, RG Pearson, RP Anderson, E Martínez-Meyer, M Nakamura, MB Araújo 2011. Ecological niches and geographic distributions Princeton Univ. Press.

Premoli AC, P Mathiasen 2011 Respuestas ecofisiológicas adaptativas y plásticas en ambientes secos de montaña: *Nothofagus pumilio*, el árbol que acaparó los andes australes. Ecología Austral 21:251-269.

Pulliam HR 2000 On the relationship between niche and distribution. Ecology Letters 3:349-361.

Pywell R F, J M Bullock, D B Roy, L I Z Warman, K J Walker, P Rothery 2003 Plant traits as predictor of performance in ecological restoration. Journal of Applied Ecology 40(1):65-77.

Rocha-M, SV Good-Ávila, F Molina-Freaner, HT Arita, A Castillo, A García-Mendoza, A Silva-Montellano, BS Gaut, V Souza, LE Eguiarte. 2006. Pollination biology and adaptative radiation of Agavaceae, with special emphasis of the genus *Agave.* Rancho Santa Ana Botanic Garden. 22: 329-344.

Rzedowski J 1978 Vegetación de México. Editorial Lumisa, S.A. México.

Sawalha MF, JR Peralta-Videa, J Romero-González, J Gardea-Torresdey 2006 Biosorption of Cd(II), Cr(III), and Cr(VI) by salt bush (*Atriplex canescens*) biomass: Thermodynamic and isotherm studies. Journal of Colloid and Interface Science300:100-104.

Scheinvar E, N Gámez, G Castellanos-Morales, E Aguirre-Planter, L Eguiarte 2016 Neogene and Pleistocene history of Agave lechuguilla in the Chihuahuan desert. Journal of Biogeography 1-13.

Schlichting CD 1986 The evolution of phenotypic plasticity in plants. Annual Reviews Ecology 17:667-693.

Shangguan W, T Hengl, J M de Jesus, H Yuan, Y Dai 2017 Mapping the global depth to bedrock for land surface modeling. Journal of Advances in Modeling Earth Systems 9:65-88.

Silva-Montellano A and LE Eguiarte 2003 Geographic patterns in the reproductive ecology of Agave lechuguilla (Agavaceae) in the Chihuahuan desert. i. floral

characteristics, visitors, and fecundity. American Journal of Botany 90: 377-387.

Soberón JM 2010 Niche and area of distribution modeling: a population ecology perspective. Ecography 33:159-167.

StatSoft 2011 STATISTICA, version 10. http://www.statsoft.com/products/STATISTICA-Features/Version-10.

Thuiller W, S Lavorel, G Midgley, S Lavergne, T Revelo 2004 Relating plant traits and species distributions along bioclimatic gradients for 88 Leucadendron taxa. Ecology 85:1688-1699.

Thioulouse J, D Chessel, S Dolédec, J Michel-Oliver 1997 ADE-4: a multivariate analysis and graphical display software. Statistics and Computing 7:75-83.

Trejo-Salazar RE, E Scheinvar, L Eguiarte 2015 Who really pollinates agaves? Diversity of floral visitors in three species of Agave (Agavoidea: Asparagaceae). Revista Mexicana de Biodiversidad 86:358-369.

Treviño-Carreón J, A Mora-Olivo, A Carreón-Pérez and A Valiente-Banuet 2011 Descubriendo el valor de los magueyes Tamaulipecos. Ciencia UAT 19:34-40.

Tsiftsis S, I Tsiripidis, V Karaginnakidou, D Alifragis 2008 Niche analysis and conservation of the orchids of east Macedonia (NE Greece). Acta Oecologica 33:27-35.

Turrocote MM and JM Levine 2016 Phenotypic plasticity and species coexistence. Trends in Ecology & Evolution 20:1-11.

Vieira MC, T Heinze, R Antonio-Cruz, A M Mendoza-Martínez 2002 Cellulose derivatives from cellulosic material isolated from Agave lechuguilla and A. fourcroydes. Cellulose 9:203-212.

Völler E, O Bossdorf, D Prati, H Auge 2017 Evolutionary responses to land use in eight common grassland plants. Journal of Ecology 105:1290-1297.

Whitford W G 2003 Ecology of desert system. Academic Press. San Diego California, USA.

Xu F, W Guo, W Xu, R Wang 2008 Habitat effects of leaf morphological plasticity in *Quercus acutissima*. Acta Biológica Cracoviensia 50:19-26.

Zhou X, A Guo, C Chen, Z Jia 2017 Soil microbial community structure and diversity are largely influenced by soil pH and nutrient quality in 78-year-old tree plantations. Biogeosciences Discussions, doi:10.5194/bg-2016-552, 2017

IV. CAPÍTULO 3: Effect of elevational gradient on the variation and structure genetic of *Agave lechuguilla* Torr and *Agave gentry* Ullrich.

Alfredo Sánchez-González[1], Ludivina Barrientos-Lozano[1], Pablo Octavio-Aguilar[2]

[1]Tecnológico Nacional de México-I. T. Ciudad Victoria, Departamento de Posgrado e Investigación. Blvd. Emilio Portes Gil No. 1301. C.P. 87010. Ciudad Victoria Tamaulipas.

[2]Universidad Autónoma del Estado de Hidalgo-Centro De Investigaciones Biológicas, Instituto de Ciencias Básicas e Ingeniería, Laboratorio de Genética. Carretera Pachuca-Tulancingo Km. 2.5. Col. Carboneras, Mineral de la Reforma, Hidalgo. México. C.P. 42184.

Abstract

This work presents a study on the genetic diversity and structure of two *Agave* species with different environmental tolerance, *A. lechuguilla* and, *A. gentryi*; generalist and specialist, respectively. It is established that genetic diversity contributes to the adaptation of species; therefore, it influences the amplitude of the niche. Similarly, environmental variation encourages genetic differentiation between populations. Therefore, it is inferred that there is a difference in the genetic diversity of *A. lechuguilla* and *A. gentryi* considering that they have a different ecological niche. However, the results show that both species exhibit high genetic diversity (*A. lechuguilla* He = 0.63±0.01; *A. gentryi* He = 0.6±0) and, unlike what was expected, the generalist species

did not show a genetic relationship with the environment, unlike the specialist species (F_{ST} = 0.084 and F_{ST} = 0.206, respectively). Therefore, environmental tolerance depends not only on genetic diversity but also on phenotypic plasticity. This work considers a different approach to studies where only genetic diversity and structure are evaluated since here, we also considered the environmental tolerance of the species.

Keywords: Generalist-specialist species, elevational gradient, genetic variation, adaptative response, *Agave.*

INTRODUCTION

Genetic diversity is a fundamental adaptive component in populations since each variation at the gene level is a different strategy that potentially allows them to face the environmental oscillations (Leisca and Allendorf 1995; Gitzendanner and Soltis 2000; Valladares *et al*. 2006; Frankham et al. 2009; Hedrick, 2010; Pauls *et al.* 2012; Arteaga *et al*. 2015). However, the current climate change scenario generates abrupt shifts in the environment which could alter the performance of the genetic component of the species (Hoffman and Sgrò, 2011; Franks and Hoffmann, 2012; Shaw and Ettersonn, 2012; Christmas *et al*. 2016); specifically in plants, due to their sessility, the effects are more direct, for example, i) change in the effective population size due to reduction, increase or contraction of geographical distribution (Felde *et al*. 2011; Courtney and Curtis, 2013; Urban, 2015; Scheinvar *et al*. 2016; Wiens, 2016) or ii) adaptation or elimination of genotypes due to natural selection conditioned by phenotypic plasticity, environmental fluctuation generates the signal to trigger the response to environmental change or this signal may be diminished or overexcited and inhibit the phenotypic plasticity process (Etterson and Shaw, 2001; Chevin *et al*. 2010; Franks, 2011). Important evaluations have been generated on the effects of climate change on genetic diversity in plant populations, to date the predictions are variable and subject to the approach used, for example, global and local effects (Sork *et al*. 2010; Beierkuhnlei *et al*. 2011; Pauls *et al*. 2013), tropical and boreal species (Alsos *et al*. 2012; Mangaravite *et al*. 2016), elevational or latitudinal gradient (Millberg *et al*. 2015; Galván-Hernández *et al*. 2015); the latter is widely accepted because it can simulate the effect of climate change more precisely. Nevertheless, their ability to use resources is not considered in detail; the niche

amplitude, generalist or specialist, explains the environmental tolerance of the species (Soberón 2010; Peterson *et al*. 2011; Blonder 2017; Sánchez-González *et al*. 2019). Therefore, analyzing the genetic diversity and structure in plant populations, considering the generalism and/or specialism of the species in an elevational gradient, can contribute in a more objective way when generating predictions about the effects of climate change on the genetic variation of the plant' species (Espíndola *et al.* 2012).

Elevation is a complex environmental gradient associated directly with temperature, atmospheric pressure, and radiation (Körner, 2007; Halbritter *et al*. 2013), which are the main environmental consequences generated by climate change (Urban, 2015). This generates significant environmental variations that could limit populations distribution or will promote genetic divergence through local adaptation in different environmental gradient (Premoli and Mathiasen, 2011; Galván-Hernández *et al*. 2015; Halbritter *et al*. 2015). Which can stimulate mutation, genetic drift, inbreeding, gene flow, selection process, and proximity to new-gene source (Gaudel *et al*. 2007; Ohsawa and Ide, 2008; Frankham *et al*. 2009). These effects may act differently depending on the performance and genetic diversity of populations. Elevational gradients provide evidence on the effect of climatic change on the genetic components in populations as the elevation increases or decreases (Ohsawa and Ide, 2008; Premoli and Mathiasen, 2011; Galván-Hernández *et al.* 2015; Wiens, 2016). Molecular markers are tools employed to evaluate genetic variation because they allow estimating parameters of interest to ecology (Selkoe and Toonen, 2006; Eguiarte and González, 2007).

Molecular tools make it possible to accurately determine the amount, distribution, and functional significance of genetic variation in natural populations. However, to make use of genetic information as a predictive tool to understand the effects of environmental variation, it is necessary to maintain an eco-evolutionary perspective, considering the short-term ecological consequences of genetic interventions such as long-term evolutionary processes related to the adaptation (Mable, 2018). Degree of environmental tolerance is an aspect that allows understanding the results of these eco-evolutionary processes; the amplitude of the ecological niche, conceptualized as the equivalent to the plastic response associated with adaptive change depending on the available environmental resources (Soberón, 2010; Blonder 2017), contributes to quantifying this parameter. Associating this with the elevational gradient, a generalist species (wide ecological niche) represents greater environmental tolerance to the changing conditions provided by the gradient; otherwise, a specialist species (restricted ecological niche) with the same resource availability represents less ecological tolerance and greater susceptibility to environmental variations is inferred (Li et al. 2014; Loxdale *et al*. 2011; Sánchez-González *et al*. 2019). Genetic diversity contributes directly to the adaptive capacity of species by the variants provided by their allelic richness (Franckham, 2005; Selechnik *et al*. 2019). However, its performance against climate change is conditioned by the time it takes to act, it must be considered that genetic adaptation works on a long-time scale. To analyze the aforementioned in a very precise way, the genetic data must be compared, at least, between two species of plants, one generalist and the other specialist, in an environment with the same availability of resources; this occurs among some species of the *Agave* L. genus.

The genus *Agave* L. (ASPARAGACEAE) is one of the most genetically studied in México (Pinero, 2008). This is due to different factors, such as its ecosystem role as a source of food by animals, nutrients to the soil, and nurse plant effect, as well as the cultural relationship with rural people of Mexico who has variate of uses for the agaves since prehistoric times, e.g., the production of alcoholic beverages, an activity that currently has a great economic impact as the Mexican tequila (García-Mendoza, 2007; Eguiarte y González, 2007; Félix-Valdez *et al*. 2015; Figueredo-Urbina, 2017).The genus *Agave* comprises 210 to 300 species, out of which 159 occur in Mexico, and 75% approximately are endemic. It is a group highly represented in arid and semi-arid vegetation, tropical dry forest, and pine-oak forest in temperate areas; even some species are distributed in diverse and contrasting environments (Eguiarte et al. 2000; García-Mendoza, 2007). This work is about two *Agave* species with different niche amplitude, *A. lechuguilla* and *A. gentry* (Sánchez-González et al. 2019). *A. lechuguilla* is distributed in an elevational range between 500-3000 masl, from the southern United States to central Mexico. This plant has been used to produce cellulose fibers and derivatives (Vieira et al. 2002), being the latter its principal use in the northern region of Mexico. According to the IUCN, *A. lechuguilla* is in a low-risk category (Least Concern), since it has a continuous and abundant distribution (IUCN, 2021). By contrast, *A. gentry* is restricted mainly to mountainous areas of northeastern Mexico, and it is associated with limestone in temperate forest and scrub, its elevational distribution is reported between 1500-3000 masl. This species has a chiropterophilous pollination syndrome, like other *Agav*e species with restricted distribution such as *A. difformis* and *A. striata*, very little is known about *A. gentryi* (Castillo-Hernández and Treviño-Carreón, 2009;

Trejo-Salazar *et al*. 2015). *A. lechuguilla* (generalist species) and *A. gentry* (specialist species) are clear examples about the contrast of niche width and environmental tolerance in the genus (Sánchez-González *et al*. 2019).

Therefore, if *A. lechuguilla* (generalist species) shows greater environmental tolerance compared to *A. gentryi* (specialist species), and if it is also established that environmental tolerance is based on a high genetic pool, which can be differentiated between populations through local adaptation to significant environmental variation such as elevation gradients. Then, *A. lechuguilla*, may exhibit high genetic diversity, greater than *A. gentry*; as well greater genetic structure associated with the environmental variation generated by the elevation gradient. This work aimed to evaluate the amount and distribution of genetic diversity of *A. lechuguilla* and *A. gentryi* in an elevation gradient with the help of neutral markers we assess basic parameters regarding genetic variability for identify of environmental variation effect on the genetic component in a generalist (*A. lechuguilla*) and specialist (*A. gentryi*) specie.

MATERIAL AND METHODS

Study area and sample collection

The study area is located in the southwestern region of Tamaulipas State between 237470 and 237240 N-latitude and, 997580 and 997350 W-longitude (Fig. 1). It encompasses two biogeographical provinces (Rzedowski 1978): *(i)* Sierra Madre Oriental and (ii) Mexican Altiplano. It is dominated by pine and oak forest and rosetophyll thicket, respectively, two vegetation types associated with agaves (Rzedowski 1978; García-Mendoza 2007). The elevation gradient in the study area is between 500 to 3500 masl, implying an important source of environmental variation.

Stratified-type systematic sampling was applied; the elevation gradient was stratified every 500 meters since significant environmental differences can be recognized according to what was reported by Sánchez-González *et al*. (2019) (Figure 1). On each gradient, individuals of *A. lechuguilla* and *A. gentryi* were identified applying an independent radius of 30 m around each localized individual to avoid coancestry effect (Flores-Abreu 2007); a tissue sample from each plant was taken for subsequent DNA extraction.

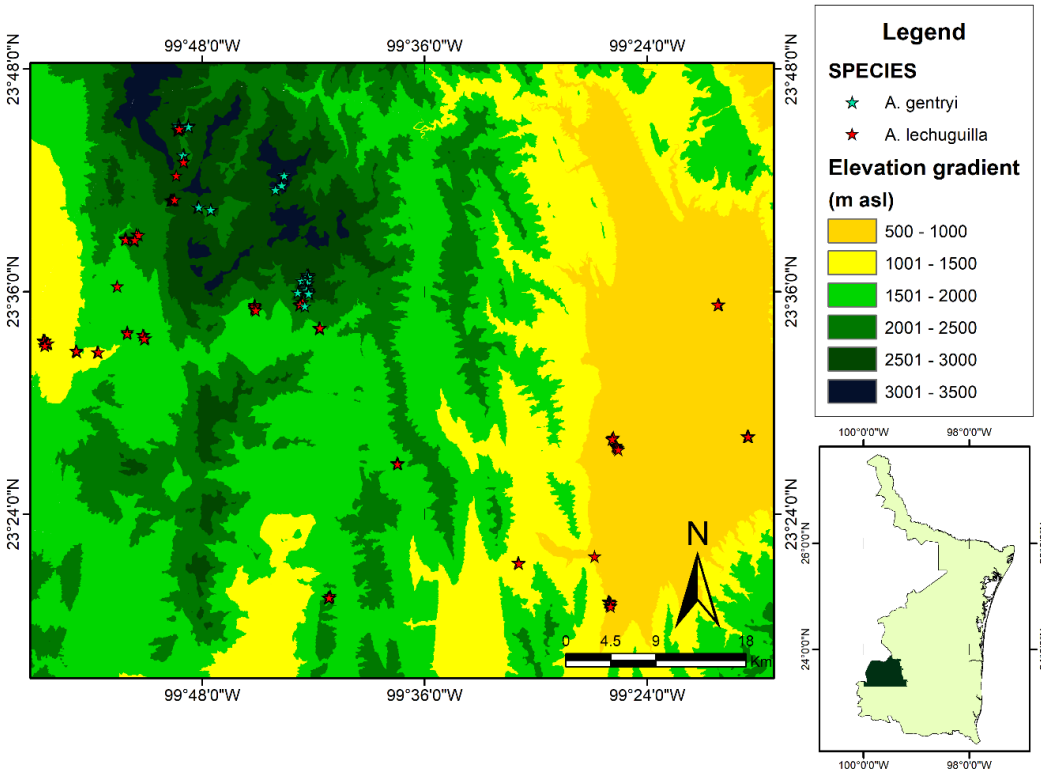

Figure 1. *Study area.* Elevation strata established every 500 m between 500 to 3000 masl, southwestern Tamaulipas, Mexico.

Microsatellite amplification and genotyping

DNA extraction was performed following the method proposed by Doyle and Doyle (1987) modified by Keb-Llanes and collaborators (2002). For PCR reactions ten primer pairs were used for polymorphic nuclear microsatellite loci; seven were designed for *Agave parryi* (Lindsay *et al.* 2012) and three for *Agave uthaensis* (Byers *et al*. 2014) (Table 1). The PCR products are visualized on a 15% acrylamide gel which was stained with ethidium bromide 10mg/ml, subsequently, the gel was visualized in a transilluminator, and finally, photographs were taken for later analysis. A matrix based on the size of amplified fragments was created, corrected by the Chybicki and Burczyk (2009) method. It sought to detect the presence of genotyping

errors due to null alleles, larges alleles, or stuttering, using Micro Checker 2.2.3 (Oosterhout, 2004) with 1000 bootstrap simulations and a 95% confidence interval. The deviation from linkage disequilibrium was estimated with Genpop software 4.7.0 (Raymond and Roussete, 1995) with the Chi-squared distribution method for each pair of loci.

Table 1. Polymorphic nuclear microsatellite loci used to analyze genetic diversity in populations of *Agave lechuguilla* at different altitudes.

Locus	Primer sequences (5'-3')	Repeat motif	Ta (°C)	Allele size range (bp)	Gen Bank No.
APAR2-12	F: CGTCACCGCCAGTTAAGAG R: GTGCCCATGATCTGTGGTTG	$(CT)_7$	59	151-205	JQ480449
APAR3-11	F: TGTGGCGGCTAAAAGAAGG R: CCGATCCGGCGTAATTCTC	$(AAC)_4$	59	158-194	JQ480450
APARLC20	F: AGTTGCTGAAATCGAAGATCCG R: TCTCGGTTGGATTCGGTCG	$(AG)_7$	60	204-240	JQ480451
APARLC21	F: TCCTCTCCACGTTCAACCC R: GGAGAGCTTGGGTACGAGG	$(CT)_9$	60	142-206	JQ480452
APARLC28	F: CCTCCTGCGTAGAGGAACC R: TCTCCGTTTGAACTCCGTG	$(AAG)_4$	58	138-195	JQ480453
APARLC34	F: ACAGGAGTCCATAACAGGAGTG R: TCAGTTCTCCATCTACTTCCG	$(ATT)_5$	57	152-206	JQ480454
APARLC35	F: TGTGTGTTTAGCAGTGCCG R: AGCGGATCTGAGCAAAATGAG	$(ATT)_4$	59	157-175	JQ480455
BYU4463	F: CGTTGAGCTTGTTGAAGTCG R: AACCACACCAAAGTCAAGCAG	**(GAG)6**	57	**178–199**	**KJ855291**
BYU5164	F: ATCGCTCCTCCAGAGTTTCA R: GTGTGTGCTTTGTTGGTTGG	**(CCA)7**	57	**155–176**	**KJ855293**
BYU8677	F: AAACCAAAAACAGGCCACTG R: TGAGTCGAGGGAATTAGGGA	**(AAT)10**	57	**171–198**	**KJ855297**

Analyses of genetic diversity

Descriptive parameters of genetic diversity were estimated; the percent of polymorphism (%P), the average alleles per locus (Na), the effective allele number per locus (Ne), and Shannon´s index (I), the observed and the expected heterozygosity (HO, HE, respectively), and Hardy-Weinberg equilibrium (HWE). These variables were calculated per strata on each elevation gradient using GenAlEx ver. 6.5 (Peakall and Smouse 2016).

Genetic differentiation

To assess the fixing index (F_{ST}), the FreeNA software was used. It follows the ENA method (excluding null alleles) considering the presence of null alleles (Chapuis and Estoup, 2007). Under the same assumption of null alleles correction, the endogamy coefficient (F_{IS}) was calculated (INEst program) using the Bayesian model IIM (Chybicki and Burzyk, 2009); every run consisted of 200,000 cycles or Markov Chains of Monte Carlo (MCMC) iterations and 20,000 burn-in.

We assigned the grade of hierarchical partitioning of genetic diversity between gradients by molecular analysis of variance (AMOVA) with the paired population method (Excoffier *et al*. 1992), this was run in GenAlex ver. 6.5 (Peakall and Smouse, 2006). A nonparametric permutation procedure was used (9999 permutations) to calculate significant differences.

It was analyzed whether there is a differentiation between gradients from models based on distances, Bayesian, and multivariate. For this, the genetic distance of Nei (1978) was estimated, a dendrogram with the Neighbor-joining method was constructed and applied a bootstrap with 1000 replicas to validate the analysis; this was calculated with Poptree2 software (Takezaki *et al*. 2010). Later, a Bayesian clustering analysis was conducted using STRUCTURE 2.3.4 software (Pritchard *et al*. 2000; Falush *et al*. 2007) with an admixture model. Ten replicates each of 50,000 Markov chain Monte Carlo (MCMC) iterations (burn-in of 50,000) were run for each number of populations (K) tested. The optimal value of K was defined with STRUCTURE software according to Kalinowski (2011) and Puechmaille (2016). The STRUCTURE output results were evaluated and visualized criterion using the webserver STRUCTURE SELECTOR (Li and Liu 2018), and K = 1 to 10 were

analyzed. CLUMPAK (Kopelman *et al*. 2015) and implemented in STRUCTURE SELECTOR (Li and Liu 2018) were used to generate a consensus Q-matrix from the replicates at optimal K and to visualize the clusters in a histogram labeled by population. Genetic groups' comparison to infer clusters under a null model of gene flow, was made with a discriminant analysis of principal components (DAPC) (Jombart *et al*. 2010) using STATISTICA v.10 (StatSoft 2011).

Isolation by distance model was analyzed by Mantel test using geographic distances (km) and genetic distances (F_{ST}) with GenePop-Web Version 4.2 (Raymond and Rousset, 1995); it is worth mentioning that the geographical distance is related to the elevational gradient, which stratified every 500m. Subsequently, we calculated migration rates (M=m/μ, where m is the migration rate per generation and μ is the mutation rate) and Theta (Θ=4Neμ where Ne is the effective population size) with MIGRATE software (Beerli, 2016), based on the maximum likelihood using the Brownian method and a constant mutation rate (μ). The effective population size (Ne) per population was estimated using an average mutation rate for microsatellites $5x10^{-4}$ (Schlötterer, 2000; Selkoe and Toonen, 2006).

Test of recent bottlenecks

For identifying populations that have recently experienced several reductions in effective population the BOTTLENECK v 1.2.02 software was used (Piry *et al*. 1999); this test evaluates the significance deviations from population mutation-drift equilibrium (Heq). Assuming a Wilcoxon signed-rank test (two tails) carried out under a Two-phase model of mutation (TPM) to determine the average standardized difference between expected heterozygous (He) and drift-mutation equilibrium

heterozygous (Heq) (Corneut and Luikart, 1996). We set stepwise mutations as 95% with a variance of 15%, with 1000 simulations for each population. The analyses were completed separately for each species.

RESULTS

Population genetic diversity

A total of 160 individuals were successfully genotyped with 10 microsatellite markers (115 individuals of *A. lechuguilla* and 45 individuals of *A. gentryi*); 113 alleles were observed after the process of standardization, and the correction of null alleles and the deviation from linkage disequilibrium to both species (69 alleles presents in *A. lechuguilla* and 44 presents in *A. gentryi*). The average polymorphic loci = 100% by two species; *A. lechuguilla* presented higher genetic diversity values compared to *A. gentryi* (Table 2); however, *A. lechuguilla* showed a lower number of individuals in a heterozygous state than *A. gentryi* (average *Ho* = 0.31±0.03 and 0.5±0.1, respectively). Populations of both species did not show variation of genetic diversity values along elevational gradient; except by population fourth of *A. lechuguilla* (Table 2). Neither stratum are in HWE to *A. lechuguilla* and *A. gentryi* (Table 2).

Table 2. Genetic diversity among elevation gradients of *Agave Lechuguilla*. GRDT: Gradient (m asl), N: sample size, *Na*: average allele by locus, *Ne*: effective alleles by locus, *I*: Shannon's index, H_O: Observed heterozygosity, H_E: Expected heterozygosity, F: Fixation index, HWE: Hardy-Weinberg Equilibrium; ChiSq: Chi-square, *: $p < 0.05$, **: $p < 0.01$, ***: $p < 0.001$, ***.

GRDT	***500-1000***	***1001-1500***	***1501-2000***	***2001-2500***	***2501-3000***	***Average***
N	25	28	30	13	19	**23±.9**
Na	5.1±0.6	4.5±0.3	5.1±0.3	4±0.4	4.7±0.4	**4.6±0.2**
Ne	3±0.2	3±0.3	3.1±0.2	2.4±0.3	3.1±0.3	**3±0.1**
I	1.2±0.1	1.2±0.07	1.2±0.06	1±0.11	1.2±0.1	**1.2±.04**
Ho	0.28±0.08	0.26±0.07	0.31±0.08	0.35±0.07	0.35±0.07	**0.31±0.03**
He	0.64±0.04	0.65±0.02	0.66±0.02	0.54±0.05	0.66±0.02	**0.66±0.01**
F	0.51±0.143	0.58±0.119	0.50±0.137	0.34±0.112	0.47±0.116	**0.48±0.125**
HWE-(ChiSq)	$50.01_{(df=12)}$ ***	$50.51_{(df=8)}$ ***	$60.10_{(df=11)}$ ***	$16.06_{(df=7)}$ ***	$41.29_{(df=9)}$ ***	

Table 3. Genetic diversity among elevation gradients of *A. gentry*i. GRDT: Gradient (m asl), N: sample size, Na: average allele by locus, Ne: effective alleles by locus, I: Shannon's index, HO: Observed heterozygosity, HE: Expected heterozygosity, F: Fixation index, HWE: Hardy-Weinberg Equilibrium; ChiSq: Chi-square, *: p < 0.05, **: p < 0.01, ***: p < 0.001, ***

GRDT	***2001-2500***	***2501-3000***	***3001-3300***	***Average***
N	11	24	10	***15±7***
Na	3.6±0.3	4.3±0.5	4.2±0.4	***4±0.4***
Ne	2.4±0.2	2.9±0.3	2.7±0.2	***2.7±0.2***
I	1±0.1	1.2±0.1	1.1±0.1	***1.1±0.1***
Ho	0.6±0.1	0.5±0.1	0.6±0.1	***0.5±0.1***
He	0.6±0	0.6±0	0.6±0	***0.6±0***
F	-0.1	0.3±0.1	0.1±0.2	***0.1±0.2***
HWE-(ChiSq)	$56.28_{(df=20)}$ ***	$113.01_{(df=20)}$ ***	$28.53_{(df=18)}$ ***	

Genetic structure

The fixing indices in *A. lechuguilla* and *A. gentryi* populations were $F_{ST} = 0.084$ and $F_{ST} = 0.206$, respectively; and the inbreeding coefficients were $F_{IS} = 0.575$ and $F_{IS} = 0.015$, respectively. The AMOVA in *A. lechuguilla* detected that the highest percentage of molecular variation was between individuals within their population (49%) and within individuals (42%) (Table 3); in *A. gentryi* the highest percentage of

molecular variation was within individuals (70%) and among populations (23%) (Table 4).

Table 4. AMOVA of *Agave lechuguilla*; (df) free degree, (SS) squared sum, (MS) medium squared; (%) Percentages of Molecular Variance.

GRADIENTS	1. 500-1000	2. 1001-1500	3. 1501-2000	4. 2001-2500	5. 2501-3000
1. 500-1000	0	3.194	4.023	1.224	1.840
2. 1001-1500	0.073	0	3.663	1.645	2.565
3. 1501-2000	0.059	0.064	0	2.249	3.758
4. 2001-2500	0.170	0.132	0.100	0	1.673
5. 2501-3000	0.120	0.089	0.062	0.130	0

Table 5. AMOVA of *Agave gentryi*; (df) free degree, (SS) squared sum, (MS) medium squared; (%) Percentages of Molecular Variance.

Source of variation	df	SS	MS	Est. Var.	%
Among Pops	4	79.745	19.936	0.328	9%
Among Individuals	110	562.664	5.115	1.790	49%
Within Individuals	115	176.500	1.535	1.535	42%
Total	229	818.909		3.653	100%

Values of pair-wise F_{ST} in *A. lechuguilla* ranged from 0.059 between 1 and 2 gradients (500-1000 and 1001-1500, respectively) to 0.170 between 1 and 4 gradients (500-1000 and 2001-2500, respectively) (Table 5); in *A. gentryi* ranged from 0.137 between 4 and 6 gradients (2001-2500, respectively) to 0.174 between 5 and 6 gradients (2501-3000, respectively) (Table 6).

Table 6. Genetic structure in *Agave lechuguilla* among strata. Below the diagonal, we show F_{ST} values, and up the diagonal Nm values are shown.

Source of variation	df	SS	MS	Est. Var.	%
Among Pops	2	52.873	26.437	0.855	23%
Among Individuals	42	129.827	3.091	0.218	6%
Within Individuals	45	119.500	2.656	2.656	71%
Total	89	302.200		3.729	100%

Table 7. Genetic structure in Agave gentryi among strata. Below the diagonal, we show FST values, and up the diagonal Nm values are shown.

GRADIENTS	4. 2001-2500	5. 2501-3000	6. 3001-3500
4. 2001-2500	0	1.448	1.578
5. 2502-3000	0.147	0	1.185
6. 3001-3500	0.137	0.174	0

In the Nei's genetic distance analysis, the Neighbor-Joining formed two groups at both *Agave* species with a significant percentage of allocation according to the Bootstrap method. *A. lechuguilla* was conformed in the first group with populations from gradients one, two and three, the second group corresponds to populations from gradients fourth and five (Figure 2); *A. gentryi* was conformed in the first group with the population from gradient four and the second group with populations from gradients five and six (Figure 3).

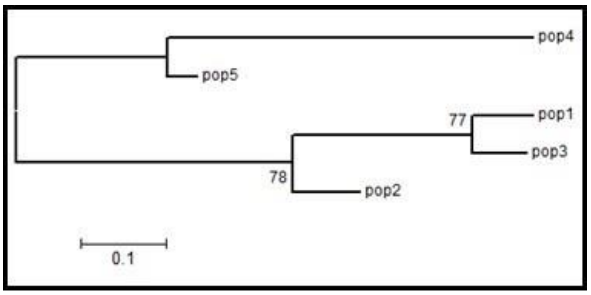

Figure 2. Neighbor-Joining cluster among elevation strata of *Agave lechuguilla* in Tamaulipas, Mexico. Note: (G1: Gradient 500-1000; G2: Gradient 1001-1500; G3: Gradient 1501-2000; G4: Gradient 2001-2500; G5: Gradient 2501-3000, masl).

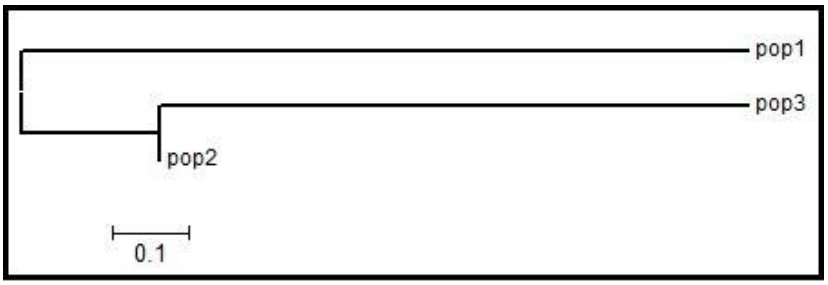

Figure 3. Neighbor-Joining cluster among elevation strata of *Agave gentryi* in Tamaulipas, Mexico. Note: (G4: Gradient 2001-2500; G5: Gradient 2501-3000, masl).

The STRUCTURE Software indicated that the maximum Δ*K* value for the two *Agave* species along the elevational gradient occurred when *K* = 3. To the individuals of *A. lechuguilla,* the assignation model by the Bayesian approach identified weak grouping; individuals distributed in gradient four showed greater differentiation of the rest of populations (Figure 4). Individuals of *A. gentryi* presented high grouping along the elevational gradient; three groups were identified, each one defined by the elevation gradient to which it belongs (Figure 5).

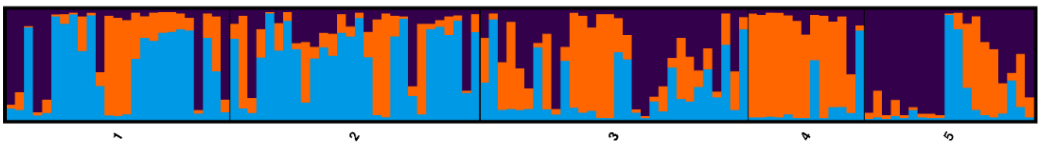

Figure 4. Estimated population structure of *Agave lechuguilla* populations along five elevational gradients, for K = 3, based on genotypes of 131 individuals using 10 microsatellite loci, obtained by Evano et al. (2005). Individuals are represented by vertical bars estimated genomic proportions corresponding to the proposed *K*.

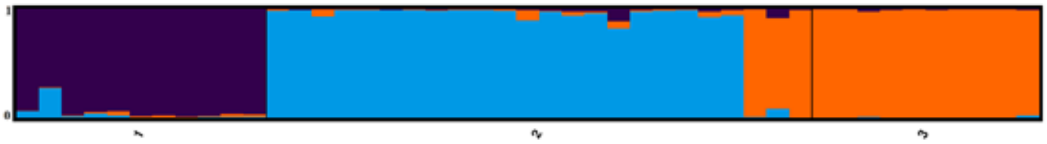

Figure 5. Estimated population structure of Agave gentryi populations along five elevational gradients, for K = 3, based on genotypes of 45 individuals using 10 microsatellite loci, obtained by Evano et al. (2005). Individuals are represented by vertical bars estimated genomic proportions corresponding to the proposed *K*.

Lineal ordination among strata, based on orthogonal distances of covariance (DAPC), explained 75% of variation with the first two components to *Agave lechuguilla* and 46% to *A. gentryi*. *A. lechuguilla* did not show a clear arrangement of its individuals (Figure 6), however, identifies divergence in the assignation of individuals at the 2001-2500 masl (G4, N = 13); by contrast, individuals of *A. gentryi* were grouped in correspondence with the elevational gradients (Figure 7).

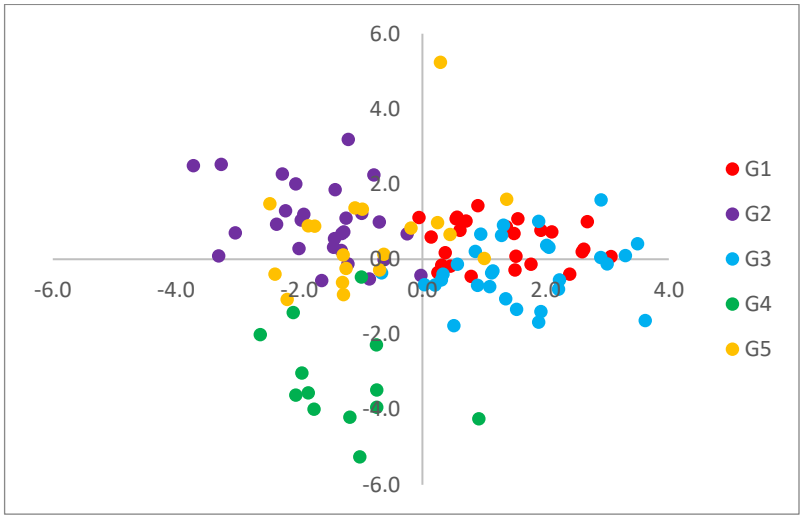

Figure 6. Discriminant Analysis of Principal Component (DAPC) contrast among elevation gradient of *Agave lechuguilla* in Tamaulipas, Mexico; a) DAPC with two roots (75% of the variation explained).

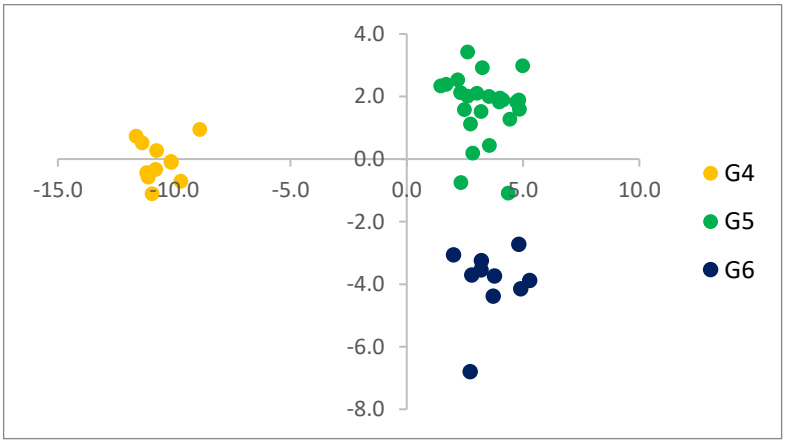

Figure 7. Discriminant Analysis of Principal Component (DAPC) contrast among elevation gradient of *Agave gentryi* in Tamaulipas, Mexico. (46% of the variation explained).

Mantel test did not show spatial correlation between geographic and genetic distance in *A. lechuguilla* and *A.* gentryi with isolation by distance model ($R^2 = 0.014$, $p = 0.47$, $R^2 = 0.502$, $p = 0.49$, respectively).

The values of effective population size (Ne) oscillate from 64 individuals in gradient fourth to 390 individuals in gradient five, which corresponds to the highest elevation gradients (2001-2500 and 2501-3000 masl), gradient one also has high values (Table 4). Gene flow (Nm) ranged from 1.15 plants migrating from gradient fourth to gradient one, to 12.44 plants migrating from gradient one to gradient two (Table 7). It should be noted that there is an important flow from gradient 5 to gradient 4 (Figure 8).

Table 8. Effective population size (Ne) and number of migrants (Nm) for the five populations of *Agave lechuguilla* in the five elevation gradients. (G1 500-1000, G2 1001-1500, G3 1501-2000, G4 2001-2500, G5 2501-3000 mals).

GRADIENT	Nm					Ne
	G1,+	G2,+	G3,+	G4,+	G5,+	
G1	-	12.44	6.27	3.72	4.33	**344**
G2	11.57	-	4.11	6.46	2.78	**240**
G3	3.44	1.71	-	1.78	2.39	**85**
G4	1.15	1.73	3.10	-	3.50	**64**
G5	4.04	6.19	6.56	11.55	-	**390**

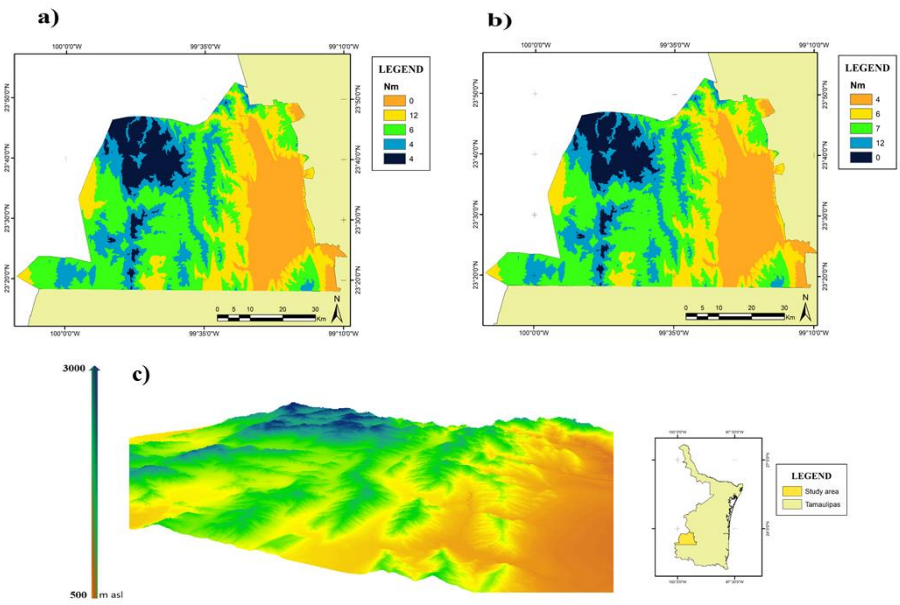

Figure 8. Map representing the number of migrants for generation in the five elevation gradients; *a)* represents Nm donate by G1 to the remaining populations along the elevation gradient? *b)* represents Nm donate by G5 to the remaining populations along the elevation gradient; *c)* three-dimensional model representing elevation-gradient and the Nm with respect to the map *a* and *b*.

No significant genetic evidence of recent demographic bottlenecks was detected for any of the *Agave* species in any of the gradients under the TPM model (Table 8 and 9).

Table 9. Bottleneck analysis for the five elevation gradients of *Agave lechuguilla* with the Two-phase model (TPM). *He*: heterozygosity, *W*: Wilcoxon 2 tails test.

Gradient (m asl)	He	W
1. 500-1000	0.66	0.70
2. 1001-1500	0.66	0.28
3. 1501-2000	0.68	0.32
4. 2001-2500	0.56	0.56
5. 2501-3000	0.68	0.49

Table 10. Bottleneck analysis to the three elevation gradients of *Agave gentryi* with the Two-phase model (TPM). *He*: heterozygosity, *W*: Wilcoxon 2 tails test.

Gradients (mals)	He	W
4. 2001-2500	0.59	0.62
5. 2501-3000	0.57	0.43
6. 3001-3500	0.55	0.1

DISCUSION

Genetic diversity of *Agave lechuguilla and A. gentryi* in an elevational gradient

Agave lechuguilla (generalist specie) and *Agave gentryi* (specialist specie) have similar values of genetic diversity (*He* = 0.66 and 0.60, respectively; polymorphic loci = 100% in both species); actually, genetic diversity of both species is higher respect to average values reported from its congeners evaluated with SSRs (*He* = 0.48 – 0.70, average = 0.54, *A. palmeryi*, Lindsay *et al*. 2012; *A. utahensis*, Bayers *et al*. 2014; *A. potatorum*, Félix-Valdez, *et al*. 2015; *A. inaquidens*, Figueredo *et al*. 2015; *A. cupreata* and *A. hookeri*, Figueredo *et al*. 2017). It also, *A. lechuguilla* was registered in five elevation gradients (500-2500 m asl) and *A. gentryi* in three (2001-3500 m asl) with respect to the parameters established in this study (Sánchez-González *et al*. 2019); species distribution is an underlying effect from the interaction of a set of environmental resources and species (the niche *sensu* Hutchinson; Hutchinson, 1957), geographic variation of key environmental factors and the degree of their ecological tolerance, conditioned by genetic pool of the population (Gibson *et al*, 2008; Frankham *et al*, 2009; Hedrick, 2010; Aguirre-Planter *et al*, 2020), determine their current distribution (realized niche; Austin and Smith, 1989). In this case, genetic diversity values of *Agave lechuguilla* and *A. gentryi* did not show variation with relation to ecological tolerance degree; therefore, contrary to expected, the ecological niche breadth does not reflect the genetic diversity of the species.

Generalist species have high diversity and allelic quality to remain in a wide environmental gradient; On the one hand, they have a genetic reservoir to produce genotypes under selection of environmental conditions, or they produce genotypes

capable of adjusting their condition (morphology, physiology, molecular) to environmental variation (Diouf *et al*. 2018). On the other hand, specialists require high genetic diversity to meet the demand for functional requirements that allow them to compete in specific environments where dynamics are more demanding.

A. lechuguilla populations were mainly found in homozygous state, contrary to *A. gentryi* populations, which expressed their genetic diversity in heterozygous state (average *Ho* = 0.31 and 0.5, respectively). Generalist species are characterized by having the ability to take advantage of a greater number of resources; while specialist species have evolutionarily exchanged this capacity to be more efficient in taking advantage of a specific set of resources, which in both cases is reflected with the ranges of distribution (Boulangeat *et al*. 2011). This ecological efficiency cost through many or few resources, can be related with the heterozygous or homozygous state of individuals; since specialism, in this case *A. gentryi* efficient in the use of specific resources, may depend on a greater number of alleles present in each individual in the population (heterozygotes); while *A. lechuguilla* generalist specie could depend on its ability to modify its morphology through plastic alleles to optimize the use of various resources. Similar data are reported with another specialist *Agave* specie; *A. kerchovei* with very restricted distribution showed high genetic diversity (*Hd* = 0.718; Aguirre-Planter *et al.* 2020); contrary to this, *A. potatorum* is found in the same region as *A. kerchovei* but with a greater range of distribution, it reported heterozygosity observed values below the average according to its congeners evaluated with SSRs (*Ho* = 0.31-0.46; Félix-Valdez, *et al.* 2015).

Both *Agave* species showed that intrapopulations genetic variation is unaffected by elevational gradient (Table 2). This pattern occurs in species that have

a significant gene flow among their populations, to such a degree that they can homogenize the distribution of genetic diversity (Oshawa and Ide, 2008). Genetic flow in angiosperms is defined, principally, by the pollination, conditioned by the relationship between floral structure and pollinator (Simon-Porca *et al.* 2017). The genus *Agave* is characterized by having a prominent spiky or paniculate inflorescence (subgenus *Littea* and *Euagave*, respectively) and its main pollinators are bats, sphincters, hymenopterans, lepidopterans, and birds (Gentryi, 1987; Rocha *et al.* 2005, 2006; Trejo-Salazar *et al.* 2015).

Genetic structure and gene flow in *A. lechuguilla* and *A. gentryi*

Patterns of genetic structure and gene flow between populations of *A. lechuguilla* (generalist) and *A. gentryi* (specialist) were opposite; *A. lechuguilla* presented low structure and high gene flow, while *A. gentryi* exhibited high structure and low gene flow (F_{ST} = 0.084 and 0.206, respectively). This indicates that, the environmental variation exerted by the elevation gradient does not generate an effect on the genetic diversity distribution of *A. lechuguilla* populations, contrary to what occurs with A. gentryi populations. This indicates that, *A. lechuguilla* produces different morphotypes without significantly modifying its genotype, presenting higher average physical fitness in different environments (Van Kleunen and Fischer, 2005; Davidson *et al*. 2011); on other hand, *A. gentryi* produce different genotypes with greater vigor by generating adaptive, selective, and reproductive advantages against environmental variation (Dobzhansky, 1950).

Populations distributed along a wide environmental gradient without showing genetic differentiation associated with the environment, may be a consequence of

mechanisms of phenotypic plasticity; natural selection of pre-existing epigenetic variation, environmental variable induction of epigenetic variation, or both (Nicotra *et al.* 2010; Lele *et al.* 2017; Fazlioglu and Bonser, 2016). Phenotypic plasticity is the property of a genotype to express different phenotypes which can be visualized as a pattern of expression in different environments without any link to fitness (Nicotra *et al*. 2010; Van Tienderen, 1997); nevertheless, in plants, it may contribute to the adaptation process by expressing morphological variation that tolerates environmental variation regulated by epigenetic process (Cubas et al. 1999; Grant-Downton and Dickinson, 2006; Richards *et al.* 2006; Nicotra *et al.* 2010; 2015). As studies explain and identify epigenetic action as a mediator of phenotypic variation, such as vernalization (Sung and Amasiano, 2004), flower chimerization (Kaing-Feng *et al.* 2018), or heterophylly (Lele *et al.* 2017). According to the genetic diversity reported to *A. lechuguilla* in the present work and morphological variation reported in flower (Silva-Montellano and Eguiarte, 2003a) and in leaves (Sánchez-González *et al.* 2019) along a latitudinal and elevational gradient respectively, these species provide indications of developing phenotypic plasticity in the face of environmental variation. Populations distributed along a wide environmental gradient and that present genetic differentiation associated with the environment, are due to their heterosis; In plants, is an important phenomenon because it is responsible for vigor in growth and increased resistance to various biotic and abiotic stresses (Fujimoto *et al*. 2018). We infer this occurs with *A. gentryi* populations.

Degree of genetic structure is due, largely, to the reproductive strategies that condition gene flow. Particularity, *Agave* reproduce sexually and asexually, in the first case it is a monocarpic state; it has a single reproductive event in its life that,

depending on the species, takes 8-20 years; on other hand, they also reproduce asexually by means of clones, cuttings of stolons (Gentry 1982; García-Mendoza 2007; Jiang *et al.* 2017). The percentage of asexual reproduction in the plant population with this ability increases when conditions become unfavorable for sexual reproduction (Ohasawa and Ide 2008). In the present work, even though the percentage of clonal and non-clonal individuals of *A. lecheguilla* and *A. gentryi* was not quantified, a significant incidence of ramets or colonies generated from a same individual was observed, it could be interpreted as inbreeding depression in populations. In relation to this, plants owe their sexual connectivity mainly to the activation of reproductive systems by pollinators. (Su *et al.* 2017; Simón-Porcar *et al.* 2018). The *Agave* genus is characterized by presenting a chiropterophilous syndrome in most of its species (Gentry, 1982; Arizaga *et al*. 2000; García-Mendoza, 2007; Trejo-Salazar *et al*. 2017). As reported for *A. gentryi* (Castillo-Hernández and Treviño-Carreón, 2009). However, to the study area of this work, the presence of nectarivore bats is reported, but its abundance is low, and the range of their elevational distribution is unknown. In addition, some studies indicate that their activity decreases with environmental factors associated with elevation such as temperature and vegetation type (Arriaga-Flores et al., 2018); this would explain the genetic structure in the *A. gentryi* populations (Figure 5, 7 and), considering the specialization of their reproductive biology. Otherwise, *A. lechuguilla* reports a variation in its pollinators, among which insects and birds stand out, they pointed out that this species expresses a general reproductive ecology (Silva-Montellano and Eguiarte, 2003b; Rocha *et al*. 2006); that is generalism in the range of receptors in pollination systems contributes to keeping greater genetic variability from genetic

connectivity (Jump *et al.* 2008; Nicotra et al, 2010; Simón-Porcart *et al.* 2018), and could explain the high genetic flow (Table 7) (Figure 8) and the absence of genetic structure among their populations (Figure 4 and 6).

Events and bottleneck

Bottleneck analysis shows no evidence of genetic drift in *Agave lechuguilla* populations distributed in the study area (Table 9); although a decrease in the population size of the species is reported. Scheinvar and collaborators (2016) report the formation of haplotypes in the *A. lechuguilla* distribution range generated by contraction and expansion events in their populations since the last ice age (transition between the Pliocene and Pleistocene, 2.6 Mya); after the decrease in population size, recovery and expansion of the species began towards the north from populations located in the Last Glacial Maximum refuges (21 thousand years). Furthermore, this plant has been used since pre-Hispanic times for the production of vegetable fibers or “ixtle”, mainly in the region of the Mexican highlands (Vieira *et al*. 2002; García-Mendoza, 2007); point data on the degree of use is not known, but it is mentioned that there was important management of the resource in the first period of the 20th century in the states of San Luis Potosí, Zacatecas, Coahuila, Tamaulipas, and Nuevo León, for local use and export to the US and Europe (Ibarra, 1938). Currently, the populations that are distributed in the Tamaulipas highlands continue to be used for local consumption, although with less intensity. Therefore, it is stated that it has significant adaptive potential and different factors are inferred, among the most important: *i)* high recruitment rate, mainly for vegetative reproduction (Gentryi, 1982; Silva-Montellano and Eguiarte, 2003a; García-

Mendoza, 2007; Cervera-Herrera et al. 2018); *ii)* competent reproductive adaptation (Rocha et al. 2006; Fazlioglu and Bonser, 2018) and; *iii)* general ecological niche (Sánchez-González *et al*. 2019), which leads to maintaining an effective populations size. *A. gentryi* did not show significant values.

CONCLUSIONS

Genetic diversity was high in *A. lechuguilla* and *A. gentryi*, generalist and specialist species, respectively. Therefore, environmental tolerance may be conditioned by the average response capacity for the phenotypic plasticity of the species. However, it should be mentioned that genetic diversity is the source of variation that can most effectively contribute to the adaptation process, especially with gradual changes in the environment. In this regard, *A. gentryi* may be more successful for its ability to use resources. On the other hand, phenotypic plasticity contributes better to sudden changes in the environment, this could give *A. lechuguilla* a greater advantage against climate change. Evaluating the genetic diversity of spices, considering their environmental tolerance (generalism and specialism), allows having a much more concrete picture of their response capacity.

REFERENCES

Aguirre-Planter, E., Parra-Leyva, J. G., Ramírez-Barahona, S., Scheinvar, E., Lira-Saade, R., & Eguiarte, L. E. (2020). Phylogeography and genetic diversity in a southern North American desert: Agave kerchovei from the Tehuacán-Cuicatlán Valley, Mexico. Frontiers in Plant Science, 11, 863.

Alsos, I. G., Ehrich, D., Thuiller, W., Eidesen, P. B., Tribsch, A., Schönswetter, P., ... & Brochmann, C. (2012). Genetic consequences of climate change for northern plants. Proceedings of the Royal Society B: Biological Sciences, 279(1735): 2042-2051.

Arizaga S, Ezcurra E, Peterson E, Ramírez De Arellano F, Vega E (2000) Pollination ecology of *Agave macroacantha* (Agavaceae) in a Mexican tropical desert. i. floral biology and pollination mechanisms. Am. J. Bot. 87: 1004-1010.

Arriaga-Flores JC, Rodríguez-Moreno A, Correa-Sandoval A, et al (2018) Spatial and environmental variation in phyllostomid bat (Chiroptera, Phyllostomidae) distribution in Mexico. Animal Biodiversity and Conservation 41: 141-159.

Beerli P, Felsenstein J (1999) Maximum-likelihood estimation of migration rates and effective population numbers in two populations using a coalescent approach. Genetics 152: 763–773.

Beierkuhnlein, C., Thiel, D., Jentsch, A., Willner, E., & Kreyling, J. (2011). Ecotypes of European grass species respond differently to warming and extreme drought. Journal of Ecology, 99(3): 703-713.

Boulangeat, I., Lavergne, S., Van Es, J., Garraud, L., & Thuiller, W. (2012). Niche breadth, rarity and ecological characteristics within a regional flora spanning large environmental gradients. Journal of Biogeography, 39(1): 204-214.

Blonder, B. (2018). Hypervolume concepts in niche-and trait-based ecology. Ecography, 41(9): 1441-1455.

Buckling A, Brockhurst MA, Travisano M, et al. (2006) Experimental adaptation to high and low quality environments under different scales of temporal variation. Journal compilation 20:296-300.

Byers C, Maughan PJ, Clouse J, Stewart R (2014) Microsatellite primers in *Agave utahensis* (Asparagaceae), a keystone specie in the Mojave Desert and Colorado Plateau. Application in plant sciences 2(9): 1400047.

Castillo-Hernández H and Treviño-Carreón J (2009). Biología floral de *Agave gentryi* (AGAVACEAE) en la localidad de La Marcela, Miquihuana, Tamaulipas. CienciUAT 4:62-66.

Cervera-Herrera JC, Leirana-Alcocer JL, Navarro JA (2018) Factores ambientales relacionados con la cobertura de *Agave angustifolia* (Asparagaceae) en el matorral costero de Yucatán, México. Acta Botánica Mexicana 124: 75-84.

Chapuis MP, Estoup A (2007) Microsatellite null alleles and estimation of population differentiation. Mol Biol Evol 24(3): 621–631.

Chevin, L. M., Lande, R., & Mace, G. M. (2010). Adaptation, plasticity, and extinction in a changing environment: towards a predictive theory. PLoS biology, 8(4), e1000357.

Chrismas, N. A., Barker, G., Anesio, A. M., & Sánchez-Baracaldo, P. (2016). Genomic mechanisms for cold tolerance and production of

exopolysaccharides in the Arctic cyanobacterium Phormidesmis priestleyi BC1401. BMC genomics, 17(1): 1-14.

Chybicki IJ, Burczyk J (2009) Simultaneous estimation of null alleles and inbreeding coefficients. J Hered. 100: 106–113.

Cornuet JM, Luikart G (1996) Description and power analysis of two tests for detecting recent population bottlenecks from allele frequency data. Genetics 144:2001–2014.

Cubas P, Vincent C, Coen E (1999) An epigenetic mutation responsible for natural variation in floral symmetry. Nature 401: 157-161.

Davis, S. C., Dohleman, F. G., & Long, S. P. (2011). The global potential for Agave as a biofuel feedstock. Gcb Bioenergy, 3(1): 68-78.

Diouf, I. A., Derivot, L., Bitton, F., Pascual, L., & Causse, M. (2018). Water deficit and salinity stress reveal many specific QTL for plant growth and fruit quality traits in tomato. Frontiers in Plant Science 9: 279.

Dobzhansky, T. (1950). Evolution in the tropics. American scientist, 38(2), 209-221.

Doyle JJ, Doyle JL (1987) A rapid DNA isolation procedure for small quantities of fresh leaf tissue. Phytochem. Bull 19: 11-15.

Eguiarte LE, González A (2007) De genes y magueyes estudio y conservación de los recursos genéticos del tequila y el mezcal. Ciencias 87:28-35.

Eguiarte LE, Souza V, Silva-Montellano A (2000) Evolución de la familia Agavaceae: filogenia, Biología reproductiva y genética de poblaciones. Boletín de la sociedad botánica de México 66: 131-151.

Etterson, J. R., & Shaw, R. G. (2001). Constraint to adaptive evolution in response to global warming. science, 294(5540): 151-154.

Excoffier L, Smouse PE, Quattro JM (1992) Analysis of Molecular Variance Inferred from metric distance among DNA haplotypes: Application to human mitochondrial DNA restriction data. Genetics Society of America 131: 479-491.

Falush D, Stephens M, Pritchard JK (2007) Inference of population structure using multiloci genotype data: dominant markers and null alleles. Mol Ecol Notes 7:574–578.

Fazlioglu F, Bonser SP (2016) Phenotypic plasticity and specialization in clonal versus non-clonal plants: A data synthesis. Acta Oecologica 77: 193-200.

Félix-Valdez LI, Vargas-Ponce O, Cabrera-Toledo D, et al. (2015) Effects of traditional management for mescal production on the diversity and genetic structure of *Agave potatorum* (Asparagaceae) in central Mexico. Genet Resour Crop Evol.

Figueredo-Urbina CJ, Casas A, González-Rodríguez A, et al. (2015) Genetic structure of coexisting wild and managed *Agave* populations: implications for the evolution of plants under domestication. AoB Plants 7.

Figueredo-Urbina CJ, Casas A, Torres-García I (2017) Morphological and genetic divergence between *Agave inaquidens, A. cupreata* and the domesticated *A. hookeri*. Analysis of their evolutionary relationships doi.org/10.1371/journal.pone.0187260.

Flores-Abreu IN (2007) Coevolución entre género *Agave sensu lato* y sus murciélagos Polinizadores (Phyllostomidae). Master's degree. Instituto de Ecología, Universidad Nacional Autónoma de México, México.

Frankham R (2005) Genetics and Extinction. Biological Conservation 126:131-140.

Frankham R, Ballou JD, Briscoe DA (2009) Introduction to conservation genetics Cambridge, NY: Cambridge University Press.

Franks SJ (2011) Plasticity and evolution in drought avoidance and escape in the annual plant Brassica rapa. New Phytologist 190:249-257.

Franks, S. J., & Hoffmann, A. A. (2012). Genetics of climate change adaptation. Annual review of genetics, 46: 185-208.

Galván-Hernández DM, Lozada-García JA, Flores-Estévez N, et al. (2015) Variation and genetic structure in *Plantanus mexicana* (Platanaceae) along riparian altitudinal gradient. International Journal of Molecular Science 16:2066-2077.

García-Mendoza AJ (2007) Los agaves de México. Ciencias 87:14-23.

Gaudel M, Stenoien HK, Agren J (2007) Landscape structure, clonal propagation, and genetic diversity in Scandinavian population of *Arabidopsis lyrate* (Brassicaceae). Am. J. Bot. 94:1146-1155.

Gentry HS, (1982) Agaves of continental North America. Arizona, Arizona: University of Arizona Press.

Gibson, J. P., Rice, S. A., & Stucke, C. M. (2008). Comparison of population genetic diversity between a rare, narrowly distributed species and a common, widespread species of Alnus (Betulaceae). American Journal of Botany, 95(5): 588-596.

Gitzendanner, M. A., & Soltis, P. S. (2000). Patterns of genetic variation in rare and widespread plant congeners. American Journal of Botany 87(6): 783-792.

Grant-Downton, Dickinson HG (2006) Epigenetics and its Implications for Plant Biology 2. The 'Epigenetic Epiphany': Epigenetics, Evolution and Beyond. Annals of Botany 97: 11-27

Halbritter AH, Alexander JM, Edwards PJ, et al. (2013) How comparable are species distributions along elevational and latitudinal climate gradients? Global Ecology and Biogeography 22:1228-1237.

Hedrick PW (2010) Genetics of populations 4th. Arizona, Arizona: Boston Jones and Bartlett Publishers.

Hoffmann, A. A., & Sgrò, C. M. (2011). Climate change and evolutionary adaptation. Nature, 470(7335): 479-485.

Ibarra R (1938) Estudio sobre el ixtle. El trimestre económico 5:103-125.

IUCN (2021) Red list of endangered species. http://www.iucnredlist.org/search. Recovered on January 30, 2020.

Jiang K, Po-Keung ET, Na-Na X, Xiao-Yong C (2017) High genetic diversity and strong

differentiation in dramatically fluctuating populations of *Zostera japonica* (Zosteraceae): implication for conservation. Journal of Plant Ecology 11:789-797.

Jombart T, Devillard S, Ballowx F (2010) Discriminant analysis of principal components: a new method for the analysis of genetically structured populations. BMC Genetics 11:94.

Jump AS, Marchant R, Peñuelas J (2008) Environmental change and the option value of genetic diversity. Trends in Plant Science 14: 51-58.

Kaing-Feng M, Qi-Xiang Z, Tang-Ren Ch, et al (2018) Substantial Epigenetic Variation Causing Flower Color Chimerism in the Ornamental Tree *Prunus mume* Revealed by Single Base Resolution Methylome Detection and Transcriptome Sequencing. International Journal of Molecular Science 19, 2315.

Kalinowski ST (2011) The computer program STRUCTURE does not reliably identify the main genetic clusters within species: simulations and implications for human population structure. Heredity 106: 625–632.

Keb-Llanes M, González G, Chi-Manzanero B, Infante D (2002) A rapid and simple method for small-scale DNA extraction in Agavaceae and other tropical plants. Plant Molecular Biology Reporter 20: 299-299.

Ketola T, Mikonranta L, Zhang L, et al. (2013) fluctuating temperature leads to evolution of thermal generalism and preadaptation to novel environments. Evolution 67:2936-2944.

Kopelman NM, Mayzel J, Jakobsson M, Mayrose I (2015) Clumpak: a program for identifying clustering modes and packaging population structure inferences across K. Mol Ecol Resour 15:1179–1191.

Körner C (2007) The use of ‘altitude’ in ecological research. Trends in Ecology and Evolution 22: 569-574.

Lindsay DL, Edwards CE, Jung MG, Bailey P, Lance RF (2012) Novel Microsatellite loci for *Agave parryi* and cross-amplification in *Agave palmeri* (Agavaceae). Am. J. Bot. 99: 295-297.

Li S, Jovelin R, Yoshiga T, et al. (2014) Specialist versus generalist life histories and nucleotide diversity in *Caenorhabditis* nematodes. Proc. R. Soc. B 281: 20132858.

Li YL, Liu JX (2018) STRUCTURE SELECTOR: A web-based software to select and visualize the optimal number of clusters using multiple methods. Mol Ecol Resour 18:176–177.

Lele L, Ning D, Cuiping P, Xiao G, Weihua G (2017) Genetic and epigenetic variations associated with adaptation to heterogeneous habitat conditions in a deciduous shrub. Ecology and Evolution 8: 2594-2606.

Lesica P, Allendorf FW (1995) When are peripheral populations valuable for conservation? 9: 753-760.

Loxdale HD, Lushai G, Harbey J (2011) The evolutionary improbability of 'generalism' in nature, with special reference to insects. Biological Journal of the Linnean Society 103:1-18.

Mabel BK (2018) Conservation of adaptive potential and functional diversity: integrating old and new approaches. Conservation Genetics 20:89-100.

Mangaravite, É., Vinson, C. C., Rody, H. V., Garcia, M. G., Carniello, M. A., Silva, R. S., & Oliveira, L. O. (2016). Contemporary patterns of genetic diversity of Cedrela fissilis offer insight into the shaping of seasonal forests in eastern South America. American Journal of Botany, 103(2): 307-316.

Millberg, H., Boberg, J., & Stenlid, J. (2015). Changes in fungal community of Scots pine (Pinus sylvestris) needles along a latitudinal gradient in Sweden. Fungal Ecology, 17, 126-139.

Nei M (1978) Estimation of Average Heterozygosity and Genetic Distance from a Small Number of Individuals. Genetics 89(3): 583–590.

Nicotra AB, Atkin OK, Bonser SP, et al. (2010) Plant phenotypic plasticity in a changing climate. Trends in Plant Science 15: 684-692.

Nicotra AB, Beever EA, Robertson AL, Hofmann GE, O'Leary J (2015) Assessing the components of adaptive capacity to improve conservation and management efforts under global change. Conservation Biology 29: 1268-1278

Ohsawa T, Ide Y (2008) Global patterns of genetic variation in plant species along vertical and horizontal gradient on mountains. Global Ecol. Biogeogr. 17:152-163.

Oosterhout CV, Hutchinson WF, Wills DPM, et al. (2006) MICRO-CHECKER: software for identifying and correcting genotyping errors in microsatellite data. Molecular Ecology Notes 4:535-538.

Pauls, S. U., Nowak, C., Bálint, M., & Pfenninger, M. (2013). The impact of global climate change on genetic diversity within populations and species. Molecular ecology, 22(4): 925-946.

Peakall R, Smouse PE (2006) GENALEX 6.5: genetic analysis in Excel. Population genetic software for teaching and research. Mol Ecol Notes 6:288–295.

Peterson AT, J Soberón, RG Pearson, et al. (2011) Ecological niches and geographic distributions. Princeton University Press, Princeton: NJ.

Piñero D (2008) La variabilidad genética de las especies: aspectos conceptuales y sus aplicaciones y perspectivas en México. In Soberón J, Halffter G,

Llorente-Bousquets J (ed). Capital Natural de México. Ciudad de México, México: Comisión Nacional para el conocimiento y Uso de la Biodiversidad.

Piry S, Luikart G, Cournet JM (1999) Bottleneck: A computer program for detecting recent reductions in the effective population size using allele frequency data. The Journal of Heredity 90: 502-503.

Premoli AC, Mathiasen P (2011) Respuestas ecofisiológicas adaptativas y plásticas en ambientes secos de montaña: *Nothofagus pumilio*, el árbol que acaparó los Andes australes 21:251-269.

Pritchard JK, Stephens M, Donnelly P (2000) Inference of population structure using multiloci genotype data. Genetics 155:945–959.

Puechmaille SJ (2016) The program structure does not reliably recover the correct population structure when sampling is uneven: subsampling and new estimators alleviate the problem. Mol Ecol Resour 16:608–627.

Raymond M, Rousset F (1995) GENEPOP (version 1.2): population genetics software for exact tests and ecumenicism. J Hered 86: 248–249.

Richards CL, Bossford O, Muth NZ, et al. (2006) Jack of all trades, master of some? On the role of phenotypic plasticity in plant invasions. Ecology Letters 9:981-993.

Rocha M, Good-Ávila SV, Molina-Freaner F, et al. (2006) Pollination biology and adaptive radiation of Agavaceae, with special emphasis on the genus *Agave*. Aliso 22: 329-344.

Rzedowski J 1978 Vegetación de México. Ciudad de México, México: Editorial Limusa, S.A.

Sánchez-González A, Octavio-Aguilar P, Barrientos-Lozano L, et al. (2019) Effect of elevation strata on morphological variation of two agave species with different niche amplitude. 180:892-901.

Scheinvar E, Gámez N, Castellanos-Morales G, et al (2016) Neogene and Pleistocene history of *Agave lechuguilla* in the Chihuahuan Desert. Journal of Biogeography.

Schlötterer C (2000) Evolutionary dynamics of microsatellite DNA. Chromosoma 109(6): 365–371.

Selechinik D, Richardson MF, Shine R, et al. (2019) Increased Adaptive Variation Despite Reduced Overall Genetic Diversity in a Rapidly Adapting Invader. Frontiers in Genetics 10:1221.

Selkoe KA, Toonen RJ (2006) Microsatellites for ecologists: a practical guide to using and evaluating microsatellite markers. Ecology letters 9: 615-629.

Shaw, R. G., & Etterson, J. R. (2012). Rapid climate change and the rate of adaptation: insight from experimental quantitative genetics. New Phytologist, 195(4): 752-765.

Silva-Montellano A, Eguiarte LE (2003a) Geographic patterns in the reproductive ecology of agave lechuguilla (agavaceae) in the chihuahuan desert. i. floral characteristics, visitors, and fecundity. Am J. Bot. 90:377-378.

Silva-Montellano A, Eguiarte LE (2003b) Geographic pattern in the reproductive ecology of *Agave lechuguilla* (Agavaceae) in the Chihuahua Desert. II. Genetic variation, differentiation, and inbreeding estimates. Am. J. Bot. 90(5): 700-706.

Simón-Porcar VI, Abdelaziz M, Arroyo J (2018) El papel de los polinizadores en la evolución floral: una perspectiva mediterránea. Ecosistemas 27: 70-80.

Soberón JM (2010) Niche and area of distribution modeling: a population ecology perspective. Ecography 33: 159-167

Sork, V. L., Davis, F. W., Westfall, R., Flint, A., Ikegami, M., Wang, H., & Grivet, D. (2010). Gene movement and genetic association with regional climate gradients in California valley oak (Quercus lobata Née) in the face of climate change. Molecular ecology, 19(17): 3806-3823.

StatSoft (2011) STATISTICA (data analysis software system), version 10. http//www.statsoft.com.

Su Z, Richardson BA, Zhuo L, et al (2017) Genetic diversity and structure of an endangered desert shrub and the implications for conservation. AoB Plants 9: plx016; doi:10.1093/aobpla/plx016.

Sung S, Amasino R (2004) Vernalization in *Arabidopsis thaliana* is mediated by the PHD finger protein VIN3. Nature 427: 159-164.

Takezaki N, Nei M, Tamura K (2010) POPTREE2: software for constructing population trees from allele frequency data and computing some other population statistics with Windows interface. Mol Biol Evol 27:747-752.

Trejo L, Limones V, Peña G, et al (2017) Genetic variation and relationships among agaves related to the production of Tequila and Mezcal in Jalisco. Industrial Crops & Products 125: 140-149.

Trejo-Salazar RE, Scheinvar E, Eguiarte LE (2015) ¿Quién poliniza realmente los agaves? Diversidad de visitantes florales en 3 especies de *Agave*

(Agavoideae: Asparagaceae). Revista Mexicana de Biodiversidad 86:358-369.

Urban MC (2015) Accelerating extinction risk from climate change. Science. 348: 571-573.

Valladares, F., SANCHEZ-GOMEZ, D. A. V. I. D., & Zavala, M. A. (2006). Quantitative estimation of phenotypic plasticity: bridging the gap between the evolutionary concept and its ecological applications. Journal of ecology, 94(6): 1103-1116.

Van Oosterhout C, Hutchinson WF, Wills DP, Shipley P (2004) MICRO-CHECKER: software for identifying and correcting genotyping errors in microsatellite data. Program Note. Mol Ecol Notes. 4: 535–538.

Van Oosterhout C, Weetman D, Hutchinson WF (2006) Estimation and adjustment of microsatellite null alleles in nonequilibrium populations. Mol Ecol Notes 6: 255-256.

Van Kleunen, M., & Fischer, M. (2005). Constraints on the evolution of adaptive phenotypic plasticity in plants. New phytologist, 166(1): 49-60.

Van Tienderen P (1997) Generalists, specialists, and the evolution of phenotypic plasticity in sympatric populations of distinct species. Evolution 5:1372-1380.

Vieira MC, Heinze T, Antonio-Cruz R, Mendoza-Martínez AM (2002) Cellulose derivatives from cellulosic material isolated from *Agave lechuguilla* and *A. fourcroydes. Cellulose 9:203-212.*

Wiens W (2016) Climate-Related Local Extinctions Are Already Widespread among Plant and Animal Species. PLos Biol 14.

Takezaki N, Nei M, Tamura K (2010) POPTREE2: Software for Constructing Population Trees from Allele Frequency Data and Computing Other Population Statistics with Windows Interface. Mol. Biol. Evol. 27(4): 747-752.

V. DISCUSIÓN GENERAL

Las poblaciones de *A. lechuguilla* (especie generalista), y *A. gentryi* (especie especialista) mostraron variación genética y morfológica relacionada con la variación ambiental provocada por el gradiente de elevación; estas variaciones fueron particulares en cada especie, lo cual se puedo asociar con la amplitud de su nicho ecológico. Los modelos sobre el efecto del gradiente elevacional y latitudinal sobre la diversidad genética de las especies, presentado en este trabajo, predijeron menor susceptibilidad de especies tropicales en comparación con especies alpinas o subalpinas (King et al. 2018; Ackerly et al, 2002; Galván-Hernández et al, 2015); esto puede representar el generalismo y especialismo, respectivamente. Este efecto, en parte, pudo apreciarse en *A. lechuguilla* y *A. gentryi*; en donde respuesta morfológica de *A. lechuguilla* es significativa, mientras que en *A. gentryi* es casi nula; no obstante, la diversidad genética es elevada en ambas especies. Esto implicaría que, el reservorio genético es importante para las especies, pero no define la amplitud de su nicho ecológico.

La variación morfológica y la diversidad y distribución de la variación genética, fue un rasgo que evidencio la amplitud de nicho ecológico de ambas especies. En el primer caso, *A. lechuguilla* reportó un patrón heterofílico de elevación, el tamaño de la hoja disminuyó con la elevación (Sánchez-González et al. 2019), y a pesar de presentar una elevada diversidad genética no mostro estructura genética asociada al gradiente; los patrones morfológicos observados a lo largo de un gradiente ambiental, que no se explican por la diversidad genética, pueden ser consecuencia de mecanismos de plasticidad fenotípica; selección natural de variación epigenética preexistente, inducción de variable ambiental de variación epigenética, o ambas

(Nicotra et al. 2010; Lele et al. 2017; Fazlioglu y Bonser, 2016). La plasticidad fenotípica es la propiedad de un genotipo para expresar diferentes fenotipos que pueden visualizarse como un patrón de expresión en diferentes ambientes sin ningún vínculo con la aptitud (Nicotra et al. 2010; Van Tienderen, 1997); sin embargo, en las plantas, puede contribuir al proceso de adaptación al expresar una variación morfológica que tolera la variación ambiental regulada por el proceso epigenético (Cubas et al. 1999; Grant-Downton y Dickinson, 2006; Richards et al. 2006; Nicotra et al. 2010). ; 2015). Como los estudios explican e identifican la acción epigenética como mediadora de la variación fenotípica, como la vernalización (Sung y Amasiano, 2004), la quimerización de las flores (Kaing-Feng et al. 2018) o la heterofilia (Lele et al. 2017). En segundo lugar, *A gengryi* no mostró una respuesta morfológica, pero si una elevada diversidad y estructura genética. La diversidad genética es uno de los tres niveles de biodiversidad que requieren conservación; una alta diversidad genética dentro de una población contribuye en su adaptación ya que subyacen variaciones fenotípicas, esto permite que las poblaciones respondan a la selección impuesta por el cambio ambiental (Frankham, 2005). Lo cual genera genotipos con alelos mucho más eficientes generando poblaciones con mayor aptitud. La diversidad genética tiende a trabajar más exitosamente con cambios ambientales graduales, lo que permite el establecimiento de genotipos adecuados; de esta manera, el generalismo se dirige evolutivamente a la especialización, ocupando nuevos nichos en condiciones ambientales específicas que contribuyen al uso eficiente de los recursos para las poblaciones (Buckling et al. 2006; Ketola et al. 2013). Por lo tanto, *A. gentriy* es una especie que posiblemente es resultado de procesos de diversificación a través de la

especialización en el uso adecuado de recursos, abandonando el generalismo y la plasticidad morfológica, para generar poblaciones con mayor vigor hibrido en ambientes de montaña (heterosis).

Los hallazgos en este trabajo muestran como la diferencia en la amplitud de nicho ecológico no es definida solamente por su diversidad genética, sino por la forma en que esta opera sobre la morfología de sus poblaciones; ya sea a partir de la plasticidad fenotípica o la heterosis.

VI. CONCLUSIÓN GENERAL

La tolerancia ecológica de las especies depende, en gran medida, de la capacidad de responder a las variaciones ambientales, dicha respuesta se define por su plasticidad fenotípica y su diversidad genética. Lo cual se observó en *A. lechuguilla* y *A. gentryi*, especies con diferente nicho ecológico (generalista y especialista, respectivamente).

El gradiente de elevación genera cambios importantes en las condiciones ambientales, los cuales pueden simular los efectos del cambio climático. Se estima, que este efecto perjudicará principalmente a especies especialistas que se distribuyen en regiones montañosas como en este caso *A. gentryi,* mientras que especies generalistas como *A. lechuguilla* podrían ampliar su rango de distribución. *Agave lechuguilla* es una especie con una tolerancia ambiental importante, esto se lo debe a, su capacidad de generar morfotipos asociados al ambiente, su diversidad genética y el generalismo reproductivo que, a pesar de ser un atributo que no se cuantificó en el presente trabajo, se pude inferir por su elevado flujo genético, esto implica una importante apertura reproductiva para polinizadores. Por otro lado, *A. gentryi* es una especie que evolutivamente pudo intercambiar la capacidad de utilizar un amplio rango de recursos ambientales por la eficiencia de explotar un conjunto particular de estos, y a pesar de no ser una especie de amplio rango cuenta con un reservorio genético importante lo que puede contribuir en su adaptabilidad a los cambios ambientales graduales.

Este tipo de evaluaciones permite tener un panorama más claro sobre la respuesta que generan las especies frente al cambio de las condiciones ambientales y tomar medidas mucho más puntuales para su manejo y conservación.

VII. REFERENCIAS BIBLIOGRÁFICAS

Ackerly, D. D., C. A. Knight, S. B. Weiss, K. Barton and K. P. Starmer. 2002. Leaf size, specific leaf area and microhabitat distribution of chaparral woody plants: contrasting patterns in species level and community level analyses. Oecología.

Buckling A, Brockhurst MA, Travisano M, et al. (2006) Experimental adaptation to high and low quality environments under different scales of temporal variation. Journal compilation 20:296-300.

Cubas P, Vincent C, Coen E (1999) An epigenetic mutation responsible for natural variation in floral symmetry. Nature 401: 157-161.

Fazlioglu, F., Al-Namazi, A., & Bonser, S. P. (2016). Reproductive efficiency and shade avoidance plasticity under simulated competition. Ecology and Evolution, 6(14), 4947-4957.

Frankham R (2005) Genetics and Extinction. Biological Conservation 126:131-140.

Galván-Hernández, D., J. A. Lozada-García, N. Flores-Estévez, J. Galindo-González and S. M. Vázquez-Torres. 2015b. Altitudinal gradient effect on morphometric variation and leaf symmetry of *Platanus Mexicana* Moric. Revista Chapingo Serie Ciencias Forestales y del Ambiente. 21: 171-183.

Grant-Downton, Dickinson HG (2006) Epigenetics and its Implications for Plant Biology 2. The ‘Epigenetic Epiphany’: Epigenetics, Evolution and Beyond. Annals of Botany 97: 11-27

Kaing-Feng M, Qi-Xiang Z, Tang-Ren Ch, et al (2018) Substantial Epigenetic Variation Causing Flower Color Chimerism in the Ornamental Tree *Prunus mume* Revealed by Single Base Resolution Methylome Detection and Transcriptome Sequencing. International Journal of Molecular Science 19, 2315.

Ketola T, Mikonranta L, Zhang L, et al. (2013) fluctuating temperature leads to evolution of thermal generalism and preadaptation to novel environments. Evolution 67:2936-2944.

King, N.G., N. J. McKewon, D. A. Smale and J Moore. 2018. The importance of phenotypic plasticity and local adaptation in driving intraspecific variability in thermal niches of marine macrophytes. Echography. 41: 1469-1489

Lele L, Ning D, Cuiping P, Xiao G, Weihua G (2017) Genetic and epigenetic variations associated with adaptation to heterogeneous habitat conditions in a deciduous shrub. Ecology and Evolution 8: 2594-2606.

Nicotra AB, Atkin OK, Bonser SP, et al. (2010) Plant phenotypic plasticity in a changing climate. Trends in Plant Science 15: 684-692.

Richards CL, Bossford O, Muth NZ, et al. (2006) Jack of all trades, master of some? On the role of phenotypic plasticity in plant invasions. Ecology Letters 9:981-993.

Sánchez-González, A., Octavio-Aguilar, P., Barrientos-Lozano, L., & Meerow, A. W. 2019. Effect of elevation strata on morphological variation of two agave species with different niche amplitude. International Journal of Plant Sciences, 180(8), 892-901.

Sung S, Amasino R (2004) Vernalization in *Arabidopsis thaliana* is mediated by the PHD finger protein VIN3. Nature 427: 159-164.

Van Tienderen P (1997) Generalists, specialists, and the evolution of phenotypic plasticity in sympatric populations of distinct species. Evolution 5:1372-1380.